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ABSTRACT

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P01
Reproductive Genetics/Prenatal Genetics

P01.001A

Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families

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Introduction: We have previously shown an extensive load of somatic copy number variations (CNVs) in the human placental genome with the highest fraction detected in normal term pregnancies[1]. Our parallel study on recurrent pregnancy loss (RPL) identified that the profile of parental CNVs may be critical for a viable pregnancy[2]. Hereby, we hypothesized that insufficient promotion of CNVs may impair placental development and lead to RPL.

Materials and Methods: We analysed placental and parental CNV profiles of idiopathic RPL trios (mother-father-placenta) and duos (mother-placenta). CNV detection pipeline included genome-wide genotyping and CNV calling using three alternative algorithms.

Results: Consistent with the hypothesis, the placental genomes of RPL cases exhibited 2-fold less CNVs compared to uncomplicated 1st trimester pregnancies[3]. This difference mainly arose from lower number of duplications. Overall, 1st trimester control placentas shared only 5.3% of identified CNV regions with RPL cases, whereas the respective fraction with term placentas was 35.1%. Interestingly, genes with higher overall expression were prone to deletions. Additionally, large pericentromeric and subtelomeric CNVs in parental genomes emerged as a risk factor for RPL.

Conclusions: Early placental development may need a burst of somatic genomic rearrangements to guarantee active proliferation, migration and invasion of trophoblasts.

[1]Kasak, L et al. Sci. Rep. 2015
[2]Nagirnaja, L, Palta P, Kasak L et al. Hum. Mutat. 2014
[3]Kasak et al. 2017 submitted

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P01.002B

Validation and application of a novel integrated genetic screening method to a cohort of 1,112 men with idiopathic azoospermia or severe oligozoospermia

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Infertility affects 10–15% of all couples and about half of the cases can be assigned to male factors. Microdeletions of the Y chromosome (YCMs) and Klinefelter syndrome (47, XXY) are common causes of severe oligozoospermia (reduced sperm count) and azoospermia (no sperm in ejaculate), but the majority of cases remain idiopathic. Here we describe a single molecule Molecular Inversion Probes (smMIP) assay, to screen infertile men for variation in known and candidate disease genes.

We designed a set of 4,525 smMIPs targeting the coding regions of causal (n = 6) and candidate (n = 101) male infertility genes. After validation, we screened 1,112 idiopathic infertile men who presented with azoospermia or severe oligozoospermia at the outpatient clinics of the Radboudumc and Monash Universities. Next to 5 patients with YCMs and 5 with chromosomal anomalies (47,XXY, 47,XYY and 46,XX male), we identified 159 patients with rare point mutations or Copy Number Variations (CNVs) affecting known infertility genes (*AURKC*, *CFTR*, *DDX3Y*, *DPY19L2*, *SYCP3*, *TEX11*). A genetic diagnosis that may explain the infertility was made in 18 patients (1.6%). Evaluation of variants in 101 candidate genes is ongoing and may increase diagnostic yield.

In conclusion, we developed a flexible and scalable method to reliably detect point mutations, CNVs and sex chromosomal anomalies causing male infertility. The assay consolidates the detection of different types of genetic variation while increasing the diagnostic yield and detection precision at the same or lower price compared to currently used methods and helps to define more genetic infertility causes to inform doctors and patients.

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P01.003C

FOG2/ZFPM2 is a noval disease gene for primary ovarian insufficiency

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Background: Premature ovarian insufficiency (POI) is a major cause of female infertility. The genetic causes of POI remain mainly unknown. We hypothesized that *Friend of GATA 2* (*FOG2/ZFPM2*) haploinsufficiency could contribute to POI pathogenesis, based on the identification of a heterozygous *FOG2* deletion in a child with an isolated 46, XY disorder of sex development (DSD) and in his 25-years old mother with low serum anti-Müllerian hormone (AMH) levels, suggestive of a decreased ovarian reserve. This hypothesis is supported by the role of *FOG2* in mouse ovarian development.

Methods: Targeted next-generation sequencing of *FOG2* was conducted in 31 Belgian and 357 French POI cases. Copy number analysis of *FOG2* was performed with array-CGH followed by qPCR. Functional validation of the identified *FOG2* variants was performed by luciferase assays in HEK293T cells.

Results: A heterozygous *FOG2* deletion was found in a child with isolated 46,XY DSD and in his mother with putative POI risk. Subsequently, seven novel, likely pathogenic *FOG2* variants were found in a heterozygous state in a large POI cohort (7/388, 1.8%), five of which are missense variants, one in-frame duplication and one frameshift. Luciferase assays showed significantly lowered transcriptional activation of the mutated FOG2/GATA4 complex for several of the identified variants.

Conclusion: We demonstrate that *FOG2* haploinsufficiency can lead to POI, supporting a role of *FOG2* in human ovarian maintenance and contributing to the molecular pathogenesis of POI. Finally, we expand the phenotypic spectrum of *FOG2* mutations, thus far associated with cardiac anomalies, congenital diaphragmatic hernia and 46,XY DSD.

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P01.004D

Detection of *de novo* structural chromosomal rearrangements with nucleotide level resolution for assessment of their clinical outcome in prenatal diagnosis

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Recognition of *de novo* (dn) balanced chromosomal aberrations (BCAs) leading potentially to congenital anomalies constitutes a huge challenge in prenatal diagnosis (PND). Therefore, cytogenetic and cytogenomic approaches, including CMA have to be complemented by methods allowing identification of breakpoints at nucleotide resolution within the actionable time frame of a prenatal diagnosis. Concomitantly with the conventional PND protocol, we apply large-insert whole-genome sequencing for identification of dnBCA breakpoints in two prenatal samples. The first fetus, referred for PND because of increased risk, is a female with a t(16;17)(q24;q21.3)dn. Sequencing identified the 16q24 and 17q21.3 breakpoints within IVS3 of *ANKRD11* and IVS1 of *WNT3*, respectively. Haploinsufficiency of *ANKRD11* causes dominant KBG syndrome (OMIM #148050), whereas of *WNT3* results in recessive tetraamelia syndrome (OMIM #165330). Although the translocation results in fusion genes no evidence of chimeric transcripts was found. The second fetus, referred for PND due to maternal anxiety, is a male with a t(2;19)(p13.3;q13.11)dn. The 2p13.3 breakpoint is localized within IVS1 of *ATP6V1B1* whereas that of 19q13.11 is proximal to *CEP89*. *ATP6V1B1* causes a recessive form of renal tubular acidosis with progressive sensorineural deafness (OMIM #192132). Clinical features of the newborns corroborate the predicted outcome in the light of chromatin topological association domains (TADs); KBG syndrome in the first case and a normal phenotype in the second case. Although prediction of the phenotypic outcomes highlights the gaps in our current knowledge, this approach allows improved genetic counselling. Therefore, we recommend inclusion of this approach into current PND care. Research grant: FCT HMSP-ICT/0016/2013.

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P01.005A

Large population cohorts reveal unrecognized adult traits of the 16p11.2 CNV syndromes

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Disorders caused by rare CNVs are a common health problem, but their phenotype associations have almost exclusively been investigated in clinically ascertained (often pediatric) patients.

The 16p11.2 600kb BP4-BP5 rearrangements have a population prevalence of about 1 in 1,000. Akin to other rare CNVs, they have so far been studied mostly in cohorts pre-selected for developmental disorders and thus adult data are lacking. To overcome such ascertainment bias, we have combined data from the pan-European and the Simons VIP cohort of 16p11.2 families (584 individuals) with the UK Biobank and the Estonian Genome Center general population cohorts (>160,000 individuals). This "disease agnostic" approach has identified a significant association between

16p11.2 dosage alterations and reproductive health. In female UK Biobank participants, 16p11.2 CNVs are associated in a dosage-dependent manner with age of menarche: compared to controls significant decrease in deletion ($\Delta = -1.33$ years, $p = 2.4 \times 10^{-4}$) and increase in duplication carriers ($\Delta = +1.42$ years; $p = 8.9 \times 10^{-4}$). The variance between the three groups remained significant even after correction for BMI ($p = 1.3 \times 10^{-7}$). We replicated these associations using the geographically distinct 16p11.2 cohorts. Importantly, in the investigated cohorts both female and male 16p11.2 CNV carriers have significantly increased prevalence of reproductive tract disorders. This is further paralleled by 16p11.2 dosage dependent alterations in the hypothalamus in mouse models and humans' brain imaging studies. Despite numerous previous studies on the 16p11.2 CNVs, the link with the reproductive axis remained overlooked, illustrating that identification of traits associated with rare variants in unselected populations provides valuable and unbiased insight into disease etiologies.

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P01.006B

Analytical Validation of a SNP-Based Noninvasive Prenatal Test to Detect the 22q11.2 Microdeletion

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Background: Non-invasive prenatal testing (NIPT) for aneuploidy using cell-free DNA in maternal plasma has been widely adopted. Recently, NIPT coverage has expanded to detect subchromosomal anomalies including the 22q11.2 microdeletion. Validation of a SNP-based NIPT for detection of 22q11.2 deletions showed high sensitivity (97.8%) and specificity (99.75%) (Wapner, 2015; Gross 2016). Here, we validated an updated version of this test in a larger cohort of pregnancy plasma samples. **Materials and Methods:** Blood samples were obtained from pregnant women at participating hospitals and contract research organizations. Ten positive control samples and 390 negative control samples were analyzed using an updated SNP-based NIPT for the 22q11.2 microdeletion. Samples were amplified and sequenced using pooled primer sets that included 1,351 SNPs spanning a 2.91Mb section of the 22q11.2 region. A risk score was assigned to all samples as previously described. The algorithm's confidence threshold was raised to 0.95 and all "high-risk" samples were reflexively sequenced at high depth of read (6×10^6 reads/sample). The sensitivity and specificity of the assay were measured. **Results:** Sensitivity of the assay was 90% (9/10), and specificity of the assay was 99.74% (389/390), with a corresponding false positive-rate of 0.26%. **Conclusions:** This validation of the updated SNP-based assay in a cohort of pregnancy-plasma samples demonstrates that it detects the 22q11.2 microdeletion with high sensitivity and specificity. Given the benefits of early intervention in patients with the 22q11.2 deletion and the high incidence of the condition, this SNP-based methodology provides a valuable addition to current population-wide prenatal screening approaches.

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P01.007C

Non-invasive prenatal testing (NIPT) for 22q11.2 deletion syndrome using a targeted microarray-based cell-free (cfDNA) test

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Objective: To determine the analytical performance of a targeted microarray based cfDNA test (Harmony Prenatal Test®) for the identification of pregnancies at increased risk for a 22q11.2 deletion.

Methods: 1736 plasma samples were studied. 122 samples had a 22q11.2 deletion ranging in size from 1.96 to 3.25 Mb. These included simulated pregnancy samples and maternal plasma from pregnancies with a fetal 22q11.2 deletion. The simulated samples were created with plasma from individuals with 22q11.2 deletions titrated with plasma from unaffected, non-pregnant women to simulate fetal fractions ranging from 4% to 33%. 1614 samples were from women with presumed unaffected pregnancies in which the maternal and fetal 22q11.2 deletion status was unknown. Cell-free DNA analysis using Digital ANalysis of Selected Regions (DANSR™) was performed as previously described with additional assays for assessment of fetal 22q11.2 deletions within a 3.0 Mb region. DANSR

products were quantified on custom microarrays and the probability of a deletion being present was determined using the FORTE™ algorithm.

Results: Of the 122 samples with a 22q11.2 deletion, 92 were determined to have a high probability of a deletion (sensitivity: 75.4%; 95% CI: 67.1–82.2%). No evidence of a deletion was observed in 1606 of the 1614 samples from presumed unaffected pregnancies (specificity: 99.5%; 95% CI: 99.0–99.7%).

Conclusions: Targeted cell-free DNA testing using microarray quantitation is able to identify pregnancies at increased risk for 22q11.2 deletions of 3.0 Mb and smaller while maintaining a low false positive rate.

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P01.008D

False 46,XX sex reversal in infertile male patient

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We report on the clinical case of false 46,XX-sex reversal. A proband is 24 years-old man referred to genetic evaluation because of infertility and non-obstructive azoospermia. Previously, proband's chromosome analysis on cultivated peripheral lymphocytes showed non-mosaic 46,XX karyotype, and a diagnosis '46,XX testicular DSD (46,XX male syndrome)' was suspected. However, the detailed questioning revealed that the patient was subjected to allogeneic transplantation of haematopoietic stem cells from his health sister. Allo-HSCT was performed because of aplastic anemia, which the patient developed at 7 years old. After that procedure, he developed chronic Graft-versus-host disease; therefore, the patient received continuous immune-suppression therapy. Because of the transplantation, genetic evaluation was needed to carry out on non-haematopoietic cell lines. Repeated cytogenetic and FISH analysis with X and Y centromeric DNA (CEPX, CEPY) probes were performed on cultivated skin fibroblasts. SRY, ZFX/ZFY loci and the Y chromosome microdeletions of AZFa,b,c regions were analyzed by multiplex PCR on genomic DNA extracted from buccal smears. The patient was found to be SRY positive and to have non-mosaic 46, XY karyotype in non-haematopoietic cells. Results of interphase FISH analysis: nuc ish (DXZ1,DYZ3) × 1[300]. No Y chromosome microdeletion was detected. Combined genetic examination allowed to reject '46,XX testicular DSD' diagnosis. Possible male infertility in the patient was due to long-term immune-suppression therapy.The presented case demonstrates the need for detailed collection of anamnestic information and factors that may affect results of genetic research, including transplantation and blood transfusion, and the successful use of combined genetic analysis in mosaics and chimeras.

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P01.009A

Comparison of variants of uncertain significance (VOUS) using different array CGH resolution platforms-implication in prenatal diagnosis

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Introduction: Over the past few years, aCGH application in prenatal diagnosis has contributed significantly in chromosomal microdeletions/microduplications detection. However, the implementation of aCGH also gave rise to the detection of variants of uncertain significance (VOUS). The present study is a comparative investigation of low and high-resolution aCGH application in prenatal diagnosis in terms of VOUS findings.

Materials and Methods: 2646 prenatal cases were referred to our center during the period 2012–2016. 2325

cases were analyzed using low-resolution aCGH (1Mb backbone resolution-Cytochip Focus Constitutional, Bluegnome), and 321 using high-resolution aCGH (500kb backbone resolution-SurePrint G3 ISCA V2, CGH 8 × 60k, Agilent Technologies).

Results: Out of 2325 cases analyzed by low-resolution aCGH, 58 cases resulted in a known chromosomal imbalance (2.5%) and 45 cases showed a VOUS (1.9%). Accordingly, out of 321 cases analyzed with high-resolution aCGH, 13 cases resulted in a known chromosomal imbalance (4.1%) and 32 cases showed a VOUS (10.0%). Parental origin of each VOUS was tested where possible (53/77 cases) and in the majority of cases the variant was inherited from one of the two parents (46/53 cases - 86.8%).

Conclusions: Application of high resolution aCGH in prenatal diagnosis led to the detection of more pathogenic aberrations in comparison to low-resolution aCGH (2.5% to 4.0%), increasing the diagnostic yield. However, VOUS detection frequency was significantly elevated (1.9% to 10.0%), making genetic counseling more complex and increasing anxiety of the future parents. The present study indicates that the implementation of high resolution aCGH in prenatal diagnosis should be thoroughly evaluated.

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P01.010B

A novel mutation causes Non Obstructive Azoospermia in infertile men

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Introduction: Infertility is defined as a failure of conception after 12 months of having unprotected intercourse, and male infertility accounts for 30–55% of infertile couples. Azoospermia, is diagnosed when sperm is completely absent in the ejaculate even after centrifugation.

Materials and Methods: Genotyping was done on four azoospermic individuals of a consanguineous Bedouin family and their parents. Exome sequencing was performed on the DNA of one patient.

Results: Assuming homozygosity of a recessive founder mutation as the likely cause of the disorder, we have genotyped 4 patients and their parents. We identified 5 shared homozygous regions larger than 2 cM, encompassing a total of 13.8Mbp on the autosomal chromosomes. In these regions only one homozygous variant with allele frequencies of less than 1% in the public databases (ExAc browser, 1000 Genomes and dbSNP) was identified. This variant segregated as expected in the family, with a calculated Lod score of 3.42. The variation was not present in 620 Bedouin controls.

The variation is a frameshift mutation in a gene encoding a protein demonstrated to be essential for silencing of Line-1 retrotransposon in the male germline. Using a commercial antibody to the N-terminus of the encoded protein, immunofluorescent studies demonstrated it is produced in patients' testes, especially in spermatogonial cells (mainly in the cytoplasm) and in spermatocysts/round spermatids (mainly in the nucleus).

Conclusions: The identification of the mutation causing azoospermia enables accurate diagnosis in the enlarged family and demonstrates the importance of repressing retrotransposon activation in the male germline in human.

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P01.011C

Using molecular karyotypes of blastocoel fluid to obtain data about the origin of aneuploidies in human pre-implantation embryo development

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Introduction: Due to cell elimination during preimplantation development, the DNA from some cells enters into cavity of blastocyst. Analysis of the blastocoel fluid apparently provides additional information about aneuploidy that presented in the embryo cells. Therefore, we can more confidently suggest the origin of aneuploidies.

Materials and Methods: Fifteen human blastocysts of good quality were analyzed on the day 5th of embryonic development. All embryos were obtained in treatment cycles of IVF, underwent blastocoel collection and separation into inner cell mass and trophectoderm cells under informed consent. Microarray-based comparative genomic hybridization was used to analyze molecular karyotypes of all trio samples.

Results: A total of 77 aneuploidies were detected in 11 from 15 embryos. Six embryos (40%) had a chromosomal mosaicism with the presence of 2 or more different aneuploidies. Four detected aneuploidies (5.2%) had apparently a meiotic origin, as they were observed in both inner cell mass and trophectoderm of the same embryo. Probably, sixteen aneuploidies (20.8%) had a mitotic origin because they were presented by reciprocal aneuploidies in inner cell mass and trophectoderm or in trophectoderm cells and blastocoel fluid. Most of the identified aneuploidies (74%) were confined by only one tissue type.

Conclusions: Our results suggest that majority of chromosomal abnormalities have mitotic origin during early stages of preimplantation development. They provide also evidence for underestimation of chromosomal mosaicism in human blastocysts.

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P01.012D

Recessive Complex Brachydactyly, Symphalangism, Zygodactyly and Male Infertility - not a Novel Syndrome but Caused by Linked Mutations in *BMPR1B* and *PDHA2*, a New Male Infertility Gene

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Introduction: We present a consanguineous family with six sibs afflicted with complex brachydactyly, symphalangism and zygodactyly plus infertility in males due to azoospermia or sperm immotility/death, which we hypothesized as a new syndrome. *BMPR1B* is known to be responsible for dominant brachydactyly type A1 and type A2 and recessive acromesomelic chondrodysplasia. Animal models indicate that many genes are involved in mammalian spermatogenesis. One such gene is exclusively testis-specific *PDHA2*, encoding the mitochondrial matrix enzyme PDHA2 expressed during spermatogenesis.

Materials and Methods: Candidate disease loci were found by homozygosity mapping using SNP data from four affected and three unaffected members of the family. Exome sequencing was applied to find rare/novel variants at the loci.

Results: We mapped the disease locus and identified novel homozygous missense variant c.640C > T (p.(Arg214Cys)) in *BMPR1B* in patients. We also detected homozygous c.679A > G (p.(Met227Val)) in *PDHA2* in infertile brothers. Structural protein modelling, protein sequence conservation and *in silico* analysis indicate that both variants are damaging.

Conclusion: The complex brachydactyly afflicting the family is the first recessive brachydactyly reported to date. *PDHA2* is a novel candidate gene for male infertility. The disease afflicting the family is a unique example of two linked variants, approximately 711 Kbp apart, in different genes that together manifest as a seemingly novel syndrome. Hence, we conclude that exome sequencing and not candidate gene approach should be employed in disease gene hunt, defining new diseases and genetic testing. Grants: TÜBİTAK grant 114Z829, Boğaziçi University Research Fund 10860 and FONDECYT no. 1150743.

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P01.013A

MPS-based carrier screening: search deeper, not wider

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Rapid development of Massive Parallel Sequencing (MPS) technologies opens wide possibilities for reproduction medicine such as carrier screening for monogenic disorders. While available MPS-based panels tend to expand including more and more inheritable conditions one may raise a concern regarding clinical proportion of such an expansion. Addition of extremely rare conditions, those of multifactorial inheritance, reduced penetrance or mild phenotype worsen technical performance of the test and complicates genetic counseling. Wider scope means decreased coverage depth leading to potential inaccuracy in residual risk assessment for severe genetic condition with childhood-onset. Expanded carrier screening sacrifices quality (clinical value) of the test for quantity (number of condition screened). We established a distinct approach to MPS carrier test for gamete donors and couples. NextGen21 genetic test includes 21 genes associated with frequent, severe genetic conditions. Target regions of the assay were selected by combining functional regions of the genes (CDSs, splicing sites and UTRs) and the hotspots (i.e. clinically significant variants). Test was verified by human DNA standards from NIST and the 1000 Genomes Project. CFTR and PAH regions were additionally verified by Sanger sequencing. The assay was applied to 42 gamete donors and 29 patients. Carriers of X-linked and autosomal-dominant conditions with late-onset were excluded from donor programme. 14 pathogenic variants (1096 unique overall) were detected. Rare pathogenic variants found in genes CFTR, PAH and GBA proves tagline "search deeper, not wider" credible in terms of residual risk assessment, therefore, clinical proportion of test overall.

R. Vasilev: A. Employment (full or part-time); Modest; NGC Research Lab. **S. Viatkina:** A. Employment (full or part-time); Modest; NGC Research Lab. **M. Strizhova:** A. Employment (full or part-time); Modest; NGC Research Lab. **N. Kornilov:** A. Employment (full or part-time); Modest; Next Generation Clinic. **A. Pavlov:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; ParSeq Lab. **T. Simakova:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; ParSeq Lab. **J. Vnuchkova:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; ParSeq Lab. **M. Glushkova:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; ParSeq Lab. **A. Bragin:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; ParSeq Lab.

P01.015C

Expanded carrier screening for recessive disorders: review of the characteristics of current internet-based offers

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Carrier screening allows identification of healthy prospective parents who are at risk of conceiving a child affected with a monogenic recessive disorder. Recently, expanded carrier screening (ECS), which can identify carriers of a large number of recessive disorders in the general population, has grown in popularity and is now widely accessible, primarily through commercial companies. We performed a review of internet-based ECS offers in January 2017 to investigate the characteristics of ECS tests currently available, such as the number and nature of conditions screened. We excluded ethnicity-based carrier screening initiatives, research-centered ECS pilot programs, and offers where test characteristics, such as the lists of included genes/disorders were unavailable. We identified fifteen providers of ECS tests: 12 commercial companies, two medical hospitals and one academic medical center. The majority (12) of the providers were based in the USA, with the remaining three located in the Netherlands, Spain, and South Korea. We observed drastic differences in the characteristics of ECS tests, with the number of conditions ranging from 41 to 1700. Only three conditions (Cystic fibrosis, Maple syrup urine disease 1b, and Niemann-Pick disease) were screened for by all providers. Where the same disease gene was analyzed by multiple providers, substantial differences existed in the mutations included and/or variant interpretation methodologies. Given the importance of carrier screening results in reproductive decision-making, the observed heterogeneity across ECS panels is concerning. Efforts should be made to ensure that clear and concrete clinical criteria are in place to help inform the development of ECS panels.

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P01.016D

Identifying unexpected carriers with expanded pan-ethnic carrier testing

	Cystic fibrosis		Spinal muscular atrophy		Fragile X		Fanconi anemia, type C		Tay-Sachs disease	
	Expected positives	Observed positives	Expected positives	Observed positives	Expected positiλves	Observed positives	Expected positives	Observed positives	Expected positives	Observed positives
African American	58.3	57	46.5	48	16.7	16	0.5	0	8.5	2
Ashkenazi Jewish	15.67	7	8.1	3	1.46	1	4.1	4	11.3	14
Asian	9.75	4	28.6	28	6.28	4	0.2	0	3.2	1
Caucasian	420	483	297.9	230*	42.35	55	1.4	15*	21.7	46*
Hispanic	123	204*	65.7	133*	32.39	21	1	2	16.7	5

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Introduction: Ethnicity-based carrier testing for single gene conditions is the current standard of prenatal care. Technological advances have allowed for rapid expanded carrier testing for hundreds of genetic mutations across a significant number of conditions, improving detection of at risk carrier couples in non-targeted ethnic groups.

Methods: A retrospective review of over 30,000 carrier test results was performed to identify positive carriers of 23 conditions included in ACOG/ACMG recommendations. Ethnicity was self-reported. For each condition and ethnicity, expected numbers of positive carriers were calculated and compared to observed rates. A Chi-square analysis was performed to calculate statistical significance ($p \leq .01$).

Results: When evaluating for Ashkenazi Jewish diseases, a higher than expected number of patients who identify as Caucasian screened positive for Tay-Sachs disease (46 individuals observed as carriers vs 21.7 expected) and Fanconi anemia type C (15 individuals observed as carriers vs 1.4 expected). Patients who identified as Hispanic screened positive for CF and SMA more often than expected as well. All of these observations were statistically significant. A select data set is represented in Table 1.

Conclusion: Testing patients with an expanded pan-ethnic panel will identify more carriers of conditions that would be missed by current ethnicity-based conventions. Expanded carrier testing will identify more carriers, allowing for more complete risk assessments and consideration of planning options.

Table 1: * indicates $p \leq 0.01$

A.E. Harbison: A. Employment (full or part-time); Significant; Progenity. **C. Terhaar:** A. Employment (full or part-time); Significant; Progenity. **C. Settler:** A. Employment (full or part-time); Significant; Progenity. **L. Dohany:** A. Employment (full or part-time); Significant; Progenity. **C. Holland:** A. Employment (full or part-time); Significant; Progenity. **R. Longman:** None.

P01.017A

CarrierTest - the expanded preconception carrier screening

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In recent years clinical genetic testing has moved from gene-by-gene direct analysis of distinct monogenic hereditary disorders to more complex approaches using high throughput methods, such as next-generation sequencing (NGS). This approach can be used for testing of carrier status of significant autosomal recessive diseases by preconception expanded carrier screening in couples without a positive family history.We have developed a NGS amplicon-based panel testing 874 key mutations of 81 genes which can influence reproductive health of prospective parents or can cause inherited diseases at offspring. CarrierTest targets (i) mutations of 72 genes causing serious recessive diseases at offspring of healthy carriers (genetic compatibility test); (ii) low penetrant mutations primarily associated with health conditions of prospective parents and influenced by external factors; (iii) mutations associated with blood hypercoagulability which can influence infertility treatment and pregnancy; (iv) inherited ovarian response to standard gonadotrophin stimulation.CarrierTest uses locally assembled bioinformatic pipeline and database implemented into laboratory information system. To replace MLPA and fragmentation analysis methods we developed coverage analysis-based CNV detection of frequent large deletions of SMN1 and CFTR genes. A software tool developed for this application generates semi-automatically

result report. The report contains comparative analysis of detected variants for evaluation of residual risk and pre-conception compatibility of couple for consideration of preimplantation or prenatal diagnostics.

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P01.018B

Surprisingly good outcome in antenatal diagnosis of severe hydrocephalus related to *CCDC88C* deficiency

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Non-syndromic congenital hydrocephalus is aetiologically diverse and while a genetic cause is often suspected, it often cannot be confirmed. The most common genetic cause is the *L1CAM*-related X-linked hydrocephalus and that explains only 5%-10% of all male cases. This underlines a current limitation in our understanding of the genetic burden of non-syndromic congenital hydrocephalus, especially for those cases with likely autosomal recessive inheritance. Additionally, the prognosis for most cases of severe congenital hydrocephalus is poor, with a majority of surviving infants displaying significant intellectual impairment despite surgical intervention. It is for this reason that couples with an antenatal diagnosis of severe hydrocephalus are given the option, and may opt, for termination of the pregnancy. We present two families with *CCDC88C*-related autosomal recessive congenital hydrocephalus with children who had severe, antenatally diagnosed hydrocephalus. Those individuals who were shunted within the first few weeks of life, who did not require multiple surgical revisions, and who had a more distal truncating mutation of the *CCDC88C* gene met their early childhood developmental milestones in most cases. This suggests that children with *CCDC88C*-related autosomal recessive hydrocephalus can have normal developmental outcomes. We recommend *CCDC88C* analysis in cases of severe non-syndromic congenital hydrocephalus, especially when aqueduct stenosis with or without a medial diverticulum is seen, in order to aid prognosis discussion.

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P01.019C

Noninvasive prenatal detection of extra chromosome abnormalities by cell-free DNA testing

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Introduction: Although noninvasive prenatal testing (NIPT) has been well studied for its efficacy in detecting common trisomies, there is a lack of clinical evaluation of its performance in detecting extra chromosome abnormalities. Herein in this study, we aimed to evaluate the performance and incremental yield of NIPT in detecting sex chromosomal aneuploidy, chromosome copy number variants, and other incidental findings.

Materials and Methods: We performed genome-wide NIPT in 15633 consecutive pregnancies prospectively collected in our center. In each case chromosomal abnormality was analyzed in all 23 pairs of chromosome. NIPT results were accordingly reported and counseled depending on the presence of other chromosome abnormalities. NIPT results were validated by invasive diagnosis of positive results and phenotypic follow-up of negative results.

Results: NIPT identified a total of 191 cases (1.2%) of chromosomal abnormality including 101 common trisomies (T21/T18/T13) and 90 extra abnormalities. By reporting extra abnormalities, NIPT increased the detection yield from 0.5% to 0.76% ($p = 0.0086$), and had the overall sensitivity of 97.14% (95%CI 91.27%–99.26%), specificity of 99.63% (95%CI 99.51%–99.72%), and PPV of 66.67 (95%CI 58.53%–73.93%). There was no significant reduction of the sensitivity and PPV by reporting extra chromosome abnormality, although specificity dropped significantly ($p = 0.0001$). Nonetheless, comparing with conventional biochemical screening, NIPT still had 60 times higher true positive rate and a small number of false positive results.

Conclusions: NIPT can detect extra chromosome abnormalities with high sensitivity and specificity. It may

offer clinical benefits due to incremental yield and outperformed efficacy than conventional biochemical screening.

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P01.020D

Evaluation of the fetal epigenetic marker *RASSF1A* for detection and quantification of cell-free fetal DNA in maternal blood

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Introduction: The aim of this study was to quantify cell-free fetal DNA (cffDNA) with *SRY* gene and the fetal epigenetic marker *RASSF1A*, in maternal plasma samples of pregnant women in the first trimester of gestation. The objective was to evaluate the potential of the universal marker *RASSF1A* for quantification of fetal DNA independently of the fetal sex.

Materials and Methods: From 102 maternal plasma samples collected between 8–12 weeks of gestation, there were 62 male pregnancies and 40 female pregnancies. Maternal plasma DNA was extracted, digested with methylation-sensitive restriction enzyme BstUI, and the fetal specific DNA was quantified by the QX200 Droplet Digital PCR (ddPCR™, Bio-Rad) system.

Results: The results observed by ddPCR revealed that; a) in male pregnancies, the median fetal fraction for *SRY* gene was 7.8% and for hypermethylated *RASSF1A* gene it was 2.9%. b) In female pregnancies, there was no amplification for the *SRY* gene in any sample, and the median fetal fraction observed for *RASSF1A* gene was 2.7%.

Conclusions: Digital PCR technology has shown a high sensitivity in the detection and quantification of cffDNA in the first trimester of gestation, since all plasma samples has been well characterized. However, the universal epigenetic marker *RASSF1A* has shown an underestimation of fetal fraction compared to *SRY* gene in male pregnancies during this early period of gestation, suggesting that *RASSF1A* could be a good marker for detection of fetal DNA independently of fetal sex, but not the most accurate method of quantification in the first trimester of gestation.

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P01.021A

Prenatal diagnosis of chromosomal mosaicism in chorionic villus and amniotic fluid samples using array comparative genomic hybridization

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Introduction: Chromosomal microarray (CMA) is gradually replacing standard karyotyping for all prenatal indications. Chromosomal mosaicism is detected in less than 1% of cultured chorionic villus samples (CVS) by karyotyping. The frequency of CMA-detected chromosomal mosaicism in uncultured prenatal samples is not known. The aim of this study was to evaluate the detection of chromosomal mosaicism in uncultured CVS and amniotic fluid (AF) samples using CMA. Materials and Methods: All patients undergoing invasive prenatal testing by CMA were included in the study. CMA was carried out on a total of 2377 uncultured samples: 906 AF and 1471 CVS. **Results:** Chromosomal mosaicism was detected in 53 cases (2.2%, CI: 1.7–2.9), of which 41 were detected in CVS (2.8%, CI: 2.1–3.8), and 12 in AF (1.3%, CI: 0.8–2.3). In 13 cases (25%) we detected sex chromosome mosaicism (45,X/46, XX (n = 5), 45,X/46,XY (n = 6), 47,XXY/46,XY (n = 2)), in 22 cases (42%) mosaicism for autosomal chromosomes (4 common and 18 other), and in 17 cases (33%) mosaicism for a copy number variation. Conclusion: This study demonstrates that CMA as a first-line test is able to identify chromosomal mosaicism. CMA on uncultured prenatal sampels may be more likely detect mosaicism for unbalanced chromosomal abnormalities than traditional cytogenetic techniques.

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P01.022B

The role of chromosomal microarray analysis in pregnancies with isolated pelvic kidney

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A single pelvic kidney is observed in up to 1:600 pregnancies. In some countries this finding mandates a routine amniocentesis for chromosomal microarray analysis testing. The objective of our study was to examine the risk for chromosomal aberrations among fetuses with apparently isolated pelvic kidney. Data from all chromosomal microarray analysis reported to the Ministry of Health between January 2013 and September 2016 were retrospectively obtained from a computerized database. Only pregnancies with sonographic diagnosis of isolated pelvic kidney and documentation of chromosomal microarray analysis result were included. Risk estimation was performed based on comparison the rate of abnormal observed CMA finding to the general population risk, based on systematic review encompassing 9272 cases of chromosomal microarray analysis for fetuses with normal ultrasound. Of 114 pregnancies with isolated pelvic kidney, two cases of known microduplication syndromes were demonstrated (absolute risk 1.75%, 95% confidence interval (CI) 0.2–6.2%). Both of the duplications were inherited from healthy mothers. In addition, six variants of unknown significance were detected (5.3%, 95% CI 1.96–11.1%). Calculation of the effect estimate for pathogenic chromosomal microarray analysis findings yielded a relative risk of 1.73 (95%CI 0.43–6.89) compared to control population ($p = 0.44$) Our study is the first report describing the rate of chromosomal anomalies in fetuses with isolated pelvic kidney. Its results question the routine recommendation for chromosomal microarray analysis analysis in such cases. Prospective well-adjusted studies are still needed to guide the optimal management of pregnancies with isolated pelvic kidney.

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P01.023C

CNV analysis of Turkish patients with congenital bilateral absence of the vas deferens:detection of a potential candidate gene

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Introduction: Congenital structural anomalies in man is one of the contributing factors of male infertility and one of these is called as Congenital Bilateral Absence of the Vas Deferens (CBAVD). According to the literature, 75–85 % of the patients with CBAVD carry at least one pathogenic *CFTR* mutation. Till date, there is no evidence showing an association of a nuclear gene associated with CBAVD. However, based on our published data, only 15,90% of Turkish CBAVD cases carry *CFTR* mutations. Therefore, we have decided to identify the candidate genomic regions that can cause CBAVD by using copy number variation (CNV) analysis.

Methods: Unrelated 19 CBAVD cases (wild genotype for *CFTR*) were included in the study. Genomic DNA was extracted from peripheral blood. Whole genome CNV analysis was performed with Affymetrix Cytoscan HD and Agilent ISCA 8×60K array-CGH platforms; analysed with the related softwares.

Results: We found 11 higly pathogenic microdeletion (in 25.5% of the cases) and microduplication (in 41% of the cases) regions in nine of the cases. Deletion in 14q11.2del is detected in three of the cases. Based on the literature, no known genes were localized in this shared deleted region (299 kb). However, Defender Against Cell Death 1 (*DAD1*) nuclear gene deletion which has also localized in 14q11.2 was detected in two of the cases.

Conclusion: In the light of these data, *DAD1* can be a potential candidate gene and 14q11.2 can be a potential chromosomal region for CBAVD cases.

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P01.024D

Beta-blocker use in pregnancy and risk of specific congenital anomalies: a European case-malformed control study

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Background: Hypertension prevalence is increasing, also in pregnant women. The most used antihypertensives during pregnancy are beta-blockers.

Objective: To investigate whether first trimester use of beta-blockers increases the risk of specific congenital anomalies in offspring.

Methods: A population-based case-malformed control study was conducted in 117,166 registrations of congenital anomalies from 17 EUROmediCAT registries. We tested associations previously reported in literature (signals) and performed an exploratory analysis to identify new signals. Odds ratios (ORs) of exposure to any beta-blocker or to a beta-blocker subgroup were calculated for each signal anomaly compared to two control groups (chromosomal anomalies and non-chromosomal, non-signal anomalies). The exploratory analyses were performed for each non-signal anomaly compared to all other non-signal anomalies.

Results: Cleft palate (CP, OR 10.0; 95% CI 1.9–52.2) and cleft lip with or without cleft palate (CL/P, OR 5.7; 95% CI 1.1–30.7) had significant increased odds of exposure to non-selective beta-blockers compared to chromosomal controls. The other literature signals (neural tube defects, congenital heart defects, hypospadias) were not confirmed. The exploratory analysis revealed that multicystic kidney disease (MCKD, OR 4.0; 95% CI 1.4–11.4) had significant increased odds of exposure to combined alpha- and beta-blockers.

Conclusion: Our study investigating first trimester use of beta-blockers confirmed the association between non-selective beta-blockers and CP and CL/P and found a possible new signal for MCKD and combined alpha- and beta-blockers, including labetalol. We did not find an increased risk for selective beta-blockers.

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De novo copy number variants and parental age

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It has long been known that older parents are at increased risk of having children with genetic disorders, however most of this relates to a maternal age effect on non-disjunction. Recent studies suggest advancing paternal age increases the risk of de novo single nucleotide mutations. The aim of our study was to investigate whether increased parental age is associated with an increased risk for de novo copy number variation (CNV) formation in offspring, since previous studies have yielded conflicting results. We investigated CNV calls from 2323 individuals referred to Signature Genomic Laboratories for clinical microarray-based comparative genomic hybridization. Overall 17% (388, 127 cases and 261 controls) were prenatal and 83% (1935, 664 cases and 1271 controls) were postnatal. The de novo CNV data was further split into de novo CNVs bound by low copy repeats (LCRs) and de novo CNVs not bound by LCRs. Despite our large sample size, we did not find any association between de novo CNV occurrence and paternal age in both of pre-natal ($p = 0.6795$) and postnatal ($p = 0.1741$) cohorts. We found advancing maternal age was associated with an increased de novo CNV occurrence in our postnatal cohort ($p = 0.0126$). Furthermore, maternal age was associated with higher rate of de novo CNVs that are bound by LCRs ($p = 0.0026$) and simple CNVs not bound by LCRs ($p = 0.0305$) but not complex rearrangements.

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Copy number variant caller evaluation in noninvasive prenatal testing

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Introduction: The clinical noninvasive prenatal testing (NIPT) is mainly focused on detecting whole-chromosome aneuploidies using low-coverage whole genome sequencing. However, various subchromosomal copy number variants such as microdeletions and microduplications (MD) are relatively common but challenging to identify. Recent articles show that these abnormalities can be sometimes detected based on few factors (sufficient fetal fraction, MD length, and coverage), but a statistical evaluation of these algorithms is missing.

Materials and Methods: We have developed a software that can identify and visualize small subchromosomal aberrations using low-coverage sequencing without prior knowledge of their location. It is based on published work and fine tuned for selected MDs. Training was performed on 790 samples with more than 7M reads.

Results: We have performed a study on pooled samples with 20M reads and the sensitivity is more than 95% when the fetal fraction is at least 10% and MD length is at least 3Mb. This is confirmed by real examples, where we have detected 1Mb, 2.6Mb, and 2.9Mb deletions with fetal fractions 12.5% and 17.5%, but not with 7.5%.

Conclusions: The algorithm for identifying MDs can be applied in clinical NIPT when fetal fraction and MD length is sufficient. The fetal fraction seems to be the most important factor - samples with 5% fetal fraction show very poor accuracy even for large MD, while small MD can be identified when the fetal fraction is high. The coverage does not appear to have such impact on detection accuracy when we carried out read count downsampling.

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Prenatal diagnosis of corpus callosum dysgenesis in 104 fetuses

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Introduction: Corpus callosum dysgenesis (CCD) is a spectrum of midline congenital anomalies, present in many monogenic syndromes. The aim of the work was the characterization of isolated and non-isolated forms of CCD and the identification of early predictors of associated anomalies, improving prenatal counseling.

Materials and Methods: We retrospectively analyzed 104 fetuses with CCD undergoing fetal magnetic resonance imaging (MRI) after ultrasonographic suspicion of CNS

anomalies. Intra and extra-cranial malformations were evaluated. Standard karyotype was obtained in 38 cases and molecular cytogenetics in 16. In 3 cases, in-depth analysis was performed by next-generation sequencing (NGS).

Results: At MRI, CCD was defined as isolated in 26.9%, the rest being associated to other abnormalities. In the group with additional findings, cortical dysplasia was the most frequently associated feature ($P = 0.008$), with a more frequent occurrence in complete agenesis (70%). Mesial frontal lobes were more often involved than other cortical regions ($P = 0.006$). Multivariate analysis confirmed the association between complete agenesis and cortical dysplasia (odds ratio = 7.29, 95% confidence interval 1.51–35.21). Genetic tests evidenced six fetuses with abnormal karyotype and three with cytogenomic defects. By NGS a Walker-Warburg syndrome, a channelopathy and a tubulinopathy were detected. In one isolated CCD case a de novo microdeletion was identified.

Conclusions: CCD is often complicated by intra and extra-cranial findings (cortical dysplasias being the most prevalent) significantly affecting postnatal prognosis. Cytogenetic analysis should always be performed in any CCD and, when imaging reveals a syndromic pattern, prenatal sequencing by NGS should be considered.

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Recurrent fetal encephalocele despite folate substitution in consanguineous couple - a severe phenotypic expression of Joubert syndrome

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Introduction: We describe a consanguineous, Indian couple with five gestations and hereof four fetuses with occipital encephalocele.

Materials and Methods: Exome sequencing was performed on both parents to identify variants, which the parents had in common and variants which in compound heterozygous form could cause encephalocele. Only formalin fixated fetal tissue, inappropriate for exome sequencing, was available. Subsequently, Sanger sequencing of two identified variants in *CSPP1* and *DHCR7* were conducted on two fetuses and on the couple's son. Tissue from the first two fetuses was not available because the terminations had occurred in India. Histopathologic examination of two fetuses was performed.

Results: Both parents were heterozygous for two variants: 1) *CSPP1* c.766C > T, p.(Gln256*). *CSPP1* is associated with Joubert syndrome 21 (JBTS21). The variant is suspected to be pathogenic in homozygous form, but has not previously been described. 2) *DHCR7* c.1091C > T, p.(Thr364Met). *DHCR7* is associated with Smith-Lemli-Opitz syndrome (SLOS). The variant has previously only been described in heterozygous form. It is uncertain if the variant in homozygote form will cause SLOS. The results of the Sanger sequencing and the histopathology are shown in table 1.

Conclusions: Based on the sequencing results it is likely that the recurring encephaloceles can be explained by the variant in *CSPP1*. Thus, we have identified a new pathogenic variant in *CSPP1* causing a severe and consistent presentation of JBTS21.

Individual		Father I.I		Mother I.II	
Genotype		CSPP1: +/- DHCR7: +/-		CSPP1: +/- DHCR7: +/-	
Individual	Fetus II.I	Fetus II.II	Son II.III	Fetus II.IV	Fetus II.V
Genotype	Unknown	Karyotype 46,XY	CSPP1: +/- DHCR7: +/+	CSPP1: +/+ DHCR7: +/-	CSPP1: +/+ DHCR7: +/+
Phenotype	Prenatal ultrasonography: Occipital encephalocele and microphthalmia	Histopathology: Occipital encephalocele, microphthalmia, bilateral cleft lip and cleft palate, and female external genitalia	1½ years old. Healthy according to the parents. Phenotypic examination is planned	Prenatal ultrasonography: Occipital encephalocele	Histopathology: Occipital encephalocele, bilateral cleft lip and cleft palate, and abdominal wall defect

Table 1. CSPP1, *CSPP1* c.766C>T, p.(Gln256*); DHCR7, *DHCR7* c.1091C>T, p.(Thr364Met).

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Molecular investigation of Cystic Fibrosis: methodological approaches and diagnostic evaluation

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Introduction: Cystic fibrosis (CF) is the second most common autosomal recessive genetic disease in Greek population. The present study shows the comparative results of 1742 cases that were referred to our center and analyzed by DGGE and NGS methodology for *CFTR* mutations.

Materials and Methods: A total of 1742 cases were analyzed using 3 different approaches: two protocols that identify 75% (505 samples) and 85% (525 samples) of the Greek known mutations by DGGE and a protocol that covers 96–98% (712 samples) of the known mutations in the Greek population by NGS technology (ION Ampliseq ™).

Results: CF-causing mutations frequencies identified by DGGE were 1.6% (8/505) for the 75% protocol and 5.3% (28/525) for the 85% protocol, while by using the NGS methodology 6.5% (46/712) of the samples were found to carry at least one CF-causing mutation. Frequencies of CFTR-Related Disease mutations were 0.8%, 5.9% and 9.6% for 75%, 85% and 96–98% methodologies respectively. Finally, mutations that were classified as variants of uncertain significance (VOUS) were detected in 0.8%, 1.5% and 7.6% of the samples depending on the method used (DGGE 75%, DGGE 85% and NGS 96–98% respectively).

Conclusions: The results indicate the analytical and diagnostic efficacy of the NGS methodology. The discrepancy between the detected and the expected frequency (4%) of CF-causing, CFTR-RD and VOUS mutations, can only be partially explained by the referral indication for the majority of the samples analyzed (infertility, IVF units).

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Clinical experience of noninvasive prenatal testing for fetal trisomies 21, 18 and 13 in high and intermediate-risk pregnancies after public combined first trimester screening

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Introduction: The objective was to evaluate the performance of noninvasive prenatal testing (NIPT) as a contingent screening model as part of a Danish public combined first trimester screening (cFTS) program (93% participation). In our clinical setting NIPT has primarily been offered to women in the intermediate-risk group (1:700 - 1:300 for trisomy 21) and secondly as an alternative to invasive testing for high-risk (>1:300).

Materials and Methods: The cohort includes consecutive on-going singleton pregnancies with intermediate or high-risk of fetal aneuploidy after cFTS that underwent NIPT from January 2015 to December 2016 in Central Denmark Region. Genome-wide massive parallel sequencing and Veriseq NIPT analysis software were used.

Results: 98% of the women were provided with a definite NIPT screening result within two weeks (Table 1).

In the high-risk group (n = 242) the sensitivity for trisomy 21 and 18 were 100% and specificity 99.6% and 100%

Noninvasive prenatal testing results, n = 810	Normal results for chromosomes 21, 18, 13 and sex, n = 783 (96.7 %)	High risk of trisomy 21, n = 6 (0.7 %)	High risk of trisomy 18, n = 3 (0.4 %)	High risk og trisomy 13, n = 0	High risk of sex chromosome aberration, n = 5 (0.6 %)	Inconclusive results and failed tests, n = 13* (1.6 %)
High-risk (>1:300 of trisomy 21) after combined first trimester screening, n = 242 (29.9 %)	230 (95.0 %)	4 (1.7 %) (confirmed, n = 3; not confirmed, n = 1)	3 (1.2 %) (confirmed, n = 3)		2 (XYY, no follow-up and monosomy X, confirmed)	3 (1.2 %) (failed tests)
Intermediate-risk (1:700 - 1:300) after combined first trimester screening, n = 568 (70.1 %)	553 (97.4 %) (false negative trisomy 21, n = 1, postnatal blood sample, 46,XX,rob(21;21)(q10;q10))	2 (0.4 %) (confirmed, n = 2)			3 (monosomy X, one not confirmed and one no follow-up and XXY, confirmed)	5 (0.9 %) (failed tests), 5 (0.9 %) (inconclusive results)
						*n = 8, repeated NIPT with normal results; n = 3, invasive diagnostics with normal results; n = 2, no follow-up

respectively. Two confirmed trisomy 21 cases and one false negative trisomy 21 (46,XX,rob(21q;21q)) were found in the intermediate-risk group (n = 568). Four follow-up invasive procedures were carried out in the intermediate risk group.

Conclusions: NIPT may be used as a valuable screening tool to increase the detection rate of fetal trisomy 21 in intermediate-risk pregnancies after cFTS. It is paramount, however, that women should be informed that it is limited to few syndromes and it is not a diagnostic test.

Table 1. NIPT results and follow-up.

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Broad multi-gene panel or whole exome sequencing in malformed fetuses reveals eight definitive and one likely diagnoses in fifteen studied fetus, in prenatal setting

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Introduction: We present the results from the first 15 fetus to which clinical exome (13) or whole exome sequencing (2) was offered in two prenatal diagnosis centers.

Methods: After diagnosis of sonographic abnormality and medical termination of pregnancy (13), or foetal death with multiple anomalies confirmed at autopsy (2), a predefined panel of 4813 genes (13) or a trio whole exome sequencing (WES) (2) were performed, following a previous extensive evaluation.

Results: A definitive diagnosis was achieved in eight cases: primary microcephaly-5 ; minicore myopathy; microcephaly, seizures, and developmental delay; hydrocephalus due to aqueductal stenosis; fetal akinesia deformation sequence; Meckel syndrome type 1; lissencephaly-1; Tosti syndrome; and a likely diagnosis of Fanconi anemia. Only one of those precise diagnoses was previously suspected in the sonogram.

Discussion: The very high diagnostic yield achieved likely derives from cohort ascertainment: recurrence was present at five of the nine families and very severe, but viable, phenotype, in other two. In the families without a diagnosis, the phenotype was always associated with intrauterine lethality, suggesting a causative gene that has not yet been described. The information was highly relevant; in four families the diagnosis was established during a pregnancy and recurrence could be excluded. At the moment, our proposal is to restrain the use of clinical exome or WES to cases with recurrence or high severity and only if collaboration with a medical geneticist is assured. In a short future, the use of these tests will increase and become part of the initial diagnostic approach.

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Systematic design and comparison of expanded carrier screening panels

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Introduction: Growth in expanded carrier screening (ECS) raises questions about design and evaluation of such panels. Design principles might improve detection of at-risk couples and facilitate objective comparisons of panels.

Materials and Methods: Guided by medical-society statements, we propose a method for the selection of genes and assays that aims to maximize the aggregate and per-disease sensitivity and specificity for Mendelian disorders considered serious by a systematic classification scheme. We evaluated this method retrospectively using results from 474,644 de-identified carrier screens. We then constructed several idealized panels to highlight strengths and limitations of different ECS methodologies.

Results: A panel of 94 diseases, properly assayed, is expected to detect 183 affected conceptuses per 100,000 US births, based on published frequency data. A screen's sensitivity is greatly impacted by two factors: (1) the methodology used (e.g., full-exon sequencing finds up to 48 more affected fetuses per 100,000 than targeted genotyping with an optimal 50 variant panel), and (2) the detection rate of the screen for diseases with high prevalence and complex molecular genetics (e.g., fragile X syndrome, spinal

muscular atrophy, 21-hydroxylase deficiency, and alpha-thalassemia account for 54 affected fetuses per 100,000).

Conclusion: The described approaches allow principled, quantitative evaluation of which diseases and methodologies are appropriate for pan-ethnic expanded carrier screening.

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P01.033A

Expanded carrier panels and the rate of identifying individuals who are heterozygous for disease-causing variants in more than one gene

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Introduction: Expanded carrier testing for inherited genetic disorders is not yet broadly recommended; however, many patients and clinicians choose this option for greater reproductive risk assessment. Through utilization of large panels, patients may learn that they are heterozygous for multiple autosomal recessive or X-linked recessive conditions.

Materials and Methods: We performed a retrospective analysis of carrier tests performed on more than 11,000 patients through the clinical laboratory at Progenity, Inc. We examined the number of patients found to be heterozygous for more than one condition when tested using either the Preparent Global (200+ disorder) or Global Plus (220+ disorder) panels.

Results: A total of 722 (6.1%) patients were found to be carriers for more than one disease. This included 619 (5.2%) patients who carried 2 diseases; 89 (0.75%) patients who carried 3 diseases; 12 (0.1%) patients who carried 4 diseases; as well as one each of patients carrying 5 or 6 diseases. Patients were found to be carriers of a total of 129 different diseases, of which 37 (28.7%) were found in more than 10 patients.

Conclusions: Expanded panels allows for testing carrier status of many conditions at one time, thus providing more information than single gene or ethnicity-based testing. With larger panels, patients may discover positive carrier status for more than one condition. Pre-test counseling and informed consent should include a discussion of this possibility. Understanding the likelihood of this outcome sets appropriate expectations for patients and ordering Providers.

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P01.034B

Couple-based expanded preconception carrier screening (ECS) offered by the general practitioner: what is the uptake and who participates?

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Introduction: The University Medical Center Groningen developed a couple-based preconception ECS test covering 50 serious autosomal recessive conditions simultaneously. As part of an implementation study, we investigated uptake, feasibility and psychological impact of this test-offer in general practice. This study describes uptake.

Materials and Methods: GPs from nine participating practices invited 4295 female patients aged 18–40 to participate in a survey study. Inclusion criteria were: having a male partner, planning to have (more) children and not being pregnant. All couples were offered a (free) ECS test, which required attending pre-test counselling by their GP.

Knowledge, attitude and socio-demographic characteristics between female test-acceptors and test-decliners were compared using Fisher's exact tests for ordinal and T-tests for continuous variables.

Results: 847/4295 (20%) women responded to the invitation. 324/847 (38%) fulfilled the inclusion criteria. Reasons for non-participation included personal medical circumstances, religious beliefs, wrong timing and over-medicalization of pregnancy. 181/324 (56%) participated in the study. 127/181 (70%) attended pre-test counselling and 115/127 (91%) had couple-testing. 89% of test-acceptors and 79% of test-decliners showed sufficient knowledge (≥ 3/5 correct answers). Test-acceptors differed significantly from test-decliners in attitude towards ECS (more positive), timing of pregnancy (less soon) and relationship satisfaction (higher). No significant differences were found in (sufficient) knowledge, age, religion or educational level.

Conclusions: Our study demonstrates an interest in couple-based ECS among couples from the general population based on actual participation. Most couples who attended pre-test counselling had couple testing. As a next step to further explain uptake, multivariate analysis will be conducted.

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P01.035C

Mendeliome sequencing as a prenatal diagnostic investigation after detecting fetal anomalies during ultrasound screening

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Introduction: Fetal anomalies are detected in about 3% of pregnancies during routine ultrasound (US) screening. Certain congenital anomalies have been associated with chromosomal aneuploidies, while other findings may indicate monogenic disorders.The aim of our study was to evaluate the efficacy of using large next generation sequencing (NGS) panel in diagnostic settings in fetuses with prenatally diagnosed anomalies.

Material and Methods: Our study sample consisted of prenatal cases in whom TrueSight One (TSO, Illumina) panel covering 4813 genes was sequenced between July 2015 and December 2016 (18 months).

Results: During this period 17 fetuses were investigated with TSO panel. A certain genetic etiology was confirmed in two cases. First, *de novo* heterozygous mutation in *TP63* gene was detected causing split-hand/foot malformation. Second, compound heterozygous mutations were identified in *DYNC2H1* confirming the diagnosis of short-rib thoracic dysplasia. In two additional cases possibly pathogenic mutations were detected - 1) paternally inherited heterozygous *COL1A1* mutation and 2) heterozygous *PTH1R* mutation (not inherited from mother, paternal carrier status unknown). Both fetuses had shortening of long bones during second trimester US screening.

Conclusions: In our study cohort of 17 fetuses with US anomaly, NGS facilitated a definite diagnosis in two cases (2/17); while in another two possible pathogenic mutations were detected (2/17). Thus NGS is a feasible method for detect genetic etiology of fetal anomalies. At the same time ethical questions remain regarding reporting variants of unclear clinical significance. This work was supported by the Estonian Research Counsel grant PUT355.

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Applying a fetal-fraction based risk score (FFBR) to SNP-based NIPT with no results due to low fetal fraction

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Background: Fetal fraction (FF) is influenced by gestational age, maternal weight, and other factors. There is a relationship between low FF and an increased risk of aneuploidy, prompting a recommendation by professional societies that women who receive 'no results' from NIPT be offered invasive testing. However, not all women with low FF are truly at risk of aneuploidy.

Objective: To determine the relative risk for FF-related aneuploidies—trisomy 13, trisomy 18, and digynic triploidy—in cases that received 'no results' due to low FF using an FFBR model that adjusts for maternal weight and gestational age

Methods: Clinical follow-up was collected on 1352 patients who received 'no results' due to low FF on NIPT. The FFBR score, computed by comparing the observed FF with three models ('normal', 'trisomy 13 or 18', 'digynic triploidy'), identifies cases with lower-than-expected FF, corrected for gestational age and maternal weight. When FFBR is <1%, the total aneuploidy rate is consistent with prior risk. High FFBR scores indicate a >1% risk of FF-related aneuploidy.

Results: Women with high FFBR scores had >10-fold increased risk of FF-related aneuploidy than women with low FFBR scores (4.8% vs. 0.4%) and a 6-fold higher incidence of pregnancy loss or aneuploidy (17.2% vs. 2.9%). Trisomy 21 risk was consistent with *a priori* risk in both FFBR groups.

Conclusions: We have validated the FFBR model for women who received 'no results' on NIPT due to low FF. The FFBR score may provide clinicians with additional information on patients' relative risk for aneuploidy.

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P01.037A

FMR1 epigenetic control can explain variable response of women ovarian reserve to clinical stimulation protocol

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Introduction: *FMR1* (*Fragile-X-Mental-Retardation-1-gene)* expression is supposed to be functional during folliculogenesis. An amplification of CGG-triplet-numbers within its promoter from normal around 30 up to 54–200 (called premutation: PM) is associated with an elevated risk (~20%) of premature-ovarian-insufficiency/failure probably due to increased *FMR1*-expression-level. Recently, first epigenetic-elements called FREE1 and FREE2 (Fragile-X-Epigenetic Element-1/2), were described, assumed to control *FMR1*-expression in leukocytes of PM-carriers and suggested a general epigenetic *FMR1*-expression-control. Granulosa cells are the putative target cells of *FMR1*-function during human folliculogenesis.

Materials and Methods: Primary granulosa cells of women undergoing IVF-/ICSI-procedure were collected and analyzed for quantitative-*FMR1*-mRNA-expression. Due to different response to controlled ovarian stimulation (COS) patients were subdivided into three groups (normal-, poor-, and high-responder). The promoter-CpG-methylation-profile in patients´ granulosa cells and leukocytes were analyzed after bisulfite-treatment of genomic DNA and sequence-analysis using sets of specific primers along the *FMR1*-promoter including intron1.

Results: FREE1 and FREE2 were present with variable patterns of CpG methylation in women granulosa cells as predescribed in leukocytes. Additionally, a third variable methylated epigenetic element distal FREE2 within intron1 was detected and named FREE3 in accordance. Specific CpG-methylation-pattern within FREE3 as well as the *FMR1*-expression-level was depending on ovarian response in granulosa cells and persisted in leukocytes.

Conclusion: The variable CpG methylation pattern of FREE3 is dependent on ovarian response to COS. Since this pattern persist in leukocytes, FREE3-CpG pattern analysis in women leukocytes prior to COS may serve as a non-invasive prognostic-tool to predict ovarian response and help to improve risk-adjusted personal protocols. DFG-founded- study RE3647/1-1

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P01.038B

High confidence preimplantation genetic diagnosis of fragile X syndrome - direct detection of the *FMR1* CGG repeat expansion mutation combined with linked multi-marker haplotype analysis

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Introduction: Fragile X syndrome (FXS) is caused by hyperexpansion of a CGG repeat within, and subsequent inactivation of, the *FMR1* gene. Trans-generational FXS transmission can be avoided using preimplantation genetic diagnosis (PGD). We describe a robust PGD strategy that can be applied to virtually any couple at risk of transmitting this disorder.

Materials and Methods: This novel strategy utilizes whole-genome amplification, followed by triplet-primed PCR (TP-PCR) for robust detection of the expanded *FMR1* allele when present. Diagnostic confidence is further increased through parallel haplotype analysis of 13 highly polymorphic microsatellite markers located within 1 Mb on either side of the *FMR1* CGG repeat, together with the *AMELX/Y* dimorphism for gender identification. The assay was optimized and validated on single lymphoblasts isolated from fragile X reference cell lines, and applied to a simulated PGD case and a clinical IVF-PGD case.

Results: In the simulated PGD case, definitive diagnosis of the expected results was achieved for all "embryos". In the clinical IVF-PGD case, an on-going clinical pregnancy was achieved after transfer of an expansion-negative blastocyst.

Conclusions: *FMR1* TP-PCR reliably detects presence of expansion mutations and obviates reliance on informative normal alleles for determining disease status in female embryos. Together with multi-marker haplotyping and gender determination, misdiagnosis and diagnostic ambiguity due to allele drop-out is minimized, and couple-specific assay customization can be avoided.

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P01.039C

Haplotyping single cells by using extended family members: an expanding option for PGD

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Genome-wide haplotyping of preimplantation embryos is being implemented as a generic approach for genetic diagnosis of inherited mutations. Previously we have developed an algorithm that allows concomitant haplotyping and copy number profiling of the embryo. To enable the phasing of the genotypes into haplotypes, genotyping direct family members of the prospective parent carrying the mutation is a requirement. Currently, the algorithm uses either (1) both parents of the affected prospective parent or (2) an affected or unaffected child of the affected couple. However, such parental genotypes are not always accessible and/or there are no offspring yet. At present, such couples cannot enter the genome-wide haplotyping-based PGD program.

Some families have access to genotypes of affected or unaffected brothers, sisters or other family members. Since the gene mutation is embedded in a local haplotype, it is possible to deduce the affected haplotype. We now expanded our algorithms by firstly determining identity by status regions (IBS), subsequently deducing the haplotype based on the shared allele between the parents and their extended family members, which can be either healthy or affected, and finally embedding this information in the genome-wide haplotype.

Twelve extended families have been analyzed. The consistency of the results as well as possible challenges will be discussed.

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P01.040D

Identifiaction and homology modeling of novel placental hemoglobin subunit alpha (HBA) isoform

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Introduction: RNA-seq, based on the next generation sequencing (NGS) technology, has recently been shown to have advantages in both sensitivity and cost. One of the primary aims of RNA-seq is to compare the gene expression levels in various tissues, and to identify alternative splicing events, and SNVs. Recent studies using massively parallel

sequencing techniques have begun to expand the knowledge of the placental transcriptional landscape, in various eutherians, e.g. humans, elephants, rats, pigs and beavers. The placenta is essential for sustaining the growth of the fetus during gestation, and defects in its functioning result in embryonic/fetal growth restriction or even embryonic and fetal mortality. It forms the interface between the maternal and fetal circulation, facilitating metabolic and gas exchange as well as fetal waste disposal.

Materials and Methods: The aim of our study was identification and homology modeling of novel placental isoforms. RNA-seq was utilized to identify global expression pattern in the placentas (N = 4) from women in single and twin pregnancies.

Results: During analyses, 228 044 transcripts were reconstructed and 6497 highly covered splice junctions unannotated in the human genome were identified and 30 were confirmed as novel. Among them, hemoglobin subunit alpha (HBA) was chosen to broaden analysis, including homology modeling using I-TASSER.

Conclusions: Examination of specific placental isoforms crucial for placental development and function can help in understanding and identifying the mechanisms underlying both normal and pathological pregnancies. Detailing the placental transcriptome could provide a valuable resource for genomic studies related to placental disease. *Supported by UWM (WNM#25.610.001-300)

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P01.041A

Role of HIF1a gene expression in physiological and uncompleted pregnancies

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Introduction: Almost all physiological pregnancies are complicated with uterus varicose veins that in some cases can lead to miscarriage, but genetic background of uncompleted pregnancy is still unclear. Several studies suggest that tissue hypoxia induced by indigenous and environmental factors can lead to spontaneous abortion and insufficient carriage. So the aim of this study was to evaluate the expression of transcription factor HIF1A gene that plays a fundamental role in the physiological response to hypoxia in normal and uncomplicated pregnancies.

Materials and Methods: Gene expression analysis was carried out on venous blood samples from 29 women with uncompleted pregnancy (UP) (I-II trimester) and 79 physiologically pregnant women (PP) collected during 22–24th, 32–34th weeks of pregnancy, separately in primapara and multigravidas. The relative gene expression level (RQ) evaluated by the 2deltaCt method and statistical significance was determined by Student t-test ($p = 0.05$).

Results: We observed that the expression level of HIF1A gene dramatically increases in III trimester of PP women (RQ=9.78, p=0.006) that can marked the hypoxia initiation in late stages of gestation. Controversially, in PP multigravidas in III trimester the activity of HIF1A gene mildly decreases (RQ=0.25, p=0.044) that can be explained by faster reactivity to hypoxia. In UP women we also observed decreasing gene expression activity (RQ=0.44, p=0.528) however it wasn't statistically significant.

Conclusions: Our results indicate a better adaptation of vascular endothelium during the first pregnancy, presumably due to changes in venous hemodynamics in comparison with each subsequent pregnancy, especially in women with uncompleted pregnancy.

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P01.043C

Ovum donation - a legal parenthesis to the only option for recurrent molar pregnancies

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Introduction: Gestational trophoblastic disease (GTD) comprises partial/complete hydatidiform mole (HM), invasive mole, gestational choriocarcinoma, placental-site trophoblastic tumor and epithelioid trophoblastic tumor, all originating from the abnormal hyper-proliferation of trophoblastic cells. HM, being the most common of GTDs, may recur in independent pregnancies, suggesting a genetic predisposition. Incidence of HM varies amongst populations, 1/600 in western countries and higher rates are observed for recurrent HMs in middle/far east countries. Familial recurrent HM is an autosomal recessive condition, with *NLRP7* and *KHDC3L* reported mutation frequencies of 48–80% and 10–14%, respectively. Women with a history of two or more molar pregnancies should be evaluated by genetic testing. We report the molecular findings of a consanguineous couple who had four HM pregnancies.

Materials and Methods: *NLRP7 and KHDC3L* genes were analysed by Sanger sequencing.

Results: The prospect mother was found homozygous for *NLRP7* IVS7 ds+1 G>A mutation, along with two heterozygous variations in *KHDC3L*. Pedigree analysis showed that her parents were first cousins and her sister also had two HM pregnancies.

Conclusions: The molecular findings confirmed HM diagnosis. The couple previously had been offered IVF and PGD which resulted with non-viable embryos. Non-directive genetic counseling should adevert all reproductive options, including ovum donation which might be the only option for this couple to have healthy off-spring. However, assisted reproductive regulations restrict ovum and sperm donation in Turkey like in many other middle-east countries. The legal issues not only complicate the clinical practice, but renders the solution based relationship between the geneticist and the patient.

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P01.044D

Next Generation Sequencing improves diagnosis for fetal hydrops

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Introduction: Classical biochemical testing for pregnancies affected by non-immune fetal hydrops has been replaced in our laboratory by a custom next generation sequencing (NGS) panel.

Method: Enzyme activities of fibroblasts cultured from amniocytes or skin were determined for 19 lysosomal enzymes known to be associated with non-immune hydrops and PMM2. Filipin staining and/or cholesterol esterification studies were performed for Niemann-Pick disease type C testing. Free sialic acid was measured for sialic acid storage disorder testing. A custom NGS panel (Illumina Trusight One) was designed to cover 52 genes associated with fetal hydrops.

Results: Classical testing of 114 samples referred from Australasian, Pacific basin and Middle Eastern centres between 1991–2013 resulted in a diagnosis rate of 11%. The commonest identified cause was MPS VII (6), with 2 cases of CDG1a and single cases of Gaucher disease, Galactosialidosis, Sialidosis and Niemann Pick C. Next generation testing of 16 samples since 2015 has resulted in 3 Noonan syndrome and one Gaucher diagnosis, a rate of 25%.

Discussion: Prior to the introduction of NGS, testing was restricted to recurrent fetal hydrops because of the complexity and expense of testing. Each of the biochemical diagnoses made was associated with a 1 in 4 recurrence risk. The commonest NGS cause of fetal hydrops, Noonan syndrome, usually represents new mutations, with a significantly reduced recurrence risk.

Conclusion: NGS testing has a higher diagnosis rate for fetal hydrops than classical testing, with a significant impact on recurrence risk as well as being both quicker and less expensive.

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P01.045A

Gene expression signatures in patients with severely impaired spermatogenesis - whole gene expression study & meta-analysis

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Introduction: A large proportion of male infertile patients are having a defect in spermatogenesis of which the underlying causes remain unknown. The main objective of this study was to identify genes that play a role in the etiology of idiopathic male infertility using experimental data derived from genetic transcriptomic studies.

Material and Methods: We performed whole gene expression analysis in 20 testis samples of patients with severely impaired and normal spermatogenesis and meta-analysis of gene expression data from three additional transcriptomic studies. The main inclusion criteria for studies were availability of raw datasets at either GEO or ArrayExpress repositories, common reference design approach towards measuring differential expression in the microarray experiment and interrogation of the same tissue samples and comparable disease states as in our experiment.

Results: Using whole gene expression testis profiles from 100 patents and 37 controls, we obtained oweral number of 3545 unique elements attaining p values below 0.01 (1283 down-regulated and 2262 up-regulated) with changed expression in infertile men. Of these, 30 down-regulated and 5 up-regulated genes were common between

all studies. Majority of the down-regulated differentially expressed genes were related with regulation and completion of germ cell maturation, whereas up-regulated genes were related to pathways associated with oxidation-reduction, response to organic substance, steroid biosynthetic process, response to wounding and regulation of apoptosis.

Conclusion: Integrated transcriptome analyses provides new insight into the pathogenesis of male infertility. This study was supported by grant P3-0326 from the Slovenian Research Agency.

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P01.046B

Stable estimated live birth prevalence of Down syndrome in the Netherlands, 2000–2013

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With the introduction of a prenatal screening programme the live birth prevalence of Down syndrome (DS) may have changed. The Netherlands lack reliable national data regarding the livebirth prevalence of DS and relevant trends since 2007, when information on the First-trimester Combined screening Test was introduced for all pregnant women. Meanwhile the next stage of prenatal screening is arriving with non-invasive prenatal testing. The aim of this study is to analyse trends in factors that influence livebirth prevalence and to estimate the prevalence of DS in livebirths for the period of 2000–2013.

Two empirical datasets were used: data on (1) livebirths according to maternal age, and (2) prenatal testing and termination of pregnancy subsequent diagnosis of DS. This data is combined in a model that uses maternal age-specific risk on DS and correction factors for natural fetal loss.

The mean maternal age has slightly increased since 2000 from 30.2 years (sd= 4.6 years) to 30.5 years (sd= 4.8 years) in 2013. The annual number of invasive tests performed decreased (from 12 047 to 5 500). In the women undergoing invasive testing, DS was diagnosed more often (1.6% in 2000 vs. 4.8% in 2013). However the proportion of induced abortions after confirmation of diagnosis has decreased. This resulted in a stable DS livebirth prevalence of 13.7 per 10 000 livebirths. Because of a decline of the total number of livebirths in The Netherlands the absolute number of livebirths with DS decreased from 284 to 244 per year.

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P01.047C

Is there any relationship between NRG1 gene duplication and cardiac findings in two fetuses with inv dup del (8p) syndrome ?

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Introduction: 8p inverted duplication deletion syndrome is a rare disorder often clinically manifests as intellectual disability, central nervous system abnormalities, congenital heart defects and dysmorphic features. To date there is a little knowledge about the clinical presentation and the submicroscopic chromosomal changes in prenatal period. Here we report two prenatally detected fetuses with this condition.

Clinical reports: The first fetus referred for increased nuchal translucency was later found to have cerebellar vermis agenesis, a solitary interhemispheric cyst and ventricular septal defect (VSD).The second fetus had VSD and discordance between right and left heart ventricles.

Results: Conventional cytogenetic, fluorescence in situ hybridization (FISH) and array-CGH (Agilent, ISCA 8×60K) analyzes revealed an inverted duplication deletion at chromosome 8p in both fetuses. Each of them have a deletion bigger than 6.5 Mb distal to the 8p23 (8p23.1-pter) region followed by an intermediate intact segment, and an inverted duplicated (approximately 30 Mb) proximal segment (8p23.1-p11.1).

Discussion: Gata-Binding Protein 4 (GATA4) has been implicated as the gene responsible for the heart defects associated with 8p23.1 deletions so far. Discordant with this finding GATA4 gene region was intact, and Neuregulin 1 (NRG1) gene, related with the trabeculation of the heart ventricle, was duplicated in both fetuses. We suggest that the overexpression of NRG1 gene could be responsible for the discordance between right and left ventricles and VSD.

More clinical reports and functional studies are necessary to clarify this hypothesis.

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P01.048D

Isodicentric chromosome 18 in the foetus and jumping translocations in the placenta

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We present one case of the foetus with idic(18) and confined placental mosaicism of various aberrations of chromosome 18, suspected jumping translocations. Jumping translocations are rare chromosomal events in which a donor chromosome segment is translocated to various recipient chromosome sites (mainly observed in haematological malignancies).

Chorionic villi sampling was indicated due to increased nuchal translucency and absent nasal bone. Array CGH was applied to uncultured material and detected 2,5Mb terminal deletion of 18p, mosaic duplication of the rest of chromosome 18 and mosaic 2p duplication. Cultured chorionic villi interphase FISH showed 18p deletion in all cells together with mosaicism of one to three 18q copies; karyotyping revealed six different cell lines: normal karyotype and five various aberrations of chromosome 18. Further ultrasound examination found choroid plexus cysts, atypical position of lower limbs, shortened upper limbs, suspected brain anomaly, cleft palate and heart defect. Amniocentesis was performed with karyotype 46,XX,idic(18)(p11.32). Interphase FISH again detected mosaic status of 18q in cultivated amniocytes. After termination of pregnancy foetal tissue DNA was examined by array CGH and its profile (18p terminal deletion and duplication of the rest of chromosome 18) confirmed idic(18) in all cells.

Phenotype of patients with idic(18) is similar to trisomy 18. Features of partial monosomy 18p are probably less specific and are covered by partial trisomy of the rest of chromosome 18. We suppose, jumping translocations including aberrant chromosome 18 may have occured in trophoblast during embryogenesis in contrast to the forming of idic(18) in embryoblast.

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Benefit of comprehensive genetic evaluation of stillbirths

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Background: Autopsy is important for evaluation of stillbirth (≥20weeks), but rates are declining due to religious/cultural belief, and conception that no useful information is obtained. We offered an alternative protocol of testing to determine cause of death for those rejecting autopsy, to reduce stillbirth-recurrence in subsequent pregnancies.

Objective: To quantify utility of placental-pathology, CT and karyotyping in identifying stillbirth-cause. STUDY DESIGN: Cohort of all 217 stillbirths from 1/2011–5/2014 at a tertiary-care-center. Records were reviewed in stepwise manner: clinical history and laboratory results, then placental pathologic evaluation, CT, and finally karyotyping. At each step, probable cause of death was determined.

Results: Clinical and basic laboratory information identified cause of death in 26/217(12%). Placental pathologic examination added probable cause in 48/119(40%, 22% of total). 46/137(34%) had CT findings, however most were result rather than cause of stillbirth. 201/217(93%) underwent karyotyping, 128 yielded results, 13/128(10%, 6% of total) had aneuploidy. 12% of couples consented to autopsy, which confirmed same etiology. Adding karyotyping resulted in identifying cause of death in 40% in total. Had comprehensive analysis been successful in all cases, yield would reach 62%, not far from ~70% yield reported in studies including autopsy. CT wasn't beneficial.

Conclusion: Adding placental pathological examination and karyotyping, beyond clinical and basic laboratory information, raises yield of finding cause to 62%. CMA and MRI are expected to further improve this yield. Comprehensive evaluation of stillbirths should be implemented, even in populations declining autopsy. It is necessary that healthcare professionals convey the value of comprehensive approach to parents.

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P01.050B

Expression of Human *Leucine-Rich Repeats and WD Repeat Domain Containing 1 (LRWD1)* by methylation

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Introduction: *LRWD*1 (*Leucine-rich repeats and WD repeat domain containing 1*) is highly expressed in the testes, but down-regulated in the testicular tissues of patients with severe spermatogenic defects, yet its transcriptional regulation remains unclear. CpG islands are located between positions -253 to $+5$ nucleotides upstream from the *LRWD1* transcription start site by CpG Plot analysis in EMBOSS.

Materials and Methods: The quantitative methylation-specific PCR and immunostaining were applied to study the effect of methylation for the transcription effect for *LRWD1*. To investigate the effect of methylation for LRWD1 expression, we used the 5-Fluoro-2′-deoxyuridine as an inducer and 5-Aza-2′-deoxycytidine as an inhibitor for DNA methylation.

Results: In the quantitative methylation-specific PCR and immunostaining showed that the inhibitor by 5-Aza-2quantitative of DNA methylation increased the *LRWD1* expression. Otherwise, the inducer of 5-Fluoro-2, the inducer for DNA methylation decreased the *LRWD1* expression in NT2D1 cells.

Conclusion: The methylation pattern of the promoter is an important factor for transcription and expression of *LRWD1* in the testicular cell under drug or environmental effect. We suggest that LRWD1 may be as epigenetic marker to detect the environmental pollution in future. Acknowledgments:This study was supported by grants from National Science Council (NSC 102-2314-B-024 -001) and Ministry of Science and Technology (MOST 103-2314-B-024 -002, 104-2314-B-024 -002 -MY2) of Taiwan. We thanks PD Dr. Michael Rehli kindly provided the pCpGL-vector or derivatives.

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P01.051C

MiR-320 regulated LRWD1 expression in human testicular cell

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Introduction: *LRWD1*(*Leucine-Rich repeats and WD repeat domain containing 1*)is highly expressed in the testes and down-regulated in the testicular tissues of the patients with severe spermatogenic defects. *LRWD1* expressed in the centrosome of sperm and involved in microtubule growth. In the Bioinformatics prediction for *miR-320*, there are binding sites in the *LRWD1* 3′UTR by miRnada software and high expression at reproduction tissue by miRnaMap software.

Materials and Methods: There is a potential binding site for *MiR-320* on the 3′UTR of the *LRWD1* transcript by the microRNA analyzing tools. We made *LRWD1*-3′UTR fused to a luciferase reporter vector to construct pMIR-*LRWD1*-3′UTR. Hydrogen peroxide (H_2O_2) or Sodium nitroprusside dehydrate (SNP) treatment to NT2D1 cell, and then to assay *LRWD1* expression by western blot and real-time PCR.

Results: The *LRWD1* 3′UTR was constructed to the pMIR plasmid and confirmed that *miR-320* increased *LRWD1* expression by Dual-Luciferase Reporter Assay. *LRWD1* expression increased by transfected *miR-320* mimic in NT2D1 cells. The expression of *miR-320* and *LRWD1* was increased by H_2O_2 or SNP treatment in TaqMan real time-PCR assay. Transfection mimic of *miR-320* enhanced cell growth.

Conclusion: The results help to understand the functions and roles of the *miR-320* in post-transcriptional regulation of *LRWD1* and may apply for further diagnosis and treatment of spermatogenic defect and male infertility diseases. Acknowledgments:This study was supported by grants from National Science Council (NSC 102-2314-B-024 -001) and Ministry of Science and Technology (MOST 103-2314-B-024 -002, 104-2314-B-024 -002 -MY2) of Taiwan.

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P01.052D

Advances of genetics in andrology and potential implications towards improved diagnostics and patient management

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Introduction: Although genetic factors are among the generally acknowledged causes for impaired male reproductive health and infertility, current genetic testing in andrology clinics is limited, including cytogenetics, Y-chr. microdeletions and *CTFR* mutations. Notably, 60% of men with impaired sperm counts remain idiopathic (Punab et al 2017 Hum Reprod 32:18-31). Studies of our clinical-basic research team have aimed to uncover clinically important genetic factors shaping male reproductive physiology and fertility potential.

Materials and Methods: All patients have been recruited at the Andrology Center, Tartu University Hospital. We have applied complimentary approach combining analysis of candidate genes encoding reproductive hormones (FSH, LH, testosterone, SHBG), and genome-wide methods (CNVs, Exome-Seq) aiming to discover novel loci implicated in male infertility.

Results: We have discovered and/or validated a number of genetic variants modulating male reproductive hormone levels and consequently, testicular and seminal parameters. Combination of these variants in consort deserves attention as a potential pharmacogenetic panel in choosing alternative management options for andrology patients. Candidate gene and exome sequencing of idiopathic infertility cases have both proved as suitable tools to detect rare patient-specific mutations. Genome-wide analysis of structural variants revealed an overall altered autosomal CNV profile as a considerable risk factor to male infertility, and revealed novel genomic hotspots and CNVs as potential causes of impaired spermatogenesis.

Conclusions: Uncovering genetic contribution to impaired male reproductive health paves the way to improved diagnostics and management in andrology clinics. This work was supported by grants from SA Archimedes (HAPPY PREGNANCY, 3.2.0701.12-0047) and Estonian Research Council (IUT34-12, PUT181).

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P01.053A

High throughput analysis revealed novel genes for male infertility

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Introduction: Approximately one out of every seven couples are infertile worldwide and male factor infertility accounts for 30–50% of the infertility cases. Previous studies from our lab have shown that about 8.5% Indian men are infertile due to Y chromosome microdeletions. Further, analysis of a few autosomal genes and mitochondrial genome accounted for additional 21% of the genetic factors responsible for infertility among Indian men. However, etiology of large proportion (71%) of infertile men still remained unknown. Therefore, we have performed exome sequencing to identify novel autosomal genetic causes of male infertility.

Materials and methods: Exome sequencing was performed in 44 idiopathic infertile men using Illumina Hiseq-2000 platform with 100X coverage. Using various bioinformatic tools, we have identified 60 novel and rare variants from 58 genes. Subsequent replication study (using Sequenom MassARRAY iPLEX platform) on 1344 samples has identified 12 variants (12 genes), which are strongly associated with male infertility. Further, we have genotyped 28 additional damaging variants from the above 12 genes in 960 case-controls. *CETN1* is one among the potential candidates identified; hence variants of this gene were characterized using biophysical and cell biology approaches.

Results: We have identified a total of 24 novel, rare, nonsense and missense variants from 12 genes that showed high expression in testis. Of which, rs61734344 (*CETN1*) showed highly significant association with male infertility ($p=1.658e^{-35}$). Functional studies have shown that rs61734344 alters calcium binding affinity, thermodynamic properties, surface hydrophobicity and disrupts spindle formation during cell division; suggesting its role in infertility.

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P01.054B

Systematic analysis of the effect of maternal cell contamination on prenatal molecular testing

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Introduction: Prenatal molecular genetic testing is available for many severe monogenic diseases. These assays mostly use amniotic fluid or chorionic villus samples, that are obtained by invasive techniques. Maternal cell contamination (MCC) in fetal samples is considered as a preanalytical risk for prenatal molecular analysis and may lead to false genotyping. It is recommended to determine the used diagnostic tests' sensitivity to MCC.

Materials and Methods: Sensitivity of three different diagnostic methods to MCC: Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) and a next-generation sequencing methodology (NGS) was tested. The experiments were performed by mixing a wild type DNA sample ('fetal') with a heterozygote DNA sample ('maternal') simulating different levels (1, 5, 10, 20, 30 and 40%) of MCC.

Results: The limit of detection of the mutant allele by Sanger sequencing was 10% (20% MCC), above this level the fetal genotype may be obscured. In the case of MLPA, up to 30% MCC had no effect on genotyping result. Fourty percent MCC resulted in a maximum of 30% reduction in relative peak height of relevant probes leading to diagnostic uncertainty. The NGS method could detect 0.5% maternal allele. Accurate quantification in the range of 0.5–20% did not lead to false genotyping.

Conclusions: Our results show that the level of MCC affecting the results of diagnostic tests is highly dependent on the applied method. These results can be interpreted correctly only in conjunction with simultaneously determined MCC level.

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P01.055C

Array CGH analysis in men with meiotic arrest

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Infertility, which affects 10–15% of all couples, is attributed to a male (co-)factor in around 50%. Male infertility is mostly caused by spermatogenetic failure, clinically noted as oligo- or azoospermia. However, the reasons for the decreased sperm production remain largely unclear. Genome-wide analyses for imbalances have been performed on infertile patients with idiopathic oligozoospermia and Sertoli-cell-only syndrome (SCOS) (Tüttelmann et al., 2011). Here we describe the analysis of 26 patients with meiotic arrest (MA) by array CGH using the 400k Array Set (Agilent). We compared the array CGH results with the results of 100 normozoospermic men. All patients and controls were selected retrospectively. The mean number of Copy Number Variants (CNVs) and the amount of DNA gain/loss were comparable between both groups (patients and controls). As previously seen in patients with SCOS, sex-chromosomal CNVs were also overrepresented in patients with MA. We selected 32 patient-specific, gene containing CNVs, which were not seen in normozoospermic men or not displayed as common variants in the Database of Genomic Variants (DGV) for further characterisation. One of these CNVs was found inside the sperm specific *CATSPERB* gene (OMIM 611169). Like the other proteins of the CATSPER ion channel subunits (CATSPER1-4 and CATSPERG) CATSPERB is localized to the principal piece of the sperm tail and the channel complex is required for sperm cell hyperactivation and male fertility. In conclusion, by studying CNVs in patients with MA we provide a number of candidate genes possibly causing or being risk factors for the men's spermatogenic failure.

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P01.056D

The effects of melatonin on oxidative stress and prevention of primordial follicle loss via activation of mTOR pathway in the rat ovary

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Introduction: Mammalian Target of Rapamycin (mTOR) signaling pathway has important roles in the regulation of puberty onset, gonadotropin secretion, follicular development and ovulation. Melatonin (*N*-acetyl-5-methoxytryptamine) is a lipophilic hormone has multiple functions in regulating the fertility. Recent studies have shown that melatonin affected the number or maturation of follicles in the ovary. The aim of this study was to investigate the effects of melatonin on mTOR expression and quantity of follicle in rat ovary.

Materials and Methods: In the present study a total of 45 female rats were randomly divided into three groups: Group 1; Control (C), Group 2:Vehicle (V) and Group 3; Melatonin (M). Melatonin was administered intraperitoneally at a dose of 50 mg/kg/day for 30 days in Melatonin group. The effects of Melatonin on expression of mTOR and downstream components were determined by Western Blot and Reverse Transcriptase PCR analysis.

Results: Upon Western Blot and RT-PCR evaluations, we detected higher expression and activation of mTOR, P70S6K, PKCalpha, PCNA and higher number of primordial follicles in melatonin group compared with V and C group. In addition to this results, melatonin decreased oxidative stress markers, such as MDA, on the contrary, levels of antioxidative markers, such as CAT and GPx, were increased by melatonin in rat ovary.

Conclusion: This study indicated that melatonin may have a significant protective effect on primordial follicles and increase the expression of mTOR and downstream components in rat ovary. Melatonin treatment may have a beneficial effect on fertility.

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P01.057A

Optimized criteria for using Microfluidics-FISH in prenatal diagnosis

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Microfluidics- FISH is a novel technique introduced to prenatal diagnosis in which a microchip device is used, consisting of glass coated with nanostructured titanium dioxide and polymeric pad with microchannels. This approach allows to combine different steps of FISH into integral process, shortening and economizing the procedure.

The aim of this study was to evaluate Microfluidics-FISH diagnostic effectiveness, turnaround time and cost in comparison to FISH as well indicate conditions in which Microfluidics- FISH might be a method of preference.

52 samples of amniotic fluid were drawn from pregnant women due to common indications, the most frequent being increased risk of chromosomal aberration (78%). Concurrently, trisomy 21, 13, 18 and sex chromosomes aneuploidy have been assessed on uncultured amniotic cells by FISH and Microfluidics- FISH method.

All aberrations that have been detected showed 100% accordance rate for both techniques. The mean turnaround time was two times shorter for Microfluidics- FISH. Also the cost of single Microfluidics- FISH assay comprised half of FISH cost due to reduced (even 13 fold) consumption of costly reagents.

Microfluidics- FISH proved to be reliable, cost-effective and rapid testing method of common aneuploidies.

The targeted group of Microfluidics- FISH test seems to be patients, in which amniocentesis was performed between 15–19 week of gestation (higher quality of amniocytes) from a smaller volume of sample, due to very specific indications, increased risk of aneuploidy being the main one.

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P01.058B

Parental origin of the retained X chromosome in monosomy X miscarriages and ongoing pregnancies

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Objective: To assess the distribution of the parental origin of the retained X chromosome in monosomy X, either in miscarriages or in ongoing pregnancies.

Method: The parental origin of the X chromosome was determined in monosomy X pregnancies, either

miscarriages or ongoing pregnancies. Microsatellite marker patterns were compared between maternal and fetal samples by Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR). Distributions of maternally and paternally derived X chromosome were assessed in miscarriages and in ongoing pregnancies using two tailed Fisher exact test.

Results: Forty monosomy X pregnancies were included in the study, 26 miscarried at 5–16 weeks, and 14 ongoing pregnancies diagnosed at 11–20 weeks. The retained X chromosome was maternally derived in 67% of the cases. In miscarriages, maternal and paternal X chromosome were retained in a similar proportion (54% (95%CI: 35–73%) vs.46%, (95%CI: 27–65%), while in ongoing pregnancies the maternal proportion was 13 times higher [93% (95%CI: 79–100%) vs. 7%, (95%CI: 0–20%)].

Conclusions: The retained X chromosome in individuals with monosomy X should theoretically be maternally-derived in 2/3 of the cases. Our study signals a significantly higher early miscarriage rate in pregnancies with a retained paternally-derived X chromosome. This may explain the observation that in 75%-90% of individuals with monosomy X retain the maternal X chromosome.

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P01.059C

Quantification of transmission risk in a male patient with a *FLNB* mosaic mutation causing Larsen syndrome: implications for genetic counselling in post-zygotic mosaicism cases

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Introduction: We report the case of a male patient with Larsen syndrome found to be mosaic for a novel point mutation in *FLNB*. Quantification of the mutation was performed in four different tissues including sperm in order to provide accurate advice on transmission risk to future offspring.

Materials and Methods: Original identification of the mutation was performed by dideoxy-sequencing of DNA extracted from buccal cells and dermal fibroblasts. To obtain accurate mutation quantification, deep next generation sequencing (NGS) was performed on DNA extracted from peripheral blood, fibroblasts, saliva and sperm samples.

Results: A low-level mosaic *FLNB* c.698A>G, p.(Tyr233Cys) mutation was initially identified in buccal and fibroblast DNA. This substitution is located within the second calponin homology domain (CHD2) of the filamin B protein, a known hotspot region for Larsen mutations, and has not been reported previously. NGS allowed accurate quantification of mosaic levels and showed that the mutation was detectable in all four patient samples tested, at levels ranging from 10.24%±0.26% in saliva, 9.79% ±0.52% in fibroblasts, 9.47%±0.13% in blood and 7.08% ±0.11% in sperm (mutation present in ~20% of diploid somatic cells, 7% of haploid sperm).

Conclusions: This report illustrates the clinical utility of performing targeted NGS analysis on multiple somatic tissues and sperm from males affected by a mosaic condition in order to accurately quantify gonadal mosaicism and provide evidence-based advice on their transmission risk to future offspring.

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P01.060D

The accumulation of vitrified oocytes is a valid strategy to increase the number of euploid available embryos for transfer after Preimplantation Genetic Testing

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Purpose: In a preimplantation genetic diagnosis for aneuploidy (PGD-A) program, the more embryos available for

biopsy, consequently increases the chances of obtaining euploid embryos to transfer. The aim was to increase the number of viable euploid blastocysts in patients undergoing PGD-A using fresh oocytes together with previously accumulated vitrified oocytes.

Methods: 69 patients with normal ovarian reserve underwent PGD-A for repeated implantation failure or recurrent pregnancy loss indication. After several cycles of ovarian stimulation, 591 accumulated vitrified oocytes and 463 fresh oocytes were micro-injected with the same partner's semen sample. PGD-A was completed on 134 blastocysts from vitrified/warmed oocytes and 130 blastocysts from fresh oocytes.

Results: A mean of 9.6% euploid blastocyst per micro-injected vitrified/warmed oocytes and 11.4% euploid blastocyst per micro-injected fresh oocyte were obtained (p>0.05). The euploidy and aneuploidy rates were comparable in blastocysts obtained from micro-injected vitrified/warmed oocytes and fresh oocytes (42.5% versus 40.8% and 57.5% versus 59.2%, p>0.05). Implantation rates of euploid blastocysts were comparable between the two sources of oocytes (56.0% from vitrified/warmed oocytes versus 60.9% from fresh oocytes, p>0.05).

Conclusions: Oocyte vitrification and warming do not generate aneuploidy in blastocysts. The number of viable euploid embryos for transfer can be increased by using accumulated vitrified oocytes together with fresh oocytes in ICSI.

S. chamayou: None.

P01.061A

Characteristics of no calls from non-invasive prenatal testing (NIPT) performed at Hvidovre Hospitals NIPT Center, Denmark

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Introduction: Main reasons for no-call results of NIPT are low fetal-fraction (FF) and technical reasons. Information on why the result cannot be reported is important, as it affects how to continue.

Materials and Methods: We performed a validation of our set-up and evaluated technical, maternal and fetal characteristics of no-call cases. NIPT analyses were performed on circulating cell-free DNA and Massive Parallel Sequencing on a HiSeq1500. Bioinformatics were performed in samples > 8 mio-reads using a pipeline for fetal fraction (SeqFF) and aneuploidy (WISECONDOR).

Results: Among 205 selected cases (incl. 73 aneuploidy cases), 13 lead to no-call results (6%). In four cases the reason was technical and in nine cases FF was below cut-off <0.02. In the group of no-calls caused by low FF two cases had a maternal BMI>40, four cases had a BMI between 30 and 40, and three cases had a BMI<30. Furthermore, we found an overrepresentation of aneuploidy cases (T21=2, T18=4, T13=1, 46XX/XY=2). Two cases of T18 and one case of T13 had a z-score >3 despite a low FF.

Conclusions: High maternal BMI and fetal aneuploidy highly influence the risk of a no-call result. Therefore, labs performing NIPT in a population with obese women might have a higher no-call rate. We found an overrepresentation of aneuploidy cases among no-calls, both T21, T18 and T13. Only T18 and T13 are described in the literature to have decreased FF. If we had not included determination of FF in our set-up, four cases might have been false negative.

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P01.062B

Determination of fetal fraction using Droplet Digital PCR for Non-Invasive Prenatal Testing (NIPT)

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Introduction: With the aim of complementing Prenatal Non-Invasive Testing (NIPT) based in Next Generation Sequencing (NGS) for the detection of chromosomal aneuploidies and fetal sex determination, we developed a new approach based on digital PCR technique for the determination of fetal fraction in maternal blood samples.

Materials and Methods: From one hundred pregnant women (gestational ages from 10 to 19 weeks), one aliquot of plasma cell free DNA (cfDNA) was selectively digested for methylated regions while a second one remained undigested. The fetal fraction was established by Droplet Digital PCR (ddPCR) based on absolute quantification of *RASSF1A* copies in digested (fetal) and undigested (fetal and maternal) samples. The ratio between both values determined the fetal fraction. The houseeking gene *β-Actin* was used as control for maternal cfDNA complete digestion. Bioinformatics analysis allowed a second assessment of fetal

fraction in male fetuses, since the analysis is based on the presence or absence of the chromosome Y, and its quantification by the number of total reads normalized respect to male control samples.

Results: The percentage of circulating cell-free fetal DNA (cffDNA) in maternal plasma was 3,42% and 31,38% regardless of gestational age. The correlation coefficient between the fetal fraction determined by ddPCR and determined by bioinformatics analysis only for male fetuses was 0,55.

Conclusions: Digital PCR is an innovative and sensitive technology compared to real time quantitative PCR as it has been published recently in the literature. This methodology is a promising tool to increase the precision of fetal fraction determination.

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P01.063C

Affordable high precision NIPT method implemented in a fully automated workflow

Vanadis team

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In several countries, there is an ongoing implementation of Non Invasive Prenatal Testing (NIPT) for detection of aneuploidies in high-risk pregnancies. A majority of the tests are performed using next generation sequencing technologies that provide both superior specificity and sensitivity compared to traditional first trimester screening. However, to enable health care providers to offer all women a high performance prenatal screening test, the NIPT assay cost and complexity need to be dramatically reduced. We present a platform that reduces the cost of the analysis substantially making the assay available for high throughput diagnostic laboratories. Our solution, called Vanadis NIPT, is a fully automated, walk-away solution, from blood sample to report, which allows one technician to process over 20,000 samples per year. The Vanadis NIPT technology uses DNA probes to specifically convert chromosomal targets of interest into DNA circles. These circles are then clonally expanded into discrete fluorescently labeled DNA objects, immobilized on a transparent nanopore filter, and finally imaged and counted through the bottom of the well. By targeting thousands of DNA fragments from each chromosome, PCR amplification can be avoided with increased assay precision and reduced contamination risks as a result. We applied the technology to analyze a large set of clinical samples including trisomy 13, 18 and 21 cases. By providing a high assay precision, >99% detection rate was achieved. In conclusion, the Vanadis NIPT platform, supported by clinical data, is a new solution that holds promise to provide NIPT to all pregnant women.

Vanadis team: None.

P01.064D

NIPT for sex chromosome aneuploidy; more harm than good?

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The reliable performance of NIPT for trisomies 13, 18 and 21 means that it is now available in many European countries; some providers also offer NIPT for sex chromosome aneuploidy. However, the clinical utility of NIPT for sex chromosome aneuploidy is debatable - most fetuses with monosomy X present with ultrasound anomalies indicating diagnostic testing, and prenatal detection of other sex chromosome aneuploidies is considered problematic. In addition, the positive predictive value (PPV) of NIPT for sex chromosome aneuploidy is reported to be as low as 38%. Within our region, NIPT is self-funded and available from a number of commercial providers; most referrals are considered low risk without ultrasound anomalies. Since January 2014 our centre received, for confirmatory diagnostic testing, 188 prenatal samples (117 amniotic fluids (AF) and 71 chorionic villus samples (CVS)) referred with a high risk (186) or inconclusive (2) NIPT result. Of these, 169 were confirmed by QF-PCR analysis; 11 cases showing mosaicism (six AF, five CVS) were classed as concordant. In 18 cases, the QF-PCR result showed normal copy number, discordant with the high risk NIPT result. PPVs were 97.9% (140/143) for trisomy 21; 90.9% (20/22) for trisomy 18; 87.5% (7/8) for trisomy 13 and 20% (3/15) for sex chromosome aneuploidy. Six of seven NIPT results showing a high risk of monosomy X were false positives. These data support the suggestion that NIPT for sex chromosome aneuploidy has little clinical utility and results in unnecessary invasive procedures and increased anxiety for some couples.

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P01.065A

A majority of chromosomal imbalances detected by NIPT are postzygotic in origin and are fetoplacental mosaics

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It has been established that cleavage-stage embryos have a high incidence of chromosomal rearrangements, with most of them being mosaics consisting of normal and abnormal cells. The majority of abnormal blastomeres are probably selected against during further development into the blastocyst stage. Nevertheless a small but significant number of trophectoderm biopsies analyzed by genome-wide aneuploidy detection methods during preimplantation genetic diagnosis or screening is poised with mosaic chromosomal imbalances. Within the PGD community there is currently a debate about the clinical importance of these imbalances and some authors even suggest that they are clinically irrelevant. Following 20,000 genome-wide NIPT analyses, we identified and reported 6 fetuses with potential clinically significant chromosomal imbalances. Further invasive follow-up unraveled the presence of complex segmental anomalies. For all 6 pregnancies we obtained invasive follow-up via amniocentesis. Additionally, for 5/6 one or more placental samples have been acquired. In 2 cases, a discrepancy was observed between the detected placental and fetal imbalances. Reciprocal and sequential events were observed in the remaining 4. Such imbalances can only be the consequence of pre-blastocystic cell lineages harboring different imbalances, which further evolved following the separation of the inner cell mass and the trophectoderm subpopulations. Hence, we demonstrate that (1) most chromosomal imbalances originate post fertilization, (2) they can pass the developmental barrier and be present at different loads in the fetus and/or the placenta, (3) detection of imbalances in trophectoderm is of clinical importance and (4) detection of segmental imbalances via NIPT provides valuable information for pregnancy management.

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P01.066B

Non-invasive prenatal diagnosis of single-gene disorders in maternal plasma: detection of paternally-inherited mutations using droplet digital PCR

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Background: To limit risks of miscarriages associated with invasive procedures of current prenatal diagnosis practice, we propose a standardized protocol for non-invasive prenatal diagnosis (NIPD) of single-gene disorders based on the qualitative detection of paternally-inherited mutations in maternal blood using droplet digital PCR (ddPCR).

Methods: Four couples at risk of transmitting paternal *NF1* mutations and four couples at risk of transmitting compound heterozygous mutations of *CFTR* gene were included in this study. NIPD was performed between 8 and 15 weeks of gestation, in parallel to conventional invasive diagnosis. Specific Taqman probes were designed to detect the paternal mutation and to control the presence of cell-free fetal DNA (cffDNA) by ddPCR. Specificity and sensitivity of each assay were determined from paternal sample, and then fetal genotype was inferred from maternal plasma sample.

Results: Presence or absence of the paternal mutant allele was correctly determined in all of cases and the accuracy of the designed protocol was determined 100%.

Conclusions: This protocol is suitable for implementation in routine clinical practice, with satisfactory accuracy in detection of paternal mutations in cffDNA, and allows considering extending the applications of these technologies in non-invasive prenatal testing of many other monogenic diseases.

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P01.068D

Prospective validation study of utilization of Trisomy test for noninvasive prenatal testing of common trisomies

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Noninvasive prenatal testing (NIPT) based on analysis of circulating DNA of pregnant women is becoming integral part of prenatal genetics. In last years different laboratories made NIPT available worldwide.

Aim of our study was prospective validation of Trisomy test, home-made NIPT test using paired-end low coverage whole genome sequencing approach and our own bioinformatical method for z score calculation.

Between September 2015 and August 2016 altogether 1863 samples of pregnant women were analyzed. In the sample cohort 21 cases of trisomy 21, 9 cases of trisomy 18 and 3 cases of trisomy 13 were present. Of these all trisomy 21 and trisomy 13 were correctly identified, in cases with trisomy 18 eight of nine cases were detected. Overall detection rate for tested trisomies was 96.97%. Two false positive cases were recorded, one for trisomy 21 and one for trisomy 13, therefore specificity of the test was 99.89% overall. Noninformative results have been recorded in less than 4% of samples, of these two thirds have become informative after second analysis performed from new blood sample that was taken two to four weeks after the original one. Higher frequency of samples with low fetal fraction was detected in pregnant women treated with low molecular weight heparin or of high body weight, what is in concordance with previously published data.

Trisomy test based on paired-end low coverage whole genome sequencing enabled simultaneous fetal fraction calculation and z score calculation with detection rate and specificity comparable with data published in larger studies and metaanalyses.

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P01.069A

Identification of viral DNA from unmapped human reads in data from NIPT

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Introduction: NIPT analysis based on whole genome sequencing is one of novel genetic test with wide worldwide use. It is based on sequencing of DNA isolated on plasma and followed by mapping of the data to reference human genome. A significant portion of reads remains unmapped to the reference and it remains unused. The purpose of this study was to identify if viral DNA can be detected in such sample data and to attempt to build longer contigs from such reads.

Materials and methods: We sequenced total DNA from plasma of pregnant women of two groups. A larger group had no indication of present viral infection and the second group contained sample positive for HIV viral infection. DNA fragments which mapped to the human reference genome (version hg38) were eliminated. We assigned each unmapped fragment a taxonomic label using metagenomic classifier Clark. Taxonomic composition of samples was compared and visualized using Krona graphs.

Results: We identified presence of viral sequences in a small number of samples in both groups of samples. In the results we present breakdown of the identified viral data.

Conclusions: Additional information can be extracted from complex data generated in NIPT tests which were originally destined to be eliminated by bioinformatic filtering. We show an example of identified viral sequences which add value to "junk" data.

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P01.070B

The clinical utility of genome-wide non-invasive prenatal screening

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Introduction Conventional cell-free fetal DNA (cfDNA)-based NIPT focuses on detection of common aneuploidies, leaving a gap of ~17% of clinically relevant chromosomal abnormalities that would go undetected. Genome-wide NIPT would greatly expand the range of chromosomal rearrangements detectable, but it could lead to a decrease of the specificity and, consequentially, to an increase in unnecessary invasive testing. In this study, we compared the performance of the genome-wide NIPT and conventional cfDNA-based NIPT in a large general population of pregnant women, in order to assess the clinical utility of the genome-wide screening.

Materials and Methods Genome-wide cfDNA analysis was offered to 12.114 pregnant women undergoing NIPT for common fetal aneuploidy. Sequencing data were analyzed using an algorithm optimized to identify aneuploidies and subchromosomal aberrations.

Results Genome-wide screening allowed detection of 12 (7.4%) potentially viable clinically relevant chromosomal abnormalities, which would have remained overlooked if only conventional NIPT had been performed. This resulted in a statistically significant higher sensitivity (100% vs92.64%,p<0.001) than did standard screening. This was achieved without sacrificing the specificity of the test, that resulted similar to that obtained with standard cfDNA testing (99.87%vs99.77%,p=0.064).

Conclusion: The clinical utility of expanding NIPT to cover the entire genome is controversial. It pertains a risk of overdiagnosis with a higher number of false positives due to chromosomal rearrangements, which are confined to the placenta, potentially leading to an increase in unnecessary invasive testing. The results of this study demonstrate that a high specificity may be maintained while extending the screen to all chromosomal abnormalities.

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P01.071C

Non-invasiveprenatal diagnosis of beta thalassaemia based on a free fetal DNA Real TimePCR-based approach

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Introduction: Prenatal diagnosis of embryos at risk for monogenic diseases, like thalassaemias, still presents the most common method of disease prevention. This approach is predominantly based on genetic analysis of material of fetal origin which is mainly obtained through invasive procedures. Since invasive procedures have a 1–2% probability of miscarriage, non-invasive prenatal testing (NIPT) approaches, based on analyzing fetal DNA circulating in the maternal plasma, were developed. Here, we present the broaden development of a novel approach for detecting beta globin gene mutations present in free fetal DNA (ffDNA). Our method is based on High Resolution Melting (HRM) combined with quantitative analysis.

Materials and methods: ffDNA is isolated from 2ml maternal plasma and screened for the presence of β globin gene parental mutations based on an in-house HRM developed approach. Previously described SNPs in the vicinity of β globin gene, for which parents presented distinct haplotypes, were also determined.

Results: Our hitherto results (27 cases) succeeded in obtaining the same diagnosis with the preceded analysis of corresponding chorionic villus sampling. Cases include male embryos that inherited either the paternal or maternal mutation and cases where the embryo has inherited both parental mutations located on the same amplicon (i.e. CD39/IVS-I-110).

Conclusions: The described approach, after further evaluation, may allow its application at the diagnostic level as a primary or secondary method for NIPT in cases where the embryo is male and has inherited either the paternal or maternal β globin gene mutation and in certain cases both parental mutations.

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P01.072D

Novel statistical model for non-invasive prenatal testing for aneuploidies

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Introduction: Recent studies showed that massively parallel DNA sequencing (NGS) of cell-free fetal DNA from maternal plasma can be used for non-invasive prenatal aneuploidy testing (NIPT). Especially desired are reliable

detections of chromosomal aberrations such as Down, Edwards or Patau syndrome. Most of the current methods in practice are based on some form of z-score analysis, usually with an assumption of normality of NGS data. By applying other probabilistic assumptions on the NGS data we derived a novel approach to z-score testing with reaches to other problems as well.

Materials and methods: We applied methods in probability and statistics to derive a sound mathematical theory of z-score calculation from the data given by non-normal distribution.

Results: Our novel method allows calculation of trisomy z-score, error of this z-score given the genetic variability in population, fetal fraction estimation with error of this estimation as well as minimal number of reads for our method to reliably work given some specified targeted fetal fraction. We demonstrate on real data from NIPT testing that our approach allows improved sensitivity and specificity for common chromosomal aneuploidies even when ultra-low read counts are used.

Conclusion: Successful application of trisomy detection in practice is becoming a reality. By proper understanding of mathematical background in such tests, we can achieve higher precision and lower cost of tests.

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P01.073A

Non invasive prenatal screening (NIPS) as indication for prenatal testing

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Non-invasive prenatal screening (NIPS) of cell-free fetal DNA (cffDNA) for fetal aneuploidy risk assessment is offered to pregnant women along with, or instead of combined maternal serum screening. It has been validated for common autosomal trisomies (trisomy 21, 18, 13), sex chromosome aneuploidies, and a selection of microdeletion syndromes.

For the past three years 2200 pregnant women with different indications (increased maternal serum screening risk, abnormal ultrasound markers and/or advanced maternal age) were referred to our department for prenatal detection of chromosomal aneuploidies by QF-PCR analysis. Among them 21 were referred for invasive prenatal diagnosis with a positive result from NIPS: 15 cases - for trisomy 21, two - for trisomy 18 and three - for sex chromosomal aneuploidies. One case was referred for amniocentesis with a positive result from NIPS for both monosomies 13 and 18.

After invasive procedures - amniocentesis and QF-PCR analysis, trisomy 21 was confirmed in all but one T21 NIPS positive cases. The remaining five NIPS -positive referred samples were found to be negative for trisomy 18 and sex chromosomal aneuploidies by use both of QF-PCR and cytogenetic analysis. Monosomies 13 and 18 were excluded too in the last case. Confined placental mosaicism was excluded for all false positive cases, newborns were with normal karyotype.

NIPS findings, discordant with the fetal karyotype can be because of confined placental mosaicism, maternal chromosome abnormalities and other maternal conditions such as occult malignancy. The lack of further, more sophisticated investigations due mainly to economical reasons leaves unanswered questions.

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P01.074B

Identification and characterization of fetal RNA biomarkers for the development of NIPT of trisomy 21

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Currently, non-invasive prenatal testing (NIPT) uses cell free fetal DNA in maternal plasma as a screening tool for fetal aneuploidy. However, pregnancies with low fetal fraction are excluded from the diagnostic screening even though high sensitivity methodologies and sophisticated bioinformatics analysis are used. RNA biomarkers can be advantageous compared to DNA biomarkers in NIPT, due to their multi-copy characteristic. We aim to identify robust RNA biomarkers, with the potential to develop a novel test with high diagnostic accuracy for the NIPT of trisomy 21 in all pregnancies. We performed whole genome expression microarrays in four normal and two trisomy 21 fetuses and

their matching maternal cells. The differentially expressed genes (DEGs) identified were confirmed with qPCR and digital PCR (dPCR) in 60 samples, 30 chorionic villi samples (CVS) of which 6 were trisomy 21, and 30 maternal cells. Further evaluation of the confirmed DEGs was performed to assess the maternal background if present. Additionally, we searched the microarray data to identify CVS specific controls with the potential to be used as normalizers in maternal plasma samples. Expression microarray data identified 386 DEGs between normal and trisomy 21 CVS, of which five were confirmed as CVS and trisomy 21 specific in qPCR. Re-evaluation with dPCR showed low but consistent expression in all maternal cells tested for three out of five DEGs identified. Additionally, two genes were identified and confirmed as CVS specific controls. The DEGs identified are promising biomarkers for the NIPT of trisomy 21 including low fetal fraction pregnancies.

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P01.075C

Nonclassical adrenal hyperplasia and polycystic ovary syndrome: evaluation of four cases

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Congenital adrenal hyperplasia is one of the most frequently seen recessive endocrine disorder. Ninetyfive percent of the patients have mutations in *CYP21A2*. Non-classical adrenal hyperplasia (NCAH) is phenotypically the milder form and usually manifests its self with hyperandrogenism, oligimenorrhea, amenorrhea, hirsutism, subfertility and in some cases with male type baldness. Patients may have polycystic overies. On the other hand, polycystic ovary syndrome (PCOS) is a extremly heterogenous and complex condition. Phenotypic findings of these two disorders may overlap. There are some PCOS patients that were detected with 21-hydroxylase deficiency and reported to be actually as NCAH. Here, we present four cases with irregular menstrual cycles, hypetrichosis, polycyctic ovaries and eleveated 17α-hydroxyprogesterone levels that were investigated for the frequent mutations of *CYP21A2*. Three of the patients had the homozygous V281L and one had the homozygous P30L mutations. The diagnosis or the rule out of NCAH in hyperandrogenic patients are of great importance. Being a monogenic condition, in NCAH providing genetic counselling is crucial both for diagnosis and reccurence.

Reference

Pignatelli D. Non-classic adrenal hyperplasia due to the deficiency of 21-hydroxylase and its relation to polycystic ovarian syndrome. Front Horm Res. 2013; 40: 158–70.

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P01.076D

The presence of cell free fetal DNA in maternal blood after single fetal reduction of trisomy 21 aneuploidy in a twin pregnancy

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Introduction: Cell-free fetal DNA is present in the maternal plasma and allows non-invasive prenatal testing (NIPT) for fetal aneuploidy screening. However, the application of NIPT to twin pregnancies is an additional challenge because DNA is available for analysis from each fetus. In this study, we estimate whether cell-free DNA is present after elective reduction in a twin pregnancy.

Methods: The woman was a 37-year-old who revealed a twin pregnancy and chose NIPT for prenatal screening of chromosomal aneuploidies. We used illumina platform and whole genome sequencing-based NIPT, and analyzed each chromosome.

Results: The normalized chromosome value (NCV) value was 8.79 for trisomy 21 and follow-up counseling and invasive testing was recommended. One fetus was trisomy 21 and another was euploid after karyotyping confirmation by amniocentesis. The karyotypes were 47,XX,+21 and 46, XX, respectively. The parents opted for selective termination of pregnancy. The pregnant woman still accepted NIPT for tracking the remaining fetus. The NCV values are 17.78, 36.71, 6.24 and 0.80, respectively for 4, 9, 12 and 15 weeks after selective reduction.

Conclusion: Cell-free fetal DNA is derived mainly from placenta. In our study, the placenta can continue to shed cell-free fetal DNA after the reduction a twin pregnancy to a singleton one and last 12 weeks. Therefore, the discordant

results for NIPT can therefore be attributed to early fetal demise.

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P01.077A

Increased nuchal translucency and additional ultrasound findings in Noonan syndrome: when is DNA testing justified?

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Background: Prenatal features of Noonan syndrome have been described, but only a few studies have performed systematic reviews or original research. Increased nuchal translucency (NT) has been one of the important factors in detecting Noonan syndrome. This study evaluates five years of prenatal Noonan syndrome (NS) testing in the Netherlands including the value of testing for NS with isolated increased NT.

Methods: More than 300 samples that were sent in for prenatal NS testing in 2011–2016 were collected. During the first years 3–5 NS/RASopathy genes were analyzed, and later 14 genes. For all mutation-positive samples the referring physician was asked to provide detailed ultrasound findings and postnatal follow up. For ~150 mutation-negative samples after testing 14 genes the clinical information was collected on the requisition form and analyzed without consulting the referring physician.

Results: In total 43 mutation positive samples were detected. 26% of these fetuses showed a PTPN11 mutation, followed by RAF1 mutations (14%) and RIT1 mutations (9%). SOS1 and HRAS mutations were found in two fetuses in each gene. SHOC2, BRAF, MAP2K1 mutations were confirmed in one fetus each. All fetuses showed a variable degree of involvement of prenatal findings. Five fetuses showed only one ultrasound finding: one with isolated increased NT and four fetuses with cystic hygroma.

Conclusion: Increased NT is rarely seen as an isolated feature in NS. However, cystic hygroma is an important sole factor. Overall, fetuses with NS show multiple ultrasound findings and NS should not be tested when increased NT is isolated.

K.E. Stuurman: None. **T. Rinne:** None.

P01.078B

Identification of *de novo* genetic targets associated with natural & accelerated ageing by single-cell whole exome sequencing

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Advanced maternal ageing in women at the age of over 35 is currently a big health concern worldwide. It links to inferior fertilization rate and higher genetic risks in offspring. External factors like hormones and follicular cells have been reported comprehensively. However, the contribution of genetic alterations in oocyte ageing remains largely elusive due to limitation on experimental technologies and the limited amount of sample materials.

In this study, we aimed to identify natural and accelerated ageing-related genetic alternations, including single nucleotide polymorphism (SNPs) and copy number variations (CNVs) in mouse oocyte ageing models Four groups of female mice consisted of a young group (6-weeks-old), a natural aging group (36-weeks-old), an accelerated aging group (treated by 4-Vinycyclohexene diepoxide) and a vehicle group (treated by sesame oil) were mated with an adult male. The DNA samples derived from single oocytes and blood samples of females, offspring and males were subjected to whole exome sequencing. Natural and accelerated aging-related *de novo* variations (DNVs) and associated genes were identified. We found mutation patterns of DNVs were similar to mutational signatures in women cancers. Finally, we detected an aberrant CNV region in natural aging oocytes, where it contained genes involved in aging diseases and reproduction.

Taken together, our study highlighted the key genetic targets associated with oocyte aging. The data allows dissecting the aging mechanisms of subfertility in women with advanced maternal age.

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P01.079C

The clinical use of determining embryonic ploidy status in couples undergoing PGD for translocations

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The use of array technology in preimplantation genetic diagnosis (PGD) for chromosomal disorders allows for simultaneous genome-wide ploidy analysis. Whether this data is useful in selecting embryos for transfer is unknown.

We performed an observational, multicentre study covering a 3-year period (2014–2016) during which 117 couples underwent 171 PGD cycles for structural chromosomal abnormalities in three IVF centres.

Comprehensive chromosome testing was performed on day 3 single blastomeres by array CGH and only embryos with a balanced karyotype for the PGD indication were eligible for transfer. Two of the three centres ranked embryos for transfer solely based on morphology. The third centre ranked embryos based on both ploidy status of non-indication chromosomes and morphology.

In total, 169 embryo transfers were performed (185 embryos). Of these, 37 involved single embryo transfer of a balanced/aneuploid embryo, leading to three biochemical pregnancies plus three children born. In the latter, aneuploidy of non-indication chromosomes involved a trisomy 19, a trisomy X and a gain of 17p combined with a loss of 17q. Seven double embryo transfers (DET) were performed with two balanced/aneuploid embryos, giving rise to three biochemical pregnancies. Six DETs comprising one balanced/euploid and one balanced/aneuploid embryo resulted in one first trimester abortion plus two children born. Compared to balanced/euploid embryos, balanced/aneuploid embryos had significantly lower implantation and higher miscarriage rates.

Data indicate that in a PGD setting, embryos with an inferred aneuploidy for non-indication chromosomes have lower implantation and higher miscarriage rates but are eligible for transfer and compatible with live birth.

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P01.080D

PGS, PGD and PGD-counselling in Russia: experience of a reference laboratory

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Introduction: Preimplantation genetic screening (PGS) for chromosomal abnormalities helps to select euploid embryos for transfer and therefore enhances pregnancy rate in IVF patients. Preimplantation genetic diagnosis (PGD) for monogenic disorders allows to prevent birth of affected child in families with high genetic risk.

Materials and Methods: PGS by aCGH with 24sure kit or by NGS with Veriseq kit (Illumina). PGD by haplotyping on ABI3130XL along with restriction analysis.

Results: A total of 3610 embryos were tested for chromosomal abnormalities and 53,7% were found to be aneuploid. Euploidy rate depended on maternal age and ranged from 59% (<35 years) to 20% (>41 years). Euploidy rate in oocyte donor cycles was 60%.

Results of PGS by NGS and aCGH were compared and the only difference was found for mosaicism detection. Isolated mosaicism was diagnosed in 3% by aCGH and in 12% by NGS.

Analysis for translocations was carried out in 34 IVF cycles and 126 embryos were analysed, 51 of them euploid. Out of 75 aneuploid embryos in 15 only anomalies in chromosomes not involved in translocation were found.

For monogenic PGD, testing systems for 23 different disorders, Rh and HLA, were developed and verified on single cells. Testing systems combined direct mutation analysis with STR haplotyping. PGD was successfully implemented in 25 IVF-PGD cycles, with or without PGS and HLA-matching.

Conclusions: PGS by aCGH and NGS has similar sensitivity for aneuploidy testing, but not for mosaicism. Both methods are compatible with PGD for monogenic disorders on the same biopsy specimen.

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Towards optimization of PGD for recurrent t(11;22)(q23;q11) carrier

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The t(11;22)(q23;q11) is the most frequent reciprocal translocation in humans. Balanced carriers often have reproductive problems, such as infertility, recurrent pregnancy loss, or the birth of offspring with congenital malformation syndrome called Emanuel syndrome. Preimplantation genetic diagnosis (PGD) is the useful solution for such reproductive problems. Since all of the t (11;22) carriers have the translocation breakpoints within the small ~500bp regions on 11q23 and 22q11, we can set up the optimal PGD condition using a sample from a single typical t(11;22) family. Experiments were performed using lymphoblastoid cell lines from a patient with Emanuel syndrome having trisomy of both distal 11q23 and proximal 22q11. Assuming the trophoectoderm biopsy (TE) biopsy, five cells were picked up using cell manipulator, and whole genome amplification was performed. For quantitative analysis of two unbalanced translocation regions on 11q23 and 22q11, we tested oligonucleotide- or BAC-based microarray as well as next generation sequencing (NGS). Translocation-specific PCR was also performed. In general, NGS detected the unbalanced region better than any of the microarray platforms, although the sensitivity was not perfect at the proximal 22q11 region possibly due to many segmental duplications. Translocation-specific PCR worked without any problems. We conclude that NGS combined with translocation-specific PCR is the best way for copy number analysis of TE biopsied samples from couples of t (11;22) carrier. (Supported by a grant-in-aid for Scientific Research from the MEXT and that from MEWL Japan.)

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P01.082B

First prenatal PIK3CA-related overgrowth spectrum (PROS) cohort

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Introduction: PIK3CA-related overgrowth spectrum (PROS) is the umbrella term used to designate phenotypes caused by PIK3CA postzygotic activating mutations. Even if prenatal manifestations are described, they rarely lead to termination of pregnancy, fetal or neonatal death.

Material and methods: We gathered 8 severe fetal cases suspected of PROS that led to fatal issues. We performed ultra-deep PIK3CA next-generation targeted sequencing in several types of fetal tissues, affected or not.

Results: We identified PIK3CA variants that affect function for six fetuses (6/8; 75%).

PIK3CA positive fetuses were classified into two spectrums: megalencephaly-capillary malformation (MCAP) spectrum with cerebral involvement, and congenital lipomatous overgrowth, vascular malformation, epidermal nevus, and scoliosis (CLOVES) spectrum, without cerebral involvement. All fetal phenotypes were detected during the second trimester US-scan (table). Autopsies permitted to precise prenatal data.

The search for mutations in all available tissues permitted to confirm that mutations are only detectable in affected tissues.

Conclusion: Most PROS fetuses in our prenatal cohort had cerebral involvement (4/6; 66.6%), likely due to a probable bias due to the greater sensitivity of prenatal screening for brain abnormalities, with a frequency of hotspot variants.

Prenatal fetal phenotypes and pregnancy outcomes						
Fetus	1	2	3	4	5	6
Sex	F	M	F	M	M	F
Megalencephaly or hemimegalencephaly	+	−	+	−	−	+
Polymicrogyria	−	−	+	−	−	+
Ventriculomegaly	−	−	+	+	−	−
Dysmorphism	+	−	−	+	−	+
Shortned long bones	+	−	−	+	−	+
Extended lymphangioma	−	+	−	−	+	−
Macrosomia	+	+	+	+	+	+
Pregnancy outcomes	Terminaison of pregnancy	Terminaison of pregnancy	Terminaison of pregnancy	Terminaison of pregnancy	Neonatal death	Death during labor
Term of birth/TOP (WG)	31+0	26+0	27+6	20+5	38+0	30+0

Molecular data						
Fetus	Phenotype	Tissue	Affected	Protein	Variant	Allternative allele count
1	MCAP	Lung	Yes	p. Phe909Leu	Rare	35%
2	CLOVES	Tumor	Yes	p. Glu545Lys	Hotspot	10%
2	CLOVES	Lung	No			0%
2	CLOVES	Muscle	No			0%
3	MCAP	Brain	Yes	p. His1047Arg	Hotspot	42%
3	MCAP	Brain	No			0%
4	MCAP	Skin	Yes	p. Glu545Lys	Hotspot	5%
4	MCAP	Lung	No			0%
5	CLOVES	Skin	Yes	p. His1047Arg	Hotspot	6%
5	CLOVES	Liver	No			0%
5	CLOVES	Skin	Yes	p. His1047Arg	Hotspot	24%
6	MCAP	Tumor	Yes	p.Glu110del	Rare	19%

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P01.083C

In vitro fertilization has no effect on prevalence of mosaic copy-number alterations in fetal and placental lineages

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Chromosomal instability (CIN) is a common phenomenon in cleavage-stage embryogenesis that leads to a mixture of euploid and aneuploid cells within the same human embryo during *in vitro* fertilization (IVF). However, the rate of CIN in naturally conceived embryos is largely unknown, because it is impossible to study human embryos *in vivo*. Here, we developed and applied a novel haplarithmisis-based method to characterize allelic architecture of DNA samples derived from the placenta and cord blood of the same pregnancy. Specifically, we scrutinized genome-wide single nucleotide polymorphism profiles in DNA from the father, mother, placenta and neonate umbilical cord blood of 55 families (quartets), of which 26 and 29 quartets were from natural and IVF pregnancies, respectively. We demonstrate that CIN is not preserved at later stages of prenatal development, and that *de novo* genomic alterations occur at similar rates in IVF and naturally conceived neonates. The findings confirm that IVF treatment has no detrimental effect on the chromosomal constitution of fetal or placental lineages.

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Fetal microsatellite in the *HMOX-1* promoter is associated with severe and early-onset pre-eclampsia

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Introduction: Pre-eclampsia is a vascular pregnancy disorder that often involves impaired placental development. Heme oxygenase-1 (HO-1, encoded by *HMOX-1*) is a stress response enzyme crucial for endothelial function and placental development. Long version of the GT_n microsatellite in the *HMOX-1* promoter decreases HO-1 expression, and is associated with late-onset pre-eclampsia. Our aim was to study whether the length of fetal repeat is associated with mother's pre-eclampsia, and whether maternal serum HO-1 level is altered in pre-eclampsia.

Methods: We genotyped the repeat in the cord blood of 609 pre-eclamptic and 745 non-pre-eclamptic neonates. Maternal serum HO-1 was measured in the first (222 cases/243 controls) and third (176 cases/53 controls) pregnancy trimester samples.

Results: The long fetal GT_n allele was associated with pre-eclampsia and early-onset pre-eclampsia, and the long fetal genotype with pre-eclampsia and severe pre-eclampsia (additive/dominant models), as well as with early-onset pre-eclampsia (additive model). Interaction analysis suggested the maternal and fetal effects to be independent. The first or third trimester maternal serum HO-1 levels were not altered in pre-eclamptics, but were lower in the carriers of the long maternal GT_n repeat. HO-1 concentration decreased towards the end of gestation, and also BMI, smoking, age and birth weight were related to HO-1 level. Experiments are underway to investigate whether the long fetal repeat decreases placental HO-1.

Conclusions: The long fetal GT_n repeat may increase mother's risk of especially severe and early-onset pre-eclampsia, and the maternal and fetal risk alleles likely predispose to different disease subtypes.

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Design and application of a diagnostic panel for preconception genetic screening

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Carrier screening of monogenic hereditary diseases is a routine practice in at risk families. In Russia, in the absence of family history, common mutation screening in genes *PAH*, *CFTR*, *SMN1* and *GJB2* is recommended due to high prevalence and frequency of carriers. Advances in genomics made possible to expand traditional preconception screening to benefit prospective parents. For this reason a diagnostic panel including 39 common mutations associated in the European population with development of more than 20 severe hereditary diseases was designed.

The presence of the mutations was examined in 465 DNA samples including 398 from the patients of IVF

clinics, 58 from sperm donors and 9 from healthy individuals. DNA was amplified in microfluidic plates with TaqMan assays and PCR product detection at the end-point. Initially, the panel included 48 mutations but 9 mutations were eliminated during the validation step due to technical reason or change of clinical status.

As a result mutations c.1521_1523delCTT, c.1545_1546delTA (*CFTR*), c.3207C>A (*ATP7B*), c.452G>A (*DHCR7*), c.1903C>T (*LAMB3*), c.657_661delACAAA (*NBN*), c.1062+5G>A (*FAH*), c.206C>T (*IDUA*), c.422G>A (*PMM2*), c.563A>G (*GALT*), c.1423C>T (*ADAMTS13*) were found in 25 individuals including a patient with mutations in 2 genes. A homozygous carrier of a mutation in the *ATP7B* gene was detected.

Thus, excluding a diagnosed case of hereditary disorder the carriage of at least one pathological mutation was identified in 5.2% of the individuals. The designed diagnostic panel can be applied for a carrier screening of severe hereditary disorders and recommended to prospective parents and donors in the assisted reproduction programs.

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Potential diagnostic value of circulating microRNA-1183 and CHURC1 gene in NIPD of preeclampsia

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Introduction: Preeclampsia is a multi-systemic disease of pregnancy and leading cause of maternal and fetal mortality and morbidity worldwide. The differential expression of circulating microRNAs were reported in maternal plasma of pregnant women and they could be stabilized in plasma. In this regard microRNAs released from placenta to maternal circulation are potential biomarkers for NIPD. The aim of this study was to identify expression profiles of circulating miR-1183 and its candidate target genes in maternal plasma.

Materials and Methods: Plasma samples were obtained at 37–39 weeks of gestation from 31 women who developed preeclampsia (cases) and 26 women with normotensive pregnancies (controls). The expression of miR-1183 was determined using RT-qPCR and comparative Ct method relative to synthetic *C.elegans* microRNA (cel-miR-39). CHURC1 was selected as putative target gene of miR-1183. Correlation of miR-1183 and CHURC1 expression levels were analyzed with Pearson's correlation coefficient (95% CI). The diagnostic performance of mir-1183 and CHURC1 were assessed by receiver operating characteristic (ROC) curve analysis.

Results and Conclusions: mir-1183 was significantly overexpressed ($p = 0.0068$) whereas its target gene CHURC1 was significantly down-regulated ($p<0.001$) in maternal plasma of cases compare to controls. Pearson correlation analysis revealed that mir-1183 expression was inversely correlated with CHURC1. The area under the ROC curve (AUC) of miR-1183 and CHURC1 were 0.90 and 0.96 respectively (95%CI) indicated that their expression can discriminate between cases and controls. These findings reveal that circulating CHURC1 can be involved in preeclampsia pathogenesis and circulating miR-1183 could contribute to disease pathology functioning through regulating CHURC1.

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X-linked CNVs in women with idiopathic pregnancy loss and extremely skewed X-chromosome inactivation

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Introduction: Skewed X-chromosome inactivation is associated with some human pathology including pregnancy loss. According to our data the population frequency of extremely skewed (threshold >90%) X-chromosome inactivation in women with miscarriage is 8%. The possible reasons for the skewed X-chromosome inactivation are lethal mutations in X-linked genes or aberrations of one of the X-chromosome.

Material and Methods: In our study, pathogenic CNVs on X-chromosomes, which may be the cause of skewed X-chromosome inactivation and pregnancy loss were analyzed. These CNVs were detected by array CGH using SurePrint G3 Human CGH+SNP 4×180K Microarray Kit (Agilent Technologies, USA) in lymphocytes of 5 women with idiopathic pregnancy loss and extremely skewed X-chromosome inactivation (>95%).

Results: One woman had dupXp22.33 (1.7 Mb), whereas other - delXq24 (239 kb) containing 15 genes (*PLCXD1, GTPBP6, LINC00685, PPP2R3B, SHOX, CRLF2, CSF2RA, MIR3690, MIR3690-2, IL3RA, SLC25A6, ASMTL, P2RY8, AKAP17A, ASMT*) and 8 genes (*SLC25A43, SLC25A5-AS1, SLC25A5, CXorf56, UBE2A, NKRF, SEPT6, MIR766*), respectively. Products of these genes are involved in cell proliferation, differentiation and post-transcriptional regulation of gene expression in multicellular organisms.

Conclusions: An excess or deficiency of gene expression can potentially affect cell cycle arrest and lead to skewed X-chromosome inactivation in maternal cells, and fetal death.

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Novel splice junction of Pregnancy-Associated Plasma Protein A (PAPPA) in human placenta

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Introduction: Human placenta finely tunes expression of various genes essential in pregnancy maintenance and development of the fetus. Any alterations in expression level of specific genes and its further processing may indicate the major molecular mechanisms underlying pathological pregnancies. Alternative splicing (AS) can generate various transcripts and hence multiple protein isoforms from a single gene. Regulation of alternative splicing events in multi-exon genes not only affects expression level or protein function, but can also lead to differences in AS between human individuals. Pregnancy-associated plasma protein A (PAPPA) is a metalloprotease belonging to the metzincin superfamily of zinc peptidase. During pregnancy PAPPA is produced at high levels by the placenta and circulates as a heterotetrameric complex. Decreased levels of this complex are associated with adverse pregnancy outcomes such as IUGR, preterm delivery, miscarriage, preeclampsia or fetal aneuploidy. The goal of this study was to identify novel sequence and splice-junction within PAPPA.

Materials and Methods: NGS was performed to identify the pattern of PAPPA expression in placentas (N = 4) from women in single and twin pregnancies. Moreover, *in silico* sequence analysis was performed to estimate potential influence of AS on structure, function and physicochemical properties.

Results: Our thorough analyses of placental transcriptome permitted the identification of novel alternative splice junction of PAPPA. In addition we predicted secondary structure of PAPPA protein.

Conclusions: We can assume that the novel identified placental transcripts may contribute to pregnancy course, but further functional analyses are required to state how they affect pregnancy outcome.*Supported by UWM (WNM#25.610.001-300).

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P01.089A

PGD to select HLA matched embryo for stem cell therapy in epidermolysis bullosa dystrophica

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Preimplantation genetic diagnosis (PGD) allows families to assure their child's health regarding genetic disorders before pregnancy and also can prevent medical abortion. this method, diagnoses perform on blastomers biopsied from 8-cell stage embryos that are created by in vitro fertilization method (IVF). PGD combine with HLA typing is a strategy to select healthy HLA-matched embryos as a potential

donor for stem cell transplantation of the affected sibling. Here we present application of molecular PGD to select healthy HLA-matched embryo as a donor for Recessive Dystrophic Epidermolysis Bullosa (RDEB). RDEB is a severe inherited skin disorder which blisters affect the whole body. A family with a 17 years old affected child with RDEB was referred to our laboratory. Peripheral blood samples were collected and genomic DNA was extracted using salting out method. Mutation detection in COL7A1 gene was carried out using direct sequencing method. Fragment analysis and haplotype mapping were performed to track the defective alleles in the family. On day 3 post fertilization one or two blastomers were removed from each embryo. Selected mutation (COL7A1:c.6994C>G) was investigated using direct sequencing and informative STR markers (17 loci for HLA and 8 for Col7A1) were checked in the family using nested PCR method. A healthy HLA-matched embryo was selected and implanted to mother's uterus. From 6 blastomeres, only one was suitable for transfer. Prenatal diagnosis was performed at 16th week of gestational age which confirmed PGD result. The child was born and she was healthy according tothe pediatric dermatologist examination.

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Perinatal follow-up of children born after preimplantation genetic diagnosis in the Netherlands between 1995 and 2014

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Introduction: Clinical studies of children born after PGD in other countries report some concern regarding adverse perinatal and neonatal outcomes. We collated data on the congenital malformation rate, misdiagnosis rate, birth parameters, perinatal mortality and hospital admissions from a cohort of Dutch children born after PGD.

Materials and Methods: Data on all Dutch PGD pregnancies between 1995 and 2014 and corresponding perinatal outcome were prospectively collected by questionnaires filled out by the couples and from the medical files of the mothers and the children. A retrospective analysis of the cohort was performed.

Results: 439 pregnancies in 381 women resulted in 364 live born children. Nine children (2.5%) had major malformations. This percentage is consistent with other PGD cohorts and comparable to the prevalence reported by the European Surveillance of Congenital Anomalies (EUROCAT). We reported one misdiagnosis resulting in a spontaneous abortion of a fetus with an unbalanced chromosome pattern. Due to twin pregnancies almost 20% of the children were born premature (<37weeks) and less than 15% had a low birth weight. The incidence of hospital admissions is in line with prematurity and low birth weight rate. One child from a twin, one child from a triplet and one singleton died at 23, 32 and 37 weeks of gestation respectively.

Conclusions: this first report of the children of the Dutch PGD cohort does not show an increased risk of major malformations or adverse perinatal outcome, compared to other PGD cohorts from the literature and naturally conceived children.

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Implementing preimplantation genetic screening: our clinical experience

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Aneuploidy is the major leading cause of age-related decline in fertility. Indeed, it has been described as the main reason for failed implantation and development to term. Preimplantation genetic screening (PGS) provides chromosomal information of each embryo avoiding aneuploid embryos transfer and increasing implantation rates.

This technique has been optimized in cleavage-stage embryo, however it has been shown that biopsies in this stage impair human embryonic implantation while blastocyst biopsy does not. Moreover, this technique has been shown to be much more robust, reliable and less harmful for embryos. For these reasons, our team sifted to blastocyst biopsies. The aim of this work was to evaluate implantation rates in cleavage-stage and blastocyst biopsies and confirm the benefits of blastocyst biopsies. A total of 208 patients went to PGS from 2013 to 2016. Of these, 183 underwent cleavage-stage biopsy and 25 experienced blastocyst biopsy. Array comparative genomic hybridization (aCGH) was used for cleavage-stage biopsy while next generation sequencing (NGS) for blastocyst biopsy. Interestingly, from 2015 to 2016 we observed a decrease in evolutionary pregnancy rate in patients >38 years (53.84% in 42 patients vs. 32.14% in 49) which is increasing thanks to blastocyst biopsy. Indeed, we reached almost the same evolutionary pregnancy rate having half of blastocyst biopsies (20% in 11 patients vs. 32.14% in 49). We also confirmed the correlation between chromosomal alterations observed in embryos and its morphology. Our results support the suitability of blastocyst biopsy in clinical practice.

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Prenatal array CGH: towards a more appropriate and targeted clinical use

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Introduction: The diagnostic value of the prenatal array testing has been well established in cases of fetal ultrasound anomalies. Despite substantial case studies are reported in the literature, some issues remain controversial.

Methods: All examined cases have been select by gynecologists very experienced in prenatal ultrasound diagnosis. After excluding karyotype abnormalities, the samples (CVS or amniotic fluid) have been analyzed with Cytosure^{TM} v2 array 180K. Array results have been subclassified in pathogenic abnormalities (causative, unexpected or susceptibility loci) and VOUS.

Results: Overall more than 400 fetuses were analyzed, of which 300 with malformations well characterized at the US examination, especially involving the cardiovascular system. In such cases the additional detection rate of genomic imbalances (CNVS) not detectable at karyotype analysis, is about 7.5% (VOUS 2.8%). The remaining 100 samples are from fetuses with NT ≥ 3.5 mm (99centile). In these cases we are in the presence of a more limited diagnostic impact, whereas it reaches a DR of about 3.6%, quite similar to that of VOUS.

Discussion: Accurate US characterization of malformations appears to highlight as the prenatal array testing is not useful in the presence of some defects such as muscular ventricular septal defects or urogenital anomalies, especially in relation to the potential finding of VOUS. Our data suggest also considerable caution in counseling path in the presence of an increased NT, also for the possible medical-legal implications for failure detection of a genomic aberration in pregnancies classified at risk even in the presence of a normal karyotype.

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Severe ultrasound abnormalities: prenatal whole exome sequencing to unravel a diagnosis before birth

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The introduction of whole exome sequencing (WES) in genome diagnostics has dramatically changed the current practice in clinical genetics. However, routine application of WES in a prenatal setting for genetic analysis of fetuses with multiple congenital abnormalities is not yet widely accepted. Since a genetic diagnosis can be extremely helpful for treatment decisions immediately after birth, we decided to use our rapid WES set-up for late pregnancy prenatal cases.

We performed a trio analysis using WES for two fetuses with severe ultrasound abnormalities (>24 weeks) and parents. After extensive counseling of the parents and getting consent, a report could be issued within two weeks. Although we were not able to make a diagnosis for these two fetuses, this study shows the feasibility and utility of WES in ongoing pregnancies.

Currently, we have introduced a WES workflow for pregnancies >24 weeks, of fetuses with severe ultrasound abnormalities which are expected being admitted to the neonatal intensive care unit (NICU). Since in the Netherlands termination of pregnancy is not an option in these

cases, reporting of variants is similar to postnatal cases. Before extending prenatal WES towards pregnancies <24 weeks, clear criteria and guidelines are necessary, mainly on the disclosure of variants of uncertain pathogenicity and incidental findings. Here, we will present the current workflow, and discuss options for a possible framework for earlier pregnancies. It is expected that WES and ultimately whole genome sequencing will replace current routine clinical practice (array based technologies), not only after birth but also during pregnancy.

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Five different 13q deletions in prenatal diagnosis

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A partial deletion of the long arm of the chromosome 13 is an unusual chromosomal aberration related with a wide spectrum of clinical phenotypes.

In prenatal a vast number of these chromosomal abnormalities are diagnosed after the detection of ultrasound alterations. In this work we report a set of five cases with different deletions diagnosed by different techniques. Only one pregnancy had an apparently normal ultrasound and was referred because of advanced maternal age, while the other four had evident findings of foetal anomalies and were, therefore, referred for prenatal diagnosis. All cases, except the one referred for maternal age, were identified and/or characterized by array-CGH 180K. The pregnancy with an apparently normal ultrasound showed to be a de novo 13q32.3 to 13qter deletion. Two other cases had interstitial deletions at 13q34 and 13q31.3. The more distal one had a high nuchal translucency measurement while the del(13)(q31.3) had cardiac abnormalities. The most severe ultrasound anomalies, including brain malformations, were associated with two de novo large terminal deletions of 40 Mb and 52Mb at 13q22.1 and 13q21.2, respectively. The literature refers 13q32 deletion as a critical region associated with serious congenital malformations, particularly central nervous system anomalies. Our cases are consistent with this data. The largest deletions, including 13q32, had ultrasounds with severe brain disorders, highlighting this region as critical for central nervous system disorders.

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Prenatal Costello syndrome as a potentially prevalent cause of intrauterine death

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Introduction: Costello syndrome (CS) is a rare condition which arises due to heterozygous germline mutations in HRAS. In CS, more than 80% of the mutations found, causes the amino acid changes p.G12S or p.G12A. These patients have a relatively homogenous phenotype characterized by developmental delay, severe failure to thrive, cardiac problems, typical coarse facial features and a predisposition to malignancies. A few reports of a very rare but far more severe and lethal neonatal CS phenotype have been published. Here, mutations causing amino acid changes p.G12V, p.G12D or p.G12C have been found. We now add to this phenotypic description and for the first time report two cases of prenatal CS causing intrauterine death.

Materials and methods: Two separate cases were diagnosed in our tertiary university hospital clinic. Although there were several differences, the similarities were striking: Both presented in first trimester with severely increased nuchal transluceny but a normal chromosomal microarray. In second trimester polyhydramnios, decreased foetal movement, macrocephaly, microopthalmus, altered cardiac axis, and increased abdominal circumference were found. Despite treatment of polyhydramnios intrauterine death was diagnosed. Autopsies confirmed the prenatal findings and described additional dysmorphic features. Subsequently trio whole-exome sequencing was performed.

Results: A de novo HRAS mutation was found in the foetuses p.G12V and p.G12D respectively.

Conclusion: Diagnosing CS prenatally is challenging and might previously have been missed as a cause of

intrauterine death. The two cases presented provide further evidence for recognisable and severe phenotype in CS caused by p.G12V and p.G12D and expands the phenotypic description.

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Mendeliome and Whole Exome Sequencing in 60 fetuses with abnormal ultrasound revealed a diagnostic yield of 30%

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Only few studies with small numbers about Whole Exome Sequencing (WES) on prenatal cases with abnormal ultrasound findings are published yet. Since we have successfully established NGS on postnatal cases with a diagnostic yield of almost 50% we performed Mendeliome-sequencing and WES on a larger cohort of prenatal and aborted cases. From a total of 60 fetuses with various malformations pathogenic mutations were found in 18 cases resulting in a diagnostic yield of 30%. Mutations were found in 17 different genes with NIPBL being the only recurrently mutated gene (2×). 56% of the diagnoses followed an autosomal recessive inheritance pattern and 44% were autosomal dominant with all but one mutations occurring de novo. Regarding the clinical presentations known from postnatal syndrome descriptions, some of the phenotypes were unexpectedly severe. Notably, in one case with suspected Neu-Laxova-syndrome we found compound heterozygosity for a deleterious missense mutation in the gene PHGDH and a CNV deleting 6.8kb of the 3′UTR including part of the last coding exon. In another case with suspected Meckel syndrome we found a 28 bp intronic deletion in-trans with a synonymous substitution affecting splicing in MKS1. Finally, in 2 cases with multiple severe ultrasound abnormalities we detected new disease causing candidate genes. In urgent cases turn-around-time was 2 weeks. We conclude that an overall diagnostic yield of 30% on prenatal and aborted samples establishes NGS as a very useful approach. Moreover, NGS screening in fetuses with severe pre- and neonatal phenotypes may reveal mutations underrepresented in postnatal observations.

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maternal and neonatal leptin and leptin receptor polymorphisms are associated with preterm birth

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Introduction: Leptin (*LEP)* and leptin receptor (*LEPR*) are suggested to play a role in female reproduction and pregnancy. Both *LEP* and *LEPR* are synthesized by the pregnant female and embryo. The link between genetic polymorphisms of *LEP* and *LEPR* and preterm birth (PTB) is unknown. We studied maternal and neonatal *LEP* and *LEPR* genetic polymorphisms and the association with PTB.

Materials and methods: Blood for DNA analysis was collected from Israeli mothers and from venous umbilical of their respected idiopathic preterm newborns (24–36 weeks, n = 102) and control term newborns (>37 weeks, n = 158). Genotypes of maternal and neonatal *LEP* (rs7799039) and *LEPR* (rs1137101(polymorphisms were analyzed by restriction fragment length polymorphism analysis. Genotype-phenotype association was assayed using SPSS program.

Results: We found a significant independent increased risk of PTB for women and neonates bearing the homozygous AA form of *LEP* genotype; where women carrying AA *LEP* genotype had 2.53 fold ([CI]1.367 - 4.685, p=0.03) and 2.38 fold ([CI] 1.150–4.915, p=0.019) increased risk for PTB compared to AG and GG genotypes, respectively. Neonates carrying the *LEP* AA genotype had a significant 2.8 fold increased risk for PTB compared to the AG genotype (CI11.040–7.577, p=0.042). Maternal *LEPR* polymorphism was significantly associated with severe PTB; where women carrying the AA and AG genotypes had a significant 4.32 and 4.76 fold increased risk for severe PTB compared to women carrying the GG genotype

(CI=1.090–17.112 and 1.332–17.027, respectively p=0.035).

Conclusion: maternal and neonatal *LEP* and *LEPR* polymorphisms are significantly associated with increased risk for PTB.

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Single Nucleotide Polymorphism (SNP)-based Products of Conception (POC) chromosome analysis identifies molar pregnancy and terminal imbalances to guide medical management

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Introduction: Molar pregnancy and chromosome rearrangements carry risks for couples, including maternal gestational trophoblastic disease/neoplasia (GTD), miscarriages, and chromosomally abnormal offspring. SNP microarray performed on POC samples identifies these at-risk couples.

Materials and Methods: Retrospective analysis of 26,102 fresh POC samples with parental samples genotyped using Illumina CytoSNP-12b microarrays with bioinformatics to identify parental origin of abnormalities. Results of full paternal UPD, paternal triploidy, or one or more terminal imbalance(s) were identified. Clinical history and parental chromosome results were requested with IRB approval.

Results: 22,452 cases had fetal results: 638 (2.8%) had paternal triploidy, 72 (0.3%) had full paternal UPD, and 403 (1.8%) had ≥1 terminal segment imbalance(s). Follow-up was obtained for 221 (31%) molar pregnancy cases and 107 (27%) terminal segment cases. 53 molar pregnancy cases (24%) were identified by ultrasound and/or pathology. Sixty-six (62%) cases at risk of parental chromosome rearrangement(s) had follow-up studies done; 30 (46%) reported parental balanced rearrangements.

Conclusions: Genetic testing of POC samples identifies molar pregnancy, which carries a risk of up to 25% for maternal GTD, and terminal chromosome imbalances, which influence recurrence risk counseling. Without genetic testing, up to 75% of women at risk for GTD might not be identified as at risk. For couples at risk for parental chromosome rearrangement(s), knowing the parental origin of the fetal finding prevents unnecessary testing in the other parent. Expanding pregnancy-loss workups beyond routine studies to include SNP-based chromosome analysis is useful for medical management.

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P01.099C

A comprehensive contribution of endothelial dysfunction genes to recurrent miscarriage susceptibility

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The roles of genetic polymorphisms in the pathogenesis of recurrent miscarriage (RM) have been intensively studied. Complex diseases, including miscarriage are believed to

have a polygenic basis and gene-gene interactions can play a significant role in the etiology of the disease. This study was conducted to investigate the association of gene-gene interaction of endothelial dysfunction genes polymorphisms and RM. A total of 592 unrelated Russian women comprising 253 RM patients and 339 healthy controls were recruited into the study. Seven functional SNPs were selected from ACE, MTHFR, SERPINE-1, NOS3, TP53 and VEGF genes based on our previous studies. Two receptor gene polymorphisms (G634C and C936T VEGF gene) were significantly associated with idiopathic RM ($P < 0.001$). We found that polymorphism C677T MTHFR gene is associated with an increased risk of RM ($P < 0.04$), whereas polymorphism G894T NOS3 of NOS3 gene showed an association with a decreased risk of disease ($P < 0.02$). By Multifactor dimensionality reduction analysis, a two-locus model (C936T and G634C of VEGF) of gene-gene interaction was the best for predicting RM risk, and its maximum testing accuracy was 67.4% and maximum cross-validation consistency was 10/10. In addition, it was shown that the interaction of ACE, SERPINE-1 and TP53 genes has a synergistic effect on the risk of RM. The gene-gene interaction between MTHFR, NOS3 and VEGF are additive. Our study was to show that gene-gene interactions of genes inducing endothelial dysfunction represent important determinants for the development of RM. This work was supported by the Russian Foundation for Basic Research.

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Restrictive dermopathy: novel *ZMPSTE24* mutation and clues for prenatal diagnosis

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Introduction: Restrictive dermopathy (RD) is a rare lethal genodermatosis caused by either heterozygous mutations in the lamin A/C gene (*LMNA*) or by biallelic mutations in the zinc metalloproteinase STE24 gene (*ZMPSTE24*).

Patients and Methods: In a couple of apparently non consanguineous parents, two consecutive pregnancies were complicated by oligohydramnios, premature rupture of membranes, preterm delivery, and similar foetal abnormalities detected by ultrasonography, including intrauterine growth restriction, dysmorphisms and reduced foetal movements. Both male babies shared at birth facial dysmorphisms, proximal and distal arthrogryposis, and a progeroid appearance suggestive of a laminopathy. The first one was a stillborn at 31 weeks of gestation, while the second one, born at 28 weeks, died at 8 days. A DNA sample of the second child and his parents was obtained to perform genetic tests, including cytogenomic microarray and next generation sequencing.

Results: A homozygous splicing mutation in the ZMPSTE24 gene was identified in the second child. Both parents were heterozygous carriers. This novel frameshift mutation, determining exon 7 skipping and introducing a premature stop codon, is compatible with the severe RD phenotype observed in both children. Analysis of *LMNA* and array-CGH were negative.

Conclusions: While the postnatal RD phenotype can be fairly recognizable, an extensive description of prenatal ultrasound findings is lacking. Comparing data of present patients with a review of the literature, we suggest that several foetal anomalies are recurrent in RD and its recognizable combination should help the prenatal diagnosis of this condition.

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Transcriptional regulation of *Septin12*

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Introduction: The human *Septin12* was originally identified by cDNA microarray as one of the genes down-regulated in the testicular tissues of patients with severe spermatogenic defects. Some studies showed that *Septin12* +/-chimeric mice suffer from severe spermatogenic defects, including immotile sperm, sperm with a bent tail, round-

headed sperm, and sperm with acrosomal defects. Those studies indicated that *Septin12* played a critical role in the process of spermatogenesis. However, the transcriptional regulation of *Septin12* in the spermatogenesis is still unclear.

Materials and Methods: The core promoter region of *Septin12* was identified using the luciferase reporter gene assay. Bioinformatics prediction showed that *Septin12* core promoter region contains binding sites of estrogen receptor (ER) and androgen receptor (AR). In addition, chromatin immunoprecipitation analysis confirmed the basal binding activity of ER and AR to the *Septin12* promoter. The transcription activity and expression of *Septin12* was assayed by reporter assay and real-time PCR under estrogen or androgen treatment.

Results: *Septin12* promoter activity was positively regulated by estrogen and androgen. Mutation of ER or AR binding sites decreased the luciferase activity; therefore, the ER and AR were very important transcription factors for the *Septin12* promoter activity.

Conclusion: Our data suggest that steroid hormones are important modulators for the transcription and expression of *Septin12* in spermatogenesis. *Septin12* could serve to develop as gene indicator for environmental endocrine disrupting and steroid hormone. Acknowledgments: This study was supported by grants from Ministry of Science and Technology (MOST 103-2314-B-024-001, MOST 104-2314-B-024-001, MOST 105-2314-B-024-001) of Taiwan.

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Preimplantation genetic testing of monogenic diseases and aneuploidies using single blastocyst biopsy approach

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Introduction: Preimplantation genetic testing (PGT) protocol development is challenging due to amplification failure, DNA contamination and allele dropout (ADO) risk. Improvements are achieved through combining direct and indirect testing, performing whole genome amplification (WGA), excluding aneuploidies for single gene disorder PGT. The aim of study was to develop robust and affordable protocol. We present our experience for families with autosomal recessive congenital ichthyosis (ALOX12B) and Huntington disease (HTT).

Materials and methods: WGA for blastocyst single biopsies were carried out by MDA technology. Gene adjacent microsatellites were selected using UCSC database, semi-nested PCR primer system was designed for STR testing. Aneuploidy testing were done by aCGH.

Results: STR haplotyping of family members revealed 14 out of 15 informative markers for ALOX12B-case and five out of 13 for HTT-case. All embryos analyzed (seven in ALOX12B-case and eleven in HTT-case) resulted in successful WGA. After STR testing in case of ichthyosis two embryos had normal genotype, seven were heterozygotes, three affected, six were mutation-free in HTT-case. ADO rate was 0.04%. HTT CAG repeat sizing confirmed results for haplotyped embryos. Single euploid embryo transfers resulted in clinical pregnancies in both cases.

Conclusions: Single biopsy WGA ensures possibility of multifactor PGT. Semi-nested testing system minimizes misdiagnosis risk due to ADO, non-specific amplification or contamination and can be used without direct mutation testing. Aneuploidy testing excludes leading PGT failed implantation cause. These are first single gene PGT cases carried out in Latvia, developed approach can be further conveniently applied for families seeking alternatives for prenatal testing.

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Single-cell RNA-seq profiling of human preimplantation 8-cell embryos reveals phenotype-dependent expressional patterns

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Assisted reproduction entails standardized preimplantation embryo grading, which typically assesses an embryo in terms of fragmentation, symmetry, multi-nucleation, vacuoles, and zona pellucida. In the era of single-cell – omics, in-depth work is needed to recapitulate the criteria by expressional profiles of each blastomere.

We apply single-cell RNA sequencing (RNA-seq) technology in retaining high-quality single-cell transcriptomes from E3 human preimplantation 8-cell embryos. In sum, 22

embryos are involved, among which 10 (45.5%), 6 (27.3%), 4 (18.2%), 2 (9.1%) are labeled 'A' to 'D' by the conventional assessment, respectively. Each blastomere is transcriptional analyzed in a step-wise way of quality control, alignment, assembly, expression level calculation, annotation in genomic context. We conduct WGCNA and IPA analyses to classify genes into functional categories.

The transcriptomes from 176 blastomeres are sequenced, and 174 cells meet with the quality criteria for further analysis. The 79 grade 'A' cells show high correlation with the published human 8-cell embryo results (Pearson correlation coefficients average 0.92). The intra-embryo correlations between blastomeres are as high as 0.97. Both results prove high reproducibility of this study. There are 14 functional modules drawn from the expression profiles, with clear enrichment for genes involved in cell division, RNA processing, and ribosome biogenesis. Intriguingly, low graded blastomeres present significant change in 3 modules. Especially, 2 genes persistently show biased expression in blastomeres with high fragmentation, a typical manifestation of low-quality. Our hitherto results shed light on the molecular mechanism behind the embryo grading and render more hints in human early development.

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P01.104D

Whole exome sequencing identifies novel SLC26A2 mutation resulting diastrophic dysplasia

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Mutations in the SLC26A2 (DTDST) gene cause rare autosomal recessive related diseases including achondrogenesis, atelosteogenesis, De la Chapelle dysplasia, diastrophic dysplasia and multiple epiphyseal dysplasia. These diseases share a number of clinical features, in particular diastrophic dysplasia (DTD) characterised by short-limb dwarfism, hearing loss, cleft palate, and normal intelligence. /

The SLC26A2 gene encodes a novel sulfate transporter. Impaired function of DTDST product would be expected to lead to undersulfation of proteoglycans in cartilage matrix and thereby to cause a clinical phenotype such as diastrophic dysplasia. Prenatal History: A 29 year old Russian woman at 19–20 weeks gestation after serial ultrasounds showed intrauterine shortened tubular bones, abnormal pes fixing, restricted knee movement, adducted thumbs, micrognathia. Platsentobiopsiya by an outside institution reported a normal male karyotype. Previous pregnancy was aborted in connection with severe skeletal dysplasia. Amniocentesis reported a normal female karyotype. Pregnancy was aborted. We performed targeted sequencing of autopsy tissue using Illumina HiSeq2500, NEBNext preparation protocol, Agilent FocusedExome panel and our own analytical pipeline. Variant calling and pathogenicity scoring were done based on ACMG guidelines. We identified a compound heterozygous mutation in the SLC26A2 gene on chromosome 5q32. The first variant is c.1957T>A that was previously identified as a pathogenic. The second variant is frameshift c.1650delG. This variant is absent from dbNSFP, Clinvar, OMIM and HGMD pathogenicity databases, and from 1000Genomes project, ExAC and Genotek frequency databases. Capillary sequencing confirmed mutations found by NGS in proband. Also both parents were confirmed to be heterozygous carriers.

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P01.106B

Identification of pathogenic single nucleotide variants (SNVs) associated with heart disease in 290 cases of stillbirth

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The incidence of stillbirth in Sweden has essentially remained constant since the 1980's, and despite thorough investigation, many cases remain unexplained. It has been suggested that a proportion of stillbirth cases is caused by heart disease, mainly channelopathies. In this study, 79 genes associated with cardiac channelopathies, cardiomyopathies and congenital heart defects (CHD) was analyzed in 290 stillbirth cases. The HaloPlex Target Enrichment System (Agilent Technologies) was utilized to prepare sequencing libraries which were sequenced on the Illumina NextSeq platform. We found that 18.6% of the 290 investigated stillbirth cases had one ($n = 47$) or two ($n = 7$) variants with evidence supporting pathogenicity, *i.e.* loss-of-function variants, evidence from functional studies, or

previous identification of the variants in affected individuals. The prevalence of the same variants in the Exome Aggregation Consortium (ExAC) and SweGen was significantly lower, 7.24% ($p<0.001$) and 8.40% ($p<0.001$), respectively. Furthermore, we identified a modest but significant difference in the overall proportion of pathogenic SNVs in relation to the total number of SNVs between the stillbirth cohort and ExAC, 3.44% vs. 2.35% ($p<0.05$). Our results give further support to the hypothesis that cardiac channelopathies might contribute to stillbirth. Additionally, our results suggest an increased frequency of pathogenic variants in genes associated with cardiomyopathies and CHD in stillbirth compared with the general population. Screening for pathogenic SNVs in genes associated with heart disease might be a valuable complement for stillbirth cases where today's conventional investigation does not reveal the underlying cause of fetal demise.

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P01.107C

In a prenatal setting we successfully applied targeted DNA sequencing to detect rare disease causing gene mutations in two families with strongly increased nuchal translucency bisides very severe additional fetal anomalies

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An increased nuchal translucency is frequently observed during pregnancies. Cytogenetic and molecular genetic analysis like array CGH following prenatal diagnosis can just provide an explanation in a proportion of these findings. If additional severe fetal anomalies can be found and the mentioned standard diagnostic tests did not allow sufficient conclusions a monogenic cause has to be considered. By using a NextSeq 550 device (Illumina) with a TruSight One sequencing panel of the same supplier covering more than 4,800 clinically relevant genes we were able to identify in a seriously affected fetus in a dizygotic twin pregnancy in a couple of South European origin a fatal skeletal dysplasia due to the homozygous missense mutation c.1639A>G, p. Asn547Asp in the LBR gene. Heterozygosity for this mutation in both parents was confirmed following amniocentesis and provided evidence that one twin showed lethal Greenberg dysplasia. Since parents did not draw any consequence the risk for the second twin might increase however it was born healthy five months later. In another young healthy Austrian couple who suffered a recurrent fetal loss in at least two pregnancies associated with comparable complex abnormalities we did find a homozygous splice mutation in an important gene essential in blood metabolism. From this experience we would propose that if recurrent similar very severe fetal anomalies are recorded in particular families an effort should be taken early to store fetal DNA from each pregnancy to allow to apply the approach mentioned here to potentially provide such couples with an appropriate prenatal diagnosis.

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P01.109A

Correlation between Z-score, fetal fraction and sequencing reads in Non-Invasive Prenatal Testing

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Introduction: A reliable NIPT result depends on sufficient amount of fetal DNA and sequencing reads. There has been a tendency not to report NIPT results from samples with a fetal fraction below 4%. Such a general cutoff-value will increase the incidence of test failures compared to study-specific cutoff-values. This study examined the combination of cutoff-values between Z-score, fetal fraction and sequencing reads in order to define the optimal study-specific cutoff-values and possibly decrease the test failure rate.

Materials and methods: Whole-genome sequencing data of plasma-derived DNA from 35 women screen-positive for trisomy 21 by NIPT was used for analysis. We computationally simulated 18 fragmented datasets containing from 5 to 95% of the original data with 5% increments for each sample. Z-scores and fetal fractions were estimated using a pipeline based on WISECONDOR and SeqFF analysis scripts.

Results: The Z-score decreased with increasing fragmentation of the original dataset. When our cutoff-values were met (SeqFF >0.02 and sequencing reads >5million) we were able to detect the trisomy samples with a Z-score >4. One sample with a SeqFF of 0.016 (below our cutoff-value) was only detected with more than 6 million reads sequenced.

Conclusion: We have shown that our current combination of cutoff-values (SeqFF >0.02, sequencing reads >5million and Z-score >4) is appropriate in order to detect

trisomy 21 pregnancies and lowering of these cutoffs in order to decrease test failures is not feasible.

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P01.110B

CLIA laboratory experience with over 330,000 samples of non-invasive prenatal testing (NIPT) using a targeted microarray-based cell-free (cfDNA) test for fetal trisomy

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Objective To describe the experience with a targeted microarray-based cfDNA test (Harmony Prenatal Test®) for fetal trisomy in a large CLIA-certified laboratory.

Methods Targeted cfDNA analysis using DANSR™ and FORTE™ with microarray quantitation was used to evaluate the probability of trisomy 21, 18, and 13 in the Ariosa CLIA laboratory (San Jose, CA, USA). The laboratory performs active follow-up of clinical and diagnostic information for patients with high-probability results. In the present study prospectively collected data on 338,365 reported samples was reviewed.

Results Of the 338,365 samples reported, 1.2% received a high-probability result for trisomy 21, 18, or 13. In the entire cohort, mean maternal age was 32.7 years; mean gestational age was 13.8 weeks. 2.5% of samples were from twin pregnancies; 6.4% of samples were from IVF pregnancies. There were 3,958 high-probability results: 3,102 for trisomy 21, 677 for trisomy 18, and 179 for trisomy 13. 1,612 of these cases were eligible for follow-up. Outcome information was available for 997 cases. In 655 cases with diagnostic information, 97.0% of trisomy 21, 85.5% of trisomy 18, and 34.1% of trisomy 13 cases were confirmed. 342 cases had other outcome information without karyotype confirmation.

Conclusions This study complements previously published validation studies that demonstrated high specificity for assessment of fetal aneuploidy using a targeted cfDNA test with microarray quantitation. High positive predictive values for trisomy 21 and trisomy 18 were observed. A lower proportion of confirmed trisomy 13 cases was not unexpected given the lower prevalence of this condition.

B. Liedstrand: A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc. **K. White:** A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc. **R. Steeke:** A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc. **J. Acuna:** A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc. **A. Batey:** A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc. **M. Schmid:** A. Employment (full or part-time); Significant; Ariosa Diagnostics Inc., Roche Sequencing Solutions Inc..

P01.111C

Whole exome sequencing in fetal structural abnormalities: experience of 8 cases

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Introduction: The underlying cause in fetuses with structural abnormalities on ultrasonography remains largely unknown, when pathogenic copy number variations are ruled out. Lack of a definitive diagnosis blurs genetic counseling for most families. Introduction of next generation techniques to the prenatal setting, improved diagnostic yield in euploid fetuses with structural abnormalities. Here we present WES-trio results of eight fetuses with structural abnormalities, and discuss impacts on genetic counseling.

Materials and Methods: Eight families were consulted due to fetal structural anomalies in second trimester ultrasound, with normal chromosomal array results. DNA was extracted from cord blood or fetal skin tissue in postmortem cases. Whole exome sequencing (CentoXome Platinum and Gold) was performed for fetus and parent trios.

Results: WES was offered all families due to structural fetal abnormalities ranging from isolated unliateral cleft lip to micrognathia with microtia. Two families chose to terminate the pregnancy, and have the test performed after the fetus was examined postmortem. WES revealed *CRYBB1* and *ASCC1* mutations in these two, ascertaining a diagnosis of Congenital nuclear cataracts and Spinal muscular atrophy with congenital fractures. Remaining six had prenatal WES, revealing mutations in three: *BBS10*, *RIT1*, and *TCOF1*, providing definitive diagnoses for Bardet-Biedl, Noonan, and Treacher-Collins syndromes. 4/6 families who had prenatal WES made choices based on the WES results.

Conclusions: Due to the small number of inconsecutive cases included in this study, the high diagnostic yield for

WES might be misleading. In our experience, WES results affected the informed choices the families made.

U. Altunoglu: None. **T. Kalayci:** None. **I.H. Kalelioglu:** None. **H. Kayserili:** None.

P01.112D

Prenatal measurement of X-inactivation (XCI): cell culture may have some influence on the result. A case report

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X inactivation (XCI) in human occurs early in development at late blastocyst stage. Structural X chromosomal abnormalities are usually tolerated in females because of the preferential inactivation of the abnormal X chromosome.

We report on a case of an unbalanced reciprocal de novo translocation with partial trisomy 6p and partial monosomy Xq detected by non-invasive-prenatal testing and confirmed by chorionic villi sampling (CVS) in a female fetus with normal ultrasound findings.

Direct analysis of chorionic villi showed the deletion to be on the paternal X chromosome and using a methylation-based test for AR completely skewed XCI (only the maternal X was active).

Long-term culture of CVS confirmed the chromosomal aberration, whereas XCI in the cultured cells showed lack of methylation on both AR-loci. A healthy baby girl was delivered at term. On cord blood the dup6p and the delXq were confirmed and the XCI in the new-born girl was completely skewed (only maternal X active).

DNA methylation is known in the literature to vary between placenta and the embryo. Peñaherrera et al., 2003, showed some correlation of XCI between embryonic and extraembryonic tissues depending on the locus tested.

Our results showed that direct analysis of chorionic villi correlated with the result of XCI in cord blood, whereas methylation in cultured CVS cells was completely lacking on the AR-locus. We suspect cell culture to influence the methylation status of AR (hypomethylation) and propose to only analyze non-cultured cells for XCI using a methylation based test for AR in prenatal settings.

D. Niedrist: None. **A. Baumer:** None. **R. Müller †:** None. **B. Oneda:** None. **A. Rauch:** None.

P01.113A

The EAA/EMQN external quality control program critically improves the molecular diagnosis of Y chromosome microdeletions

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Introduction: The molecular diagnosis of Y chromosome microdeletions is a routine genetic test in the workup of infertile male patients. Owing to its diagnostic and prognostic value, it is crucial that it is performed according to the highest possible standards. For more than 15 years the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have been supporting the improvement of Y chromosome microdeletion testing by offering an external quality control program.

Aim: To quantify the impact of external quality control in the molecular diagnosis of Y chromosome microdeletions.

Methods: Analysis over a 16-year period of the efficiency of the EAA/EMQN external quality control program in: i- decreasing genotyping errors; and ii- improving reporting practice.

Results: The EAA/EMQN external quality control program has had a critical impact in reducing diagnostic errors: at the start of this program the overall diagnostic error rate was almost 8% and is currently 0.74% of all reported cases. Furthermore, the program has significantly improved reporting practice: the number of analyses with a full interpretation score has clearly increased since the start of the program to the current 68% of all analyses. Nevertheless, there is still room for improvement: several labs have yet to adopt the best practice multiplex PCR set-up, as well as to offer the now mandatory deletion extension analysis.

Conclusion: Participation in the EAA/EMQN external quality control program has lead to a critical improvement

in the molecular diagnosis of Y chromosome microdeletions and is strongly recommended to all labs.

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P01.114B

The role of *LRWD1 in the* embryonic development of zebrafish

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Introduction: The human *Leucine-rich Repeats and WD repeat Domain containing 1* (*LRWD1*) was identified by cDNA microarray as one of the down-regulated genes in the testicular tissues of patients with severe spermatogenic defects and highly expressed in the testicular tissues. However, the role of *LRWD1* in the embryonic development is still unclear.

Materials and Methods: In order to understand the role of *LRWD1* in embryonic development, we use zebrafish (Danio rerio) as an animal model. We analysis the *LRWD1* expression in different organs (eyes, brain, heart, liver, muscle, testis, ovary) and embryogenesis (4, 8, 12, 24, 48, 72 hpf) of zebrafish by Real-Time PCR and immunofluorescence. To investigate the role of *LRWD1* in embryo and germ cells, we used gene knockdown by Morpholino nucleotide to disrupt the *LRWD1* in the embryo of zebrafish.

Results: *LRWD1* was in different organs (eyes, brain, heart, liver, muscle, testis, and ovary), but majorly in the testis of zebrafish. We found that knockdown *LRWD1* caused the tail and spine bending of zebrafish embryo and early derth.

Conclusion: In addition to the role of *LRWD1* in the reproduction, we suggested that *LRWD1* has an indispensable role in the germ cell and embryonic development for zebrafish. Acknowledgments:This study was supported by grants from National Science Council (NSC 102-2314-B-024 -001) and Ministry of Science and Technology (MOST 103-2314-B-024 -002, 104-2314-B-024 -002 -MY2) of Taiwan.

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P01.115C

An overlooked phenomenon: female-biased sex ratio among carriers of Robertsonian translocation in consecutive newborns, presumably due to female-specific trisomy rescue

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Examination of the sex ratio (SR, male-to-female ratio) among carriers of Robertsonian translocation (*rob)* in newborns in the general population has not previously been given due attention, probably because of focusing on the striking female preponderance among fertile carriers explained by sterility of male carriers. Meta-analysis of published studies on 68,212 newborns (35,027 males and 33,185 females) showed differences in SR depending on the type of rearrangements: similar rates of male and female reciprocal translocations carriers (34 males/33 females, for a rate of 0.97‰ and 0,99 ‰, correspondingly), but female preponderance among carriers of *rob*, regardless of their parental origin (27M/41F, 0,76 ‰ and 1,24 ‰). Similar results were obtained in carriers identified prenatally for indications other than familial rearrangement. Collectively, among carriers of *rob* with known parental origin, there were 66 males and 97 females (SR=0.68), different from the ratio of 1.05, p = 0,0023, for carriers of reciprocal translocations and inversions where a typical slight male prevalence was found. Female-biased SR was demonstrated for carriers of the most frequent *rob*, t(13;14), with 50 males и 85 females, SR=0,58, but not for carriers of other *robs* (28M/27F, SR=1.04). A mechanism of female-specific rescue of translocation trisomy resulting in female preponderance among carriers of balanced translocation, along with a male preponderance among carriers of unbalanced translocation, could explain the observed phenomenon. Both female-biased SR among carriers of balanced 45,der (13;14),upd(14) with 4M/12F and male-biased SR among carriers of unbalanced 46,+13,der(13;14) with 16M/2F, support the proposed hypothesis.

N.V. Kovaleva: None.

P01.116D

Comparative system genetics view of uterine leiomyoma

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The results of comparative system genetics studies of uterine leyomyoma (LM) - common benign tumor affecting almost half of all women are reported. Candidate gene sequencing, methylation and expression profiles studies were applied to paired samples of normal and fibroid tissues of the same LM patient. Significant variations in DNA methylation and hydroxymethylation in different LM nodules was shown. Multiple chromosomal rearrangements mostly partial monosomies of different chromosomes were registered by array- CGH analysis. A sequencing of exon 2 of MED12 gene demonstrated typical mutations in 59% of all LM samples. Combined studies of MED12 and AR gene advocated for independent origin of the most multiply fibroids in the same uterus . Increased expression of PR gene and no change in expression of ER gene were also found. Total DNA methylation in a single LM nodule was reduced if compared to this one in multiple fibroids whereas high hydroxymethylation was more common in MED12 positive fibroids. Significant (FDR<0.05) expression decrease of 9 genes with its concomitant increase in 16 genes was shown by comparable expression profiles studies of paired LM and normal myometrium samples. The origin of LM fibroids from mesenchymal stem cells storage within endometrium/ myometrium junctional zone of the uterus is hypothesized. According to our studies personal genetic background, unique epigenetic landscape as well as detrimental (provoking) external factors make principal contribution to the origin, clinical manifestation and progression of LM. RS F Grant 14-15-00737

V.S. Baranov: None.

P02 Sensory disorders (eye, ear, pain)

P02.01A

Next Generation Sequencing (NGS) followed by *in vitro* and *in vivo* functional studies revealed new genes for both Hereditary (HHL) and Age Related Hearing Loss (ARHL)

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Introduction: Given the high genetic heterogeneity of hearing disorders, it is extremely important to look for new genes involved in HHL and in ARHL, a complex disease affecting millions of people. To reach this goal 70 HHL-families and 464 ARHL-patients were screened by NGS technologies (i.e. Targeted Re-Sequencing and Whole Exome Sequencing). Genes/alleles identified were then functionally analyzed.

M&M: TRS: Panel 1) 113 HHL-genes (4.356-amplicons,~92,6% coverage), Panel 2) 46 ARHL-candidate genes (1942-amplicons,~96,55% coverage).

WES: 293.903-amplicons,~>99% coverage.

Filtering of NGS data analysis: allele frequency (checked in public and in our internal databases), pathogenicity prediction and segregation within the HHL families.

In vitro studies: expression vectors containing the wt or the mutant cDNA sequence, RT-PCR, Western Blot.

In vivo studies: expression studies in mouse/zebrafish models, KI/KO generation.

Results: Three new HHL-genes and 22 ARHL-candidate genes have been recently identified. Table 1 displays the results of the first series of functional studies of the most promising six genes (prioritized according to literature updates and type of mutation).

Gene	Phenotype	Mutation	In vitro studies	Pathogenicity	in vivo studies	Animal model hearing phenotype
TBL1Y	HHL	Missense	+	high	NA	NA
PLS1	HHL	Nonsense	NA	NA	+	in progress
SPATC1L	HHL/ ARHL	Missense/ Nonsense	+	high	NA	NA
SLC9A3R1	ARHL	Missense	+	moderate	+	yes
SLC44A2	ARHL	Missense	+	moderate	+	in progress
SLC28A3	ARHL	Nonsense	+	high	+	in progress

Conclusions: This multi-step approach proved to be extremely powerful for uncovering the molecular mechanism of hearing loss. Updated results will be presented and discussed.

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P02.02B

Congenital hearing impairment as an early sign of mild phenotype of alpha-mannosidosis : report of five new cases

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Alpha-mannosidosis (AM) is a very rare (1/500 000) autosomal recessive lysosomal storage disorder. It is characterized by multi-systemic involvement, with the association of intellectual disability, hearing loss, skeletal anomalies, and coarse facial features. The spectrum is wide, from very severe and lethal condition to milder phenotype, usually slowly progressive. AM is caused by deficiency of lysosomal alpha-mannosidase, MAN2B1, that activity can be measured in the leucocytes to establish the diagnosis. The molecular confirmation is obtained by the identification of the *MAN2B1* mutations. Enzyme replacement therapy is currently on trial. Here, we report on five individuals from four families, aged 3 to 8 years at the diagnosis, referred to a clinical geneticist for etiologic exploration of syndromic hearing loss. The deafness was associated with moderate learning disabilities in three individuals. The sib pair, presented with atypical clinical presentation and were referred for exome sequencing after negative genetic workup for the association of long habitus, intellectual disability and deafness. The diagnosis has been established by whole-exome sequencing in three cases including the two sibs. Whereas systematic screening of urinary oligosaccharides excretion led to the diagnosis in the two other individuals. Through these observations, we would like to emphasize that AM is a probable underdiagnosed rare cause of syndromic hearing loss, whose clinical diagnosis can be challenging. There is a major input of whole-exome sequencing to the early diagnosis of these mild phenotypes. Notably, there is a real benefit for the patients to have an early diagnosis in term of treatment and management.

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P02.03C

Genetic characterization of patients with Congenital Aniridia in Spain

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Introduction: Congenital aniridia is a panocular genetic disease with autosomal dominant inheritance, caused by *PAX6* haploinsufficiency. It is characterized by iris hypoplasia, and may appear isolated or as part of a syndrome. Our goal is to define the mutational spectrum of aniridia in Spanish population.

Patients & Methods: In 73 families with aniridia, genetic analysis of *PAX6* and 11p13 region was performed by a combination of techniques, including Sanger and targeted next-generation sequencing, MLPA, FISH, karyotype and array-based CGH.

Results: 52% of cases presented 33 different mutations in the *PAX6* coding sequence, including 30 loss-of-function mutations and 3 missense/in-frame deletion variants. Five well-known *PAX6* hotstop mutations were identified in 2 unrelated families, respectively. 15 of these mutations had not previously been described. Additionally, 7 families carried 5 variants of unknown significance in non-coding regions, its potential pathogenicity is currently being assessed by *in vitro* and *in vivo* analysis. In 23% of cases, 11p13-14 genomic rearrangements were identified: 3 multiexonic deletions, 4 deletions of *PAX6* upstream enhancers, 9 contiguous gene deletion syndromes and one translocation tr.(6,11). The use of personalized high-resolution array-CGH allowed specifying the size, breakpoints and involved genes.

Conclusions: Molecular study of *PAX6* has allowed identifying the cause of Aniridia in 75% of our patients, and the possible cause in 7 additional families. This study demonstrates the usefulness of new genomic techniques in the study of Aniridia, allowing increasing the diagnostic rate through a more precise analysis of 11p13 and non-coding *PAX6* regions.

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P02.04D

New insights into understanding the genetic basis of severe bilateral eye anomalies

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Introduction: Anophthalmia and microphthalmia (AM) syndromes are a genetically heterogeneous group of disorders with overlapping phenotypes reflecting the complexity of eye morphogenesis. Here we present our analysis of a large cohort with severe bilateral AM.

Methods: Detailed phenotyping of probands and parents was performed with genetic analysis using arrayCGH, MLPA, targeted and high-throughput sequencing.

Results: SOX2 (18/91) and *OTX2* (7/91) were the most commonly identified variants; while both are associated with severe eye and pituitary phenotypes, *OTX2* is linked to milder systemic manifestations and the ocular findings can exhibit variable penetrance. *FOXE3* (3/91) and *VSX2* (3/91) variants cause ocular phenotypes segregating in both recessive and dominant fashion, albeit more severe recessively. An X-linked recessive *BCOR* variant was found in one male with anophthalmia and atypical normal intelligence. We also identified variants in genes normally associated with milder eye phenotypes, *GJA8* (cataracts) and *PAX6* (aniridia), in two individuals with severe microphthalmia. Other genetic diagnoses included *ALDH1A3 (3), TFAP2*A (2), *BMP7*, *CHD7*, *OLFM2*, *STRA6* and partial chr13 trisomy (all 1).

Conclusion: We have made genetic diagnoses in 44 families (48%) from our cohort with severe eye anomalies and will provide detailed descriptions. Although *SOX2* and *OTX2* are still the most frequent diagnoses, with increasing availability of routine testing for these genes, many cases are being diagnosed and no longer referred for research. This means our cohort is continually evolving to consist of a high preponderance of cases where a diagnosis is more challenging, and highlights the likely existence of new AM genes.

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P02.05A

ear atresia: is there a role for apoptosis regulating miRNAs?

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Introduction: MicroRNAs (miRNAs) are highly conserved, small non-coding RNA molecules approximately 21–24 nucleotides in length that control post-transcriptional gene expression in a wide variety of cellular processes including proliferation, differentiation, development, tumorigenesis and apoptosis.

The molecular events underlying ear development implies numerous regulatory proteins that the role of miRNAs have not previously been explored in patients with ear atresia.

Materials and Methods: We investigated 12 miRNAs, regulating different pathways such as apoptosis, angiogenesis and chondrogenesis. miRNAs were isolated from serum samples of ear atresia patients (n = 7) and control group (n = 8) using the miRNeasy Serum/Plasma Kit (Qiagen) according to manufacturer's instructions. After complementary DNAs were randomly primed using miScript II Reverse Transcription (Rt) Kit (Qiagen), miRNA

expressions were analyzed by real time PCR (RotorGene Q, Qiagen).

Results: We found apoptosis regulating miRNAs were significantly down regulated in ear atresia patients. Among them, miR-126 (MIR126), miR-146a, (MIR146), miR-222 (MIR222) and miR-21 (MIR21) were significantly 76.2 ($p=0.041$), 61.8 ($p<0.001$), 30.5 ($p=0.009$) and 71.21 ($p=0.042$) fold decreased compared to controls, respectively.

Discussion/Conclusions: **Significant down-regulation of apoptosis controlling miRNAs in ear atresia patients could possibly be the cause of abnormal ear development. This might be by enhancing intensity of apoptosis or anticipating the timing of apoptosis during ear development. Our report is the first to provide clues of relationship between apoptosis regulating miRNAs and ear atresia/microtia, up to the literature.**

Reference: Nishizaki K,(1998) Programmed cell death in the development of the mouse external auditory canal. Anat Rec 252:378–382Grant #TSG-2013–333 Research Fund of Istanbul Medeniyet University

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P02.06B

The lack of *LAT2* induces damage in the cytoarchitecture of the inner ear

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LAT2/CD98hc has a direct role in age-related hearing-loss (ARHL). Identification of rare variants in LAT2 gene together with amino acid transport loss-of-function in ARHL patients supports the concept that LAT2 (SLC7A8) has a role in the auditory system.

A detailed inspection of *Lat2* knockout inner ear (Lat2KO) showed altered stria vascularis with down-regulation of channel Kir4.1 expression cells and increased presence of gaps between strial cell-layers. In addition, a loss of both hair cells in the organ of Corti and neurons of the spiral ganglion (SGN) were observed. Alterations in the stria vascularis, SGN and hair cells seem to be at the basis of the hearing loss phenotype in the absence of LAT2 although the molecular mechanisms are at present unknown. Our hypothesis is that LAT2 loss-of-function might render alterations in the cell content of bulky neutral amino acids like branched chain amino acids or glutamine, which affect proteostasis and renewal of cell structures causing cell stress. Moreover, Lat2KO cochlea presents signs of unsolved chronic inflammation with up-regulation of IL1b and IL6 mRNA. Currently we are analyzing oxidative damage in Lat2KO cochlea.

The description of *LAT2* as a novel gene involved in ARHL demonstrates the importance of amino acid homeostasis in preserving hearing function and suggests that genetic screening may enable the identification of individuals susceptible to developing ARHL, allowing for early treatment or prevention of the disease.

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P02.07C

Genome wide sequencing (WGS) reveals new insights into Age Related Hearing Loss (ARHL): cumulative effects and the role of selection

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Introduction: To unravel the genetic determinants of ARHL, a highly heterogeneous disease, a WGS study of cases and controls was conducted.

Materials and Methods: Samples recruitment: a)discovery cohort of 156 subjects and b)independent replication cohort of 56, both from Italian villages. Samples phenotyping: individuals aged more than 50 were defined as cases or controls based on their hearing thresholds at high frequencies. Linear regression: Total variation load per gene was compared between cases and controls to detect outlier genes. Enrichment: Gene Ontology (GO) was analyzed with PANTHER web-tool. Natural Selection: a PCA-based method was used to investigate adaptation along the Europe-EastAsian axis. Expression studies: RT-PCR was performed in mouse cochlea cDNA.

Results: Two groups of outlier genes were detected: A) 375 more variable in cases and B)371 less variable in cases. The largest GO enrichment for both groups (fold>5,$p<0.05$)

was the "sensory perception of sound", suggesting cumulative genetic effects involved in ARHL. 141 genes were replicated in the independent cohort, among which we identified 21 genes putatively under selection. After expression studies in the inner-ear, 20 out of 21 genes were positively expressed and two of them (CSMD1 and PTPRD), were previously described as involved in hearing function [Girotto et al.2011&2014].

Conclusions

We present a novel multistep strategy, providing major insights into the molecular characterization of complex diseases such as ARHL and could be applied to other phenotypes/diseases, where the paucity of samples makes the GWAS approach not feasible.

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P02.08D

A first report of copy number variations in *CNGB3* implicated in achromatopsia

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Introduction: In hereditary retinal degenerations, up to 50% of cases remain genetically undiagnosed even after whole exome sequencing analysis. This may be attributed to as yet unknown disease genes or elusive mutations including genomic structural variations. Here, we provide a first report on copy number variations (CNVs) in *CNGB3*, the major gene for autosomal recessive achromatopsia.

Materials and Methods: We identified heterozygous CNVs in 16 of 43 unrelated achromatopsia patients harboring a single mutant *CNGB3* allele by quantitative real-time PCR (qRT-PCR) using predesigned TaqMan Copy Number Assays or custom designed (amplicon-based) qRT-PCR assays applying the QuantiTect SYBR Green PCR Kit. A homozygous deletion encompassing 15 exons was identified by conventional PCR during routine diagnostics. All identified CNVs were validated either by CytoSure arrays or arrEYE. Breakpoint mapping was performed for all intragenic CNVs (14) by long-distance PCR using Phusion High-Fidelity DNA Polymerase. Segregation analysis was done in 12/17 cases depending on availability of family members.

Results: Applying qRT-PCR, we detected heterozygous CNVs encompassing 1 to 10 consecutive exons in 16 unrelated patients previously shown to carry a second clearly pathogenic *CNGB3* allele. We observed more patients with duplications (10) than patients with deletions (6). The largest duplication and all identified deletions were unique, while 6 unrelated patients showed an identical duplication of exon 7. Another duplication encompassing 4 exons was identified in 3 unrelated cases.

Conclusions: These findings add to the *CNGB3* mutation spectrum and demonstrate the importance of heterozygous CNVs in achromatopsia patients missing a second clearly pathogenic allele.

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Missense mutation in CEP78 in a family with cone-rod dystrophy, sensorineural hearing loss, obesity and subfertility

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Introduction: Bi-allelic truncating mutations in CEP78 were recently found to cause cone-rod dystrophy (CRD) with sensorineural hearing loss. Here, we aimed to identify the causal mutation in a family with CRD and sensorineural hearing loss, and to explore other ciliary phenotypic features.

Methods: Whole exome sequencing was performed in 2 affected siblings and segregation analysis was done in 5 additional family members. Skin biopsies and nasal brush

samples were obtained from both affected individuals and their parents to study ciliary structure and length.

Results: We identified a novel missense mutation in CEP78 in a homozygous state in both affected individuals: c.449T>C, p.(Leu150Ser). This mutation segregates with disease in 7 individuals. The affected amino acid is highly conserved, and the change is predicted to be damaging by several *in silico* prediction tools. Phenotyping revealed the presence of additional features reminiscent to a ciliopathy such as recurrent airway infections, obesity and subfertility. Morphology of primary cilia in fibroblasts was evaluated and induced cilia in patient's fibroblasts were significantly longer in comparison to control cells. Functional studies are ongoing on nasal epithelial cells and semen samples to investigate if this CEP78 mutation affects ciliary structure, and if it is associated with the ciliary phenotypic features observed.

Conclusions: In conclusion, we identified the first missense mutation in CEP78 causing CRD and sensorineural hearing loss, a recently identified syndrome distinct from Usher syndrome. The family studied here displayed additional features, suggesting a potential involvement of CEP78 in more complex ciliopathies.

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P02.10B

Genetic analysis of 100 consanguineous families to identify the molecular cause of mendelian visual impairment

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Consanguineous populations have a higher prevalence of autosomal recessive disorders due to the higher probability of homozygosity for alleles identical by descent. By studying the spatial distribution of runs of homozygosity (ROH) across the genome, we have observed that the average ROH size of inbred population (215MB) was significantly higher than outbred populations (25MB). In order to decipher the genetic basis of visual impairment, the current study was designed to identify the known or likely pathogenic variants in known or novel candidate genes in consanguineous families with mendelian visual impairment.

By using a strategy that combines exome sequencing and genotyping, we have analyzed 100 highly consanguineous families from Pakistan (where consanguinity rate is >60%), manifesting undiagnosed likely autosomal recessive visual impairment. We have identified known or novel likely pathogenic variants in genes which are already reported to cause visual impairment in 65% of the families. In 18% of the cases we have found likely damaging variants in novel candidate genes. In 17% families, we failed to identify any causative or candidate variants in the coding regions covered by exome sequencing.

We conclude that the discovery of novel autosomal recessive genes for visual impairment can be accelerated by analyzing large cohorts of consanguineous families. This has important implication in correct molecular diagnosis, family planning, and may reduce the incidence of autosomal recessive disorders in consanguineous couples. Gene discovery of novel candidates is the first step in understanding the molecular pathophysiology of a disease and may contribute to innovative treatment.

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P02.11C

Application of Next Generation Sequencing Upon the Diagnosis of Deafness

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Introduction: There are 15 000 hearing-impaired children are born in Vietnam every year. Early detection of common deafness mutations is a key factor for diagnosing, helping hearing-loss children to develop their language and awareness normally.

Research's Objects and Methods: 50 hearing-impaired and 100 normal children from Viet Nam. Apply technology of next generation sequencing to detect 100 mutations of 18 deafness genes, namely GJB2, GJB3, SLC26A4, MT-RNR1, MT-CO1, MT-TL1, MT-TS1, MT-TH, DSPP, GPR98, DFNA5, TMC1, MYO7A, TECTA, DIABLO, COCH, MYO15A and PRPS1.

Result: Identify 12 mutations of deafness genes of 11 hearing-impaired patients (account for 22% in total), including 7 cases of heterozygous mutations of genes GJB2 (c.299-300delAT (3 cases), c.512insAACG); SLC26A4 (c.2168A>G); TMC1 (c.1334G>A and 5 cases of homozygous mutations of genes GJB2 (c.512insAACG, 2 cases), MT-RNR1 (m.827 A>G, m.961delTinsC); MT-TH (m.12201T>C); MT-TL1 (m.3243A>G). No *mutations* were identified in the *control group*.

Conclusion: The incidence of deafness mutations in hearing-loss group is 22%. Mutations of GJB2 cover the largest proportion (12%) among 18 genes investigated. In this review, we describe commonly used genomic technologies as well as the application of these technologies to the genetic diagnosis of deafness and to the discovery of novel genes for syndromic and nonsyndromic deafness.

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Molecular Inversion Probe-Next Generation Sequencing to identify genetic markers in painful neuropathies - The PROPANE study

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Introduction: Neuropathic pain is a frequent feature of peripheral neuropathy causing a significant impact on patients' quality of life and health care costs. Current inability to identify high-risk individuals hinders development and application of therapies to counteract neuropathic pain. The purpose of this study is to resolve the genetic architecture of painful neuropathy by 1) constitution of a DNA databank of patients with painful and painless diabetic and idiopathic neuropathy, 2) targeted sequencing of sodium channel genes expressed in the nociceptive pathway.

Materials and Methods: DNA was extracted from peripheral blood of 1258 patients (Idiopathic n = 726, Diabetic n = 532) recruited in different partner centres. Targeted sequencing of sodium channel genes has been performed by Molecular Inversion Probe-Next Generation Sequencing (MIP-NGS). Variants' pathogenicity was classified according to the ACGS practice guidelines.

Results: In a cohort of 859 patients with diabetic (n = 367) and idiopathic neuropathy (n = 492), sequence data of SCN3A, SCN7A-11A and SCN1B-4B revealed 116 different potentially pathogenic variants in 181 patients (Idiopathic n = 83/111 patients, Diabetic n = 58/70 patients). Hundred variants were unpublished, and 91 were specific for a painful phenotype. Variants with strong evidence for pathogenicity are selected to undergo cell electrophysiology testing. Screening of the ten sodium channel genes has been extended with 97 candidate genes known to be involved in neuropathic pain, their interacting partners, and genes selected by integrative genomics.

Conclusion: MIP-NGS is relatively inexpensive and flexible technique, allowing a wide genetic characterization of painful neuropathy patients. This study was funded by the European Union 7th Framework Programme (grant n° 602273).

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P02.13A

Diagnostic exome sequencing in 266 Dutch patients with visual impairment

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Inherited eye disorders have a large clinical and genetic heterogeneity, which makes genetic diagnosis cumbersome. An exome-sequencing approach was developed in which data analysis was divided into two steps: the vision gene panel and exome analysis. In the vision gene panel analysis, variants in genes known to cause inherited eye disorders were assessed for pathogenicity. If no causative variants were detected and when the patient consented, the entire exome data was analyzed. A total of 266 Dutch patients with different types of inherited eye disorders, including inherited retinal dystrophies, cataract, developmental eye disorders and optic atrophy, were investigated. In the vision gene panel analysis (likely) causative variants were detected in 49% and in the exome analysis in an additional 2% of the patients. The highest detection rate of (likely) causative variants was in patients with inherited retinal dystrophies, for instance a yield of 63% in patients with retinitis pigmentosa. In patients with developmental eye defects, cataract and optic atrophy, the detection rate was 50%, 33% and 17%, respectively. An exome-sequencing approach enables a genetic diagnosis in patients with different types of inherited eye disorders using one test. The exome approach has the same detection rate as targeted panel sequencing tests, but offers a number of advantages. For instance, the vision gene panel can be frequently and easily updated with additional (novel) eye disorder genes. Determination of the genetic diagnosis improved the clinical diagnosis, regarding the assessment of the inheritance pattern as well as future disease perspective.

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P02.15C

CTG18.1 trinucleotide repeat expansion in Polish patients with Fuchs endothelial corneal dystrophy

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Introduction: Fuchs endothelial corneal dystrophy (FECD) is the most common, genetically determined degenerative disease of the cornea. The molecular basis of FECD is complex and heterogeneous. The latest and the most predisposing genetic factor for the development of FECD is a trinucleotide repeat expansion CTG18.1, which is located in the second intron of the *TCF4* gene.

Materials and Methods: Clinical evaluation was based on slit-lamp examination, in vivo confocal microscopy (IVCM) and anterior segment optical coherence tomography (AS-OCT). Genomic DNA was isolated from peripheral blood samples of unrelated FECD patients (n = 236) and control subjects (n = 58). We genotype the CTG18.1 repeat expansion to determine an association between the genetic variant and FECD development and to analyse possible relationships between the different CTG18.1 genotypes and clinical picture of the patients. For this purpose a combination of methods, i.e. analysis of short tandem repeats (STR), triplet repeat primed PCR (TP-PCR) and statistical analysis were performed.

Results: The results showed that the repeat expansion CTG18.1 is currently the strongest predisposing factor for the development of FECD in a group of Polish patients (OR=43,72; CI:13,20-144,83; $\chi 2$=80,77; p<0,0001). There were no statistically significant associations between the CTG18.1 genotype and the best corrected visual acuity (BCVA), central corneal thickness (CCT) or the density of the corneal endothelium.

Conclusions: Our study confirms association of CTG18.1 repeat expansion with FECD by testing a novel previously not analysed population. This genetic determinant has an important predictive value and may be beneficial in clinical practice.

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P02.16D

Molecular diagnosis of hereditary hearing impairment by high throughput sequencing of targeted exome in patients from Jordan and North Africa

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After the exclusion of the most prevalent mutations, the identification of causal mutations is difficult due to the high genetic heterogeneity and high cost of the Sanger technique. We used a targeted exome sequencing strategy (Hear Panel) to analyze 137 known deafness genes and some candidate genes in 268 unrelated patients, belonging to families with at least two affected individuals, originated from Jordan, Tunisia, Algeria, Morocco, and Mauritania. Molecular diagnosis was established in 206 patients (77%), with a total of 194 mutations being identified in 52 different genes, among which 140 (72%) had not been previously reported. This makes Hear Panel a powerful tool to identify rare HI mutations in populations with high rates of consanguineous marriages. Six genes were involved in 41% (110/268) of the patients: *MYO15A* (26/268), *MYO7A* (25/268), *SLC26A4* (18/268), *OTOF* (16/268), *CDH23* (13/268) and *TMC1* (12/268). Among the 206 diagnosed patients, 178 (86%) carried biallelic mutations in autosomal recessive deafness (DFNB) genes (159 patients were homozygote and 19 were compound heterozygote), 27 (13%) carried monoallelic mutations in autosomal dominant deafness (DFNA) genes and one patient carried a mutation in an X chromosome-linked deafness (DFNX) gene. In the 36 patients (13%) carrying biallelic mutations in genes underlying Usher syndrome, sight impairment was subsequently identified by ophthalmological examination. This establishes the Hear Panel as an efficient and reliable tool for an early diagnosis of Usher syndrome before the onset of the sight loss which is of utmost medical importance as these patients should benefit from early cochlear implantation

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P02.17A

Genetic background of sensorineural hearing loss in Slovakia: data from Slovak nation-wide survey

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Introduction: Goal of our study was to perform nationwide screening for hereditary deafness and elucidate the genetic background of sensorineural hearing loss (SNHL) in our

population composed of ~90% Caucasians and ~8% of Roma ethnicity.

Patients and methods: Between 2010–2016, we collected 1003 DNA samples from 682 families with bilateral SNHL. Probands were screened for *GJB2*; negative and heterozygous subjects were tested for *GJB6*-D13S1830 and D13S1854 deletions. Roma patients were further investigated for *MARVELD2* gene. Subjects with matrilineal inheritance of SNHL and diabetes or history of aminoglycoside treatment were tested for mitochondrial pathogenic variants m.3243A>G and m.1555A>G. 20 families with familial nonsyndromic deafness were analysed using whole exome sequencing (WES).

Results: In case of *GJB2*, 201 probands carried biallelic pathogenic variants (29.5%), 50 heterozygous (7.3%) and 431 no pathogenic variant (63.2%). The most frequent variant was c.35delG in Caucasians and c.71G>A in Roma population. One subject was compound heterozygote for *GJB2/GJB6*-D13S1830. In *MARVELD2* gene c.1331 +2T>C pathogenic variant was detected in three families accounting for 3.5% of SNHL cases in the Roma cohort. Mitochondrial variant m.3234A>G was detected in four families and one family carried the m.1555A>G variant. WES analysis in 20 families identified causative gene in 11 families (55% of the WES group). These included seven known genes: *COCH, EYA4, MYO6, OTOG, TECTA, TMC1* and *TMPRSS3* with five novel variants.

Conclusion: We identified causal genotype in about one third of unrelated patients with SNHL. As we further demonstrated, WES may dramatically increase precise diagnostics of deafness etiology.

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P02.18B

Expanding the etiological work-up of congenital hearing loss by targeted resequencing approach

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Objective: Infants identified with congenital hearing loss through a newborn hearing screening program undergo an etiological work-up to identify an underlying cause. We routinely exclude congenital infections and anatomical abnormalities through magnetic resonance imaging and/or computerized tomography. Unless clinical findings or family history dictate otherwise, until recently, genetic analysis was limited to a search for mutations in the GJB2 genes (encoding Connexin 26). Based upon current protocols, an etiological diagnosis may be established in about 50% of infants/children with congenital hearing loss (Declau et al. Pediatrics 2008).

Methods: Targeted next generation sequencing of known hearing loss causing genes was proposed to 59 children with confirmed bilateral congenital hearing loss of unknown cause (GJB2 mutations excluded). Two gene panels (one for non-syndromic hearing loss and one for syndromic hearing loss) were employed covering more than 100 hearing loss genes.

Results: Informed consent was obtained for 20 children with bilateral moderate to profound hearing loss. A likely genetic cause for the hearing loss was found in 11 children (mutations in Whirlin (DFNB31), Marveld2 (DFNB29), MYO15A (DFNB3), SOX10 (Waardenburg syndrome type 2E), TRIOBP (DFNB28), Oto-ancorin (DFNB22), TMPRSS3 (DNFB8/10), MITF (Waardenburg syndrome type 2A), TMC1 (DFNB7/11), OTOG (DFNB18B), and GPR98 (USH2C).

Conclusions: Targeted next generation sequencing of known hearing loss causing genes may expand the diagnostic yield of currently available protocols for the etiological work-up of congenital hearing loss. The identification of a genetic cause may guide treatment decisions and allow for genetic counselling.

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P02.19C

Identification of candidate pathogenic variants in patients with hearing loss using extended gene panel containing 120 disease associated genes

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Hearing loss is with incidence higher than 1:1000 one of the most common hereditary diseases. Routinely GJB2 gene sequencing is performed as in this gene pathogenic variants are found the most frequently, with population specific predominant mutations like c.35delG in Caucasians or p. W24* in Gypsies. In cases without GJB2 associated hearing

loss high level of genetic heterogeneity could be a problem. Gene panel resequencing bring the possibility to comprehensively analyze such patients and could help to solve previously unsolvable cases. Customized 120 genes containing gene panel was designed to cover the most relevant genes associated with syndromic as well as nonsyndromic deafness. Gene resequencing of 32 samples that were negative for the most common pathogenic variants, as previously screened for the most common and/or population specific mutations of GJB2, GJB6 and MARVELD2 genes, were analyzed with the designed gene panel. After filtering and annotation with Ingenuity Variant Analysis altogether 151 variants were identified to have relevant biological context. Of these 125 were classified as variants of unknown significance or likely pathogenic or pathogenic. Surprisingly also in case of such well studied disease like hereditary hearing loss only 2 likely pathogenic and 4 pathogenic variants were in this group. These 6 variants found in USH2A, TECTA, SLC26A4, USH1C and surprisingly also in GJB2 genes. The next 119 identified variants need more comprehensive research, represented by detailed literature search and if possible with additional family members targeted genetic analyses for segregation analysis. Study was supported by grant VEGA-1/0048/14.

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P02.20D

Next-Generation Sequencing to Decipher the Genetic Heterogeneity of Deafness in Palestinian Arab Families

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Analysis of multiplex families from the Palestinian Arab provides a rich source for discovery and characterization of hearing loss (HL) genes. Primary clinical evaluations and audiological analyses were undertaken for each family, and *GJB2* gene mutations and other common founder alleles were tested. Families with no such mutations were analyzed by hybridization and multiplexed sequencing, using our panel of all known human and mouse hearing loss genes. SNVs, indels, and CNVs were identified in exons and proximal regulatory regions. Mutations were inferred as damaging only if they co-segregated with deafness in their families; and were truncating, completely deleted the gene, or were missense mutations with confirmed functional effect. Of 85 families evaluated by our panel thus far, 53 families (62%) carried a pathogenic or likely pathogenic mutation in a known deafness gene. A total of 44 different mutations, most not previously reported, were identified in 22 genes and submitted to LOVD and ClinVar. Genes responsible for non-syndromic HL in 41 families were *CDH23*, *CLDN14*, *ESRRB*, *ADGRV1*, *GPSM2*, *LOXHD1*, *MYO15A*, *MYO6*, *MYO7A*, *OTOA*, *OTOG*, *PCDH15*, *PTRH2*, *TBC1D24*, *TMC1*, *TRIOBP* and *USH1C*; genes for syndromic or other HL phenotypes in 12 families were OTOF, *PAX3*, *SLC26A4* and *USH1G*. The 38% of families with no mutation in any known deafness gene represent a highly informative resource for identification both of additional HL genes and of distant non-coding regulatory mutations. We are evaluating these unresolved families with whole exome and/or whole genome sequencing.

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P02.21A

Siblings with Perralut syndrome and *LARS2* mutation who presented with neurologic abnormalities

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Perrault syndrome represents a genetically heterogeneous disorder characterized by sensorineural hearing loss and ovarian dysgenesis in females. Causative genes include *HARS2, HSD17B4, CLPP*, *C10orf2*, and *LARS2 (*mitochondrial leucyl-tRNA synthetase). Some patients exhibits neurologic features including learning disability, cerebellar ataxia, and motor or sensory peripheral neuropathy. No patients with neurological symptoms have been reported among 15 patients in 8 families with *LARS2* mutations reported to date. Here we report two female siblings with biallelic mutation in *LARS2*, p.Glu 294Lys and p.Thr

519Met. The proposita developed progressive sensorineural hearing loss at 18 months and pervasive developmental disorder at 8 years with cognitive and behavioral problems included repetitive behavior, insistence on sameness, attention deficit, chic, and, irritability, and ataxic gait. She later was diagnosed as having primary amenorrhea with elevated FSH, LH and decreased estradiol and small uterus and no detectable ovaries on MRI at the age of 15. The proposita's younger sister presented with neonatal sensorineural hearing loss, mild delay in motor and speech development. She was diagnosed as having primary amenorrhea with comparable endocrinologic and radiographic findings that were comparable to her sister. Present observation of PDD in one of the siblings and mild developmental delay in the other sibling with classic symptoms of Perrault syndrome and LARS2 indicates that patients with LARS2 mutations are at risk for neuro-behavioral symptoms as Perrault syndrome patients with other causative genes. Variable expressivity between the two siblings could be ascribed to general tendency of mitochondrial diseases in which intra-familial variation is common.

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P02.22B

Mutation study of 69 genes in 100 Thai patients with non syndromic hearing loss detected by next generation sequencing

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Introduction: Hereditary hearing loss (HHL) is one of the most common birth defects. One-fourth of HHL is syndromic hearing loss (SHL), in which deafness is accompanied by other signs and/or symptoms whereas the remaining is non-syndromic hearing loss (NSHL). About 80% of NSHL is inherited in autosomal-recessive mode, almost 20% of NSHL is inherited in autosomal-dominant pattern, and very small fractions are sex-linked inherited. The present study attempts to find the genetic variants underlying congenital NSHL in Thai patients.

Materials and methods: A targeted sequencing panel, using Ion Torrent™ systems, including 69 genes for HHL were designed and performed in 100 specimens from unrelated Thai individuals with congenital NSHL, including 11 familial and 89 sporadic index cases. Single-nucleotide variations (SNVs) and insertions/deletions (INDELs) were analysed.

Results: We identified 930 genetic variants (SNVs/INDELs) in 59 genes studied. We filtered out SNVs/INDELs with Thai minor allele frequencies >0.03 as well as synonymous nucleotide substitutions. The overall filtering process led to 144 to Sanger sequencing validation. Sanger sequencing were confirmed 21 reported pathogenic, 104 promising variants in 52 patients, and 19 false positive variants. Usher syndrome associated genes (CDH23, MYO7A, USH2A) are the most common cause (23.1%) of Thai apparently NSHL, followed by SLC26A4 (13.5%), COL11A2 (11.5%), and GJB (9.6%). However, the 104 promising variants require segregation analysis in family members and further study.

Conclusions: Targeted exome approach for identification of causative genes in Thai NSHL population is highly effective and results in high detection rate at ascend 52%.

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P02.23C

HRC imputations reveal some of the missing heritability of endophenotypes of glaucoma

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Introduction: Primary open-angle glaucoma (POAG) is the main cause of irreversible blindness worldwide. Intraocular pressure (IOP), central corneal thickness (CCT) and the morphology of the optic nerve head are important heritable endophenotypes of the glaucomatous eye. Genome-wide association studies (GWAS) using the HapMap or 1000 Genomes imputation panels have identified numerous loci associated with these traits. The recently developed imputation panel by the Haplotype Reference Consortium (HRC) allows a better quality of rare variants imputations which will result in refining causal variants in known loci and revealing additional loci for IOP, CCT and optic disc parameters.

Materials and Methods Meta-analysis GWAS results from 17000 to 31000 European individuals participating in the IGGC has been carried out using the HRC as a reference set for imputations. We explored the associations between all single genetic variants with IOP, CCT and three optic disc parameters: cup area (CA), disc area (DA), and vertical cup disc ratio (VCDR).

Results We replicated previous findings and identified 13 novel loci (2 with CA and VCDR, 2 with IOP, 3 with CCT and 6 with CA only). Among the new loci associated with VCDR and CA is a variant in *MEF2C*, a gene involved in synaptic development.

Conclusions The study shows that the HRC panel is a powerful tool for further enhancing our understanding of the genetic basis of these traits. The numerous identified variants in our study show the genetic complexity of this disease and increase our knowledge in the pathways leading to this disease.

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P02.24D

A large multi-ethnic genome-wide association study identifies novel loci influencing intraocular pressure and central corneal thickness

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Introduction: Intraocular pressure (IOP) and central corneal thickness (CCT) are associated with glaucoma, a common eye condition which can lead to vision loss. Family studies indicate that IOP and CCT have a moderate to strong genetic component, with heritability estimates of up to 0.67 and 0.95, respectively. Genetic studies have reported 11 and 29 loci associated with IOP and CCT, respectively, with one locus associated with both traits. This suggests that additional loci remain to be discovered and some of the genetic risk of IOP and CCT might be shared.

Materials and Methods: We first conducted a genome-wide association study (GWAS) of IOP in 76,937 individuals with 496,897 IOP measurements from four race/ethnicity groups (non-Hispanic White, Hispanic/Latino, East Asian, and African American) in the Genetic Epidemiology Research in Adult Health and Aging (GERA) cohort. We then conducted a GWAS of CCT in a GERA subset (N = 17,730) with both IOP and CCT measurements available.

Results: We identified 51 genome-wide significant IOP-associated loci ($P < 5 \times 10^{-8}$), of which 36 were novel, that is, not previously-reported to be associated with IOP, CCT, or glaucoma. We also identified 23 CCT-associated loci, of which 8 were novel. Importantly, 11 loci were associated with both IOP and CCT at a genome-wide level of significance.

Conclusions: Our results confirm that these two traits partly share a common genetic background. Investigating genetic variation influencing IOP and CCT might help elucidate their role in glaucoma pathophysiology.

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P02.25A

Whole exome sequencing (WES) identified COL6A5 variants in familial idiopathic itch

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Introduction: To date, no molecular markers have been identified in patients with idiopathic spontaneous chronic itch (SCI). Here we describe the first gene variants identified in eight patients, all suffering from idiopathic SCI and neuropathic pain.

Materials and methods: Family1 is 3 generations, 1 member per generation complaining of SCI. Family2 is diagnosed with JHS/EDS-HT Syndrome, 2 affected members referring also daily SCI attacks. In family3 three affected members are distributed over two generations. WES was performed on affected and unaffected samples. Libraries were obtained either by Agilent technologies, run on 2500Hiseq (Illumina), or by LifeTechnology approach, run on IonProton platform (LifeTechnologies). Immunofluorescence microscopy, western blotting and null-allele test were performed on fibroblasts cultures. Skin biopsies were examined assessing intraepidermal nerve fiber density and protein expression.

Results: We compared the rare genetic variants selected in the affected samples and found 3 mutations (two *in-cis* in family-1; one in family-3) affecting the COL6A5 gene, encoding a collagen subunit expressed at epidermal level. WES data were inspected for same variants also in family-2, recruited in a different project, and the same 2 *in-cis* mutations were found to segregate with itch phenotype. Haploinsufficiency were demonstrated by null-allele test on cDNA from patients' fibroblasts carrying the nonsense variant. Immunofluorescence microscopy and Western blotting revealed disorganization and reduced synthesis of the specific collagen fiber, immunohistochemistry showed reduced amount around dermal vessels.

Conclusion: Our findings revealed that WES on families with common phenotype could reveal new variants involved in the individual susceptibility to chronic itch.

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P02.26B

Whole exome sequencing profiling indicates inter-tissue genetic variation in keratoconus patients

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Introduction: Evidence linking somatic variants to complex diseases is rising. Keratoconus (KTCN) is a

multifactorial ocular disease, observed in 1 per 375 individuals, and its genetic background are not fully understood. Majority of KTCN molecular studies were performed using DNA derived from blood. The purpose of this study was to assess potential inter-tissue genetic variations which may be involved in KTCN etiology.

Materials and Methods: Corneal tissues as well as blood samples from five non-related Polish patients after KTCN keratoplasty procedure were collected to perform deep whole exome sequencing (WES) in tissue-blood sample pairs. DNA samples were prepared into paired-end libraries and underwent whole exome capture, followed by deep exome sequencing.

Results: Exome capture discovered somatic variants in protein-coding regions of each corneal tissue donors. A number of cornea-specific variants has differed between samples and was in range of 6–22, including potential pathogenic variants in *CACHD1* and *ZNF467*. The most somatic variation was observed at chr7, including mucins genes *MUC3A* and *MUC17*. No common somatic variant was observed in the examined samples. Variants in both blood and matched corneal tissue from the same donor were also considered. The assessed sample pairs had variants simultaneously detectable in both tissues, in range of 94–143, including rare variants in *RNF19B* and *ERICH2*.

Conclusions: Somatic variants may represent an additional component in KTCN etiology. This was the first WES profiling of human KTCN corneas.

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P02.27C

Missense mutations substituting Arg51 of *Mab21l1* cause a spectrum of murine panocular malformations

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The highly-conserved male abnormal 21 (Mab21)-like genes are known to regulate eye development. Previously-reported homozygous *Mab21l1*-deficient mice have small eyes with a rudimentary lens (PMID 12642482), whilst *Mab21l2* nulls are embryonic lethal with only a rudimentary lens and retina (PMID 15385160). Mice heterozygous for either gene show apparently normal eyes.

We previously identified heterozygous missense variants substituting Arg51 of MAB21L2 as a cause of severe eye malformations with skeletal dysplasia. Homozygous loss-of-function mutations in *MAB21L1* have recently been reported in a single family with congenital glaucoma and genital anomalies (PMID 27103078). The crystal structure of human MAB21L1 was recently determined, with the protein appearing to pack into a decamer (PMID 27271801). Modelling a mutation of Arg51 was predicted to disrupt fold stabilisation.

We used CRISPR/Cas9 genome editing to knock-in a p.Arg51Leu substitution into *Mab21l1* resulting in a viable and fertile heterozygous line on a C57Bl/6 background. The eyes of adult mice were examined using binocular indirect ophthalmoscopy, slit lamp and histological examination. Mab21l1 p.Arg51Leu heterozygotes showed anomalous optic discs. Homozygous p.Arg51Leu mice showed small eyes with a spectrum of panocular eye malformations, including a disorganised anterior segment with abnormalities of the cornea, iris, ciliary body, lens, retina and optic nerve; the most severely affected eyes had only a rudimentary lens, retina and optic nerve.

In combination with protein structural support of homomultimerisation, the heterozygous phenotype in the mice may be consistent with a mild dominant negative effect of this mutation, whereas the homozygotes phenocopy null animals.

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P02.28D

Rare variants with unknown significance in candidate hearing loss genes for Meniere disease in Spain

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Introduction: The genetic architecture in Meniere's disease (MD), an inner ear disorder defined by episodic vertigo, sensorineural hearing loss (SNHL) and tinnitus, is not

known. We have sequenced a panel of 69 hearing loss genes to search for rare variants in MD.

Materials and Methods: Nine hundred thirty DNA samples (890 MD cases and 40 controls) were pooled and libraries were generated by HaloPlex PCR target enrichment system. Paired-end sequencing of was performed in a Nextseq500 instrument. BWA and GATK were used for alignment and quality control. Variant calling was made through VarScan2. False positives were filtered by similar strand calling and variants with ≥2 calls in both strands were examined, prioritized and estimated minor allelic frequencies were compared with public references values in multiple populations, including Spanish variant server database.

Results: An enrichment of certain rare variants (18–335 times more frequenctly observed than in gnomAD) was observed in the DFNB31, GJB2, ESPN, ESRRB, and MYH14 and RDX genes in our cohort of patients with MD. Some variants with unknown significance showed a higher MAF compared with Spanish population datasets. Prioritizing tools suggest that some of them should be consider as candidate for MD.

Conclusions: Spanish population has a specific enrichment of rare variants in some hearing loss genes. The role of these variants in SNHL and MD remains to be established.

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P02.29A

Novel *GJA8* mutations and CNVs extend the phenotypic spectrum to include microphthalmia and coloboma

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GJA8 encodes Connexin50 (Cx50), a transmembrane protein involved in the formation of lens gap junctions. Connexins play a critical role in intercellular communication and mutations in *GJA8* have been linked to congenital and early onset cataracts in humans and animal models. Interestingly, missense mutations and homozygous deletions of *Gja8* in mice also cause microphthalmia, as well as cataracts, suggesting that *Gja8* may be involved in ocular growth in addition to lens development and homeostasis.

To better understand the range of developmental ocular anomalies associated with *GJA8*, we screened this gene in a cohort of patients with severe congenital eye abnormalities, primarily anophthalmia, microphthalmia and coloboma (AMC). Four likely pathogenic variants (c.116C>G, p.(Thr39Arg); c.151G>A, p.(Asp51Asn); c.208T>C, p.(Phe70Leu); c.290T>G, p.(Val97Gly)) were identified in five families with cataracts, microphthalmia and other ocular anomalies. These rare or novel mutations were all *de novo* or dominantly inherited from an affected parent. Four rare or novel missense variants of unknown significance were also found in four additional families.

Heterozygous 1q21 deletions involving *GJA8* were identified in three families with microphthalmia, cataracts and/or coloboma. Although these structural variants showed incomplete penetrance, suggesting that modifiers may be important, their identification in our cohort supports their potential contribution to AMC conditions.

The data presented in this study expand the spectrum of human phenotypes associated with *GJA8* variants to include microphthalmia and coloboma, demonstrating the role of this gene in early eye development. This highlights the importance of including *GJA8* in the genetic screening of patients with AMC.

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P02.30B

Anterior segment dysgenesis with microphthalmia, microcornea, and spontaneously reabsorbed cataract is associated with a novel mutation in *CRYAA*

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Introduction: Proband with a sporadic complex ophthalmic phenotype was initially tested for mutations in *PAX6* gene and 11p13 locus due to congenital iris hypoplasia, microphthalmia, and microcornea and was found to have no changes. Obscure ophthalmic phenotypes could arise from clinical and genetic heterogeneity that requires a complex approach to diagnosis.

Materials and Methods: Affected proband (a 26-year-old woman) and her healthy relatives underwent detailed clinical and ophthalmic examination with subsequent molecular analysis. Whole exome sequencing (WES) was performed on Illumina NextSeq 500. Sanger sequencing was used to confirm found changes. The populational study was performed with PCR-RFLP.

Results: Detailed clinical and ophthalmic examination of proband revealed microphthalmia, microcornea, anterior segment dysgenesis, congenital iris hypoplasia, spontaneously reabsorbed cataract, complicated glaucoma, fovea and optic nerve disc hypoplasia, nystagmus and strabismus. WES analysis identified heterozygous novel single nucleotide variant (SNV) in *CRYAA*:c.521A>C leading to C-terminal extension (CTE) p.*174Serext*41. *CRYAA* mutations are described in patients with autosomal dominant congenital cataract type 9 (OMIM #604219). The proband's family analysis showed no mutation in healthy members. The populational study did not find this SNV in 210 control chromosomes of the same ethnic background. Analysis of closely linked SNP rs112855370 identified mutation occurred *de novo* in the paternal allele.

Conclusions: The in-depth molecular analysis allows clarifying the diagnosis. Mode of *CRYAA* damage through CTE suggests the severity of observed phenotype and additional defects in ocular development.

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P02.31C

Identification of microRNAs associated with primary open-angle glaucoma using GWAS data

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Introduction: Genetic polymorphisms in microRNAs (miRNAs) or miRNA-binding sites (BS) within target genes are expected to contribute to disease risk. We aimed to identify miRNAs and target genes that are involved in glaucoma using data from the largest available GWAS for glaucoma endophenotypes.

Methods: Polymorphisms in miRNA-encoding sequences and in miRNA-BS were retrieved from online databases and their associations were investigated with intraocular pressure (IOP), cup area, disc area and vertical cup-disc ratio (VCDR). Target genes for the associated miRNAs were ranked according to their association with the studied traits and tested in cell lines by transfection experiments for regulation by the miRNAs. The most likely functional miRNA-BS SNPs were prioritized based on various criteria (e.g. eQTL, miRNA and gene expression, interaction score).

Results: Out of 412 miRNA-SNPs available in IGGC GWAS data, two SNPs were associated with VCDR and cup area (significant threshold 1.2×10^{-4}). One of the SNPs is located in the pre-miRNA sequence, and has been shown to decrease expression of the miRNA. The other is located in the miRNA-seed region and we showed that the SNP reduces interaction between the miRNA and its target *CARD10*, a gene associated to disc area. Out of 72,052 miRNA-BS SNPs present in IGGC GWAS data, 47 SNPs (in 21 genes) were significantly associated with glaucoma endophenotypes (p-value$<6.9\times10^{-6}$). These include two SNPs in *CDKN2B*, a known glaucoma-associated gene, and have been experimentally confirmed to disrupt the miRNA-binding sites.

Conclusion: Our findings support the contribution of miRNAs to the genetic susceptibility to glaucoma.

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P02.32D

Comprehensive genetic testing of 214 French hearing impaired patients

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During the last 5 years, Massively Parallel Sequencing (MPS) has deeply modified routine practices in diagnostic laboratories. In the field of highly genetically heterogeneous rare diseases, such as non-syndromic hearing loss (NSHL), MPS has provided the ability to study dozens of genes in a single assay while only one was analysed just a few years ago. Since 2015, the Laboratory of Molecular Genetics in Montpellier University Hospital studies human hearing impairment with a 70 NSHL genes panel which also includes 10 Usher syndrome genes. Samples from patients are referred from all over France. Samples are multiplexed and 12 samples are sequenced per assay. Considering the 2015-2016 period, we analysed 214 index cases referred for NSHL. Prior to MPS, we screen for mutations at the *DFNB1* locus (*GJB2* gene and *GJB6*-D13S1830 deletion). Fifty-five probands were carriers of a *DFNB1* pathogenic genotype. The remaining 159 probands were analysed using MPS and 49 of them reported a clear pathogenic genotype in 24 different genes. Twelve of them carried in addition a potentially or clearly pathogenic variant in a second gene. Moreover, several variants, e.g. in *ACTG1, TJP2* or in *CDH23*, arose *de novo*. We report here an overall diagnostic rate of 48% for NSHL in France. We identified pathogenic genotypes in several Usher genes, which in some cases implies critical outcomes for the patient's follow-up and outlines the urges to better understand the frontlines between syndromic and non-syndromic hearing loss. This is the first report of such a large cohort in France.

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P02.33A

First implication of a hypophosphatemia gene in familial cases of dominant hearing loss

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The first etiology of hereditary non-syndromic hearing loss is the deletion of a Guanine (del35G) in the *GJB2* gene, coding for the Connexin 26 protein. Here we report on a family with an unusual story of hereditary hearing loss. The two parents are deaf, as their two daughters, but their son has normal audition.

Firstly, we performed the molecular analysis of *GJB2* in this nuclear family. The mother was found homozygous for 35delG, her three children were heterozygous, whereas the father had no *GJB2* variant. An enlarged search for *GJB2* mutation in the grandparents showed that they were heterozygous for 35delG, with a normal hearing function. As the genotype-phenotype segregation in this family did not match with the implication of *GJB2* 35delG, we performed a New-Generation-Sequencing analysis using a deafness panel of 146 genes, with no additional informative result. Finally, an Exome analysis (Integragen) identified a candidate pathogenic variant at the heterozygous state shared by the deaf father and his two deaf daughters, but absent in the mother and her son.

This variant concerns *SLC9A3R1* gene, that codes for a Na+/H+ exchanger regulatory factor (NHERF1) expressed in basal renal cells, and implied in dominant hypophosphatemia with nephrolithiasis (OMIM *604990). Animal models research proved that this gene is also expressed in neurons and auditory outer hair cells, with an association with hearing loss phenotypes.

Our hypothesis is that this gene could be responsible of a dominant form hearing loss in this family, which has never been described before in human pathology.

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P02.34B

From hero to (almost) zero: the hidden dangers of whole-exome sequencing in disease-gene discovery

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Introduction: Whole-exome sequencing (WES) represents a powerful approach to identify genes/mutations responsible for genetically heterogeneous diseases. We performed WES on two affected siblings from a consanguineous family with recessive nonsyndromic hearing loss (NSHL).

Results: Standard WES data analysis for SNVs/indels excluded point mutations in known NSHL genes and highlighted a homozygous variant in *HCN1* (NM_021072: c.1250A>G:p.Y417C). The variant segregates with the phenotype, is absent in ethnically-matched controls and public databases, and is predicted to be damaging. *HCN1* encodes a sodium/potassium channel expressed in spiral-ganglion neurons and cochlear cells. Moreover, Hcn1 deficient mice show mild auditory deficits. Dominant mutations in *HCN1* were recently associated with early-infantile epileptic encephalopathy (MIM#615871). Patch-clamp recordings of HEK293 cells overexpressing the wild-type or mutant recombinant protein showed a strikingly decreased current in the presence of the p.Y417C, suggesting a loss-of-function effect. The p.Y417C channel showed a different current amplitude and kinetics also compared to the gain-of-function epilepsy-causing p.D401H mutant. Despite these encouraging results, pointing to *HCN1* as a candidate deafness gene, a re-analysis of WES data for structural variants, pointed out a 250-kb homozygous deletion of the *OTOA* gene, which was confirmed by segregation analysis and PCR.

Conclusions: We characterized a novel loss-of-function *HCN1* variant which is likely functional, although it is clearly not the primary cause of deafness in our patients. We are exploring its potential role as phenotype modifier. Our case report strengthens the importance of performing both point mutation and structural variant analysis to avoid false hypes in WES.

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P02.35C

Simultaneous analysis of single nucleotide and structural variants through NGS using a targeted panel of genes involved in ocular congenital malformations

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Objectives: Implementation of CNVs detection using a targeted NGS gene panel for the characterization of ocular congenital malformations (OCM).

Materials & Methods: A cohort of 88 patients with OCM was analyzed, including 6 control cases with previously known pathogenic variants and CNVs. The remaining 82 uncharacterized patients had variable phenotypes: 31 with anophtalmia-microphtalmia (A/M), 22 with coloboma, 17 with optic nerve anomalies and 12 with anterior segment dysgenesis. A set of 150 genes were selected and included in a custom gene panel (Haloplex). SNVs were analyzed following an in-house bioinformatic pipeline. CNVs identification was performed prioritizing statistically significant regions in the read depth. Custom CGH-arrays (180K, Agilent) are being used for validation purposes.

Results: A total number of 19 pathogenic SNVs spanning 14 different genes and 13 additional VUS were identified, validated and segregated in the families. Our specific bioinformatic method in combination with the high depth of coverage (>400×) allowed the detection of the 2 control cases included in the panel, carrying multi-exon deletions of *PAX6*. Additionally, 5 new structural variants were detected: a duplication of *GDF3* and complete deletions of *FOXC1, GJA8, HPS5* and *OTX2-SIX6*.

Conclusions: This study allowed the characterization of 24% of the patients (40% of the patients with A/M). Our results have shown that a NGS panel is a good strategy for the genetic analysis of OCM patients since it permits the co-detection of SNVs and CNVs in a single design when a homogeneous coverage between samples is ensured.

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P02.36D

Functional analyses of the effect of seven mutations in PAX6 in severe congenital ocular anomalies

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Introduction: Congenital aniridia is a rare ocular malformation mainly characterized by aplasia or severe hypoplasia of the iris. Loss of function mutations in *PAX6*, the paired box-6 gene essential in eye development, are involved in >90% of patients with aniridia. We have identified seven heterozygous mutations encompassing the PAX6 DNA binding domain in patients with severe microphthalmia (reduced eye size) or anophthalmia (absence of the eye) (MA), developmental delay, microcephaly and/or hypoplasia of the *corpus callosum*. Subsequently, we hypothesized that this atypical phenotype should be the result of dominant negative mutations. We thus studied *in vitro* the functional effects of these mutations and compared them to the mutations associated with classical aniridia.

Materials and methods: We performed site-directed mutagenesis in an expression vector containing the PAX6 coding sequence to create the different mutant clones containing the seven mutations associated with the atypical phenotype and other three which gave rise to classical aniridia. Afterwards, we analyzed their transcriptional activation activity by using the luciferase assay. In addition, we performed a western blot and a qPCR with the aim of evaluating the amount of protein and mRNA produced, respectively.

Results and Conclusions: In this study, we report that mutations in *PAX6* are also involved in MA with a neurological component. We successfully created all mutated vectors and optimized the luciferase assay, western blot and qPCR. Preliminary results showed that luciferase activity profile might be affected by these two types of mutations, however, we are currently in the process of confirming this data.

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P02.37A

Recurrent substitutions in the PAX6 paired domain are associated with severe microphthalmia, coloboma and related ocular defects

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Haploinsufficiency of *PAX6* causes a pan-ocular disease termed classical aniridia (absent iris associated with foveal hypoplasia and keratopathy). Missense mutations in *PAX6*

have been recognized as a cause of milder or more focal phenotypes such as partial aniridia, Peters anomaly, isolated foveal hypoplasia and optic nerve hypoplasia. Here we show a distinct class of *PAX6* missense mutations that are associated with the structural ocular phenotypes of severe microphthalmia and coloboma. These recurrent substitutions of specific residues are primarily in the PAX6 paired domain alpha helices I and III of the N-terminus subdomain and helix VI of the C-terminus subdomain: the critical residues are Arg26, Gly51, Ser54 and Asn124, together with Arg38 in the linker region between alpha helices I and II.

In an attempt to understand the molecular mechanisms underlying these exceptional cases we have used quantitative phenotypic analysis of the differences between >150 individuals with loss-of-function mutations compared to a matched number with missense variants. This has allowed us to create rigorous phenotypic classes to inform the structural modeling of the PAX6 paired domain and the interpretation of the consequence of substitutions of these critical residues.

We estimate that this subset of *PAX6* missense mutations account for ~2% of severe eye malformations. It seems likely that a proportion of these missense mutations result in a "worse than null" effect via a dominant negative mechanisim related to altered DNA-protein, or disturbance of protein-protein, interactions within the bound complex.

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P02.38B

Novel neuro-audiological findings and further evidence for *TWNK* involvement in Perrault syndrome

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Introduction: Hearing loss and ovarian dysfunction are key features of Perrault syndrome (PRLTS) but the clinical and pathophysiological features of hearing impairment in PRLTS individuals have not been addressed. Mutations in one of five different genes *HSD17B4*, *HARS2*, *LARS2*, *CLPP* or *TWNK* cause the autosomal recessive disorder but they are found only in about half of the patients. Materials and

Methods: We report two siblings with neurological type of PRLTS. Neuroimaging with volumetric measurements and objective measures of cochlear hair cell and auditory nerve function (otoacustic emissions and ABRs) were performed. Whole-exome sequencing was applied to identify the genetic cause of the disorder. Protein 3D structure modelling was used to predict the deleterious effects of the detected variants on protein function.

Results: We found two rare biallelic mutations in *TWNK* gene. Mutation c.1196A>G (p.Asn399Ser) recurred for the first time in a patient with PRLTS and the second mutation c.1802G>A (p.Arg601Gln) was novel for the disorder. In both patients neuroimaging studies showed diminished cervical enlargement of the spinal cord and for the first time in PRLTS partial atrophy of the vestibulocochlear nerves and decreased grey and increased white matter volumes of the cerebellum. Morphological changes in the auditory nerves, their desynchronized activity and partial cochlear dysfunction underlay the complex mechanism of hearing impairment in the patients.

Conclusions: Our study unveils novel features on the phenotypic landscape of PRLTS and provides further evidence that the newly identified for PRLTS *TWNK* gene is involved in its pathogenesis. Grants No.: 2011/03/D/NZ5/05592, 2014/15/B/ST6/05082 and 2013/09/B/NZ2/00121

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P02.40D

Clinical utility of *MYOC* predictive genetic testing for primary open-angle glaucoma

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Introduction: *MYOC* variants are associated with primary open-angle glaucoma (the most common subtype of glaucoma) in an autosomal dominant manner with high penetrance, and can lead to severe disease if left untreated. Early interventions can reduce irreversible vision loss due to glaucoma, emphasising the importance of identifying at-risk individuals. This study explored the clinical utility of predictive genetic testing for *MYOC*.

Material and Methods: *MYOC* variant carriers were identified through the Australian and New Zealand Registry of Advanced Glaucoma. Individuals were classified based on how they retrospectively presented to an ophthalmologist into Clinical cases (referred by their general practitioner or optometrist) and Genetic cases (referred following positive genetic results). Individuals were further classified according to the glaucoma severity at the time of their first examination into 4 groups (unaffected, glaucoma suspect, glaucoma, advanced glaucoma).

Results: 73 *MYOC* carriers were identified, comprising 43 Clinical and 30 Genetic cases. At presentation, 83% of Genetic cases were unaffected and 17% were glaucoma suspect whereas among Clinical cases, 44% were glaucoma suspect, 28% had glaucoma and 28% had advanced glaucoma. Genetic cases were significantly younger at presentation than Clinical cases (40.6 ± 12.5 vs. 47.5 ± 16.7 years, $p = 0.018$). All clinical parameters related to glaucoma were worse at presentation among Clinical cases compared with Genetic cases.

Conclusions: Our findings show that *MYOC* predictive genetic testing leads to early identification of at-risk individuals who can benefit from early interventions to prevent irreversible blindness. Grants: National Health and Medical Research Council (APP1023911).

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P02.41A

Targeted NGS analysis of 124 genes in 602 individuals with retinal dystrophies

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Introduction: Retinal dystrophy encompasses a range of diagnoses and modes of inheritance, and more than 250 genes have been associated so far. The molecular diagnostic strategy has previously depended on the specific diagnosis as well as laborious screening of individual genes.

Materials and Methods: We aimed to delineate the diagnostic outcome and mutation spectrum, by sequencing 124 genes in 602 adult individuals clinically diagnosed with retinal dystrophy. The individuals had one of the following diagnoses: autosomal recessive retinitis pigmentosa; autosomal dominant retinitis pigmentosa; X-linked retinitis pigmentosa; Leber's congenital amaurosis; Usher syndrome; Bardet-Biedl syndrome; cone- or cone-rod dystrophy; unspecified macular dystrophy or Stargardt disease; congenital stationary night blindness or age-related macular degeneration. All individuals gave written informed consent for genetic analysis. Variants were interpreted using an in-house system based on the ACMG 2015 guidelines and classified as class 1 (benign), class 2 (likely benign), class 3 (variants of unknown significance, VUS), class 4 (likely pathogenic) or class 5 (pathogenic).

Results: The results from 602 individuals identified a likely molecular genetic explanation of their retinal dystrophy in 344 (57%) individuals. Variants in nine genes (*ABCA4, EYS, USH2A, BEST1, RHO, RP1, RPGR, CRB1,*

PRPH2) can explain the retinal dystrophy in about half of the 344 individuals, rendering these as major disease genes.

Conclusion: These findings confirm that a targeted NGS panel analysis can provide molecular genetic diagnosis in a large part of individuals with retinal dystrophies and form the basis for a rational genetic diagnostic strategy.

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P02.42B

CNGA3-associated autosomal recessive achromatopsia caused by uniparental isodisomy of chromosome 2

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A single female patient with a clinical diagnosis of achromatopsia was referred to our laboratory for genetic research testing. Achromatopsia is a rare, genetically heterogeneous, autosomal recessive disorder characterized by color blindness, photophobia, nystagmus, and low visual acuity.

Sanger sequencing revealed an apparent homozygous missense mutation c.778G>C;p.D260H in *CNGA3*, predicted to be disease-causing, and affecting a conserved amino acid residue in transmembrane domain S3 of the CNGA3 polypeptide. Segregation analysis proved the father to be a heterozygous mutation carrier, while the mutation could not be displayed in the mother. A heterozygous deletion in the mother explaining the apparent homozygosity of the mutation in the patient was ruled out by long-distance and qPCR-based copy number analysis of exon 7. Genotyping of microsatellite markers revealed homozygosity for all tested markers on chromosome 2 in the patient for the paternal haplotype, indicative for paternal uniparental isodisomy (UPD) of chromosome 2.

UPD is a rare phenomenon due to inheritance of both chromatids of a single chromosome from one parent - here the paternal chromosome - while the maternal chromosome is lost. UPD thus can uncover a recessive mutation present in just one parent, here the missense mutation c.778G>C;p.D260H in *CNGA3* in the father, causing ACHM in the index patient. It is one of very few examples for this mechanism observed and described in inherited retinal dystrophy, and the first for achromatopsia. The detection of UPD highlights the importance of segregation analysis, and has important implications for counseling and recurrency risk assessment in this family.

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P02.43C

Relevance of X-linked and autosomal dominant inheritance patterns in simplex Retinitis Pigmentosa cases

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Introduction: Retinitis Pigmentosa (RP) is a group of inherited retinal dystrophies (IRD) in which the photoreceptors and retinal pigment epithelium cells degenerate leading to progressive visual loss. With a great clinical and genetic heterogeneity, RP can be inherited in autosomal dominant (ad), autosomal recessive (ar), or X-linked (xl) trait. Nevertheless, a major proportion of patients (41% in Spain) represent simplex cases (sRP) in which the pattern of inheritance is *a priori* unknown. Traditionally, sRP patients have been considered recessive cases; however a precise inheritance pattern can only be assigned via further diagnostic and analytical efforts.

Materials and Methods: We performed a comprehensive genetic study of 106 Spanish patients with sRP using a high-throughput targeted sequencing panel of 68 IRD-genes. Sanger sequencing and MLPA was conducted to validate and to segregate candidate variants in available family members.

Results: Application of our data analysis pipeline allowed the identification of 96 potential causative mutations in 66 patients (62.26%), of which 37.8% are non-arRP cases (24.2% adRP and 13.6% xlRP). The most frequent mutated gene for adRP was *PRPF31* and for xlRP, *RPGR*.

Conclusions: These findings represent a significant advance in understanding the

genetic basis of sRP. This study highlights that, although most families harbour mutations in arRP genes, an unexpectedly high proportion of cases harbour mutations in adRP or xlRP genes which implies consequences for counselling of patients and families.

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P02.44D

Targeted Next Generation Sequencing for non-syndromic hearing loss

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Hereditary sensorineural Non-Syndromic Hearing Loss (NSHL) is characterized by clinically indistinguishable phenotypes and genetic heterogeneity, that until recently have hampered effective early etiological diagnoses.

With the aim of developing advanced diagnostic tools to investigate the genetic bases of these conditions in a population of individuals, mainly of pediatric age, we designed a customized targeted NGS panel of 59 genes, strongly associated, in Caucasians, with NSHL or syndromes (i.e. Pendred and Usher) with onset as seemingly isolated deafness.

Ion Torrent PGM™ platform and bioinformatic data analysis pipeline have been used for the analysis of DNA samples collected from clinically selected *GJB2* negative patients. Our panel has an average depth coverage of 235X and a mean of 506 variants/subject.

48 cases have been completed, with a diagnostic yield of 20/48 (42%). We have identified 18 novel mutations in the *CDH23, MYO7A, COCH, EYA4, PTPRQ, TMPRSS3, GJB3, SLC17A8, TPRN, OSBPL2, ACTG1, GRHL2, MYO15A* genes. The novel *EYA4* mutation, identified in two related subjects with post-lingual progressive deafness was also found to co-segregate in the same family with a new *PAX3* gene mutation in two members with Waardenburg syndrome phenotype. *CDH23* resulted the most frequently mutated gene in our series (20% positive cases).

The high diagnostic rate demonstrated by the developed gene panel, is extremely promising for our analysis, which is currently being extended to other 50 highly selected cases, and confirms the power of this NGS approach in combination with appropriate clinical information, to reach precise etiological diagnoses and provide appropriate counseling.

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P02.45A

SLC6A6 taurine transporter: a novel autosomal recessive candidate gene for progressive visual impairment

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SLC6A6 (OMIM#186854) encodes a known taurine transporter, whose knockout in a reported mouse model is associated with progressive retinal degeneration at three months old and very low taurine levels in blood plasma and other tissues. Taurine plays a key role in retinal development, and taurine deficiency in rats and cats leads to retinal degeneration as one of the main pathological features. To date, *SLC6A6* pathogenic variants have not been linked to any pathology in humans.

Autosomal recessive genetic disorders, including visual impairment, are more common in consanguineous populations. By exome analysis and genotyping data within a consanguineous Pakistani family, with two children suffering from progressive visual loss, we have identified a recessively segregating likely pathogenic homozygous missense variant (NM_003043:c.1196G>T:p.(Gly399Val))

in the 8th transmembrane domain of *SLC6A6*. The Gly399 residue is very well conserved down to zebrafish. Interestingly, the fasting blood taurine levels in all tested family members correspond to the genotypes of the identified variant: the two affected children 'homozygous mutants' have very low blood taurine levels (6 and 7 μmol/l), while their parents 'heterozygous' have intermediate levels (24 and 34 μmol/l), and an unaffected sibling 'homozygous wild-type' has normal levels (71 μmol/l) (normal values: 37-127 μmol/l). Further studies to characterize the mutation and to assess its impact on taurine transport, as well as thorough clinical evaluation of the patients, are currently in progress.

In summary, *SLC6A6* is a promising novel candidate gene for progressive visual loss in humans and provides a potential target for nutritional therapeutic intervention.

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P02.46B

GWAS of smell recognition identifies rare variants underlying numerous cell processes

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Aim: Olfaction plays an important role in daily functioning, influencing dietary habits, safety, emotions and overall well-being, while its' impairment was implicated in several neurological diseases. The aim of this study was to investigate the genetic background of smell recognition.

Materials and methods: 1,966 subjects from two settlements from the Island of Korcula, Croatia, within 10,001 Dalmatians cohort, were involved in the smell recognition measurement using Sniffin' Sticks with 12 scented probes. Subjects were given four options to choose from for each of the scent, resulting in the percent of correct responses, which were used in the HRC imputed GWAS, controlling for the effects of age, gender, smoking and kinship. Lastly, a fixed effect meta-analysis was performed, reflecting separate genetic structures of the two sub-sets. Bonferroni corrected threshold of 2.5E-9 was applied.

Results: Smell recognition was strongly associated with 14 rare intron variants (MAF≤1.3%) involved in various cell functions, differentiation, structure and chemical sensation. The most significant SNP was rs181890080 (p=2.14E-11, within STK33 gene) associated with sweet taste signaling. There were additional 39 SNPs under the significance threshold (p<8.8E-8), and the most relevant gene was NTN4 responsible for neurite elongation from olfactory bulb explants.

Conclusion: This study confirms the great complexity of the genetic basis of smell recognition, having identified genes from chemical sensation, but also various other cell functions that were not previously implied for this trait. Further refinement of these results in increased sample sizes is under way.

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P02.47C

Antisense oligonucleotide-based splice correction of two neighboring deep-intronic *ABCA4* mutations causing Stargardt disease

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Introduction: Stargardt disease (STGD1) is one of the most frequent autosomal recessive retinal dystrophies. STGD1 can be caused by over 600 mutations in *ABCA4*, which explain 70% of the cases. We and others hypothesize that the remaining 30% can be largely explained by non-coding mutations of the *ABCA4* locus. Recently, we identified two neighboring, deep-intronic mutations in intron 30 of *ABCA4* that create a cryptic splice donor site, leading to the inclusion of a 68-bp pseudo-exon.

Materials and methods: We designed 2′-O-methyl phosphorothioate antisense oligonucleotides (AONs) targeting the cryptic donor site in intron 30. AONs were tested using minigene assays, by co-transfection of AONs and minigenes containing exon 30 to 31 of *ABCA4* in HEK293T cells, subsequent RNA-isolation and RT-PCR analysis. Patients' fibroblasts were tested for the presence of the pseudo-exon.

Results: Both deep-intronic mutations activate a cryptic donor site, resulting in the use of a nearby strong acceptor site and the inclusion of a 68-bp pseudo-exon in the *ABCA4* mRNA. AONs directed against SC35 motifs in this pseudo-exon prevent insertion of this exon by blocking the binding of splicing factors. A nearly full restoration of normal splicing was observed in a dose-dependent manner. The 68-bp pseudo-exon was confirmed on patients' fibroblasts. Testing of AONs on patients' fibroblasts is ongoing.

Conclusions: The designed AONs induced skipping of the aberrant pseudo-exon and resulted in restoration of normal splicing events of *ABCA4* minigenes. Overall, we demonstrated the efficacy of AON-based splice correction of two different, neighboring deep-intronic mutations, using the same AON.

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P02.48D

Next Generation Sequencing of the complete *COL2A1* gene and Association Studies in cases of Rhegmatogenous Retinal Detachment

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Retinal detachment (RD) is a serious sight threatening disorder with approximately 10,000 cases in the UK annually. Stickler syndrome is the commonest genetic cause of RD with the type 1 sub-group (mutations in *COL2A1*) at particularly high risk but other loci include *COL9A1*, *COL9A2*, *COL9A3* and *COL11A1*. Interestingly, mutations in *COL2A1* have been identified in dominantly inherited RD, while the SNP rs1635532 has been found to affect alternative splicing efficiency of exon 2 in *COL2A1*, possibly conferring an increased risk of RD. Next generation sequencing (NGS) was used to identify rare and unique variants in *COL2A1*, in a subset of cases with either a giant retinal tear, bilateral RD or have a family history of RD. Twenty-seven rare and four unique variants were found, with six rare variants appearing in three separate samples. A further 6 cases and 2 controls, from our case/control cohort, were heterozygous for the variant, c.2356-62 C>T. Ninety-two SNPs across five collagen genes (*COL2A1*, *COL9A1*, *COL9A2*, *COL9A3* and *COL11A1*) were genotyped using the Sequenom iPLEX assay, including four SNPs in high linkage disequilibrium with five rare variants discovered from NGS. Three SNPs were significantly associated with incidence of RD, rs1635547 (p=0.04747), rs1623332 (p=0.0369) and rs1973953 (p=0.0270). Taqman genotyping assay confirmed the significant associations with rs1635532 and rs1793953 (p=0.04773 and p=0.02822 respectively). As rs1635532 has previously been found to affect the alternative splicing efficiency of exon 2 in *COL2A1*, these results further strengthen its involvement as a risk factor for RD.

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P02.49A

Comprehensive study of the STRC gene for the diagnosis of autosomal recessive deafness-16 (DFNB16) non-syndromic hearing loss

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Introduction: DFNB16, caused by biallelic variants in STRC, has recently been described as a frequent cause of non-syndromic hearing loss (NSHL), but the existence of a pseudogene (pSTRC) with 99.6% coding sequence identity and the frequency of large deletions in STRC (CNV) make the study of this gene a challenge. We present a combined approach for the comprehensive study of STRC.

Materials/methods: 69 NSHL patients negative for the screening of GJB2/GJB6 and mitochondrial m.1555 and m.1494 mutations were studied. STRC-specific digital-droplet PCR (ddPCR) excluding pseudogene (Mandelker et al, 2014) was used to detect CNVs. Exome sequencing by TruSight-One NGS panel (Illumina) was performed to detect point mutations in STRC and variants were confirmed by Sanger sequencing after a STRC-specific LR-PCR (Vona et al, 2015), in order to prevent false-positive results derived from pseudogene contamination. In addition, 228 normal-hearing individuals where studied to estimate the general population frequency of STRC deletion carriers.

Results: 4 patients showed homozygous deletion of STRC and 4 presented heterozygous deletion. From the latter, 2 presented also a hemizygous single nucleotide variant, being one pathogenic and the other uncertain but

predicted deleterious by bioinformatic tools. Two heterozygous deletion carriers where found in the normal population (0.88%).

Conclusions: 5 individuals (7.2%) previously negative for GJB2/GJB6 and m.1555/m.1494 screening were diagnosed using this approach, increasing the diagnostic rate from 17% to 23%. This data and the frequency of the deletion carriers in general population underline the convenience of including the comprehensive study of STRC in the screening of NSHL.

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P02.50B

Repair of the prevalent c.2299delG mutation in the USH2A gene using CRISPR/Cas9 nucleases

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Introduction: Usher syndrome (USH) is a rare autosomal recessive disease combining sensorineural hearing loss and retinitis pigmentosa (RP). Up to 13 genes are associated with this disorder, although *USH2A* presents a higher prevalence, partially owing to the c.2299delG mutation recurrence. Hearing aids or cochlear implants are used by patients to palliate the hearing impairment. But, up to date, there are no treatments for RP.

Objetive: To repair the c.2299delG mutation by gene editing.

Methods: We use the CRISPR/Cas9 system targeting c.2299delG on fibroblasts from an USH patient carrying the mutation in homozygosis.

Results: We show successful *in vitro* mutation repair when using *locus* specific RNA-Cas9 ribonucleoproteins with subsequent homologous recombination repair induced by engineered simple strand oligonucleotides (ssODNs) template supply.

Conclusions: The proven effectiveness of this correction tool, applied to the c.2299delG pathogenic variant of *USH2A*, discloses the CRISPR/Cas9 system future treatment prospects for Usher syndrome. Financial Support: Fundación Telemaratón "Todos somos raros". Fondo de Investigaciones Sanitarias (FIS): proyecto PI13/0068. CFG is recipient of a fellowship from the FIS:IFI14/00021

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P02.52D

A novel variant in NLRP12 gene in familial Meniere's disease suggests NLR mediated inflammation in autoinflammatory inner ear disease

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Introduction: Meniere's disease (MD) is a spectrum of rare disorders characterized by recurrent vertigo attacks, low frequency sensorineural hearing loss and tinnitus, with a strong familial aggregation. We have performed whole-genome sequencing (WGS) analysis in a family with autosomal dominant MD and autoimmune background.

Materials and Methods: WGS data were processed in a HiSeqX platform. Scripts developed in house at Luxembourg Centre for Systems Biomedicine based in Annovar were applied to annotate genetic variants with 87 databases, including allele frequency, protein stability, or regulation sites. We filtered SNVs by high-quality and MAF &lt 0.001 from 1000 Genomes Project, EVS, ExAC and gnomAD. We also filtered against a Spanish-database with 790 unrelated individuals to exclude polymorphisms and local variations. Then we prioritized according different pathogenic scores including combined scores as CADD, Revel and FATHMM. All variants were validated by Sanger sequencing.

Results: After filtering and prioritizing, we have identified two novel heterozygous variants in the VHL and NLRP12 genes. Linkage analysis and clinical data discarded VHL as causal gene in this family. We also showed the expression of NLRP12 in the human inner ear tissue. Since NLRP12 negatively regulates T cell responses, we suggest that NLRP12 may cause autoinflammatory inner ear disease in this family.

Conclusion: Our results support that mutations in NLPR12 may cause autoinflammatory inner ear disease in patients with MD and autoimmune background.

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P03 Internal organs & endocrinology (lung, kidney, liver, gastrointestinal)

P03.01A

17q24.3 Duplication In A Patient Presenting With SRY-Negative 46,XX Disorders of Sex Development

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Introduction: Disorders of sex development (DSD) are a group of disorders affecting gonad and/or genitourinary tract development. The underlying genetic causes of 46,XX *SRY*-negative ovotesticular DSDs are poorly understood. Recently, 46,XX DSD patients with large duplications upstream of *SOX9* have been described. A region located upstream of *SOX9* was assumed to contain gonad specific enhancer(s), gain or loss of which results in activation or inactivation of gonadal *SOX9* expression, respectively.

Materials and Methods: We here report on clinical and molecular findings of a 19-month-old patient with ambiguous genitalia. Karyotype, SRY deletion and array CGH analyses were performed.

Results: The patient was the product of a non-consanguineous marriage and was born at term with a birthweight of 3100 g. Developmental milestones and growth parameters of the patient were normal. Physical examination revealed bilaterally palpable gonads in labioscrotal folds, a phallus of 4 cm in length, a severe chordee and a single urogenital sinus opening. The patient was 46, XX and SRY was negative. Array CGH analysis using Agilent SurePrint® G3 CGH 8×60K Human Microarray Kit revealed Arr[hg19]17q24.3(69151003-69970418)X3, a duplication of 819 kb located upstream of *SOX9* on chromosome 17q24.3.

Conclusion: This report adds literature a new case of SRY-negative 46,XX DSD with a duplication located upstream of *SOX9*. Identification of new CNVs and delineation of the associated phenotypes in detail will help us understand better the mechanisms involved in sexual development and uncover the genetic defects underlying DSDs.

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P03.02B

Molecular karyotyping in ten patients with isolated anorectal malformation

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Introduction: Anorectal malformations (ARM) are classified in the group of multifactorial diseases. Because of the complexity of the pathological changes, etiology of ARM is still not clear. In the present study, we aimed to investigate the molecular cytogenetic factors in ARM etiology.

Materials and Methods: Ten ARM patients with normal karyotype and not associated with known genetic syndrome were included in this study. Array-CGH analysis was performed using the Agilent 60K ISCA Array platform.

Results: Chromosome 22q11.2 deletions were detected in two patients. One of this patients had anal stenosis, minor cardiac abnormalities, mild dysmorphic features, and a small 0.89-Mb deletion. The second patient had anal atresia, immune deficiency, inguinal hernia and 2.7-Mb cryptic deletion. Overlapping genes in the deletion regions of two patients are DGCR5, DGCR6 and PRODH. Of the remaining 8 patients, two had variant of unknown significance and six had benign CNVs.

Conclusions: To our knowledge, this region is one of the smallest interstitial deletion on chromosome 22q11.2 region which has been published up to now. DGCR5, DGCR6, PRODH genes may be candidate for one of the etiologic factors of the ARM. This view is also supported by the existence of ARM patients with 22q11.2 deletion published earlier. Especially DGCR6 gene which is a crucial gene for neural crest cell migration, could be responsible of anal atresia. We believe that further research on this gene will clarify this point.

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P03.03C

Sequence variant at 4q25 near *PITX2* associates with appendicitis

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Introduction: Appendicitis is one of the most common conditions requiring acute surgery and can pose a threat to the lives of affected individuals.

Materials and methods: We performed a genome-wide association study of appendicitis in 7,276 Icelandic and 1,139 Dutch cases and large groups of controls.

Results: In a combined analysis of the Icelandic and Dutch data, we detected a single signal represented by an intergenic variant rs2129979 [G] close to the gene *PITX2* associating with increased risk of appendicitis (OR = 1.15, $P = 1.8\times10^{-11}$). The association can only be observed in patients diagnosed in adulthood. The marker is very close to, but distinct from, a set of markers reported to associate with atrial fibrillation, which have been linked to *PITX2*.

Conclusion: *PITX2* has been implicated in determination of right-left symmetry during development. Anomalies in organ arrangement have been linked to increased prevalence of gastrointestinal and intra-abdominal complications, which may explain the effect of rs2129979 on appendicitis risk.

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P03.04D

Complex genetic analysis approach in selected patients with congenital anomalies of the kidney and urinary tract

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) are the commonest cause of chronic kidney disease in children. Defects can be unilateral or bilateral, and in many cases different anomalies coexist in one individual. Some forms are associated with positive family history which indicates the genetic component in the pathogenesis. The development of CAKUT is a complex process where the interactions of many factors are involved.

Materials and methods: In the present study 71 patients from 56 families were included. All patients were screened for mutations in HNF1B, PAX2, SALL1, SIX1 and EYA1 genes via Sanger sequencing. MLPA was used for detection of possible deletions/duplications in HNF1B. Cases, in which no genetic variants were identified during the initial screening were subjected to aCGH and NGS assays in order to extend the targeting of genetic defects, responsible for the pathology.

Results: During the initial screen, point mutations in HNF1B, SALL1 and PAX2, as well as deletion of the entire HNF1B gene were detected. The higher throughput approaches (aCGH and NGS) allowed us to identify a low level mosaic aneuploidy, large deletions as well as oligogenic inheritance.

Conclusions: Application of high throughput techniques, such as aCGH and NGS, could contribute to understanding the molecular causes in cases with composite clinical phenotype. The genetic screening even with extended genetic panels cannot isolate the molecular pathology in every CAKUT patient. For those cases an implementation of exome and transcritptome analysis may be required.Funding: MU-43/2009, DMU03/73-2011, 18-D/2014, 17-D/2015; DUNK01-2/2009

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P03.06B

NGS-panel consisting of genes with cholestatic potential identified pathogenic and predisposing variants in 10 of 33 patients with unexplained cholestatic liver disease

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Introduction: Variants in genes involved in bile transport and formation may be an important disease-related factor in cholestatic liver disease. In a clinical setting we examined this impact by testing a panel of 8 genes (*ATP8B1, ABCB11, ABCB4, JAG1, NOTCH2, ABCC2, ABCG5* and *UGT1A1*) known to cause cholestatic disease in patients with chronic unexplained cholestasis after standard diagnostic examination.

Materials and Methods: From 2011-2015 33 patients with unexplained cholestasis were included. Sequence variations in the coding regions of the 8 genes and the promoter region of *UGT1A1* were examined by using targeted Next Generation Sequencing of DNA extracted from whole blood. In close collaboration clinical geneticists and hepatologists assessed the possible causal relationship of genetic variants after state-of-the-art clinical genetic work-up.

Results: Variants considered a major factor or of predisposing importance to the cholestatic condition were discovered in 10 of 33 cases (30%). To state this association evaluation of family history was often necessary. In women with prolonged symptoms after intrahepatic cholestasis during pregnancy a genetic cause or predisposition was identified in 8 of 13 (62%).

Conclusion: The gene panel provided an effective tool to investigate unexplained cholestatic liver disease and suggests that in at least 30% of the cases genetic variants are involved in the pathogenesis. Larger cohorts should be examined in the future to justify genetic testing and the panel should be expanded. Classical clinical genetic work-up and hepatological expertise combined are necessary to interpret results.

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P03.07C

Development of the most comprehensive genetic test based on next generation sequencing for diagnosis of congenital hypothyroidism

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Congenital hypothyroidism (CH) is the most common neonatal metabolic disorder and, if untreated, results in severe neuro-developmental impairment and infertility. Genetic diagnosis not only allows definite diagnosis of the disease but also reveals the pathogenic mechanism by which CH develops. CH is genetically heterogeneous and to date 11 causative genes have been identified for the development of the disease. Next generation sequencing (NGS) technology enables sequencing of may genes at the same time in many patients at a reasonable cost. Thus, we aimed to develop a new method for efficient genetic diagnosis of CH based on NGS. To achieve this aim we carried out following work on DNA samples of 100 CH cases born especially to consanguineous families: (i) determination of the known-genetic CH locuses by linkage analysis; (ii) detection of mutations in patients linked to a known CH gene by conventional sequencing; (iii) mutation screening in patients with no linkage by using NGS. By this approach we were able to detect mutations in 73% of the cases, doubling the pick-up rate with conventional methods. The determinations of causative genes and mutations in this study contributed to the diagnosis and classification of CH and enhanced understanding the thyroid physiology as well as underlying molecular mechanisms leading to the development of CH.

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P03.08D

COPA syndrome in an Icelandic family caused by a recurrent missense mutation in *COPA*

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Introduction: Rare missense mutations in the *COPA* gene have recently been established as a cause of autoimmune interstitial lung, joint and kidney disease, also known as COPA syndrome, under dominant mode of inheritance. Here we describe an Icelandic family with three affected individuals over two generations (index, her son and daughter) with a rare clinical presentation of lung and joint disease and a histological diagnosis of follicular bronchiolitis.

Materials and Methods: We performed whole-genome sequencing of the three affected as well as three unaffected members of the family, and searched for rare genotypes associated with disease using 30,067 sequenced Icelanders as a reference population. We assessed all coding and splicing variants, prioritizing variants in genes known to cause interstitial lung disease.

Results: We detected a heterozygous missense mutation, p.Glu241Lys, in the *COPA* gene, private to the affected family members. The mutation occurred *de novo* in the mother and was absent from 30,067 Icelandic genomes and 141,353 individuals from the genome Aggregation Database (gnomAD). The mutation occurs within the conserved and functionally important WD40 domain of the COPA protein.

Conclusions: This is the second report to present the p. Glu241Lys mutation in *COPA*, indicating the recurrent nature of the mutation. The mutation was reported to co-segregate with COPA syndrome in a large family from the USA with five affected individuals, and classified as pathogenic. The two separate occurrences of the p. Glu241Lys mutation in cases and its absence from a large sequencing set confirms its role in the pathogenesis of the COPA syndrome.

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P03.09A

Multi-omics approach to assess genetic susceptibility of COPD in never-smokers

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Introduction: Although a striking proportion (25-45%) of patients with chronic obstructive pulmonary disease (COPD) are never-smokers, most genetic susceptibility studies have not investigated this group exclusively. We therefore performed a Genome-Wide Association Study (GWAS) on COPD in 5,070 never-smokers of the LifeLines study and further assessed the identified genetic variants using a multi-omics approach.

Methods: COPD was defined as the ratio of Forced expiratory volume in one second over Forced vital capacity (FEV_1/FEV) <70%. The GWAS was adjusted for sex, age and height. SNPs with a p-value<10^{-5} were validated in a meta-analysis of never-smokers from the Vlagtwedde-Vlaardingen study and Rotterdam Studies I-III (n = 1,966). Subsequently, we performed quantitative trait loci (QTL) analyses for expression in lung tissue (*cis* eQTL, n = 1,087) and for blood DNA methylation (meQTL, n = 1,561) within the LifeLines study.

Results: Four SNPs were associated with COPD in never-smokers (p<10^{-5}). Two SNPs were suggestively replicated: rs7519348 (*NFYC*, p=0.08) and rs6913003 (*FABP7*, p=0.07), with the same direction of effect in all cohorts. Further, rs7519348-A was associated with higher expression of *NFYC-AS1* and a meQTL for 20 CpGs (12 showed lower methylated). The *FABP7* SNP was not an eQTL in lung tissue, but was associated with lower methylation of 2 CpGs. *NYFC* is a transcription factor that binds promoters of genes previously associated with airway obstruction, and *FABP7* may be involved in abnormal pulmonary development.

Conclusions: The genes *FABP7* and *NFYC(-AS1)* could play a role in the pathogenesis of airway obstruction in never-smokers through epigenetic regulatory mechanisms. **Support:** Longfonds grant 4.1.13.007 (Netherlands).

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P03.10B

Options for CRISPR/Cas9 editing of the *CFTR* p.F508del in cystic fibrosis

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Cystic fibrosis is one of the commonest monogenic diseases all over the world. Worldwide the most frequent mutation causing CF is *CFTR* p.F508del - deletion of phenylalanine in the ion channel protein. This deletion results in abnormal folding and degradation of the protein immediately after its synthesis. One of the most promising therapeutic approaches for monogenic diseases is gene therapy. CRISPR/Cas9 provides best opportunities to edit mutations directly in the host genome. We develop CRISPR/Cas9 based correction of *CFTR* p.F508del. One of the main drawbacks of genome editing in vivo is unnecessary editing of the normal allele. This can be overcome by designing sgRNA targeting mutated allele only. However, the obligatory PAM sequence limits choice of target sequences for Cas9/sgRNA complexes. We performed *CFTR* editing and compared four sgRNAs, two different Cas9 enzymes and two cell lines including CFTR29o- culture homozygous for p.F508del. We also analyzed possible reasons why sgRNA activity may depend on cell line and sgRNA sequence. Several sgRNAs in the region of *CFTR* p.F508del are active and induce indels by NHEJ after Cas9 editing, but not the sgRNA#1 targeting the mutated allele. Different model cells demonstrate dramatically different editing efficacy. Immune response which was initially hypothesized to attenuate activity turned out as unlikely to be responsible. Another hypothesis why sgRNA#1 may be inefficient include high AT content and, hence, overall low stability of the sgRNA#1-DNA interaction. Overall efficacy of CRISPR/Cas9-induced NHEJ depends greatly on cell type, genome locus and sgRNA sequence.

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P03.11C

SNP rs16857259 near gene CACNA1E predicts time to progression from uncomplicated to complicated form of Crohn's disease in Slovenian patients

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Introduction: Crohn's disease (CD) is usually diagnosed as uncomplicated form of the disease. Nevertheless, a majority of patients will progress to complicated stricturing and penetrating form during their lifetime. The aim of the present study was to identify DNA polymorphisms (SNPs) associated with time of the disease progression using a comprehensive association study.

Materials and Methods: 111 patients with uncomplicated form of the disease at the time of diagnosis were enrolled in this study and were followed-up for 10 years. This study was approved by the Regional Ethics Committee and written informed consent was obtained from all patients. DNA was extracted from peripheral blood mononuclear cells. Genotyping was performed using a custom made Illumina iCHIP. Data analysis was performed using PLINK v1.07 and IBM SPSS Statistics 22.0.

Results: Obtained results have shown that patients with CC (2 years) or AC (5.1 years) genotype at SNP rs16857259 near gene *CACNA1E* progressed to complicated form earlier as compared to patients with genotype AA (8.8 years) ($p=3.82\times10^{-7}$). Furthermore, suggestive association with disease progression ($p<10^{-5}$) was found for 9 additional SNPs near genes *RASGRP1*, *SULF2*, *XPO1*, *ZBTB44*, *HLA DOA/BRD2*, *HLA DRB1/HLA DQA1*, *PPARA*, *PUDP* and *KIAA1614*. Moreover, construction of genetic risk score (GRS) for associated SNPs with subsequent multiplicative cumulative GRS score for individual patient confirmed strong correlation with time to progression ($\rho=-0.719$; $p=2.95\times10^{-18}$).

Conclusions: Our study found strong association and correlation between locus on chromosome 1 near gene *CACNA1E* with time to progression from uncomplicated to complicated form of CD in Slovenian patients.

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P03.12D

Structure-based prediction of *CYP21A2* novel variants and a survey of gene variations

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency accounts for 90-95% of CAH cases. In this work we performed an extensive survey of mutations and SNPs modifying the coding sequence of the *CYP21A2* gene. Using bioinformatic tools and two plausible CYP21A2 structures as templates, we initially classified all known variants (343) according to their putative functional impacts, which were either reported in the literature or inferred from structural models. We then performed a detailed analysis on the subset of variants believed to exclusively impact protein stability. Using the FoldX software, we initially calculated the free energy difference between 30 mutants with experimental residual enzymatic activity (REA) reported and the wild-type counterparts and plotted against the natural logarithm of the REA. We obtained a correlation $R^2=0,79$ for the bovine model and $R^2=0,60$ for the human crystal. We validated our method by estimating the *in silico* REA of other 10 mutations with known experimental activities. A high concordance was obtained when comparing our predictions with *in vitro* REA and/or the patient's phenotype. The predicted stability and derived activity of all reported mutations and SNPs lacking functional assays ($n = 108$) were assessed. As expected, most of the SNPs (52/76) showed no biological implications. Moreover, this approach was applied to evaluate the putative synergy that could emerge when two mutations occurred *in cis*. In addition, we propose a putative pathogenic effect of five novel mutations: p.L107Q, p.L122R, p. R132H, p.P335L and p.H466fs, found in 21-hydroxylase deficient patients of our cohort.

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P03.13A

Investigation of androgen receptor (AR) gene mutation spectrum in the turkish patients with disorder of sex development

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Introduction: Androgen insensitivity syndrome (AIS) is an X-linked recessive condition resulting in a failure of normal masculinization of the external genitalia in chromosomally 46,XY individuals. This failure of virilization can be either complete androgen insensitivity syndrome (CAIS) or partial androgen insensitivity syndrome (PAIS), depending on the amount of residual receptor function. Mutations in the AR gene on chromosome Xq12, cause androgen insensitivity syndrome. In this study, it was aimed to investigate the mutation spectrum in Turkish patients who had AR mutation analysis with suspected gender development disorder and AR insensitivity syndrome.

Materials and Methods: The AR gene from the DNA material isolated from the peripheral blood of patients was amplified using appropriate primers and sequenced using the new generation sequence analysis technique on the Mi-Seq device.

Results: In this study, molecular analysis results of 383 individuals who underwent AR genetic analysis in Ege University Medical Genetics Department between 2011-2016 were evaluated retrospectively. There were 44 mutations in these cases. Of the 44 cases detected in the mutation, 16 were affected and the karyotypes were 46,XY. 28 of them are the 46, XX carrier mothers, carrier relatives or siblings of the affected cases.

Conclusion: New mutations detected in our studies between 2011-2016; L57Q, T576I, D691Y, P672R, Q739E, p.R544KfsX8, c.1745_1747delTCT, F726S, L881V, R102G, L863F. Different mutations can be detected in AR gene in Turkish society. In cases with disorder of sex development, AR should be examined.

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P03.14B

Improved diagnosis and management of patients with Disorders of Sex Development (DSDs) Using Next Generation Sequencing

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Disorders of sex development (DSDs) refer to a range of congenital disorders where the chromosomal, gonadal or anatomical sex is atypical. Genetic testing is key in establishing diagnosis, allowing for personalised patient management. Prior to Next Generation Sequencing (NGS), genetic tests were only available for a few DSD genes, which required sequential testing. Pinpointing the molecular cause of a patient's DSD can significantly impact patient management by informing future needs or altering management strategies.

Using the TruSight One technology to screen 30 DSD related genes (listed below), we have demonstrated a 32% pick-up rate in the first 75 patients tested. Pathogenic mutations in *AR, SRD5A2, HSD17B3, NR5A1, AMH, AMHR2, WT1, LHCGR & MAMLD1* were identified, some of which have resulted in changes to patient management. For example, we reported a girl with an *AR* mutation who was subsequently spared surgical intervention and identified a *WT1* mutation in a baby who was consequently referred for Wilms tumour screening. This data also highlights some

of the challenges around variant interpretation in DSD diagnostics such as incidental findings and mosaicism.

46, XY DSD		46, XX DSD	
Disorders of Testicular Development	Disorders of Hormone synthesis or action	Disorders of Ovarian Development	Androgen Excess
WT1 (11p13)	DHCR7 (11q12-q13)	SRY (Yp11.3)	HSD3B2 (1p13)
CBX2 (17q25)	LHCGR (2p21)	SOX9 (17q24)	POR (7q11.2)
NR5A1/SF1 (9q33)	StAR (8p11.2)	RSPO1 (1p34.3)	CYP11B1 (8q21-q22)
SRY (YP11.3)	CYP11A1 (15q23-24)	WNT4 (1p35)	CYP19A1 (15q21)
SOX9 (17q24-q25)	HSD3B2 (1p13.1)		Glucocorticoid receptor (5q31)
DHH (12q13.1)	CYP17A1 (10q24.3)	**Table listing the genes covered by our panel**.	
ARX (Xp22.13)	POR (7q11.2)		
TSPYL1 (6q22-23)	CYB5A (18q23)		
MAMLD1 (Xq28)	HSD17B3 (9q22)		
DMRT1 (9p24.3)	SRD5A2 (2p23)		
ATRX (Xq13.3)	AR (Xq11-q12)		
NR0B1/ DAX1 (Xp21.3)	AMH (19p13.3-p13.2)		
WNT4 (1p35)	AMHR2 (12q13)		

We have demonstrated that an NGS strategy can improve molecular diagnosis of DSDs, resulting in quicker and cheaper tests as well as a more accurate prediction of recurrence risk. For complex multigenic disorders such as DSD, identifying the molecular cause is crucial for effective management and counselling.

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P03.15C

Molecular diagnostics of familial intrahepatic cholestasis by targeted-NGS

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Introduction: Familial intrahepatic cholestasis (FIC) refers to a group of rare autosomal recessive diseases present in infancy or childhood. Mutations in *ATP8B1*, *ABCB11*, *ABCB4* and *TJP2* genes are associated respectively to FIC1, 2, 3 and 4 forms. To improve the diagnostics of FIC, we developed and validated a targeted-NGS to test these genes simultaneously.

Materials and Methods: We studied 13 FIC1-2, 73 FIC3 and 14 unclassified patients. The amplicon-based NGS was developed by Ion Torrent PGM, data analysis was performed by Variant Caller and predictions were made on SIFT, PolyPhen-2 and Human Splicing Finder. Validations were performed by Sanger sequencing.

Results: We identified 13 likely pathogenic variants, 11 variants of uncertain significance and 1 splicing variant: 3 in *ATP8B1*, 8 in *ABCB11*, 10 in *ABCB4* and 4 in *TJP2* genes. In *ATP8B1* the novel P23L was in compound heterozygosis with a known mutation. In *ABCB11* we identified the novel variants Y93S, V597L and S1100Qfs*38 and in *ABCB4* the novel Y403C, L672* and 2925-2A>G. In *TJP2*, only the A287V novel variant was detected. 4 among 13 FIC1-2 patients showed defects in *ATP8B1* or *ABCB11* genes. Only 19 among 73 FIC3 patients had alterations in *ABCB4* gene, while 7 cases showed variants in *ABCB11* or *TJP2*. 39 benign polymorphisms were also detected.

Conclusions: Our analysis yielded a low detection rate (25%), confirming the great heterogeneity of FIC phenotypes. Additional genes and/or a non-Mendelian transmission may contribute to the disease, making up a predisposing genetic profile.

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P03.16D

Genomic profiling in apparently sporadic renal disorders and secondary Focal Segmental Glomerulosclerosis

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Focal segmental glomerulosclerosis (FSGS) is a morphologic pattern of glomerular injury directed at the glomerular visceral epithelial cell, podocyte, and defined by the presence of sclerosis involving parts (segmental) or some (focal) glomeruli, identified by a renal biopsy. FSGS is characterized by nephrotic syndrome and it is more frequent in children and adolescents. About 30-50% of affected individuals don't respond to steroid therapy, leading to end-stage renal disease. So far, our understanding of FSGS pathogenesis derived only from instrumental studies, focused to identifying structural alterations of the slit diaphragm and the actin cytoskeleton of podocytes, considered critical to maintaining glomerular function. Up to now, literature data agree on the genetic heterogeneity of the FSGS, indicating monogenic disorders (e.g.SRNS) as crucial of this condition.We analyzed the whole-exome sequencing of 50 individuals diagnosed, by biopsies, for early onset "primary" FSGS. In more than 50% of cases we identified for each sample two or more candidate pathogenetic variants not only in podocyte genes, but surprisingly also in different genes involved in kidney morphogenesis. Taken together, the pathogenic variants explain the patient's clinical picture suggesting that digenic/polygenic inheritance, due to variants transmitted in various combination from each healthy parent, is underlying the renal disorder that determines the FSGS development. The knowledge of FSGS molecular basis can contribute to the refinement of focused clinical management and could be used to counsel patients and/or parents regarding prognosis.

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P03.17A

Incidence of GANAB in polycystic kidney disease

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Autosomal Dominant Polycystic Kydney Disease(ADPKD) is a genetically heterogeneous disease with two genes PKD1 and PKD2 responsible of respectively 75% and 15% of ADPKD patients .Recently a new gene,GANAB has been reported to be involved in polycystic diseases(Porath et al, Am J.Hum Genet 2016) .For many years we have completely analyzed a large cohort of more than 3000 ADPKD patients and performed a genotype/phenotype correlations (Audrezet et al, Hum Mut,2012 ;Cornec-Le Gall et al, J.Am Soc Nephrol, 2013) in this disorder. In about 10% of our cohort, no mutation were found either in PKD1 or PKD2.Here we sequenced the coding sequence of the gene in a large cohort (291 patients) of non PKD1 non PKD2 mutated patients . Results : We identified 4 families with a deleterious mutation in GANAB .The following mutations were found :c.2723del ;c.39-1G/C ;c.334 C/T ; c.2176 C/T). A genotype/phenotype correlation was performed and we showed that the patients with a GANAB mutation display both a renal and a liver polycystic disease . Most of the time the size of the kidney is not increased and these patients display a mild form of the disease . Conclusion : GANAB is a new gene associated with an autosomal dominant transmission of poly cystic kidney and liver disease .The incidence of the disease is rare with a frequency of 1.6/1000 of the ADPKD diseases .However the

molecular diagnosis of GANAB patients is important to include or not these patients in the future clinical trials .

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P03.18B

Creation and characterisation of induced pluripotent stem cells from Hirschsprung disease patients and their promise for future therapeutic strategies

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Introduction: Hirschsprung disease (HSCR) is a complex genetic disorder affecting the Enteric Nervous System and is characterised by a failure of enteric neural crest cells to fully colonise the developing gut tube. HSCR is currently treated by removal of the affected aganglionic tissue. Therefore, current therapies do not alleviate the underlying cause. This might be overcome by cell therapy, using an allogenic patient source. However the genetic variants that prevented normal development would still be present. HSCR is often the result of multiple genetic disturbances shifting the balance of a molecular network from normal to impaired. Correcting one major genetic variant could restore this balance.

Materials & Methods: Fibroblasts were cultured from skin biopsies taken at the time of corrective surgery of HSCR patients. Their DNA was exome screened and fibroblast lines with deleterious variation in HSCR disease genes were selected for creation of induced pluripotent stem cell (iPSC) lines. iPSC lines are established and proliferation, migration and differentiation to vagal neural crest lineage were assessed. Ongoing work concerns using CRISPR-Cas9 to correct the variants and restore impaired functions.

Results: Patient specific iPSC lines were induced from fibroblast samples with deleterious variants in *RET*, *ZEB2*, *PHOX2B* and *GFRA1*. Proliferation, migration and differentiation to vagal neural crest lineage were assessed.

Conclusions: Patient specific iPSC lines have been established, in order to study the balance of genetic involvement in HSCR. These cells are, to our knowledge, the first HSCR patient-derived iPSC lines containing deleterious variants for HSCR implicated genes.

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P03.19C

Missing heritability in Hirschsprung Disease: somatic mosaicism

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Introduction: Hirschsprung disease (HSCR) is a developmental neuropathy in which enteric ganglia are absent in the distal colon. This is the result of either impaired cell migration, proliferation or differentiation of enteric neural crest cells (ENCCs) and as a consequence, ENCCs fail to fully colonize the intestine. HSCR is considered a complex inherited disorder for which so far fifteen genes and six loci have been described. However, variants in these genes explain no more than 20% of the total disease-risk. Explaining this missing heritability proves difficult. One possible explanation is that somatic changes, in early development, contribute to HSCR genetics.

Materials & Methods: The presence of somatic changes was determined by comparing whole exome sequence (WES) data, determined in DNA isolated out of blood, fibroblasts and flow sorted ENCCs of five patients taken from intestinal biopsies just proximal to the aganglionic segment.

Results: We could detect putative deleterious inherited variation in known disease genes (*EDNRB*, *EDN3*, *GFRA1*, *NRG3*) in four out of five patients. Moreover, several putative somatic mutations (with a coverage above 30X and a variant quality above 300) in ENCCs were detected with WES. Currently, we are confirming these changes with conventional technologies. Moreover, we are evaluating the functional consequence of the variations in the known disease genes.

Conclusions: Using WES, we could detect putative somatic mosaicism which might point towards a role for somatic mutations in HSCR development.

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P03.20D

Whole genome sequencing implicates rare variants in sporadic Hirschsprung disease

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Sporadic Hirschsprung disease (HSCR), representing ~80% of the HSCR cases, is the most common form of the disorder and is believed to be genetically complex. Thus far, studies of rare mutations have discovered more than 10 genes (e.g. *RET*, *EDNRB* and *GDNF*) associated with the disease. The major HSCR gene, *RET*, have both rare coding mutations and common regulatory variants contributing to the disease. The differential contributions of these rare and common, coding and noncoding variants tend to vary with length of aganglionosis. In view of this, we performed a high coverage whole genome sequencing (~30×) of 11 trios of sporadic patients with the rarer subtype (long segment HSCR; L-HSCR), aiming to identify rare *de novo*, recessive and compound heterozygous mutations causal to HSCR. Our data show that the combined contribution of the *de novo* and inherited, both coding and non-coding, variants contribute to the development of ENS and thereby to HSCR. The discovery might shed light on pathways relevant to the etiology of HSCR. This work has been supported by HMRF grant 01121516 to MMGB.

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P03.21A

Genetics of fatty acid profile abnormalities in Inflammatory Bowel Diseases

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Introduction: Inflammatory bowel diseases (IBD) are defined by chronic and relapsing inflammation of the gastrointestinal tract. IBD is thought to arise from inappropriate immune responses to gut microbes in a genetically susceptible host. Fatty acid profile abnormalities have been associated with IBD independent of nutritional status or disease activity. However, no study so far has attempted to look for overlap between IBD and fatty acid associated loci or investigate the genetics of fatty acid profiles in IBD.

Materials and methods: Blood samples from 55 controls and 77 IBD patients were fractionated to lymphocyte and erythrocyte fractions. DNA was isolated from lymphocyte fraction. Genotyping was performed using iCHIP, a custom microarray platform designed for fine mapping of susceptibility loci in immune-related diseases. Lipids in erythrocyte fraction were processed and analyzed using gas chromatography with flame ionization detector to obtain fatty acid profile data. Genotype and fatty acid profile data was analyzed using R, PLINK and SPSS software packages.

Results: We identified 6 loci (near CBS, LRRK2, FRMPD4, TMCOA5, ZNF767P, ABCA12) significantly associated with fatty acids in IBD. Most significant loci is located near the CBS gene ($p = 7.62 \times 10^{-8}$). Of note, we also replicated the fatty acid associated *FADS* locus. Furthermore, we replicated previously observed changes in fatty acid profiles in IBD and inverse correlation between oleic and arachidonic acid levels.

Conclusions: Our results encourage further investigations of the genetics of fatty acid profile changes in IBD and the role of fatty acids in IBD pathogenesis.

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P03.23C

Mutations in *LAMB2* are associated with focal segmental gomerulosclerosis and septo- optic dysplasia

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Introduction: Mutations in *LAMB2*, the gene encoding laminin-β2, a multidomain protein are associated with Pierson Syndrome, an autosomal recessive disorder

characterized by congenital nephrotic syndrome, ocular abnormalities including microcoria and neurodevelopmental delay. Very few patients survive to adolescence, with only 3 reported to date.

Materials and Methods: We present an 11 years old male born to a non-consanguineous pedigree, who presented at 5 years of age with steroid resistant nephrotic syndrome and focal segmental glomerulosclerosis together with poor vision, growth hormone deficiency and seizures. MRI of his brain showed a global lack of white matter, a small anterior pituitary and bilateral hypoplastic optic nerves. Using whole exome sequencing, we identified compound heterozygous missense mutations in *LAMB2* [c.737G>A p. Arg246Gln, c.3982G>A p.Gly1328Ser]. Dual immunofluorescent histochemistry revealed reduced glomerular laminin-β2 expression compared to control biopsies [time zero renal transplant]. Interestingly, laminin β2 is expressed during murine anterior pituitary morphogenesis and analysis of murine *Lamb2* mutant pituitary morphogenesis is currently underway.

Discussion: Septo-Optic Dysplasia [SOD] is a heterogenous condition that is characterized by the presence of two or more of the following: hypopituitarism with isolated or combined hormone deficiencies, optic nerve hypoplasia and/or midline brain defects. Our case exhibited both isolated GH deficiency and optic nerve hypoplasia and raises the possibility that mutations in *LAMB2* may be associated with SOD-related phenotypes.

Conclusion: We propose that patients presenting with genetically undefined SOD should be screened for albuminuria.

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P03.24D

Biallelic variants in methionyl-tRNA synthetase associated with anemia, hypothyroidism, cholestasis, and interstitial lung disease

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Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed enzymes responsible for charging tRNA with cognate amino acids during protein translation. Non-canonical functions are becoming increasingly recognized, and include transcription and translation control and extracellular signaling. Mutations in various ARSs, with a predilection for the catalytic and C-terminal domains, lead to tissue-specific disorders. ARSs are mostly specific to either the cytoplasm or the mitochondria, with a few being bifunctional. Monoallelic mutations in cytoplasmic and bifunctional ARSs have been identified in axonal Charcot-Marie-Tooth (CMT2) disease (i.e., *AARS, YARS, GARS, KARS* for alanine-, tyrosine-, glycine-, and lysine- ARSs, respectively). Biallelic mutations in these genes have been associated with systemic disorders, variably involving the central nervous system, lung, and liver. We report a 6-month old male infant of non-consanguineous origin, presenting with successive onset of transfusion-dependent anemia, hypothyroidism, cholestasis, interstitial lung disease, and developmental delay. Liver biopsy demonstrated fibrosis, cholangiolar proliferation, macrovesicular steatosis and hemosiderosis. Bronchoalveolar lavage showed foamy, lipid-laden macrophages. Whole exome sequencing (WES) identified compound heterozygous variants (p.Y307C and p.R618C) in *MARS*, encoding methionyl-tRNA synthetase. Interestingly, the p.R618C variant, inherited from an unaffected father, was previously reported in a family with autosomal dominant late-onset CMT2, with functional studies in yeast supporting pathogenicity. Biallelic *MARS* mutations have been reported in a single patient with remarkable phenotypic overlap to that reported here, as well as in a cohort with pulmonary alveolar proteinosis. Our finding underscores the phenotypic variability associated with ARS mutations, and implicates modifying genetic or environmental factors in the onset of monoallelic *MARS*-associated CMT2.

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P03.25A

MERTK polymorphism rs4374838 (A/G) influences the risck of hepatocellular carcinoma in patients with HCV cirrhosis through PI3K regulation

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Introduction: MERTK is a member of TAM receptor tyrosine kinases, mainly expressed in M2 macrophages. GWA studies reported that the rs4374383 (A/G) SNP of MERTK is associated with the risk of developing liver fibrosis in patients with hepatitis C virus (HCV) chronic infection.

Materials and Methods: Genotyping was performed by TaqMan genotyping allelic discrimination method. Gene expression was performed on liver samples by custom RT^2 Profiler PCR Array (Qiagen).

Results: In a cohort of 349 patients with compensated HCV cirrhosis treated with Peg-interferon alfa-2b and ribavirin we found that the homozygosity AA, associated with protection against fibrosis progression in chronic hepatitis C, is associated with a higher risk of developing hepatocellular carcinoma (HCC) in subjects not responding to treatment.

We evaluate the signaling pathways downstream to MERTK on liver biopsy from patients (20) with AA and GG genotypes in absence of HCC. Interestingly, we found that the AA omozigosity is associated with higher MERTK expression which leads to up-regulation of phosphatidylinositol-3-kinase (PI3K) (two folds each). On the contrary, in AA subjects we found a downregulation of SOCS3, TRAF3,TRAF6 (2.9, 2.5, 2.2 fold respectively), involved TLR signaling inhibition TAM receptor mediated.

Conclusion: Since the MERTK is a regulator of tumor-associated macrophages involved in the modulation of inflammatory responses and in the tumorigenesis, its polymorphic status AA could be related to: the direct overexpression of PI3K, activating proliferation/survival pathways; the maintenance of inflammatory chronic state by the deficiency of the negative feedback usually played by TAM receptors on TLR signaling.

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P03.26B

Mutation spectrum of GCK, HNF1A and HNF1B in MODY patients and 40 novel mutations

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Introduction: MODY (maturity onset diabetes of the young) is a monogenic diabetes mellitus, caused by pancreatic beta cell dysfunction. Mutations in the genes encoding the nuclear transcription factor 1 homeobox A (*HNF1A*) and the enzyme glucokinase (*GCK*) are the most common causes of MODY. Additionally *HNF1B* gene is responsible for 5% of the disease. The aim of this study is to investigate the mutation spectrum of *GCK, HNF1A* and *HNF1B* genes in MODY patients.

Material and Methods: This study included 152 MODY patients carrying a mutation in one of three MODY genes; *GCK, HNF1A* and *HNF1*. Molecular analysis was performed using Sanger sequencing or next generation sequencing. Mutation spectrum of the genes analyzed were evaluated separately.

Results: Eighty four percent of mutations detected were in *GCK*, 13% in *HNF1A* and 3% in *HNF1B* genes. Fifty seven different mutations (40 missense, 8 nonsense, 7 frameshift, 1 inframe deletion, one splice site) in *GCK*, 15 different mutations (11 missense, 3 frameshift, one 3′ UTR) in *HNF1A* and 4 different mutations (2 missense, one frameshift, one indel) in *HNF1B* were found. Thirty three, 5

and 2 mutations were detected as novel mutations in *GCK*, *HNF1A* and *HNF1B* genes, respectively.

Conclusion: Definition of molecular etiology in MODY patients is important for giving appropriate genetic counseling and disease management. The most common affected gene has been found to be GCK gene among the MODY patients studied. In the genes *GCK*, *HNF1A* and *HNF1B* 40 mutations have been defined for the first time in this study.

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P03.27C

Gender differences in phenotype of severe early-onset obesity associated with congenital leptin receptor deficiency in a large consanguineous family

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Introduction: Congenital deficiency of the leptin receptor is a rare cause of severe early-onset obesity caused by biallelic mutations in *LEPR* gene. However, it is the most commonly mutated gene in monogenic obesity from consanguineous families. *LEPR* encodes leptin receptor and is involved in the regulation of fat metabolism, energy homeostasis and varieties of other neuroendocrine functions including reproduction.

Materials and Methods: Members of an extended consanguineous family comprising 10 affected individuals aged 0.2 to 36 years old with severe early-onset obesity across six interlinked nuclear families were studied. Their pedigree show an autosomal recessive pattern of inheritance. We employed genome-wide single nucleotide polymorphism (SNP) array analysis to map the location of the causative gene and performed Sanger sequencing of obesity-associated gene located in the sizeable homozygous region shared by affected individuals.

Results: The clinical presentation of the condition in the affected members of the family characterised by extreme early onset obesity occurring soon after birth, marked hyperphagia with ravenous hunger, increased susceptibility to infection, particularly among the females and hirsutism. Delayed and failure of pubertal development was only presented in female adults. Unlike females, affected males started losing weight around puberty at the age of 13–15 years with preserved fertility. Homozygosity mapping and Sanger sequencing of *LEPR* located in a single region of homozygosity revealed a nonsense homozygous mutation segregated within the family.

Conclusion: Gender discrepancy observed among member of a same family suggest leptin possibly plays different roles in male and female reproduction and body weight regulation.

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P03.28D

Severe acute liver failure with hepatic encephalopathy due to *NBAS* mutations significantly improved after total blood exchange transfusion

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Introduction: Acute liver failure (ALF) is a severe and life-threatening condition, which about 50% remains unknown etiology. Vary prognosis and treatment options has been reported. Recent publications discovered *NBAS* mutations causing recurrent infantile ALF, typically triggered by fever, with/without extrahepatic phenotypes (eg. short stature, facial dysmorphism, optic atrophy, and Pelger-Huet anomaly of granulocyte). Most reported patients with *NBAS*

mutations achieve complete recovery, however, some of their siblings died with severe ALF. Treatment with total blood exchange transfusion (TBET) has not been reported in these patients. Here, we described dramatic improvement after TBET in severe ALF with *NBAS* mutations.

Materials and methods: Clinical courses were reviewed. Whole exome sequencing was performed, and followed by Sanger sequencing in the patient.

Results: A 14-month-old girl presented with drowsiness after 3 days of fever with rash. We noticed widening anterior fontanel, frontal bossing, prominent eyes, hepatomegaly, coma, and pale optic disc. She had pre- and postnatal growth restriction with delayed bone age. Markedly elevated liver transminases, coagulopathy, mild hyperbilirubinemia, and hyperammonemia were noted. Metabolic and infectious investigations were unremarkable. A trial of TBET with supplemental high caloric intake was provided. In one day, liver transminases and coagulopathy were significantly improved. The patient regained full consciousness in 2 days, and did not suffer from recurrent ALF till current age at 3.5 years. Novel compound heterozygous mutations, c.5752A>C (p.Thr1918Pro) and IVS12+2T>G in *NBAS*, were identified.

Conclusion: We proposed that TBET along with high caloric intake is an alternative treatment for severe ALF caused by *NBAS* mutations.

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P03.29A

Genetic heterogeneity in nephronophthisis and related syndromes

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Introduction: Nephronophthisis (NPHP) is a rare tubulointerstitial autosomal recessive cystic kidney disease and represents one of the most common genetic causes of end-stage renal disease in children and adolescents. 15% of NPHP patients show additional extrarenal manifestations and are classified as NPHP-related ciliopathies. Homozygous deletions in the *NPHP1* gene account for approximately 21% of all cases, whereas other 20 causative genes contribute to less than 3% each.

Materials and Methods: 16 unrelated patients with NPHP phenotype, including 2 with Senior-Løken syndrome, were analyzed in this study. *NPHP1* deletions were investigated using MLPA; the negative-cases for the homozygous deletion were sequenced by a NGS custom panel including the most common NPHP-genes and genes involved in other renal diseases.

Results: Molecular defects were detected in 14 (87%) patients. Five cases showed the homozygous deletion of the entire *NPHP1*; one case presented a deletion of entire *NPHP1* in one allele and a deletion encompassing 12–15 exons region in the conserved one. In three of the four patients with heterozygous deletions, NGS identified nonsense mutations in the conserved allele. A nonsense homozygous mutation in *NPHP5* and a missense heterozygous mutation in *UMOD* gene were identified in two cases. Finally, in one patient a heterozygous deletion of *HNF1B* was identified.

Conclusion: Our study shows a great genetic heterogeneity in patients with NPHP-like phenotype and suggests extending the analysis to additional genes related to other cystic renal diseases.

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P03.30B

Human liver transcriptomes reveal tight co-regulation of cholesterol synthesis pathway and *DERL3* as a potential liver cancer driver

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Introduction: Obesity may perturb cholesterol synthesis and other key liver functions by inducing ectopic fat deposition to the liver, which can cascade to non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, and cancer.

Materials and Methods: To identify genes affecting NAFLD progression, we RNA-sequenced liver biopsies from 259 obese bariatric surgery patients. Using 15,670 expressed genes, we performed weighted co-expression network analysis to identify co-regulated genes critical to liver's main functions as well as differential expression (DE) analysis between healthy and NASH livers (n = 69 and 43) to uncover disease-perturbed genes.

Results: Among the 19 co-expression modules, a 75-gene module significantly correlates with LDL cholesterol (LDL-C) (adjusted $P=1.71\times10^{-6}$), with enrichment for cholesterol biosynthesis pathway (FDR=9.68×10^{-14}) and protein-protein interactions (P<0.00001), implying a tight co-regulation of cholesterol synthesis at both mRNA and protein levels. The module encompasses known cholesterol genes (*HMGCR, SREBF2, LDLR* and *PSCK9*) in high correlation with new candidates for cholesterol regulation (*DERL3, SLC29A2, C14ORF1, and DNHD1*). The flanking (±500kb) variants of the 35 LDL-C associated module genes ($P<6.67\times10^{-4}$) are highly enriched for LDL-C signal ($P=1.24\times10^{-4}$), accounting for 2.6% of heritability based on previous GWAS statistics using LD Score. One new LDL-C associated gene, *DERL3*, displays significant DE (adjusted P=0.01) with a 36% reduced expression and decreased module membership (P=0.037) in NASH versus healthy, suggesting disrupted co-regulation.

Conclusions: The reduced expression of *DERL3*, a tumor suppressor, might contribute to hypercholesterolemia in NASH and cancer progression from NASH. Overall, the liver transcriptome reveals tight cholesterol regulation with perturbations in liver disease.

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P03.31C

Rare copy number variants are enriched and highlight novel pathways in individuals with early-onset severe obesity

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Introduction: Only a handful of monogenic drivers underlying childhood obesity have been pinpointed to date. In particular, copy number variants (CNVs) are known to contribute to obesity, both syndromic (15q11. deletions, Prader-Willi syndrome) and non-syndromic (16p11.2 deletions). We have studied the contribution of CNVs in early-onset obesity and evaluated the expression of candidate genes in adipose tissue from BMI discordant siblings.

Materials and methods: CNVs were analyzed in 90 obese subjects and 67 normal-weight controls, using a custom high-density array comparative genomic hybridization with exon resolution in 1989 genes, including all known obesity loci and genes implicated in intellectual disability and ciliopathies. Expression levels of candidate genes were assessed using microarray analysis of RNA from adipose tissue.

Results: We identified 17 (19 %) obese individuals with rare CNVs, of which three were known syndromic lesions (22q11.21 duplication, 1q21.1 deletion and 16p11.2 deletion). Only two (3%) controls carried rare CNVs (p<0.001, Chi-Square test). In ten families where parental DNA was available, eight CNVs were inherited and seven segregated with obesity. Expression analysis of 37 candidate loci showed discordant expression for ten genes (*PCM1, EFEMP1, MAMLD1, ACP6, BAZ2B, SORBS1, KLF15, MACROD2, ATR*, *MBD5)*.

Conclusions: Our data suggest that CNVs contribute possibly pathogenic alleles to a substantial fraction of children with early-onset obesity, and pinpoint candidates for further study. Identification of three individuals with known syndromic lesions that influence care and follow-up, highlights the importance of CNV screening in early-onset obesity.

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P03.32D

A novel nonsense variant in *TJP2* cause divergent phenotypes in homozygous siblings

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Introduction: Several distinct forms of "Progressive Familial Intrahepatic Cholestasis" (PFIC1-PFIC4) have been described. Variants in *TJP2* have recently been connected to autosomal recessive PFIC4. These patients often require liver transplantation early in life. Known disease-causing TJP2-variants are predicted to prevent TJP2-protein expression through premature termination codons inducing nonsense-mediated mRNA decay (NMD). We describe a novel nonsense-variant in *TJP2* showing divergent phenotypes in three siblings.

Materials and Methods: The proband is the 7th child of 13 to consanguine parents. Two other siblings with possible liver affection were also examined. DNA was analysed by NGS-panel targeting 9 cholestatic liver disease-related genes (NimbleGen, Illumina MiSeq). Data was analysed by CLC Biomedical Genomics Workbench (Qiagen). Variants were confirmed by Sanger sequencing.

Results: NGS detected a novel nonsense-variant in *TJP2* in a homozygous state in all three siblings (Table 1). Several common missense-variants in *ABCC2* and *ABCB11* associated with less severe conditions were also detected. The CNV-analysis was normal. Parental segregation analysis and histological investigation of TJP2-protein expression in liver biopsies are pending.

Conclusions: This TJP2-variant is predicted to induce NMD and subsequent prevention of TJP2-protein expression. We suspect that it explains cholestasis and liver fibrosis in the proband. Surprisingly, two siblings with identical *TJP2* genotype only display minor liver affection. These divergent phenotypes suggest a reduced penetrance of PFIC4 progression.

Table 1. Genetic and phenotypic description of 3 siblings harbouring a novel nonsense variant in *TJP2*

Patient Age	TJP2 [NM_004817.3]	ABCC2 [NM_000392.3]	ABCB11 [NM_003742.2]	Phenotype
Proband 21 years	c.[3334C>T](;) [3334C>T]	c.[3563T>A](;) [4544G>A]	c.[1331T>C](;) [1331T>C]	Icterus as newborn. Liver disease since childhood. Liver biopsy shows cirrhosis, non-specific, maybe caused by cholestasis. Candidate for liver transplantation.
Brother 24 years	c.[3334C>T](;) [3334C>T]	c.[3563T>A](;) [4544G>A]	c.[1331T>C](;) [1331T>C]	Icterus as newborn, normal liver biopsy. Marginally increased liver parameters.
Sister 19 years	c.[3334C>T](;) [3334C>T]	c.[3563T>A](;) [4544G>A]	c.[1331T>C]; [=]	Icterus as newborn. Intrahepatic cholestasis of pregnancy (ICP). Liver biopsy not performed yet.

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P03.33A

Complex DNA analysis of polycystic kidney disease

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Introduction: Polycystic kidney disease is the most frequent hereditary nephropathy. The most severe forms are caused by mutations of following genes: PKHD1, HNF1β, PKD1, PKD2. The results of molecular genetic analysis of 58 families with clinically suspected ARPKD are presented.

Materials and methods: The molecular analysis of the PKHD1 and HNF1 β genes was carried out using next-generation sequencing method. In patients without 2 causal mutations of PKHD1 or 1 mutation in HNF1 β, the subsequent MLPA analysis of PKHD1 and HNF1 β was performed. The cohort of probands was divided into 2 groups on the basis of their fulfillment of clinical criteria of ARPKD (Group A- patients fulfilling all criteria, n = 25; Group B-others, n = 35).

Results: The detection rate in PKHD1 amounted to 84% in Group A, and 26% in Group B. Moreover, 8 families within Group B without mutation in the PKHD1 harbored mutations in other genes: 5 families had mutations in HNF1β, 2 families in PKD1 and 1 family in PKD2. The lowest detection mutation rate was found in fetuses with renal cysts from terminated pregnancies.

Conclusions: The detection rate of PKHD1 mutations in children who fulfilled all 3 of the clinically diagnostic criteria of ARPKD is high, reaching 84%. Because of the etiologic heterogeneity of polycystic kidney disease phenotype, the complex mutational analysis of several genes is needed for reliable differential diagnosis, especially in affected fetuses with cysts.

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P03.34B

A novel mutation in *PROP1* c.109+1G>A identified by target gene panel in patients with congenital growth hormone deficiency

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Background: Congenital GH deficiency (GHD) can be isolated (IGHD) or combined with other pituitary hormone deficiencies (CPHD). The identification of mutations has clinical implications for the treatment of patients and genetic counseling, yet most of the genetic etiologies remain currently unknown. Mutations in *PROP1* gene are the most frequently implicated in CPHD associated to orthotopic posterior pituitary lobe (OPP).

Objective: to screen for mutations in genes associated with IGHD or CPHD.

Patients and Methods: Forty patients with IGHD (n = 8) or CPHD (n = 32) were studied using target gene approach. Targeted regions (involving 26 genes associated with GHD) were captured using Agilent Sure Select technology. Sequencing was performed with Illumina NextSeq. Variants were analyzed and classified as according to recommendations of ACMG. Copy number analysis for targeted resequencing method was used to evaluate gains or losses in the regions of interest.

Results: We identified 31 rare allelic variants (excluding synonymous) in 17 of 26 genes associated with GHD in 19 patients. One patient with CPHD and OPP was compound heterozygous for *PROP1* variants: c.[109+1G>A]; [301_302del]. The former a novel mutation in a consensus splice site location and the latter the already described 2-bp deletion leading to p.Ser101fs*8 and classified as pathogenic. The implications of the remaining variants for the phenotype are still under analysis.

Conclusion: The target gene panel revealed a novel mutation in *PROP1* in a patient with CPHD further expanding the list of mutations related to this condition. The patients with negative results are candidates for whole exome sequencing.

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P03.35C

Loss of function mutations in ttc7a cause hereditary multiple intestinal atresia in patients and disrupt intestinal lumen in zebrafish

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Introduction: Multiple intestinal atresia (MIA) results in neonatal intestinal obstruction. MIA is characterized by multiple intestinal discontinuities throughout the intestine. Often MIA patients are also immunodeficient. Deleterious variants in the tetratricopeptide-7A (*TTC7A*) gene were identified. *TTC7A* encodes a protein with an important role in iron haemostasis. *In vitro* studies suggest importance of *TTC7A* in regulation of intestinal polarity and cell adhesion.

Materials and methods: Two siblings with MIA and immunodeficiency from a consanguineous family were studied. Nucleotide sequence of *TTC7A* was determined by Sanger sequencing. Human intestinal tissues from both patients and controls were immunostained and analyzed. A ttc7a knockout zebrafish model has been generated by TALEN and characterized. Sections of 5dpf mutant fish were stained and analyzed. Fluorescent pellets were fed to a ttc7a genetically mixed population of fish for *in vivo* analysis of gut function using intravital microscopy.

Results: In both siblings, a homozygous deletion of 13.123 bp from intron 1 to intron 2, including exon 2, was identified. Immunostainings revealed abnormal intestinal lumens and mucosa. Intestinal stenosis was observed in homozygous mutant ttc7a zebrafish and they present with

narrowing of the intestinal lumen. They also show a statistically significant slower gastrointestinal transit time.

Conclusion: The homozygous deletion of exon 2 confirms *TTC7A* to be the disease-causing gene in these patients diagnosed with hereditary MIA and immunodeficiency. The intestinal phenotype is the result of disturbed intestinal lumen formation. The homozygous mutant ttc7a zebrafish closely mimics the human phenotype.

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P03.36D

The promise and challenge of high throughput sequencing to genotype-phenotype correlation in salt loss tubulopathies

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The Bartter-Gitelman syndromes (BS-GS) are two different salt loosing tubulopathies. Both diseases are characterized by inability to reabsorb NaCl in the thick ascending limb (TAL) of Henle's loop and distal convoluted tubule (DCT). The main clinical features are common to both diseases including hyperaldosteronism, hypokalemic metabolic alkalosis, failure to thrive; hypercalciuria characterizes the majority of BS, while hypocalciuria and hypomagnesemia characterizes GS. BS is due to defect in almost 5 genes, while the *SLC12A3* is responsible for GS. We performed molecular analysis in 25 patients with diagnosis of BS-GS using a NGS panel in which are included genes associated and potentially involved in different type of tubulopathies. Our patients showed mutations in almost all genes associated to different type of BS. In one case we found a homozygous deletion involving both *CLCNKA* and *CLCNKB* genes. Cases already described with digenic form of BS showed early deafness, while our case did not showed this features. Moreover among our cases, 5 with BS diagnosis, showed mutation in *SLC12A3*. Our results demonstrate that this approach can contribute to a correct genetic diagnosis when it is not always possible a precise discrimination of the clinical phenotypes. Patients with salting loss tubulophaties are often treated similarly, with little consideration of individual characteristics that might affect clinical outcome and therapeutic response. Our study provides a highly sensitive method for identification of variants in different causative genes associated with a salting loss, drawing the best way for a tailored medicine.

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P03.37A

Clinical and molecular characterization of Wilson's disease in the Valencian Region

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Introduction: Wilson's disease (WD) is an autosomal recessive disorder caused by mutations in the liver's copper-transporter protein ATP7B, which results in an impaired biliary excretion of copper and its accumulation mostly in liver and brain. We have performed an in-depth clinical and molecular characterization of a cohort of WD patients from the Valencian Region (Eastern Spain), with the purpose of improving clinical diagnosis and prognosis of these patients.

Methods: Clinical-diagnosed WD series of 42 index cases. Differential diagnosis based on routine test for hepatic dysfunction. Neurological involvement was evaluated with the Global Assessment Scale (GAS) for WD. Genetic analysis of *ATP7B* included the study of the promoter, coding exons and its flanking intronic regions by Sanger sequencing, and detection of large deletions and duplications by multiplex ligation-dependent probe amplification (MLPA) analysis. The whole gene, included introns, is investigated in patients with only one or no mutations by massive sequencing.

Results: Genetic diagnosis was achieved in 24 cases; only one mutation was detected in 6 cases, and no candidate mutations were identified in 12 cases. The most common mutation in our cohort is p.M645R (21.4% of the alleles). Studies related to the sequencing of the whole *ATP7B* are in progress. Strikingly, apart from hepatic dysfunction, most of the patients present with neurological signs (tremor, dystonia and parkinsonism).

Conclusion: The genetic studies performed in our cohort contribute to improve our knowledge about the genetics and the clinical picture associated with Wilson's disease.

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P03.38B

Microdeletions in the 11p13 region involving WT1 may result in genitourinary malformations without WAGR syndrome

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Introduction: Large deletions affecting the 11p13 chromosomal region have long been known to cause WAGR syndrome: Wilms' tumor, aniridia, genitourinary malformations and mental retardation. Recently, some patients carrying different microdeletions within 11p13 have been documented in the literature with varying clinical symptoms. We hereby report a patient with Phelan-McDermid syndrome carrying a heterozygous deletion in the 11p13 region in addition to the 22q13 deletion.

Materials and Methods: The patient was diagnosed with low muscle tone at birth and later developed a classic form of the Phelan-McDermid syndrome. Additional findings at birth included a stricture and dysfunction of the left ureter. The patient was enrolled in WT1-screening on the basis of unusual genitourinary findings, and after a deletion involving WT1 was detected, copy number analysis of the region was performed by both qRT-PCR and dPCR.

Results: In addition to the 22q13 deletion, a heterozygous microdeletion was confirmed in the 11p13 region including WT1 and EIF3M, but not affecting PAX6.

Conclusions: Aniridia is currently thought to be the main clue to the diagnosis of the 11p13 deletion syndrome, as it is seen in classic cases of WAGR. However, if we compare our findings to similar recent cases reported in the literature, it seems likely that many 11p13 deletions without PAX6 involvement are unnoticed. Rather than thinking of WAGR syndrome as a single entity, we should consider it as a spectrum of clinical features as different genes may be affected by each particular deletion.

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P04 Skeletal, connective tissue, ectodermal and skin disorders

P04.01A

Postzygotic dominant-negative mutations of *RHOA* cause a mosaic neuroectodermal syndrome

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Hypomelanosis of Ito (HI) is commonly seen as a non-specific manifestation of mosaicism. Because of its frequent association with various extracutaneous anomalies, especially cerebral involvement, HI is often considered as a neurocutaneous syndrome. Apart from rare reports of non-recurrent mosaic chromosomal anomalies, and recent descriptions of linear hypopigmentation in MTOR-related hemimegalencephaly, the genetic causes of HI have remained largely unknown. We studied six unrelated patients with a previously undescribed mosaic neuroectodermal syndrome combining linear hypopigmentation, facial, ocular, dental, and acral anomalies, alopecia, and apparently asymptomatic leukoencephalopathy. We performed whole-exome sequencing (WES) in affected skin from two parent-case trios, and identified the same postzygotic change of *RHOA* in both probands. This variant was also found in two unrelated individuals by targeted ultra-deep sequencing of *RHOA*. The fifth proband carries a different postzygotic variant, detected by trio WES. On the sixth patient, no changes were detected. *RHOA* encodes RhoA, a RAS-related Rho GTPase, which controls morphogenesis, chemotaxis, axonal guidance, and cell cycle progression. RhoA is a highly conserved protein particularly intolerant to amino acid substitutions, supporting the idea that *RHOA*-related mosaic ectodermal dysplasia should be added to the list of disorders resulting from lethal mutations surviving only by mosaicism. Transfected NIH 3T3 cells with mutants RhoA plasmid displayerd reduced cell spreading and decreased stress fiber formation, suggesting a dominant-negative effect of both variants. Our findings pave the way towards elucidating the etiology of pigmentary mosaicism and highlight the role of *RHOA* in human development and disease.

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P04.02B

Plastin 3 regulates bone development and maintenance through the NFκB pathway in osteoclasts

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Introduction: Osteoporosis affects a large proportion of people, particularly women after menopause. Plastin 3 (PLS3) mutations cause familial osteoporosis and osteoporotic fractures in men and an increased risk of fractures in elderly women. In about 5% of the general population PLS3 is overexpressed, raising the question whether PLS3 overexpression (OE) can act protective against osteoporosis. Here we investigated PLS3 OE mice to unravel the impact on bone structure and remodelling and to identify the underlying molecular mechanism.

Materials and Methods: Micro-CT and histology were performed on femora of the animals. Co-immunoprecipitation and mass spectrometry let to novel PLS3-interacting partners. Bone resorption assay, immunofluorescence staining, WB, Co-IP and qRT-PCR were performed from primary differentiated osteoclasts.

Results: Femora of PLS3 OE mice showed significantly increased cortical and trabecular thickness compared to controls. Primary osteoclasts of PLS3 OE compared to WT mice presented evidently larger and differently shaped osteoclasts with disrupted podosome formation and severely decreased resorptive activity. NFκB expression was strikingly upregulated in PLS3 OE osteoclasts. An unbiased proteomics approach identified NFκB repressing factor (NKRF) to interact with PLS3, which we confirmed by an independent Co-immunoprecipitation assay. We hypothesized that NFκB transcriptional regulation is inhibited through NKRF and PLS3 interaction, which was supported by the finding of downregulated NFATc1, an NFκB downstream target important for osteoclastic function, as well as altered NKRF localization in PLS3 OE osteoclasts.

Conclusions: We identified a novel cellular pathway, by which PLS3 acts on bone development and remodelling, which might open new therapeutic possibilities.

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P04.03C

Retinoic acid catabolizing enzyme CYP26C1 is a genetic modifier in SHOX deficiency

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Mutations in the homeobox gene SHOX cause SHOX deficiency, a condition with clinical manifestations ranging from short stature without dysmorphic signs to severe mesomelic skeletal dysplasia. In rare cases, individuals with SHOX deficiency are asymptomatic. To elucidate the factors that modify disease severity/penetrance, we studied a three-generation family with SHOX deficiency. The variant p.Phe508Cys of the retinoic acid catabolizing enzyme CYP26C1 co-segregated with the SHOX variant p. Val161Ala in the affected individuals, while the SHOX mutant alone was present in asymptomatic individuals. Two further cases with SHOX deficiency and damaging CYP26C1 variants were identified in a cohort of 68 individuals with LWD. The identified CYP26C1 variants affected its catabolic activity, leading to an increased level of retinoic acid. High levels of retinoic acid significantly decrease SHOX expression in human primary chondrocytes and zebrafish embryos. Individual morpholino knockdown of either gene shortens the pectoral fins, whereas depletion of both genes leads to a more severe phenotype. Together, our findings describe CYP26C1 as the first genetic modifier for SHOX deficiency.

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Chemical modulation of Hedghehog signaling in the abnormal osteogenic niche of non-syndromic craniosynostosis

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Nonsyndromic craniosynostosis (NCS), is a heterogeneous congenital anomaly due to premature fusion of one or more calvarial sutures with largely unknownetiopathogenesis. Our previous data highlighted the upregulation of the Bardet Biedl Syndrome-associated gene 9 (BBS9) expression at the site of premature suture fusion, along with a dysregulation of the Hedgehog (HH) signaling and aberrant primary cilium expression.

The aim of this study was to investigate the effect of HH chemical modulation on the osteogenic commitment of somatic stem cells residing in the calvarial suture niche.

Cells isolated from fused and unfused sutures of NCS patients, were cultured either in standard growth or in osteoinductive media. Subconfluent cells were treated either with the HH modulators cyclopamine (inhibitor) or with purmorphamine (inducer) at 5μM concentrations in time course. The expression of *BBS9* and bone specific transcription factor genes (*RUNX2*, *OSX*) was assessed by qPCR. Matrix mineralization was evaluated with AlizarinRed staining (AR).

In standard conditions, *BBS9* expression was increased in fused- as compared with unfused-suture derived cells, and underwent a further increase during osteogenic differentiation. Following treatment with cyclopamine and purmorphamine, both osteogenic differentiation rates and the expression levels of *BBS9, RUNX2*, and *OSX* correlated positively with HH modulation.

These data confirm the involvement of the Hedghehog pathway in the osteogenic commitment of suture-derived cells, and provide preliminary hints towards the implementation of HH chemical modulation through small molecules, as a potential molecular-targeted approach to counteract premature suture ossification.

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A novel recessive connective tissue disorder is caused by *AEBP1* deficiency

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Introduction: The *AEBP1* gene encodes the aortic carboxypeptidase-like protein (ACLP) that associates with collagens in the extracellular matrix and has a number of roles in development and adult tissue repair. ACLP is expressed in bone, blood vessels, and skin, and *Aebp1*$^{-/-}$ mice have deficient wound repair and have poor fibroblast proliferation. We recently reported two siblings born with severe joint and skin laxity, severe osteopenia, and facial dysmorphisms, who had a homozygous splice site variant (c.1630+1G>A) in *AEBP1*. In this study, we describe two additional families and provide evidence supporting the role of *AEBP1* in disease.

Materials and Methods: Patient 1 is an adult male with joint laxity, redundant skin, poor wound healing, and osteopenia. As his phenotype is reminiscent of Ehlers-Danlos syndrome (EDS), extensive clinical evaluations were undertaken including a vascular workup that was normal. Electron microscopy identified variation in collagen fibril size and the presence of "collagen flowers," which are seen in several variants of EDS. Patient 2 presented with congenital hip dislocation, bowel rupture, bone degeneration, decreased dermal collagen, and abnormal scarring.

Results: Exome sequencing revealed novel compound heterozygous variants in *AEBP1* (c.1470delC, p.Asn490LysfsX6 and c.1743C>A, p.Cys581Ter) in patient 1 and a homozygous variant (c.1320_1326del, p.Arg440SerfsX3) in patient 2.

Conclusions: Given the phenotypic overlap of patients with loss-of-function variants in *AEBP1* and concordance with the phenotypes described in *Aebp1*$^{-/-}$ mice, we propose that truncating loss-of-function variants in this gene are the cause of a novel recessive connective tissue disorder.

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Glutathione S-transferases M1/T1 and P1 polymorphisms in patients with alopecia areata

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Alopecia areata (AA) is a common, recurrent, chronic autoimmune and inflammatory disease resulting from T cell-induced damage to hair follicules. The disease can appear in both sexes at any age. Although the aethiopathogenesis of AA is unclear, many factors including genetic predisposition, emotional and environmental stress are thought to play important roles in its development. Since antioxidant/ oxidant balance disturbance is a common feature in autoimmune, emotional and environmental stress, current evidences support the association between oxidative stress and AA. Glutathione S-transferases (GSTs) play a major role in the detoxification of various compounds. The present study was aimed to determine the frequencies of GSTM1-GSTT1-GSTP1 polymorphisms in 176 patients with AA and 196 age- and sex- matched healthy controls. GSTM1 and GSTT1 genotypes were determined by multiplex PCR whereas GSTP1 polymorphisms were analyzed by using PCR-RFLP technique. Genotype frequencies of the GSTM1, GSTT1 and GSTP1 polymorphisms showed significant differences between AA patients and healthy controls (Table). In conclusion, our results suggested a significant association between the GSTP1,GSTT1 and/or GSTM1 null genotypes and AA that is the first report showing the relation with the disease. However, the potential role of GSTs as markers of susceptibility to AA needs further studies in larger patient groups. Table. Genotype frequencies and P value of the GSTM1, GSTT1 and GSTP1 polymorphisms.

Genotype	AA (n = 176) n (%)	Control(n = 196) n(%)	P value
GSTM1 Null (-)	16(9)	0(0)	<0,01
Present (+)	160(91)	196(100)	
GSTT1 Null (-)	28(16)	0(0)	<0,001
Present (+)	148(84)	196(100)	
GSTM1/ GSTT1 +/+	112(63,6)	196(100)	<0,001
-/-	10(5,6)	0(0)	<0,05
GSTP1 Ile/Ile	0(0)	30(15)	>0,05
Ile/Val	98(55,6)	166(84,6)	<0,001
Val/Val	34(19)	0(0)	<0,001

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Gene panel testing for Alport syndrome reveals inheritance patterns to inform risks and management

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Alport syndrome is a nephropathy, often resulting in end-stage renal failure; also associated with sensorineural hearing loss and ocular anomalies. Estimated prevalence is 1:50,000 live births. Approximately 65–80% of cases are X-linked (*COL4A5)*, 10–15% autosomal recessive (*COL4A3*, *COL4A4*); up to 20% dominant inheritance (*COL4A3*, *COL4A4*), while recently Mencarelli et al. described cases of digenic inheritance, involving *COL4A3* and *COL4A4* genes. All three genes encode subunits of type IV collagen, which is the major structural component of basement membranes of the kidney, inner ear and eye.

The last 4 years we tested 89 unrelated patients initially by Sanger sequencing switching to gene panel testing using Illumina TruSightOne. A genetic diagnosis was achieved in 34 patients (diagnostic yield 38%), while another seven patients had a variant of uncertain significance. A total of 36 pathogenic and likely pathogenic variants identified, 16 were novel. 28 patients had X-linked disease, four autosomal dominant, one autosomal recessive and one patient had two variants in different genes, *COL4A3* and *COL4A4*, indicating a possible case of digenic inheritance. 52 follow up tests were performed across 18 families, including prenatal diagnosis. Interesting cases will be presented.

A genetic diagnosis can establish the mode of inheritance which is important in assessing potential clinical outcome, allows for improved surveillance and effective treatment and identification of at risk relatives as well as members that can be live kidney donors. Massively parallel sequencing of the genes simultaneously enables identification of complex inheritance patterns; these should be considered when reviewing prognosis and recurrence risks.

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Integration of GWAS data and hair follicle miRNA and mRNA expression profiles yield novel insights into male-pattern baldness pathobiology

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Male-pattern baldness (MBP) is characterised by a progressive loss of androgen-sensitive hair follicle (HF) subpopulations in the frontal and vertex scalp. HFs in the occipital scalp remain unaffected.

To gain insights into the underlying pathobiology, we analysed for a differential micro(mi)RNA and mRNA expression in HFs from affected (frontal) and unaffected (occipital) scalp regions and integrated these data with the results from a large GWAS meta-analysis (N = 22,518) on MPB. Our analyses revealed differential expression (DE) of 144 miRNAs and 3,230 mRNAs. Of these, only one miRNA and 49 mRNAs mapped to known MPB risk loci ($P<5\times10^{-8}$), suggesting that the remaining DE genes may help to pinpoint novel risk loci and relevant pathways.

Indeed, DE mRNAs and target genes of DE miRNAs not only showed an enrichment in previously implicated pathways (WNT- and oestrogen-signalling) but also provided evidence for the involvement of additional pathways such as mTOR- and ephrin-receptor-signalling in MPB-aetiology. Moreover, the comparison with suggestive association findings ($5\times10^{-8}<P<10^{-5}$) from the meta-analysis revealed an overlap with DE miRNAs and mRNAs at nine genomic regions, that had not previously been implicated in MPB. Among them a locus at 3q22.2 comprising the genes for ephrin-receptor-B1 (*EPHB1*) and the prostaglandin-D2-transporter SLCO2A1, which may contribute to the characteristic hair loss pattern via mediation of androgen-sensitivity and hair growth inhibition, respectively.

In summary, mRNA and miRNA transcriptome profiling and subsequent integration with genetic association data identified novel candidate genes (*EPHB1*,*STAT1*,*DLL1*) and pathways (mTOR-,ephrin-signalling), and highlights nine novel loci that are likely to contribute to MPB-development.

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Arterial tortuosity syndrome: 37 new families and literature review

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Introduction: Arterial tortuosity syndrome (ATS, MIM#208050) is a rare autosomal recessive connective tissue disorder, characterized by elongation and tortuosity of the large and middle-sized arteries. ATS is caused by mutations in *SLC2A10*. Aim To delineate ATS clinically and molecularly in 37 newly identified ATS families (46 individuals) with ultrastructural characterization of skin biopsies. Results All patients (mean age 11.1, median age 9) harbor biallellic *SLC2A10* mutations of which ten are novel. Initial manifestations were cardiovascular (aortic coarctation and cardiac murmurs) in 50%, aspecific connective tissue findings (hernias, stretchy skin) in 25%, pulmonary manifestations (neonatal respiratory distress, dyspnea) in 15%, and familial segregation in the remaining 10% of patients. Aggressive aortic root dilatation (ARD) occurred in four infants without dissection or rupture, five patients had slowly progressive ARD. Stenoses, tortuosity and aneurysm formation occurred widespread, necessitating extensive imaging of the aorta and renal arteries. Vascular surgeries and catheterizations are well tolerated. Other severe but sporadic complications included neonatal intracranial bleeding, ischemic stroke and gastric perforation. Novel findings include prenatal manifestations (intra-uterine growth retardation, prenatally detected aortic tortuosity and oligohydramnios) and corneal thinning. Transmission electron microscopy shows a patchy and irregular deposition of elastin at the periphery of the elastic fibers and variable diameters of the collagen fiber. Conclusion We confirm a less severe natural history than initially reported, but respiratory distress, aggressive aneurysm formation and cerebrovascular accidents occur. In electron microscopy, skin biopsies show specific elastic fiber and collagen anomalies discernable from other elastic fiber diseases.

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P04.10B

c.961C>T Sequestosome1 gene mutation, associated to Paget´s disease of bone, causes a blockade of autophagy and an activation of NF-kB pathway

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Paget´s disease of bone (PDB) is a disorder characterized by a bone turnover increase in a disorganized way. The strong tendency to familial aggregation supports the genetic hypothesis of PDB and the most important known genetic factor predisposing to the disease is mutation in Sequestosome1 (SQSTM1) gene.

We have characterized three PDB patients that carry the c.961C>T mutation that it is localized in exon 6 of SQSTM1 gene. It causes the p.R321C mutation, localized in the LIR domain of p62 protein. LIR domain is crucial in autophagy process. Our functional analysis showed that p. R321C mutation caused a blockade in autophagy and increases the activation of NF-kB pathway. In cells that over-expressed the p62 321C protein variant we observed cytoplasm aggregates similar to autophagosomes, which were unable to fuse with the lysosomes probably due to the p.R321C a lack of interaction between p62 and LC3 proteins. We have confirmed it by immunofluorescence and immunoprecipitation assays.

The blockade of autophagy increased the p62 protein levels that caused IKKα/β phosphorylation and NF-kB pathway activation. The NF-kB activation induced cell proliferation. Western blot analysis confirmed IKKα/β phosphorylation.

We report, for the first time, that c.961C>T SQSTM1 gene mutation causes a blockade in autophagy process that contributes to a higher intracellular expression of p62 protein, NF-kB pathway activation and an increase of cell proliferation.

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Precocious prenatal phenotype of BHLHA9 duplication: a case report

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Ectrodactyly, also known as split hand-foot malformation (SHFM), is a rare developmental disorder characterised by malformations of the distal limbs affecting the central rays of hands and-or feet. It can occur as an isolated malformation or as part of various complex syndromes. Among these syndromes, a distinct entity associates SHFM and long bone deficiency (SHFLD). Duplication on chromosome 17p13.3 has recently been associated with SHFLD. In this region, duplication of BHLHA9 has been incriminate in the physiopathology of limb anomalies. This CNV is not associated with other organ malformation or developmental delay. Here, we present a case of ectrodactyly and tibial agenesia diagnosed in the first trimester by two-dimensional and three-dimensional ultrasonography, leading to the choice by the patient to make a precocious termination of pregnancy. Array-Cgh, performed after termination of pregnancy, found a 157 kb duplication on chromosome 17p13.3. This CNV only includes the BHLHA9 gene. To our knowledge, this is the earliest diagnosis of BHLHA9 duplication reported to date. Parental analyses proved de novo character of this CNV. Fetal limb abnormalities are now being encountered at increasingly earlier gestational ages due to improvements in image quality of ultrasound in the first trimester. Early identification of these types of malformations without a definitive diagnosis present a challenging clinical problematic. In these cases, patients regularly may opt for earlier termination of pregnancy rather

than wait for additional information, including chromosomal testing, to help decision-making.

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Identification of mutations in the RMRP gene in patients with Cartilage-Hair Hypoplasia

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Introduction: Cartilage-Hair Hypoplasia (CHH, #250250), is an autosomal recessive disease characterized by metaphyseal and other skeletal abnormalities. Immune deficiency and predisposition for some types of cancer can also occur. This pathology is caused by mutations in the *RMRP* gene, responsible for coding a non-translated RNA, member of riboproteic RNase MRP complex. *RMRP* gene is involved in mitochondrial DNA replication, in pre-5.8S rRNA processing and in cell cycle progression through cyclin B2 cleavage. The goal of this study was to identify mutations in the *RMRP* gene in patients with CHH clinical and radiographic suspicion.

Material and Methods: Genomic DNA was extracted from peripheral blood by salting-out method. Promoter and transcribed regions of *RMRP* gene were PCR amplified, cloned and sequenced.

Results: In this study, we report twenty-two distinct alterations including duplication, triplication, insertion and/or point mutations, among which 11 are new putative mutations found in a group of 20 different patients. All genotypes occurred in compound heterozygosity. Pathogenic mutations were concomitant to polymorphisms already described in the literature. It is noteworthy that g.71A>G, the most worldwide frequent described mutation in CHH patients, was not found in our cohort. In contrast, more than 50% of our patients presented the g.196C>T mutation, a frequency not found previously in other countries.

Conclusions: Molecular results confirm disease etiology and allow genetic counseling of the families, anticipating clinical interventions in patients and avoiding serious clinical complications.

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Genetic architecture of early growth phenotypes gives insights into their link with later obesity

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Introduction: Early growth in childhood is associated with adult metabolic health, but the underlying mechanisms are unclear.

Materials and Methods: We performed genome-wide meta-analyses and follow-up in up to 22,769 European children for six early growth phenotypes derived from longitudinal data: peak height and weight velocities, age and body mass index (BMI) at adiposity peak (AP ~9 months) and rebound (AR ~5–6 years). We then performed expression quantitative trait loci (eQTLs), pathway enrichment and genetic correlation analyses for functional characterization.

Results: We identified four associated loci ($P< 5\times10^{-8}$): *LEPR/LEPROT* with BMI at AP, *FTO* and *TFAP2B* with Age at AR and *GNPDA2* with BMI at AR. The common AR-associated SNPs at *FTO, TFAP2B* and *GNPDA2* represent known BMI-associated variants. The common variant at *LEPR/LEPROT* associated with BMI at AP is independent of the early onset obesity-associated SNPs at the same locus, and was significantly associated with both *LEPR* and *LEPROT* gene expression levels especially in subcutaneous fat ($P<2\times10^{-51}$). Analysis of the full discovery stage results for Age at AR revealed enrichment for insulin-like growth factor 1 (IGF-1) signaling and apolipoprotein pathways. We also identified strong positive genetic correlations between early growth and later adiposity traits.

Conclusions: We report the first genome-wide association study of six childhood growth phenotypes, contributing to the understanding of genetic influences on early growth in humans. Our results suggest mechanistic links between these phenotypes and increased adiposity in later childhood and adulthood, highlighting the need to identify modifiable casual factors that determine these early growth phenotypes.

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P04.14B

Implication of syndromic cleft genes in non syndromic forms : towards translational phenotypes ?

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Cleft lip with or without cleft palate (CL/P) and cleft palate (CP) are the most common craniofacial birth defects with an approximate incidence of ~1/700. In 30% of cases, clefts are seen in syndromic forms likely caused by genetic factors. Non syndromic forms (NS) are believed to be caused by a combination of genetic and environmental factors. Several genome-wide association studies have proposed a few loci in NSCL/P. A few genes have been implicated in both syndromic and non-syndromic forms of CL/P. Moreover, there are families with NSCL/P following Mendelian transmission with low penetrance and variable expressivity. These two points suggest that a strategy of studying genes or pathways associated with syndromic forms as the cause of NS clefts could be productive. To this end we decided to perform whole exome sequencing on patients with NS cleft, after having ruled out *IRF6* mutations and cytogenetic anomalies. Patients from 12 families with CP, 10 families with CL/P, 2 families with velopharyngeal insufficiency, and 6 sporadic CP cases were selected. Several likely causative variants were identified in five families: in *GHRL3, TP63, LRP6* and *TBX1*. Clinical reassessment confirmed the isolated occurrence of cleft with variable expressivity in affected patients, as well as low penetrance, given the number of unaffected carriers. Our study illustrates the involvement of genes known to be mutated in syndromic clefts, in non syndromic CL/P. It raises the question of an important part of the "missing heritability" of NSCL/P possibly being explained by modest-to-medium penetrant variants in such genes

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P04.15C

A novel heterozygous duplication of RUNX2 gene in a Hungarian family with cleidocranial dysplasia

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Introduction: Cleidocranial dysplasia (CCD) is an autosomal dominant skeletal dysplasia characterized by hypoplastic or aplastic clavicles, persistence of wide-open fontanelles and sutures and tooth anomalies. The prevalence is estimated 1:1000000. It may be underdiagnosed because of the extremely wide range of clinical manifestation. CCD is caused by mutations in the RUNX2 gene which plays an important role in bone maintenance and ossification.

Materials and Methods: We investigated a currently 5 year-old boy with features of CCD. Our proband is the third child of non-consanguineous Hungarian parents, has had normal cognitive development and his craniofacial features included hypertelorism, broad nasal bridge, open anterior fontanelle, frontal bossing and short stature. Radiological findings were: hypoplastic clavicles, spina bifida occulta and lack of coccygeal vertebral segments. Mother, grandmother and aunt have the same symptoms in the family.

Results: The molecular genetic test was performed within the framework of international collaboration at Gendia. A pathogenic heterozygous duplication of 4 nucleotides RUNX2:c.906_909 dupTTAC was identified in exon 7 of the RUNX2 gene. This frameshift causing duplication results in a premature stop codon which leads to truncating protein production or diminished mRNA due to mRNA decay. To the best of our knowledge this variant is novel not previously described in other individuals.

Conclusions: This case report represents a novel mutation of RUNX2 in the background of classical CCD. Although the diagnosis is based on clinical signs and on characteristic radiographic findings, molecular genetic testing is recommended in order to get appropriate management and adequate treatment if neccessary.

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P04.16D

The first report of a severe classical Ehlers-Danlos syndrome phenotype in two siblings, caused by a homozygous hypomorphic *COL5A1* mutation

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Introduction: Classical Ehlers-Danlos syndrome (cEDS) is a heritable disorder of connective tissue caused by defects of type V collagen. Major diagnostic criteria include fragile, hyperextensible skin with atrophic scars and joint hypermobility. Aortic root dilation and mitral valve prolapse are reported associations, but tend to be of little clinical significance, whereas vascular events have been reported rarely in cEDS patients.

Methods: Clinical history and examination was performed in five individuals from a consanguineous family. Collagen biochemical analysis was done on collagens I, III and V produced by cultured dermal fibroblasts. Punch biopsy of non-lesional skin was examined by electron microscopy (EM) for abnormalities of collagen fibrils. Mutation analysis was performed by direct sequencing of *COL5A1*..

Results: Two siblings had hypermobile joints, hyperextensible skin, bruising, atrophic scars and a history of inferior vena cava dissection at age 7 and mesenteric artery dissection age 8 respectively. Biochemical analysis suggested deficiency of type V collagen. EM showed numerous collagen cauliflowers. Sequencing of *COL5A1* detected a homozygous c.5317_5319del, p.(Glu1773del) mutation. A half-sibling had hypermobile joints and joint dislocation, the mother only hypermobile joints, and both had fewer collagen cauliflowers on EM. They were heterozygous for the familial mutation. A clinically unaffected sibling was negative for this mutation.

Conclusion: Homozygosity for a hypomorphic mutation in *COL5A1* is associated with a high risk of vessel dissection. Although heterozygote family members did not meet the clinical criteria for diagnosis, they have EM findings consistent with cEDS. This case widens the known phenotypes associated with mutations in *COL5A1*.

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P04.17A

Phenotypic heterogeneity of craniostenosis syndromes associated with coronal suture - 14 cases from 9 families

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Introduction: Craniosynostosis, premature fusion of cranial sutures, results in abnormal shape of head and dysmorphic facial features. Coronal synostosis is observed in 20–25% of patients with craniosynostosis and related mainly to *TWIST1* and *FGFR3* mutations resulting in Saethre-Chotzen and Muenke syndrome, respectively.

Patients and methods: We present phenotypic manifestation in 14 patients with mutations in *TWIST1* (9 patients, including 3 familial cases) and *FGFR3* (5 patients, 2 familial) genes in comparison to cases from medical literature. The *TWIST1* and *FGFR3* genes were analysed using classic Sanger sequencing technique and chromosomal rearrangements - using array CGH technique (CytoSure, ISCA 60K v2, OGT, hg18). In single case, clinical exome was sequenced with TruSight One (Illumina) panel.

Results: In two cases, a 7p21.1 deletion encompassing *TWIST1* gene was identified and in eight patients known point mutations in *FGFR3* (p.P250R) or *TWIST1* (c.G349T, c.417–438ins21, c.283_285dupAGC). In three families, a novel mutations p.Ser140Ter, c.177dupG in *TWIST1* and p.Cys613Phe in *FGFR3* were found.

Ten patients presented coronal suture synostosis, 5 of them had cranial surgery. Four patients had wide fontanelles without synostosis. All patients demonstrated dysmorphic features, 10 - skin syndactyly of digits 2/3, wide halluces and 4 - simian creases. Single patients had: congenital heart defects (2), speech delay (4), mild deafness (2), Arnold-Chiari malformation type I (1) and lacrimal ducts's agenesis (1).

Conclusions: Our results confirm presence of clinical heterogeneity observed in patients with *TWIST1* and *FGFR3* mutations, including familial cases. There were no significant clinical differences depending on gene and type of mutation.

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P04.18B

A de novo duplication on chromosome 1q22 in a patient presenting with craniosynostosis and psychomotor retardation

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Craniosynostosis refers to the group of craniofacial malformations characterized by the premature fusion of one or more cranial sutures. The disorder is clinically and genetically heterogeneous and occurs in a syndromic form or more frequently as an isolated trait. The significant proportion of cases is caused by genetic factors, however the molecular origin of 40–70% of craniosynostoses remains unknown.

Here we report on a sporadic male patient affected by complex craniosynostosis phenotype (metopic and unilateral lambdoid synostosis), muscular hypotonia, and severe psychomotor retardation. In order to identify the genetic cause of the disorder we performed high-resolution array based comparative genomic hybridization (array-CGH).

Array-CGH revealed the presence of a previously unreported de novo 1.25 Mb duplication at chromosome 1q22 encompassing a strong candidate gene, *LMNA* encoding A-type lamins. Mutations in *LMNA* cause a wide spectrum of disorders called laminopathies. Those include diseases of striated muscles, that could explain muscular hypotonia observed in our patient. Interestingly, homozygous missense mutation in *LMNA* gene is associated with delayed closure of the cranial sutures, an opposite phenotype to the craniosynostosis reported in our proband.

We hypothesize that the congenital defects presented in our patient result from either increased gene dosage of *LMNA* or its misexpression caused by the disruption of *LMNA* regulatory landscape. The exact pathomechanism underlying the complex phenotype of the presented patient is yet to be elucidated.

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P04.19C

Targeted sequencing of sagittal nonsyndromic craniosynostosis in candidate regions on chromosomes 3, 7, and 20

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Craniosynostosis (CS) is a common congenital malformation in which one or more of the infant skull sutures (metopic, coronal, sagittal, or lambdoid) fuse prematurely. Sagittal non-syndromic craniosynostosis (sNCS) is the most common type of CS, with an estimated prevalence of 2 per 10,000 live births. We previously performed the first genome-wide association study (GWAS) for sNCS, and discovered highly significant associations to a 120 kb region located 345kb downstream from *BMP2* on 20p12.3 and within a 167kb region intronic to *BBS9* on 7p14.3. Additionally, analyses of 52 imputed variants in *DLG1* on 3q29 were found to be genome-wide significant. In the current study, targeted Next Generation Sequencing (NGS) methods were used to sequence the regions on 3q29, 7p13.2, and 20p12.3, as well as two additional genes in close proximity to some of these associated regions (*BMP2* and *BMPER)* in 100 case-parent trios, in order to identify variants in the coding and noncoding regions responsible for the sNSC phenotype. Single nucleotide variants and insertions/deletions available from the NGS study were analyzed for *de novo* mutations, rare variants in coding regions, and over-transmission of rare variants in non-coding regions. Candidate variants present in more than one trio and predicted to be damaging to gene function are prioritized for functional studies in zebrafish.

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P04.20D

Clinical and molecular study of craniosynostosis in Bulgarian patients

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Craniosynostosis (CS), the premature fusion of one or more skull sutures, is a congenital anomaly occurring in 1 of 2,500 newborns. The majority of the patients present with an isolated nonsyndromic CS (NCS). The aim of this study is to investigate the genetic and non-genetic factors contributing to the risk of NCS in Bulgarian patients. A total of 39 patients (28 males and 11 females) with different types of CS were recruited and characterized through standardized clinical, epidemiological and molecular protocols. Based on clinical evaluation the patients were characterized as syndromic (9) or NSC (30). Epidemiological questionnaire was administered to collect data regarding past medical and family histories, lifestyle and possible environmental exposures. Patient's DNA was analyzed by Sanger sequencing for mutations in targeted "hot spot" regions of FGFR1 (exon 7), FGFR2 (exons 8 and 10), FGFR3 (exons 6 and 7) and the entire TWIST1 gene. A previously described pathogenic mutation c.1024T&gtC, p.C342R in exon 10 of FGFR2 was identified in a patient with clinical manifestation of Crouzon syndrome. Chromosomal microarray analysis is being considered for the remaining patients with syndromic CS. A subgroup of patients and their parents was included in a cohort of 415 case-parent trios with metopic NCS that are currently being analyzed by genome-wide association study (GWAS) in a search of new genes and loci, in the frame of the International Craniosynostosis Consortium. In conclusion, the etiologic deciphering of this birth defects requires coordinated and complex research efforts combining clinical, epidemiological, molecular and modern genomic approaches.

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P04.21A

Novel mutation in *HOXC13* expand the mutation spectrum of pure hair and nail ectodermal dysplasia

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Ectodermal dysplasias (EDs) are a large group of heterogeneous genetic disorders characterized by abnormal development in ectoderm-derived tissues and organs including skin, hair, and nails. Among the EDs, pure hair and nail ectodermal dysplasia (PHNED) is a rare genodermatosis characterized by nail dystrophy and sparse or absent hair on the scalp.

A family of Iranian origin was enrolled in this study. Two children from a consanguineous marriage are affected from PHNED. In addition, the father has alopecia areata (AA) but does not show any nail dysplasia. The mother is unaffected. The paternal and maternal grandfathers had nail dysplasia almost similar to the siblings but did not manifest any hair loss or AA. Homozygosity mapping and Genedistiller analysis were performed to identify candidate genes.

In total, we identified 10 homozygous regions with almost 700 candidate genes. Among these genes were also *KRT85* and *HOXC13*, already known to be related to the phenotype of our patients. Sanger sequencing showed a novel homozygous insertion of 28 bp in exon2 of *HOXC13*.

We identified an unknown mutation for PHNED which expands the spectrum of mutations for PHNED.

The hair loss of the father seems rather be due to a distinct type of hair loss, namely AA, which is quite common in the general population. However, the nail dysplasia from both grandfathers is unclear and cannot be examined anymore. It still remains unclear if the nail dysplasia in the grandfathers was due to the same mutation in *HOXC13* or is based on a different mutation.

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P04.22B

Vascular Ehlers Danlos syndrome as part of 2q32.2q33.1 microdeletion syndrome

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Introduction: Interstitial deletions involving 2q32q33 result in clinical features including learning disability, growth retardation, dysmorphic features, thin and sparse hair, feeding difficulties and cleft or high palate. We present two patients referred to the Ehlers Danlos Syndrome (EDS) National Diagnostic Service with 2q microdeletions that encompass *COL3A1* and *COL5A2* genes associated with vascular EDS and classical EDS respectively. Features of vascular EDS include arterial or hollow organ rupture, extensive bruising, thin, translucent skin and characteristic facial features. Major diagnostic criteria for classical EDS include; joint hypermobility, fragile hyperextensible skin and poor wound healing.

Material and Methods: Array CGH testing was performed due to developmental delay in these patients by local genetics services. Upon referral to the EDS National Diagnostic Service patients were clinically examined for features of classical and vascular EDS.

Results: These patients both have clinical features consistent with vascular EDS but neither fulfilled diagnostic criteria for classical EDS. Haploinsufficency of *COL3A1* has been well described as a disease mechanism whereas dominant negative effects have been the focus of *COL5A2* reports.

Conclusions: These cases highlight the importance of pre-test genetic counselling to prepare patients for possible incidental findings. Early identification of vascular EDS has been shown to lead to improved survival and quality of life for patients. Referral to specialist services ensures that patients are informed about the condition and signposted to appropriate surveillance and management. These cases also add evidence to the theory that null allele *COL5A2* mutations result in no or mild clinical phenotype.

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P04.23C

New variants in COL5A1 gene among 44 Polish patients with Ehlers-Danlos syndrome - analysis of nine cases

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Introduction: The Ehlers-Danlos syndrome (EDS) is a non-inflammatory, heritable connective tissue disorder. One of the subtypes of EDS, classic type (cEDS) is characterized by hypermobile joints, hyperextensibility of skin and widened atrophic scars, which are the major criteria of the Villefranche nosology. cEDS is caused mainly by mutations in *COL5A1*, encoding the type V collagen. Material and methods: The investigated group consisted of 44 patients, adults and children, from all regions of Poland. Patients presented clinical symptoms of classic type of Ehlers-Danlos syndrome, according to Villefranche nosology. Control group consisted of 100 healthy persons from general Polish population. Mutation analysis of selected parts of *COL5A1* gene was performed by Sanger sequencing. Results and conclusion: In 9 among all tested patients new mutations were detected, 8 were missense mutations, one was splice site mutation. They were not described to date in LOVD, ClinVar and HGMD databases. Pathogenicity of the mutations was evaluated using SIFT, PolyPhen-2, AlignGVGD, RNAfolding for missense mutations and Human Splicing Finder, NetGene2 for splice site mutation. All used tools indicated pathogenicity of the variants. For all found mutations control group was also tested. Among 100 healthy individuals variants were not found. Additionally, in 4 patients (9.09%) previously described alteration c.1588G>A (p.G530S) was detected. In control group frequency of this variant was assessed as 4%. This investigation was the first one in Poland on such a large scale and was the first one among cEDS patients in Central-East Europe.

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P04.25A

New splice-site mutation in KRT5 suspected to cause localized Epidermolysis Bullosa Simplex

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Background: Epidermolysis Bullosa Simplex (EBS) is characterized by fragility of the skin resulting in non-scarring blisters due to minor trauma and is caused mainly by mutations in the genes, KRT5 and KRT14. The phenotypic features of localized EBS are infancy-onset blistering of mainly the hands and feet, but blistering can occur anywhere if the trauma is significant.

Pathogenic variant detection rate in individuals with biopsy-diagnosed EBS is 75%. The genes are expressed in keratinocytes. Their protein products, keratin 5 and keratin 14, form heterodimeric molecules that assemble into the intracellular keratin intermediate filament network.

Methods and Results: We present a family with biopsy-diagnosed EBS and autosomal dominant inheritance. The phenotype is mild. Sequencing analysis of the *KRT5* and *KRT14* revealed heterozygosity for a splice variant (c.927 +2T>C) in *KRT5* in affected family members. The mutation has not previously been reported, but is predicted to cause a splice-variant of keratin 5.

RT-PCR of flanking sequences of the mutation followed by gel electrophoresis revealed a prolonged RNA transcript. Sanger sequencing of the cDNA showed that the point mutation resulted in aberrant splicing leading to incorporation of 30 intronic nucleotides into the mRNA transcript, which results in incorporation of 10 extra amino acids into keratin 5.

Conclusion: Our finding supports the prediction of the *KRT5* c.927+2T>C mutation to be pathogenic. The alteration is located at the terminal end of the coiled coli 1B and is predicted to affect polymer assembly and consistent with a dominant negative effect. The phenotype is mild and localized EBS.

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P04.26B

Combined approach for finding susceptibility genes for DISH&Chondrocalcinosis families

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Introduction: Twelve families with early onset of calcium pyrophosphate dehydrate (CPPD) chondrocalcinosis (CC) and diffuse idiopathic skeletal hypersostosis (DISH) were identified in Terceira Island, the Azores, Portugal.

Material and Methods: After clinical and radiological characterization, 92 individuals from 12 unrelated families, were selected for a combined gene-mapping strategy study. First, a whole genome wide linkage analysis was performed, then an identity-by-descent analysis in 10 individuals from 5 of the investigated pedigrees.

Results: The maximal LOD score obtained was for an area of chromosome 16 (D16S1000) with a LOD score of 1.32 (p=0.007). Because this area was not replicated by the IBD/IBS analysis, it was not further investigated. From the IBD/IBS analysis, two candidate genes, *LEMD3* and *RSPO4*, were selected for sequencing analysis. Nine genetic variants were identified in the *RSPO4* gene. No statistically significant differences in the occurrence of these genetic variants was observed in DISH/CC phenotype relative to the controls. However, two regulatory variants (rs146447064 and rs14915407) are significantly more frequent in controls than in DISH/CC patients (p=0.03). Four variants were identified in *LEMD3*; variant rs201930700 was further investigated through segregation analysis. The 10 genetic variants in *RSPO4* and in *LEMD3* did not segregate within the families studied.

Conclusions: The results of the present study revealed that two *RSPO4* gene regulatory variants, may have a protective role against the DISH/CC phenotype, possibly by altering gene expression of the *RSPO4* gene. Variant in *LEMD3* is extremely rare and its effect is difficult to ascertain at this point.

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P04.27C

High bone mass due to *LRP5* and *AMER1* gene defects - two patients with novel mutations

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High bone mass (HBM) refers to excessive bone accumulation. Here we describe two patients with novel mutations in *LRP5* or *AMER1*, leading to increased WNT signaling and subsequently substantial bone mass and skeletal complications.

Patient I

A 51-year-old male with severe osteopetrosis (DXA T-scores + 7.2 – +10.1). He has undergone several surgeries for complications of cranial hyperostosis (narrow ear canals, bilateral jaw exostoses, and mandibular tori). Radiography showed cranial hyperostosis and long bone cortical thickening. Bone formation marker PINP was elevated. Genetic studies revealed a novel gain-of-function mutation c.592A > T (p.N198Y) in *LRP5* (LDL Receptor Related Protein 5).

Patient II

A 16-year-old female with osteopathia striata and cranial sclerosis (OS-CS). She has marked macrocephaly (head circumference +6 SD), and dysmorphic features (triangular face, micrognatia, and hypertelorism). Broncoscopy revealed laryngomalasia and subglottic stenosis. Motor development was delayed and she later developed epilepsy. Radiography showed severely thick, sclerotic, and longitudinally striated long bones. Biochemistry was normal. Genetic studies identified a novel *de novo* X-chromosomal heterozygous frameshift mutation c.655del (p.G219Afrs*63) in *AMER1* (APC Membrane Recruiting Protein 1).

Conclusions: WNT signaling is essential to bone health and defective WNT signaling leads to skeletal disorders. LRP5 is a co-receptor for WNT-ligands and *LRP5* mutations can cause HBM. AMER1 enables degradation of intracellular β-catenin and thus down-regulates WNT target gene expression and can cause OS-CS. These two novel mutations highlight the crucial role and pathogenic effect of increased WNT signaling in skeletal development.

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P04.28D

Holt-Oram syndrome: clinical and molecular insights

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Holt-Oram Syndrome (HOS) is an autosomal dominant condition characterised by the association of radial and congenital heart defects (CHD), with or without conduction disturbances, due to *TBX5* mutations. Within syndromic radial dysplasia, HOS is a challenging diagnosis by its variability of expression and the large phenotypic overlap with other conditions, like Okihiro syndrome (*SALL4* mutations), TAR syndrome or Fanconi disease.

In this study, 228 patients addressed for suspicion of HOS between 2002 and 2014, and whose *TBX5* gene has been studied by Sanger sequencing and MLPA, have been retrospectively reviewed. A *TBX5* mutation has been identified in 78 patients, forming the largest molecular series ever described. The patients' phenotypes were sorted in 2 categories: "typical" (with or without heart defect) or "uncertain". 22% of the mutated patients had an "uncertain" presentation. The genotype-phenotype study highlights the importance of some critical features in HOS: the bilateral and asymmetric characteristics of the radial defect, shoulder or elbow mobility defect and the septal characteristic of the CHD. Thorax, spinal or lung deformities do not rule out the HOS hypothesis. Besides, 21 patients presented with another overlapping condition, including 13 "typical" presentations. Thirty percent of the typical patients had no *TBX5* or *SALL4* mutations.

We propose a molecular workflow in HOS and overlapping conditions diagnostic approach, based on the results of an exhaustive clinical examination and systematic complementary evaluations. Finally we discuss the different strategies that can be adopted to improve the molecular delineation of the remaining "typical" patients.

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P04.29A

A family sample for primary focal hyperhidrosis

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Introduction: "Sweating" in a narrow sense exists solely in a few groups of mammals, namely equines and primates. Hyperhidrosis is a condition characterized by excessive sweating. Two forms are described, 1, primary hyperhidrosis with causation unknown so far, and 2, secondary hyperhidrosis caused by organic malfunction such as hormonal imbalance. Subjects affected by hyperhidrosis may suffer from psychosocial stress due to ostracism or self-imposed avoidance of physical contact, and subsequently experience depressive symptoms, as we have demonstrated recently. Here, we focus on primary focal hyperhidrosis (OMIM %114110), which is characterized by excessive sweating at distinct body regions, for example palms, feet, or face.

Material and Methods: A total of 15 multiplex families with 78 affected and 102 non-affected subjects were recruited. Diagnoses were made by experienced clinicians at the *Deutsches Hyperhidrosezentrum*, Munich. DNA samples await genotyping and Next Generation Sequencing (NGS) strategies to identify causative mutations.

Results: Preliminary results of our study using microsatellite markers to cover previously described candidate loci (Higashimoto et al., 2006; Chen et al., 2015) point to confirmation of linkage to chromosomal regions 2q31.1 and 14q11.2 - q13.

Conclusions: Primary focal hyperhidrosis is a genetically heterogeneous hereditary condition with at least two chromosomal loci involved, and follows an autosomal-dominant pattern of inheritance. Linkage studies and NGS will be performed to identify causative genetic variants.

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P04.30B

A novel *EDA* gene mutation in two Turkish brothers with X-linked hypohidrotic ectodermal dysplasia

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Introduction: Ectodermal dysplasia is a rare congenital hereditary disorder characterized by deficiency in development of structure derived from the ectoderm. The disease encompasses more than 200 clinically distinct features, which affect the skin, hair, nails, teeth, sweat glands, and sebaceous glands. Phenotypes caused by mutations in the genes *EDA, EDAR*, or *EDARADD* have similar clinical features with different inheritance patterns. Mutations in *EDA* gene cause X linked Hypohidrotic Ectodermal Dysplasia (HED), which is the most common form of the ectodermal dysplasia. HED is characterized by the typical triad that includes hypodontia, hypotrichosis and hypohidrosis that can lead to hyperthermia episodes. Affected males typically exhibit all the typical features of HED, but heterozygous carriers may show mild to moderate clinical manifestations. Materials and Methods: Two affected brothers applied to our clinic with the complaints of HED with a chief complaint of missing tooth in upper and lower anterior region. For mutation analysis, the coding region of *EDA* of 2 patients was sequenced.

Results: We report two affected siblings with HED with a novel heterozygous mutation, c.641_668del in the *EDA* gene. To our knowledge, the mutation we describe has never been reported. The mutation was located in exon 4 and consisted of a frame-shift mutation at codon 215, which gave rise to an abnormal protein with a premature stop codon after 56 residues. Conclusion: Genetic analyses in families with HED are useful for checking carrier status, but they also provide information for genetic counseling and prenatal diagnosis.

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P04.31C

The COL1A2 SNP rs42524 is associated with adult Hypophosphatasia in heterozygotes for *ALPL* mutations

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Dominant inheritance of hypophosphatasia (HPP), a rare skeletal dysplasia, is assumed to be due to the dominant negative effect (DNE) of missense mutations deriving from the functional homodimeric structure of TNSALP, the protein encoded by the *ALPL* gene. However, there is no biological argument excluding other causes of dominant HPP, for instance haploinsufficiency. We determined *in silico* that 97% of the mutations with an experimentally proved DNE affected residues of particular domains of TNSALP (DNE domains), allowing to define 2 classes of mutations, class 1 (possible DNE) and class 2 (no DNE). Then we localized *in silico* the mutations of 124 patients with various forms of dominant HPP. Class 2 mutations were found in 50% of adult patients (26/52) but only 19% of patients with other forms of dominant HPP, suggesting that a large subset of adult dominant HPP is due to mutations presumably with no DNE. These patients had a mean AP level and an age of first symptoms slightly higher. We looked by NGS for coding SNPs that could contribute to haploinsufficiency. We detected in adult HPP patients an association between class 2 alleles and the GG genotype of the SNP rs42524 in COL1A2 ($\chi 2$ test, $p=1.8\ 10^{-4}$). In conclusion our results suggest that dominant adult HPP may be due either to DNE or to another mechanism, presumably haploinsufficiency triggered by the GG genotype in COL1A2 gene, suggesting that this gene is a modifier gene

of HPP. This result need to be confirmed with a larger cohort.

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P04.33A

Homozygous p.Arg1014Cys mutation in *COL1A1* may be responsible for a more severe form of infantile cortical hyperostosis (Caffey disease)

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Infantile cortical hyperostosis, or Caffey disease (OMIM 114000) is a disorder characterised by subperiosteal bone proliferation associated with an inflammatory process. Signs and symptoms appear spontaneously in the first months of life and the course is generally self-limiting. The disease is caused by a recurrent heterozygous *COL1A1* mutation and is usually transmitted in an autosomal dominant pattern. Until now, the same heterozygous mutation has been identified in several unrelated families. We now present an 11-year old male patient from a family with 10 individuals diagnosed with Caffey disease. The boy was born with bone lesions that were already observed *in utero* during prenatal ultrasonographic examination. Inflammatory process was severe, but it regressed gradually over several years. At this point the patient requires orthopaedic surgeries to correct deformities of lower extremities. The previously described heterozygous missense mutation c.3040C > T (p.Arg1014-Cys) in *COL1A1* was detected in the affected members of this family. However, the patient was found to be homozygous for the mutation. Detailed family history revealed that homozygosity is most likely due to consanguinity. We assume that the more severe phenotype of the disease is associated with homozygosity for the mutant allele. This is most likely the first report of a homozygous p. Arg1014Cys *COL1A1* mutation in a patient with Caffey disease.

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P04.34B

Diagnosis implications of the whole genome sequencing in a large Lebanese family with hyaline fibromatosis syndrome

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Background: Hyaline fibromatosis syndrome (HFS) is a recently introduced alternative term for two disorders that were previously known as juvenile hyaline fibromatosis (JHF) and infantile systemic hyalinosis (ISH). These two variants are secondary to mutations in the anthrax toxin receptor 2 gene (*ANTXR2)* located on chromosome 4q21. The main clinical features of both entities include papular and/or nodular skin lesions, gingival hyperplasia, joint contractures, and osteolytic bone lesions that appear in the first few years of life, and the syndrome typically progresses with the appearance of new lesions.

Methods: We describe five Lebanese patients from one family, aged between 28 and 58 years, and presenting with nodular and papular skin lesions, gingival hyperplasia, joint contractures and bone lesions. Because of the particular clinical features and the absence of a clinical diagnosis, Whole Genome Sequencing (WGS) was carried out on DNA samples from the proband and his parents.

Results: A mutation in *ANTXR2* (p.Gly116Val) that yielded a diagnosis of HFS was noted. **Conclusions:** The main goal of this paper is to add to the knowledge related to the clinical and radiographic aspects of HFS in adulthood and to show the importance of Next-Generation Sequencing (NGS) techniques in resolving such puzzling cases.

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P04.35C

STAT4 gene polymorphism is associated with polyarticular juvenile idiopathic arthritis in Belarusian patients

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Introduction: Juvenile idiopathic arthritis (JIA) is a multifactorial disease predominating in pediatric rheumatology, with varying prevalence among different geographically distinct populations. As understanding of the disease mechanisms and genetic predisposition is still insufficient, the investigation of molecular-genetic basis of the JIA could shed some light on this problem.

Materials and Methods: A total of 94 patients diagnosed with JIA (mean age 8.78±5.21, 66% females), 95 children with articular syndrome (mean age7.90±4.81, 43% females) and 164 hospital controls with no sign of autoimmune or inflammatory diseases (mean age13.99±2.68, 51% females) were recruited to this study. The JIA patients were divided into subgroups according to IIAR classification criteria. The samples were genotyped for the *STAT4* rs7574865 and *CTLA4* rs5742909 using PCR-RFLP.

Results: There were no differences between the frequencies of the *CTLA4* rs5742909 alleles and genotypes across all three groups. On the contrary, the minor T allele of *STAT4* rs7574865 was associated with polyarticular JIA when compared with hospital controls (p=0.03 and p=0.01 for genotypes and alleles accordingly). Besides, TT genotype was 8,4 times more frequent in polyarticular JIA patients (p=0.004; OR=9.96; 95% CI=[1.53–65.00]) and the frequency of the risk T allele was also 2,3 times higher (p=0.001; OR=3.37; 95%CI=[1.56–7.28]) when compared with the group of children with other articular pathology.

Conclusions: *STAT4* rs7574865 polymorphism demonstrated a subtype-related association with JIA owing to increased frequency of the minor T allele in patients with polyarticular JIA as compared to both hospital controls and other articular pathology.

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P04.36D

A dominant-negative mutation in the BTB domain of KCTD15 in a family with frontal lipoma, congenital heart disease and cutis aplasia of the scalp defines a novel syndrome

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The BTB (Broad-complex, Tramtrack and Bric-a-brac) domain proteins have diverse functions that include potassium channel transport, DNA replication, protein degradation and transcriptional repression. Heterozygous mutations in BTB domains within the potassium-channel tetramerisation domain-containing proteins (KCTD) were previously described in scalp-ear-nipple (SEN) syndrome (*KCTD1*), characterised by cutis aplasia of the scalp and anomalies of the external ears, digits/nails, and breasts, and myoclonus-dystonia (*KCTD17*). We describe a father and daughter with a distinct phenotype combining large frontal lipoma, cardiac defects (tetralogy of Fallot or patent ductus arteriosus) and cutis aplasia of the scalp. In the father we identified, by trio exome sequencing, a *de novo* heterozygous c.310G > C (p.Asp104His) mutation in *KCTD15*, the closest paralogue of *KCTD1*, that was transmitted to his affected daughter. We show *in vitro* that the p.Asp104His substitution destabilises the pentameric assembly of both KCTD15 homodimers and KCTD15-KCTD1 heterodimers by interaction via their BTB domains, indicating a specific dominant negative mode of action. Modelling based on the available KCTD1 structure predicts that this is caused by disruption of key intermolecular contacts made by the conserved Asp104 residue within the pentamer. KCTD15

was previously demonstrated to repress the neural crest transcription factor AP2, and we are currently investigating the effects of the mutation using transactivation assays. This is the first report of a mutation identified in KCTD15, highlighting the importance of the Asp104 residue in maintaining the correct structure and function of the protein, and the critical role played by KCTD15 in tissues of neural crest and ectodermal origin.

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P04.37A

Lethal skeletal dysplasias: molecular investigation in a series of 62 fetus, including a novel phenotype

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Introduction: Around 50 skeletal dysplasias (SD) are lethal or semi-lethal. The precise diagnosis for this group of SD is necessary for the correct genetic counseling and consequently for reproductive decisions, including PGD. While the radiological diagnosis is sufficient for the most frequent lethal SD (ex: Tanathophoric D, and OI-IIA), usually for the most rare types the molecular investigation is necessary for the differential diagnosis. Here we present the results of a molecular investigation in a cohort of 62 fetus.

Materials and Methods: We used both, the classical Sanger sequencing (41 cases) and the NGS technology for the remaining (21 cases). For the NGS we used a custom panel (TruSeq Custom Amplicon - Illumina) including 39 genes. All mutations found by NGS were confirmed by Sanger sequencing. We also used WES for studying two novel phenotypes.

Results: The molecular investigation confirmed the radiological diagnosis in 54 fetus (87%). The most frequent diagnosis were: Tanathophoric D (25), collagenopathies type-2 (8), ciliopathies (7), and Campomelic D (7). The two fetus with a novel phenotype are still under the exome analysis. Although these two fetus present a clinical phenotype of achondrogenesis (hydrops with severe micromelia), the radiological findings of both fetus are identical and not related to any known skeletal dysplasia.

Conclusions: The mutation rate detection was very high (87%), and in all defined cases except one, the molecular analysis confirmed the radiological diagnosis. Only in one fetus the radiological evaluation was doubtful (Torrance D).

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P04.38B

Identification of binding partners of the LPAR6 protein involved in hypotrichosis simplex

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Introduction: Hypotrichosis simplex (HS) is a genetically heterogeneous hair loss disorder characterized by progressive hair loss. Mutations in more than 10 genes have been identified for HS, including the *LPAR6* gene encoding for the human lysophosphatidic acid receptor 6 (LPAR6). This protein belongs to the same signalling network of lipase H (LIPH), which is also a causative gene for HS. Considering the importance of this pathway in the determination of proper hair growth, we speculated that more genes related to the same pathway could be a cause of HS. Therefore, our goal was to identify new interactors of LPAR6.

Materials and methods: Membrane yeast two hybrid (MYTH) assay and bait dependency test were performed to spot putative binding partners (BPs) of LPAR6. The results revealed 72 putative interactors from a skin/hair follicle library. The interaction was further assessed by pull-down assay, to discard false positive results and confirm the real interactions between LPAR6 and the candidate proteins.

Results: Six true BPs have already been confirmed, including the U11/U12 small nuclear ribonucleoprotein 35 kDa protein (SNRNP35), the 40S ribosomal protein S28 (RPS28), the emopamil-binding protein (EBP), the transcription factor AP-1 (JUN), the lysosomal amino acid transporter homolog 1 (PQLC2) and the lymphocyte antigen 6 complex locus D (LY6D). Furthermore, many false positive BPs could be discarded.

Conclusions: We were able to identify some of the BPs of LPAR6. Many more putative interactors have been cloned and await to be studied.

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P04.39C

Diagnostic value of NGS for primary lymphedema in Slovene patients

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Background: Primary lymphedema is a rare, genetically diverse disease. Since next generation sequencing (NGS) is an ideal approach for the diagnosis of such diseases, we wanted to establish a diagnostic value of NGS in a cohort of Slovene primary lymphedema patients.

Methods: Index patients of five unrelated Slovene families with pedigree typical for primary lymphedema (lymphedema of upper and/or lower extremities and/or neck region; autosomal dominant pattern of inheritance) were sequenced with clinical exome sequencing. We searched for mutations in coding regions of genes associated with lymphedema (HP:0001004). Synonymous variants and variants with the frequency exceeding 1% in a control population (esp6500) were not analyzed. The mutations found were confirmed with Sanger sequencing (in patients and additionally in at least one of the affected family member(s)).

Results: The female to male ratio of our index cases were 2/3. Mean age of lymphedema presentation was at birth, however the oldest patient was 25 years old, when she first got lymphedema symptoms. In 2 patients an already described, missense mutation causative for primary lymphedema, was identified in the FLT4 gene (c.3489G>A). Two affected family members of the index cases, one from each family, were also harboring this mutation. In three patients, we were not able to identify any mutation associated with primary lymphedema.

Conclusions: With clinical exome sequencing, we were able to identify the genetic cause in 40% of patients in a cohort of well-defined Slovene primary lymphedema patients.

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P04.40D

Genotypic and phenotypic evaluation of 40 patients with marfan syndrome: 7 novel mutations

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Marfan syndrome is an autosomal dominant genetic condition that affects the connective tissue in many parts of the body. Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems. The diagnosis of Marfan syndrome relies on defined Ghent criteria, outlined by international expert opinion to facilitate accurate recognition of this syndrome and to improve patient management and counselling. However, it may not be possible to make definitive diagnosis according to these criteria in each patient, thus molecular genetic confirmation is necessary in such group of patients. Marfan syndrome is caused by mutation of *FBN1*. Here we review clinical and genetic evaluation of 40 patients with marfan syndrome diagnosed at our center between 2014 and 2017. We identified mutations that were not previously reported in the literature in 7 of these patients. There are very few studies on the genotype-phenotype correlation of Marfan syndrome and no clear genotype-phenotype association has been shown to date. We have identified c.5917+5 G>A and c.5917+5 G>C heterozygous mutations of the same locus in two different patients. And we found that both patients showed a more severe clinical phenotype than the other patients. We think that these newly detected mutations we described will be a positive contribution to the literature.

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P04.41A

Quantitative genetics of Marfan syndrome

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Marfan syndrome (MFS) is a connective tissue disorder with an autosomal dominant inheritance which mainly affects cardiovascular, skeletal and ocular systems. It displays considerable inter- and intra-familial clinical variability, but its environmental are genetic determinants are still poorly known. The contribution of inherited factors to this variability has not been quantified yet, and might be different for each clinical feature.

In a cohort of 1306 MFS well-phenotyped patients carrying *FBN1* mutation, we analyzed the distribution of 23 clinical features and found strong correlations between those interesting the same system, suggesting that they have common underlying determinants. On the other hand, features from different systems appeared to be largely uncorrelated.

Moreover, classical quantitative models were adapted to estimate heritability of clinical features in MFS and to screen the influence of the major locus *FBN1* and the existence of dominant effects. Most clinical features showed strong familial aggregation and high heritability. Only *Ectopia lentis* showed a significant contribution of the major locus on the phenotypic variance. Among MFS patients whose transmitting parent had aortic surgery or dissection, we found that the prevalence of aortic surgery or dissection is significantly higher in the case of maternal transmission. This phenomenon is a further argument to support genetic susceptibility for cardiovascular phenotype in MFS.

Taken together, our results encourage further research to identify probable genetic modifiers.

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P04.43C

Novel *TRPV4*-mutation causing severe metatropic dysplasia

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Background: Metatropic dysplasia is a skeletal dysplasia characterized by craniofacial dysmorphia (frontal bossing and midface hypoplasia), thorax abnormalities (short ribs and development of severe scoliosis/kyphosis) and short limbs (metaphyseal abnormalities result in short limbs and prominent joints) and may present with a tail-like coccygeal appendage.

It is caused by mutations in the *TRPV4*-gene. *TRPV4* is implicated in autosomal dominant diseases in two systems - the skeletal and the peripheral nervous system demonstrating an extraordinary pleiotropism of *TRPV4* mutations.

TRPV4 is expressed in many tissues and is required for normal development of bone and cartilage.

Material and methods: We present a two years old girl born with a prominent forehead, short neck, small bell-shaped thorax, short limbs with prominent joints, long hands and feet, and a tail-like coccygeal appendage. The narrow thorax necessitates respiratory support. Spinal stenosis due to columnar deformity has resulted in partial tetraplegia despite decompression surgery.

The severe phenotype raised suspicion of metatropic dysplasia and the entire coding region of *TRPV4* and flanking intronic regions were sequenced by direct sequencing of DNA extracted from a blood sample.

Results: A novel heterozygous missense variant in exon 5 of *TRPV4* (c.838G>A, p.Gly280Ser) was detected. The variant is located in a highly conserved nucleotide and amino acid position. Software prediction predicts the variant to be probably damaging. The parents do not carry the variant in blood.

Conclusion: We believe the c.838G>A variant in *TRPV4* is the cause of severe metatropic dysplasia in this patient. The mutation has not previously been reported.

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P04.44D

Cutaneous mosaic syndromes associated with early postzygotic activating *BRAF* mutations

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Background: Postzygotic *BRAF* variants result in congenital linear syringocystadenoma papilliferum (SCAP) or phacomatosis pigmentokeratotica (PPK). We previously identified a *BRAF* p.Lys601Asn variant in PPK.

Methods: We performed targeted and whole exome sequencing (WES) in DNA from affected skin in two patients with cutaneous mosaicism. Patient 1 had a right-sided congenital SCAP and aplasia cutis of the scalp and forehead, a vascular tumour and coloboma of the eyelid, an epibulbar dermoid and an intracerebral vascular aneurysm resulting in early stroke, hemiparesia and seizures. Patient 2 had PPK with a right-sided epidermal nevus of the neck and arm, extensive café-au-lait macules of upper and lower limbs, with superimposed agminated melanocytic naevi on her left arm, and an extensive speckled lentiginous naevus on her left leg.

Results: In both patients, *HRAS/KRAS/NRAS* targeted sequencing was negative. Using WES, we identified in patient 1 a postzygotic *BRAF* p.Val600Glu variant in 31% of reads, already reported in SCAP. In patient 2, *BRAF* targeted sequencing identified a postzygotic *BRAF* p.Gly596Arg variant in 3% of reads. Both variants were not found in blood.

Conclusions: *BRAF* p.Val600Glu has been reported in SCAP, possibly associated with ocular coloboma or cerebral anaplastic astrocytoma. In addition, our patient had vascular cerebral involvement. *BRAF* p.Gly596Arg had not been reported in mosaic syndromes, but was found in melanoma, myeloma, colon, and bladder cancer. Both mutations have never been reported in the germline.

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P04.45A

The challenge of mosaicism

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Introduction: Mosaicism has implications for clinical genetics practice. Depending on which tissues harbor the variant and to what extent, mosaic individuals may be affected, display a milder phenotype, or exhibit no signs of the related genetic disease. The associated risk for offspring may be as high as 50%.

Materials and Methods: Here we present three cases identified on DNA extracted from blood by next-generation sequencing (NGS) targeted for connective tissue disorders. Patient #1 (61-year-old man) had a thoracoabdominal aortic aneurysm. Patient #2 (78-year-old man) presented with aortic valve insufficiency and aortic dilatation (from the root to the arch). Patient #3 (29-year-old woman), with a family history of aortic root dilatation, displayed joint hypermobility and reported two preterm deliveries.

Results: Patient #1 carried in $12/286 = 4\%$ of the reads a likely disease-causing *ACTA2* variant previously identified in his son, who suffered from aortic dissection aged 26. Sanger sequencing had failed to detect this variant, which was filtered out by the NGS evaluation pipeline. Patient #2 carried a likely disease-causing mosaic *NOTCH1* variant (10.5% of the reads). Patient #3 carried a mosaic *TGFBR1* variant of uncertain clinical significance (13.5% of the reads).

Conclusions: mosaicism should be considered in cases of inconsistent cosegregation (e.g. affected parent of proband with apparent *de novo* variant, or healthy parent who carries the proband's variant). Moreover, low-level mosaicism may be missed by Sanger sequencing and filtered out during NGS analysis. Finally, one should avoid testing first a potential mosaic individual when assessing a family with multiple affected members.

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P04.46B

Pathophysiology of large subcutaneous hematomas in Musculocontractural EDS-*CHST14*: an iPS cells-based comprehensive investigation

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Dermatan 4-*O*-sulfotransferase-1 (D4ST1) deficiency caused by loss-of-function mutations in *CHST14* represents a clinically recognizable form of Ehlers-Danlos syndrome (EDS), named as musculocontractural type EDS-*CHST14*. It is clinically characterized by multiple congenital malformations (craniofacial features, multiple congenital contractures) and progressive multisystem fragility-related complications (skin hyperextensibility and fragility, recurrent dislocations and progressive talipes or spinal deformities, large subcutaneous hematomas). Pathological and glycobiological studies on affected skin specimens suggested multisystem fragility to be caused by impaired assembly of collagen fibrils resulting from loss of dermatan sulfate in the decorin glycosaminoglycan side chain that promotes electrostatic binding between collagen fibrils. Large subcutaneous hematomas are frequent and one of the most serious complications, typically occurring after minor traumas, spreading in several hours with severe pain, and sometimes accompanying hemorrhagic shock. Hypothesizing that these large subcutaneous hematomas would be attributable to impaired contraction followed by rupture of small-sized arteries caused by the structural fragilities, we performed induced-pluripotent stem cells (iPSCs)-based pathophysiological studies. After validating undifferentiation status and pluripotency of iPSCs derived from cultured skin fibroblasts of three patients and healthy subjects, vascular smooth muscle cells (VSMCs) were induced. Significantly reduced contraction of VSMCs from affected iPSCs was observed after stimulation by a muscarinic agonist or a calcium agonist, compared with VSMCs from normal iPSCs. Impaired vascular formation was observed on a Matrigel where affected iPS-derived VSMCs were transplanted, compared with normal iPS-derived VSMC transplantation. These results would support the hypothesis, which also implicates an indispensable role of DS in the maintenance of arterial structure in humans.

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P04.47C

The nightmare of pediatric orthopedic surgeons: cafe -au-lait spots

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, manifested with neurofibromas and cafe´-au-lait spots. NF1 present tibial dysplasia, with anterolateral bowing of the leg and subsequent fracture and nonunion (pseudarthrosis). In our study, we present two cases of congenital pseudarthrosis of tibia (CPT) that suffered multiple surgeries before the correct diagnosis was completed. The management of pseudarthrosis of the tibia is one of the most challenging problems in pediatric orthopedics. The treatment aims to obtain a long term bone union, to prevent limb length discrepancies, and to avoid pathological fracture. Surgery is based on: pseudarthrosis resection, biological bone bridging of the defect and the correction of angular deformity. The most common complication is refracture. Both our cases have been operated for 4, respective 5 times before obtaining a good result. This is

why café-au –lait spots should require a multidisciplinary approach with meticulous clinical examination and genetic counseling that might lead to an earlier diagnosis of NF1 and CPT.

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P04.48D

High success in the etiology determination of skeletal dysplasias from a custom NGS panel and identification of a gene related to Beemer-Langer syndrome

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Introduction: The huge breakthroughs driven by next generation sequencing (NGS) technology, allowed not only identifying a great number of new genes associated with skeletal dysplasias (SD) in the last years, but also to fast reaching a precise diagnosis in the practical clinical. However, most of the reported results so far have shown a detection rate of mutation around or less than 50%. Here we present the results of a cohort of 37 previously and carefully classified patients with SD, for which we used the NGS technology to establish the precise diagnosis.

Materials and Methods: 37 local patients with SD were analyzed on the MiSeq sequencer using a customized NGS panel (TruSeq Custom Amplicon - Illumina) with 39 genes related to SD. Sanger sequencing confirmed all pathogenic variants.

Results: We identified a total of 32 different pathogenic variants (22 novel) in 26 patients giving a detection rate of 70.3%. In 14 lethal SD and 23 non-lethal patients we found the following detection rates - 78,61% and 60,9%, respectively. One of the identified mutations (*PCYT1A*) was only found by Sanger sequencing of a bad covered region. Another interesting and novel result was the identification of a cilia-related gene associated with Beemer-Langer syndrome.

Conclusions: Our results showed a high detection rate (70,3%) of mutations using NGS; a novel association with a cilia-related gene and Beemer-Langer syndrome; and we put in evidence the importance to analyse by Sanger sequencing the bad covered regions by NGS.

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P04.49A

Molecular characterization of patients with Neurofibromatosis Type1

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Introduction: Neurofibromatosis Type I is one of the most common autosomal dominant disorders. It is caused by mutations of the *NF1* gene. The aim of the current study is to identify the genetic causes underlying the disease and possible phenotype/genotype correlations. Materials and Methods A protocol based on genomic DNA was established in 135 patients fulfilling the NHI diagnostic criteria and in 32 children (<4 years old) strongly suspected of having NF1. It included multi-step PCR and sequencing of all exons and adjoining introns. In several cases deletions/duplications were detected by high resolution aCGH and MLPA. Novel findings were evaluated with bioinformatic tools and family segregation analysis. Results We identified the germline mutation in the majority of cases; 87 known and 66 novel variants in coding and non-coding regions. We also found three unrelated patients with whole and partial gene deletion. Partial gene duplication was found in one patient with B- Acute Lymphoblastic Leukemia. Various mutations were identified in 80% of children under the age of 4 years with only multiple café-au-lait macules (CALM) as a reason for referral. Different clinical manifestations were observed in two brothers with the same mutation (p.Tyr2264X). Conclusions Reduced penetrance of specific mutations suggests the implication of modifier

genes, epigenetic or environmental factors. Genetic testing of children with only CALM is recommended in order to exclude or establish clinical diagnosis. Understanding the nature of an identified novel mutation along with *in silico* analysis is essential for genetic counseling and clinical management.

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P04.50B

Next generation sequencing of osteogenesis imperfecta related genes in Slovene patients

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Introduction: Osteogenesis imeperfecta is characterised by bone fragility frequently associated with blue/gray discoloration of the sclera and dentinogenesis imperfecta. The disease is classified in several types according to the severity of the bone fragility or genetic aetiology. OI is genetically heterogeneous disease with dominant mutations in *COL1A1* and *COL1A2* being the most common genetic cause. Molecular genetic testing of OI is important, as it can prompt the treatment.

Materials and Methods: 15 subjects (3 males, 12 females) aged between 1 month and 18 years were evaluated at a tertiary paediatric outpatient clinic due to the bone fragility and referred to genetic testing with next generation sequencing (NGS). Fourteen patients had clinically mild OI, one had clinically severe OI. All had blue sclera and none had additional hearing loss or dentinogenesis imperfecta. We performed targeted NGS with TruSightOne Sequencing Panel on the MiSeq platform (Illumina, USA) followed by interpretation of variants in the OI associated genes (*ALPL, BMP1, COL1A1, COL1A2, CRTAP, FKBP10, IFITM5, LEPRE1, LRP5, PLOD2, PPIB, SERPINF1, SERPINH1*, SP7) and subsequent Sanger sequencing confirmation.

Results: Ten different mutations in two genes (*COL1A1* and *COL1A2*) were detected in ten patients (66% success rate). Among them, two variants, namely *COL1A1*, NM_000088.3:c.1853delC (p.Ala618ValfsTer148) and *COL1A1*, NM_000088.3:c.740C>T (p.Pro247Leu), were not previously reported in OI patients.

Conclusion: NGS enables fast and reliable identification of causal mutations in several genes related to OI simultaneously. Presented subject group had mutations in genes commonly associated with OI, where each patients had his own private mutation.

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P04.51C

Implementig non-invasive prenatal diagnosis of achondroplasia on free fetal DNA

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Achondroplasia (ACH) is the most common autosomal dominant non-lethal skeletal dysplasia with an incidence of 1 in 6,500 – 20,000 live births. Non-invasive prenatal diagnosis (NIPD) of ACH is an ideal application of cell-free fetal DNA (cffDNA) analysis because in about 99 % it is caused by a single mutation (G380R) mostly arised *de novo (*in approximately 80 % of cases), and there is a correlation with advanced paternal age.

Methods: cffDNA occurs in maternal plasma in form of short fragments. By targeting the shorter DNA molecules, cffDNA was isolated from maternal plasma by the QIAamp DNA Mini Kit and Clean Circulating DNA Kit. Quality of the cffDNA isolation was evaluated by automate capillary electrophoresis on Fragment AnalyzerTM. For confirmation of cffDNA in a sample, we evaluated three approaches: fetal gender determination, comparison of SNPs in maternal and fetal genome, and methylation analysis. In cooperation with TIBMolBiol, we designed HRM-based LightSNiP assays to detect the G380R. Real-Time PCR is performed by using the DyNamo ColorFlash Probe qPCR Kit, Xceed qPCR Probe 2x Mix No-ROX and SensiFAST™ Probe No-ROX Kit.

Results: Based on our results, we concluded, that the cffDNA isolation performed by the evaluated kits does not differ in cffDNA yield. We concluded that there is no significant difference between performances of three Real-Time PCR master mixes used.

Conclusions: As the NIPD of ACH does not bring any danger for the mother or the fetus, it can be offered as a screening test for pregnancies where the father is older.

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P04.52D

A novel frameshift mutation causing NOG-symphalangism spectrum disorder (NOG-SSD)

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The NOG gene encodes noggin, a negative regulator of bone morphogenic proteins (BMPs), transcription factors important for development of nerve tissues, muscles and bones. Noggin is implicated in morphogenesis and body patterning, middle ear formation, and apoptosis in digital and interdigital regions. Heterozygous mutations in NOG are associated with multiple disorders, such as stapes ankylosis with broad thumbs and toes (SABTT), proximal symphalangism 1A, multiple synostoses syndrome, and carpal-tarsal coalition. Prior to gene identification, these disorders were previously classified as distinct conditions. These conditions are now collectively known as NOG-related symphalangism spectrum disorder (NOG-SSD). However, genotype-phenotype correlation is unclear.

We review the NOG-SSD literature, and report a 17 year old male patient with a novel heterozygous truncating mutation in NOG, c.155dupC, p.(Ile53Tyrfs*3). Our patient has many symptoms of the NOG-SSD, including a hemicylindrical nose, stapes ankylosis with conductive hearing loss, hyperopia, fused cervical vertebrae, 5th digit symphalangism, and cutaneous syndactyly. In addition, he has 13 ribs and thoracic-type vertebrae, and spina bifida occulta, which to our knowledge, have not been previously reported in this condition.

Our report provides further expansion of the understanding of NOG-SSD. While our patient's presentation could be classified as SABTT, symphalangism is also present, which was classically thought to be absent in SABTT. His features overlap several NOG related conditions, further supporting that NOG mutations can result in a spectrum of craniofacial and skeletal manifestations, and that not all patients fit within previously described discrete phenotypes.

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P04.53A

Bi-allelic loss-of-function mutations in the gene encoding the natriuretic peptide receptor C (NPR3) result in enhanced growth and connective tissue abnormalities

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The natriuretic peptide signaling pathway has been implicated in many cellular processes including endochondral ossification and growth. More precisely, different mutations in the NPR-B receptor and the CNP ligand have been identified in patients with either short or tall stature. With this study we show that the NPR-C receptor is also regulating bone growth, however by a different mechanism. Using WES in two affected brothers with tall stature, long fingers and rapidly growing halluces, we identified bi-allelic inactivating mutations in the *NPR3* gene, encoding the NPR-C receptor. Our data indicated that the first mutation (c.442T>C, p.Ser148Pro) hampered proper trafficking of the mutant protein to the cell membrane and that the second mutation (c.1524delC, p.Tyr508*) resulted in nonsense mediated mRNA decay. Biochemical assays in the affected sibs revealed a reduced clearance of the bio-active natriuretic peptides by NPR-C and an increase of bone turnover biomarkers in the serum. The presence of joint laxity in both sibs and a mild aortic dilatation in the proband suggests that NPR-C may also be important in connective tissue homeostasis. The observation of extra epiphyses in the tubular bones of hands and feet in both patients is intriguing. This remarkable radiographic finding was in retrospect also visible in the family with an activating NPR2 mutation reported by Miura K et al. It suggests that enhanced growth due to defects in either the NPR2 or NPR3 controlled signaling pathways may be partially mediated through the creation of extra growth plates in tubular bones.

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P04.54B

Mosaic somatic *KRAS* gene mutation in Oculo-cerebro-cutaneous syndrome (OCCS): a seventh patient report

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Introduction: OCCS is a multisystemic condition with choristome, epibulbar cyst as ophthalmic involvement; aplasia cutis congenita, lipomatosis on subcutaneous regions; intra medullary and cerebral lesions. Fibroma on jaw during first decade and on long bones throughout life may occur (Moog 2005). Applying whole genome shotgun sequencing to DNA extracted from femoral fibroma allowed to select variants in 2 genes. *KRAS* gene was selected since found in other tissues and absent in unaffected relatives (Peacock 2015). These original findings were confirmed on 4 patients with various degrees of mosaicism as hot spot mutations on codon 13,19,146 (Boppudi S. 2016). Expressivity is variable, prognosis factors are not yet available.

Patient Report: Present patient from birth developed aplasia cutis on left parieto-occipital region, epibulbar dermoid kyst of left conjonctiva and a cervical subcutaneous mass. At the age of 18 months, milestones as behaviour are normal. Cervical mass ultrasound correlated to lipoma. Extracted DNA from this lesion, targeted *KRAS* gene sequencing identified presence of the c.38G>A change (p. Gly13Asp) in 47% of the cells. This mutation is one of the 3 (hotspot) reported so far.

Conclusion: Present patient is the 7th patient with OCCS identified carrier of somatic mutation in *KRAS* gene. Appropriate genetic counselling is now available. Precise gain-of-function effect of the missense change in the RAS pathway to phenotype and indication for mTOR inhibitor therapy remain to be evaluated.

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P04.55C

Novel and highly accurate method to detect *de novo* copy number deletions from targeted resequencing data identifies potentially causal variant in *TRAF3IP3* in an oral cleft proband

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Copy number variants (CNVs) are defined as gains or losses of large genomic segments that alter the copy-neutral diploid state of DNA. CNVs can be major contributors of genome variability in humans, and frequently underlie the etiology of disease, including craniofacial disorders. *De novo* CNVs delineated in case-parent trios, albeit very rare, are of particular interest for their potential to have a functional role in the etiology of structural birth defects. We developed a novel method based on the MinimumDistance statistic (PMID: 23234608) for delineating *de novo* copy number deletions simultaneously across multiple trios from targeted sequencing data, dramatically lowering the false positive rate while maintaining high sensitivity. We applied our method to 1,305 case-parent trios with targeted sequencing data of 13 regions previously implicated as possibly causal for orofacial clefts, the most common form of craniofacial malformations. Across the 6.3Mb of the genome sequenced here, we detected one *de novo* deletion in the gene *TRAF3IP3* on chromosome 1q32 (adjacent to *IRF6*, a recognized causal gene for Mendelian malformation syndromes which can include orofacial clefts) in a Caucasian proband with a cleft lip. In addition, we found one rare inherited deletion and two copy number polymorphic regions. These calls are further supported by paired end reads spanning the putative deletions.

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P04.56D

Oro-facio-digital syndrome caused by a novel mutation in *DDX59*

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Introduction: Oro-facio-digital syndrome type 5 (OFD5, OMIM: 174300) is characterized by lobulated tongue, cleft palate and polydactyly; additional features include frontal bossing and intellectual disability. We have investigated a Pakistani family with autosomal recessive OFD. Earlier, a single report of two homozygous mutations of *DDX59* (encoding a DEAD-box-containing RNA helicase) has shown that mutations of this gene are a rare cause of OFD.

Methods and Results: Exome sequencing combined with Sanger sequencing was used to search for a genetic cause of OFD. Exome sequencing revealed a missense variant (c.1430C>T; p.Ser477Leu) in the helicase C terminus domain of DDX59. This variant lies in a 28Mb region

of homozygosity on chromosome 1. This *DDX59* missense variant is predicted "probably damaging" by Polyphen-2 and "not tolerated" by Sift. Sanger sequencing of 3 affected and 6 unaffected (3 parents and 3 siblings) family members revealed that this variant co-segregates in the family with the OFD phenotype. All three affected members were showing features of OFD5 including lobulated tongue, polydactyly and intellectual disability. An additional feature of absent maxillary lateral incisors was observed in one affected female.

Conclusion This is the third report of a homozygous variant of *DDX59* causing OFD, strengthening the role of this RNA helicase family member in the pathogenesis of OFD5.

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P04.57A

Comprehensive analyses of genome-wide data reveal novel insights into distinct etiologies of cleft lip with/without celft palate, and cleft palate only

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Nonsyndromic orofacial clefts are among the most common human birth defects and can be subdivided into nonsyndromic cleft lip with/without cleft palate (nsCL/P) and nonsyndromic cleft palate only (nsCPO). We here present results from two large genome-wide imputation studies on nsCL/P and nsCPO, respectively. While we identified four previously unknown risk loci for nsCL/P (at 2p21, 14q22, 15q24, and 19p13), we did not identify any common risk variant associated with nsCPO (beyond a previously identified missense variant in the *GRHL3* gene). Furthermore, we demonstrate that there is no polygenic component of nsCL/P detectable that is shared with nsCPO. In total this suggests that, while common variants are strongly contributing to nsCL/P risk, they do not seem to be involved in nsCPO. On a systematic level, we show that the association signals within the nsCL/P imputed dataset are enriched in functionally-relevant genomic regions that are active in both human neural crest cells (hNCC) and mouse embryonic craniofacial tissue. Notably, this enrichment is also detectable in hNCC regions that are primed for later activity. Our data suggest that 30% of the nsCL/P-risk variance can be attributed to common variants, with 25.5% assigned to the 24 risk loci known to date. Finally, we performed credible SNP analyses using a Bayesian refinement approach and identified two loci (at 2p21 and 17q13) that each harbour only one likely causative variant. Our study generates novel insights into both nsCL/P and nsCPO etiology and provides a systematic framework for research into craniofacial development and malformation.

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P04.58B

Nonsyndromic cleft palate only - evidence for a limited contribution of common variants in contrast to nonsyndromic cleft lip +/- palate

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Nonsyndromic cleft palate only (nsCPO) is a common malformation with multifactorial etiology. One genome-wide significant nsCPO locus has been identified so far. To discover more risk loci, we increased the marker density of GWAS data from 550 case-parent trios by genome-wide imputation, followed by independent replication of suggestive findings. Eighty-three SNPs at 26 loci showed $5\times10^{-08}<P<1\times10^{-05}$ in the imputation of (i) 272 European trios and/or (ii) 550 European and Asian trios. Of these, loci containing minimally one variant with info-score>0.8, were chosen for replication in two case/control cohorts from Central Europe (94 cases,339 controls) and Yemen (38 cases,232 controls), and one European trio cohort (EuroCran study, 224 trios). Genotyping of 25 SNPs (19 loci) was performed using MALDI-ToF analysis. One SNP (rs6809420) showed a nominal significant association ($P = 0.024$ in Yemen cohort) to nsCPO in the same direction as in the imputed GWAS dataset. This locus might harbour common risk variants with low effect size. Of note, for nonsyndromic cleft lip with/without cleft palate (nsCL/P), the other common form of orofacial clefting, 25 risk loci have been identified by GWAS so far, with some detected in samples much smaller than 550 cases. In our dataset none of the known nsCL/P risk loci showed a P-value$<1\times10^{-05}$, supporting previous molecular and epidemiological findings, that nsCPO is genetically distinct from nsCL/P. Our findings indicate that common variants may contribute to nsCPO, but suggest that the individual effect sizes might be limited and too small to be detected with current sample sizes.

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P04.59C

Analysis of mRNA profiles in extracellular microvesicles from cultured chondrocytes

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Chondrocytes are responsible for producing the extracellular matrix of cartilage, a tissue in which inter-cellular signaling plays an important role in maintenance. Signaling within cartilage has been studied from the viewpoint of growth factors, with pathways such as TGF-beta, FGF and BMP among those characterized. However, intracellular communication involving extracellular microvesicles (ECM) has not been extensively studied in cartilage. Recently, ECM, including exosomes have been shown to be important in communication between cells, both in normal processes such as differentiation, in disease pathologies. The present study aimed to characterize microvesicles isolated from culture media of chondrocytes grown *in-vitro*, with a focus in identifying transcripts encapsulated in the ECM. Microvesicles were collected by ultracentrifugation, RNA was extracted, and transcripts analyzed by qRT-PCR. An initial analysis of mRNA and microRNA present in these microvesicles identified a set of transcripts associated with chondrocyte functions. Subsequently, ECM were collected at different time points to study the dynamics of mRNA encapsulation during culture. We observed variation in mRNA expression for transcripts Sox9, Wdr5, Fgf3, Ihh, Acan, Col2a1, Fgfr2, Bmp4 and Col10a1 over 22 days. It was concluded that the transcripts present in chondrocyte-derived microvesicles carry factors that participate in the development and maintenance of cartilaginous tissue, potentially influencing chondrogenesis and osteogenesis through intercellular communication. These data are of relevance to the study of cartilage development, and may also be relevant in degenerative diseases such as osteoarthritis, due to the potential for microvesicles to be used in the treatment of lesions, and in-vitro production of chondrocytes.

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P04.60D

Targeted next-generation sequencing in Brazilian individuals with Osteogenesis Imperfecta

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Osteogenesis imperfecta is a clinically and genetically heterogeneous group of connective tissue disorders mainly characterized by bone fragility. The severity in OI varies widely, ranging from lethal cases to mild bone alterations. Extra-skeletal findings such as dentinogenesis imperfecta (DGI) are also described. OI is considered a collagen-related disorder and most of the cases (80–90%) are related to dominant mutations in *COL1A1* and *COL1A2*, the genes encoding the collagen type 1α chains. In the last decade, at least 16 other genes, linked to autosomal recessive OI and one X-linked OI have been reported.

The aim of the present study was to identify mutations in 32 unrelated Brazilian children with moderate or severe OI referred to the Oral Center for Inherited Diseases, University of Brasilia. For this purpose, a next generation sequencing panel was designed with 14 known genes associated with OI and DGI and the analysis was performed using the Ion AmpliSeq ™ platform.

A total of 18 heterozygous mutations in *COL1A1* and *COL1A2* were identified in this study. Nine previously reported mutations (7 missense; 1 nonsense; 1 frameshift) in *COL1A1* as well as were identified total of 9 missense mutations in *COL1A2* including three novel mutations in *COL1A2* (c.1612G>A, c.1657G>T and c.3142G>A). Four homozygous missense mutations in *SERPINF1*, *P3H1*, *CRTAP* genes were also identified in four patients with history of consanguinity. We did not identified pathogenic mutations in 10 patients. This was the first study in our hospital that allowed an accurate molecular diagnosis of OI.
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P04.61A

FAM46A mutations are responsible for Osteogenesis imperfecta

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Stuve-Wiedemann syndrome (SWS) is characterized by bowing of the lower limb, respiratory distress and hyperthermia often responsible for an early death. Survivors developed progressive scoliosis and spontaneous fractures. We previously identified *LIFR* mutations in SWS. The absence of *LIFR* mutations in 5 cases prompted us to perform exome sequencing analysis. We identified, in one case, a homozygous mutation in *FAM46A* [p.Ser205Tyrfs*13]. The two parents originated from Italy, were related and heterozygous for this mutation. The clinical findings in this case included bowing of the lower limbs at birth, hyperthermia only in the first year of age, spontaneous fractures and abnormal teeth, reminiscent of osteogenesis imperfecta (OI). We therefore screened OI patients from 25 unrelated families with no mutations in the OI genes. In one family (affected brother and sister), we identified a homozygous variant in *FAM46A* [p.His127Arg]. The two parents were related and heterozygous for the mutation. Clinical manifestations included at birth bowing of the lower limb and blue sclerae and then spontaneous fractures (> 5 at 2 years of age). FAM46A is a member of the superfamily of nucleotidyl-transferase fold proteins but its exact function is unknown. By RT-PCR analysis, we found a specific expression in human control osteoblasts. Moreover, a nonsense mutation in *Fam46a* has been recently identified in an ENU-derived mouse model characterized by short stature, skull deformities and reduced cortical bone thickness in long bones. We conclude that *FAM46A* mutations are responsible for a form of osteogenesis imperfecta with bowing of the lower limb.

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P04.62B

Genetic transmission of osteogenesis imperfecta type 5 by a healthy mosaic carrier father

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Background: OI-V is an autosomal dominant OI, which is characterized by recurrent fractures, hyperplastic callus formation and forearm interosseous membrane calcification. Less than 5% of patients of OI are diagnosed with OI-V. The 5'-UTR *IFITM5* mutation is a single recurrent heterozygous mutation reported in the majority of patients.

Presenting problem: The 2 years old girl was born at term, BW 2880g(P25–50), L 48cm (P25–50), OFC 33cm (P3). Motor developement was delayed, sitting age 15 months, standing without support at 2 years, whereas cognitive development seemed to be normal. At the age of 7 months the girl complained with pain after bending sitting at the mothers womb. X-ray revealed a fracture oft he right femur. A second low impact femur fracture occured at 13 months . Osteogenesis imperfecta (OI) type 1 was excluded by normal COL1A1/COL1A2 testing.

Fracture healing was noticed to be abnormal with delayed and hypertrophic callus formation. The child was treated with Neridronate 1mg every 3 months with good response. In follow up care a limitation in forearm supination/pronation was noticed at 1 ½ years. A recurrent *IFITM5* mutation was identified in the proband c.-14c>T, 5'UTR. The mutation was present and confirmed by NGS in the mosaik state of about 34% in the healthy father.

Discussion: OI-V caused by the 5'-UTR *IFITM5* mutation was confirmed in our patient. There are few reports of families with autosomal dominant inheritance from an affected parent. To our best knowledge a transmission from an unaffected parent was not reported before.

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P04.63C

A novel mutation in TCIRG1 gene in a Turkish patient with malignant autosomal recessive osteopetrosis

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Malignant autosomal recessive osteopetrosis is a rare and severe genetic bone disease characterized by increased bone density due to failure bone resorption by osteoclasts. About 50% of patients have mutations in the *TCIRG1* (T-cell immune regulator 1) gene that encodes the osteoclast-specific a3 subunit of human V-ATPase.

In this report, we present a 5-month-old female infant born as the second child of the first degree cousin marriage. She was born at term by normal vaginal delivery. Her sister died at 2 years with an unknown diagnosis, and the second pregnancy ended with spontaneous abortion. Clinical findings include moderately pale appearance, developmental delay, short stature, frontal bossing, depressed nasal bridge, hepatosplenomegaly, abdomen distention, hearing loss and nystagmus. Skeletal survey and limb radiographies showed calvarial thickening with diffused increase in density of the bones. X-ray of vertebrae showed sclerosis of vertebra. Hemoglobin level was 7.7 gr/dl and platelet level was 35.000. Our patient died at nearly 2-years-old with diagnosis of osteopetrosis and infection. DNA isolation from blood sample of the patient and Sanger sequencing analysis of the all coding exons *TCIRG1* gene were performed. A novel homozygous c.1778_1779delTG mutation (V595LfsX74) on exon 15 was detected. Analysis of parents' DNA confirmed heterozygous carrier status for this mutation in both parents.

According to The Human Gene Mutation Database *TCIRG1* gene mutations include approximately 30% splice-site mutations, 37% missense/nonsense and 30% deletion/insertion. To our knowledge, c.1778_1779delTG mutation has not been reported previously in malignant autosomal recessive osteopetrosis.

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P04.64D

Unclassifiable pattern of hypopigmentation in a patient with mosaic partial 12p tetrasomy without Pallister-Killian syndrome

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Pallister-Killian syndrome (PKS-#OMIM601803) is a multisystem developmental disorder typically due to the presence of an aneuploidy cell line, consisting of a supernumerary tetrasomic chromosomal marker (SCM) arisen from the short arm of chromosome 12 (12p isochromosome). The clinical phenotype, which is strictly related to the percentage and tissue distribution of aneuploid cells, is characterized by craniofacial dysmorphisms, pigmentary skin anomalies, limb shortening, congenital heart defects, diaphragmatic hernia, hypotonia, intellectual disability, and

epilepsy. We report on a 4 year-old girl harboring a 12p partial isochromosome, involving the PKS critical region, affecting about 70% of circulating lymphocytes, urine and saliva cells and fibroblast from a hyperpigmented skin spot, and 100% of fibroblasts from a hypopigmented skin spot. Interestingly, despite the high proportion of affected cells this patient was not presenting PKS, with a pattern of disseminated pigmentary mosaicism being the sole clinical manifestation. The present observation suggests that partial 12p SCM can also result in mild phenotypes, and its prevalence in the human population could have been underestimated. Accurate dermatologic evaluation could be a major handle for genetic testing.

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P04.65A

Identification of genetic modifying factors responsible for the development of the distinct Papillon-Lefévre syndrome and Haim-Munk syndrome clinical phenotypes

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Introduction: Papillon-Lefévre syndrome (PLS; OMIM 245000) and Haim-Munk syndrome (HMS; OMIM 245010) are characterized by overlapping clinical symptoms including palmoplantar hyperkeratosis and severe periodontitis. Besides these symptoms, HMS is also featured by pes planus, arachnodactyly, acroosteolysis and onychogryphosis. Both PLS and HMS develop as the consequence of mutations in the cathepsin C (*CTSC*) gene and exhibit autosomal recessive inheritance.

Patients and methods: In this study, we have investigated a Hungarian PLS and a Hungarian HMS patient carrying the same disease-causing, homozygous, nonsense mutation (c.748C/T; p.Arg250X). Whole exome sequencing (WES) of the two patients was performed in order to identify putative genetic modifier factors responsible for the development of the distinct PLS and HMS clinical phenotypes.

Results: The bioinformatic analysis of the WES results highlighted that the putative genetic modifier factors might be present in the human leukocyte antigen (HLA) genes. Rare, putative damaging variants of the *HLA-DQA1, HLA-DQB1, HLA-DRB1* and *HLA-DRB5* genes were present only in the patient with HMS, but not in the PLS patient.

Conclusion: Our results demonstrate that the development of the different clinical symptoms in patients carrying the same disease-causing nonsense mutation (c.748C/T; p.Arg250X) of the *CTSC* gene, are the consequence of genetic modifiers present in the *HLA-DQA1, HLA-DQB1, HLA-DRB1* and *HLA-DRB5* genes. These results have great importance, since the elucidation of genetic modifiers could further improve the understanding of genotype-phenotype correlations and the development of rare diseases.

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P04.66B

Phacomatosis pigmentokeratotica and precocious puberty associated with *HRAS* mutation

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We report the recurrent co-presentation of precocious puberty (PP) and phacomatosis pigmentokeratotica (PPK) is associated for the first time with mosaic *HRAS* mutation. We investigated a 34-year-old male that first presented at the age of 2 years with an extensive epidermal naevus, multiple melanocytic naevi and the onset of puberty. Endocrine investigations confirmed central precocious puberty with adult levels of luteinising hormone and testosterone. The patient had an extensive Blaschkoid epidermal naevus affecting the face, neck and upper torso. Clusters of dark naevi were noted on the torso and limbs within café-au-lait patches. Using next generation sequencing targeting H*RAS, KRAS, NRAS and BRAF* we demonstrated the presence of mosaic *HRAS* mutation in both clinically affected skin as well as in apparently unaffected tissues, including blood, saliva and skin. This finding supports the view that mosaicism is likely to exist beyond visible tissues such as birthmarks, and is now demonstrable with the increased sensitivity of detection offered by next generation sequencing. It also supports the presence of mosaic mutation in inaccessible compartments such as the pituitary gland. Recently a patient with PPK and PP was reported to carry a mosaic *BRAF* mutation. Taken together with our finding, this strongly implicates Ras-Raf-MEK-ERK signalling in the development of central precocious puberty which is important as the mechanisms causing

central precocious puberty are poorly understood. Furthermore, this pathway is now druggable, and this work may influence novel treatment strategies of central precocious puberty with MEK inhibitors. NR is a Wellcome Intermediate Fellow (WT097163MA).

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P04.67C

Mild manifestations of Proteus syndrome (PS) can be challenging to diagnose

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Proteus Syndrome (OMIM176920) is a highly variable disorder with asymmetric and disproportionate overgrowth, connective tissue nevi, epidermal nevi, dysregulated adipose tissue, and vascular malformations, caused by a somatic activating *AKT1* mutation.

We report on three unrelated individuals who showed similar clinical findings that not fulfilled the rigorous clinical criteria for PS (Biesecker, 1999). Beside an asymmetric hyperostosis of the skull or facial bones, all three had an ocular dermoid, two individuals developed alveolar hyperostoses and intracranial calcifying meningiomas, only one individual showed skin changes. All three had normal feet and no vascular lesions.

Molecular analyses in individual1 performed in blood revealed normal results for array karyotyping and no relevant variant in whole exome sequencing.

After the working diagnosis PS had been established, molecular analyses regarding the recurrent *AKT1* mutation (p.Glu17Lys) were performed by Sanger sequencing in available affected tissue specimen of all three individuals. This revealed a high level of mosaic state for the *AKT1* mutation c.49G>A, (p.Glu17Lys) in affected tissues from bone and in meningiomas. Re-evaluation of the NGS data from blood (individual1) confirmed the absence of that mutation in all reads, and no mutation was detected by Sanger sequencing in DNA from blood in individuals 2+3.

Thus, a somatic mosaicism leading to a mild Proteus phenotype could be confirmed as the underlying genetic cause in all three affected individuals.

In conclusion, mild forms of Proteus syndrome caused by the recurrent *AKT1* mutation in patients with limited regional involvement may be particularly difficult to diagnose and might be underdiagnosed.

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P04.68D

Using Capture Hi-C to identify novel candidate genes in psoriasis-associated genetic loci

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Introduction: Psoriasis is a common, complex autoimmune condition affecting the skin. Genome-wide association studies have identified many psoriasis-associated loci, but most lead SNPs are non-coding and the target gene remains unknown. Capture Hi-C (CHi-C) has previously demonstrated the presence of long-range chromatin interactions between disease-associated loci and distant gene promoters. The aim of this work is to apply CHi-C in psoriasis-associated loci and relevant cell types.

Methods: Duplicate CHi-C libraries were generated from a CD8+ T cell line (MyLa) and will also be generated from a keratinocyte cell line (HaCaT) that has been stimulated with the psoriasis-relevant cytokine IFNγ. RNA capture baits were designed to target all known psoriasis-associated loci, defined by a set of SNPs in tight linkage disequilibrium with the lead SNP. The data was analysed using the HiCUP and CHiCAGO pipelines to call significant interactions.

Results: Interaction data was collected for 64 psoriasis-associated loci across European and Chinese populations. In some cases interactions occurred between the psoriasis association and the nearest gene candidate, for example *NFKBIZ* and *KLF4*. Many other regions demonstrated complex interactions implicating multiple gene candidates, for example the SNP set at *REL* interacted with fragments

containing the promoters of several distant genes such as *VRK2*, *USP34* and *CCT4*.

Conclusions: This is the first instance of CHi-C applied in psoriasis research. As such, this data provides vital clues towards genes involved in psoriasis pathways that may be targets for downstream functional analysis and drug targeting.

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P04.69A

The phenotype-genotype correlation of RASopathies in 33 patients from Turkey

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Introduction: RASopathies are a group of disorders caused by mutations in genes encoding RAS/MAPK pathway and share overlapping clinical features like facial dysmorphism, congenital heart defects, variable degree of intellectual disability and ectodermal abnormalities. The aim of this study is to define clinical and molecular features of Noonan syndrome and related disorders. Method:33 patients with a clinical diagnosis of Noonan syndrome and related disorders were enrolled. All exons harboring known mutations were analyzed for RAS/MAPK pathway genes. 10 patients whose molecular testing have not been completed yet was regarded as unknown mutation group (five of them had normal results in first step analysis). **Results:**23 heterozygous missense mutations were identified in 70% of the patients (seven in PTPN11, five in SOS1, five in BRAF, two in LZTR1, one in RAF1, one in HRAS, one in RIT1, one in MAP2K2 gene). All patients had typical or suggestive facial dysmorphism. Short stature was observed in 45% of patients (PTPN11 7/7, SOS1 1/5, BRAF 2/5, LZTR 1/2, RAF1 1/1, unknown 3/10). The most common cardiac anomaly was pulmonary valve stenosis, observed in 60% (PTPN11 5/7, SOS 4/5, BRAF 3/5, LZTR1 2/2, RAF1 1/1, HRAS 1/1, RIT1 1/1, unknown 3/10) and second most common was hypertrophic cardiomyopathy observed in 27% (BRAF 2/5, LZTR1 1/2, RIT1 1/1, MAP2K2 1/1, unknown 4/10). Intellectual disability was in 63% of the patients (PTPN11 4/7, SOS1 1/5, BRAF 4/5, LZTR1 1/2, RAF1 1/1, MAP2K2 1/1, unknown 9/10). Conclusion: Phenotype-genotype correlation allow us to prioritize the genes to be tested in each patient.

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P04.70B

RSPRY1-associated skeletal dysplasia: Spondylo-epi-metaphyseal dysplasia with cono-brachydactyly and craniosynostosis

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Introduction: The number of genetic skeletal disorders has steadily increased, as application of high-throughput sequencing technology has expanded. One of new comers is RSPRY1-associated skeletal dysplasia identified in four affected siblings from a consanguineous Saudi family. Affected individuals manifested with intellectual disability, facial dysmorphism, short stature, and spondyloepiphyseal dysplasia (SEMD). However, the skeletal phenotype of these patients were not delineated in detail. Materials and Methods: We report on clinical, radiographic and molecular features of two siblings with this disorder from a consanguineous family. **Results:** The siblings showed intellectual disability, facial dysmorphism (brachycephaly, hypertelorism, proptosis, short nose, malar hypoplasia), short stature, and progressive difficulty in walking. The skeletal hallmarks included; mild spondylar dysplasia, epi-metaphyseal dysplasia of long bones (flat and irregular epiphyses, metaphyseal flaring, and mild metaphyseal irregularities) associated with coxa vara and genu valgum, brachymesophalangy with cone-shaped epiphyses, and craniosynostosis. Craniosynostosis was overt in the elder sister who also showed unilateral shortening of the 4th metatarsal, as were in previously reported patients. Whole exome sequencing revealed a novel homozygous [c.377delT] [p.Ile126fs*] mutation in *RSPRY1*. Conclusion: RSPRY1-associated skeletal dysplasia shows distinctive

skeletal phenotype composed of SEMD, cono-brachydactyly, and craniosynostosis, along with typical facial features and intellectual disability. The triad of the skeletal changes may be confused with the skeletal alterations in an other rare skeletal dysplasia termed osteoglophonic dysplasia. However, metaphyseal cortical defects in osteoglophonic dysplasia are absent in RSPRY1-associated skeletal dysplasia. RSPRY1-associated skeletal dysplasia is yet another type of SEMD, characterized by distinctive clinical and skeletal findings.

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P04.71C

Functional implications of RUNX2 evolutionary sweep in nonsyndromic craniosynostosis

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Introduction: Runt related transcription factor 2 (*RUNX2*) encodes a key transcription factor acting downstream of skeletal developmental pathways. *RUNX2* gene dosage affect skull ossification, with opposite trends in cleidocranial dysplasia and craniosynostosis. A previous study reported signs of positive selection in *RUNX2* genomic sequence in anatomically modern humans (AMH). We aimed at inferring the functional consequences of such sequence changes in suture patterning, using nonsyndromic craniosynostosis (NCS) as a model.

Methods: *RUNX2* sequences from extinct hominins (Neanderthal and Denisova) and AMH genomes were compared, and their functional consequences were predicted *in silico*. The expression of *RUNX2* isoforms and of miRNAs binding to the 3′UTRs of the gene, were then analyzed in NCS suture tissues and cells.

Results: Sequence changes map within the P1 and P2 promoters and the two alternative 3′-UTRs. Accordingly, gene expression showed a site-related trend in NCS sutures: *RUNX2* P1-isoforms were upregulated in all but metopic sites, suggesting a selected contribution of P2 functional activation in frontal patterning. Changes in the 3′UTRs of *RUNX2* were predicted to affect miRNA binding. Expression profiling showed that miR134, miR3118, miR4728, miR1249, miR6797, miR4700 and miR8089 are modulated during the osteogenic differentiation of suture-derived cells, coherently with transcript expression.

Conclusion: Most evolutionary changes in the *RUNX2* locus map within the P2 promoter, which affect early skull development, and selectively neural crest-derived sites. Changes at 3′UTR in AMH modified the epigenetic regulation of *RUNX2*, by altering the binding of selected miRNAs and binding on a selected isoform that lost a functional domain.

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P04.72D

Clinical variability of Sensenbrenner syndrome in Polish patients with *WDR35* mutations

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Introduction: Sensenbrenner syndrome (Cranioectodermal dysplasia, CED) is a very rare autosomal recessive and genetically heterogeneous ciliopathy. CED is characterized by craniofacial, skeletal and ectodermal abnormalities. The clinical symptoms include: dolichocephaly, craniosynostosis, brachydactyly, limb shortering, narrow thorax, small and widely spaced teeth, skin and joint laxity, renal and liver insuficiency. To date five genes: *IFT122*, *WDR35*, *WDR19*, *IFT43* and *IFT52* have been associated with this disorder. All known genes encode proteins that are part of the intraflagellar transport machinery, which plays an important role in the assembly of cilia. Methods and results: Analysis by whole exome sequencing and sequencing of known genes revealed that compound heterozygosity for a nonsense (c.1922T>G, p.(Leu641*)) and a missense change (c.2522A>T, p.(Asp841Val)) in the *WDR35* gene is the most common cause of Sensenbrenner syndrome in Polish patients. The nonsense mutation p.(Leu641*) has been

previously reported in two CED patients. The missense p.(Asp841Val) change is a novel variant and was not listed in the 1000 Genomes, the NHLBI Exome Variant Server or the Exome Aggregation Consortium browsers. This change is predicted to have a damaging effect on IFT121 protein. These variants have been identified in three independent families with Sensenbrenner syndrome. Clinical picture of CED individuals is variable suggesting that the expressivity and the severity of patient's phenotype can be modified by genetic and non-genetic factors. **Conclusions:**
Our results suggest that there is an intrafamilial and interfamilial clinical variability among the patients with Sensenbrenner syndrome and WDR35 mutations. Granted by Poznan University of Medical Sciences (502-14-01126186-10642).

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P04.73A

Expanding the spectrum of *SHOX* regulatory region alterations in Idiopathic Short Stature patients (ISS)

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Mutations and deletions within *SHOX* (Xp22.33/ Yp11.3) and its enhancer are reported in 2–15% of children diagnosed as ISS. Currently molecular diagnosis for *SHOX* deficiency is carried out by sequencing the coding regions of *SHOX* and MLPA for the identification of deletions/duplications. We screened 469 ISS patients for *SHOX* defects. Thirty-four patients (7%) were identified with mutations involving *SHOX* coding region or its enhancers (3 point mutations and 31 deletions/duplications). In five patients we identified 4 variants c.-55C/T, c.-51G/A, c.-19G/A, and c.-10delG within the promoter. In a functional analysis with a dual luciferase assay in U2OS, the variants c.-51G/A, c.-19G/A and c.-10delG showed 60% ($p = 0.00967$), 35% ($p = 0.02147$) and 40% ($p = 0.0262$) level of luciferase activity compared to the wild type respectively, suggesting that mutations in the promoter might alter the expression of *SHOX*. In order to identify if there were some patients remained undiagnosed, thirty-seven patients tested negative with the standard methods presenting the most severe phenotype were analysed using a high resolution custom oligo array-CGH platform. Two small deletions of 12.3 kb (1 patient) and 7 kb (2 patients) downstream *SHOX* not reported in the general population were identified. To understand if these sequences affect the gene expression, we are currently performing a functional assay by cloning the two sequences along with the *SHOX* promoter in an expression vector. In conclusion, our findings suggest that mutations interfering with *SHOX* regulation might remain undisclosed with standard methods and could expand the spectrum of *SHOX* alterations associated to Short Stature.

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P04.74B

Comparison of the distribution of duplicated regions associated with *SHOX* gene between LWD/ISS patients and population sample

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Heterozygous deletion or aberration of *SHOX* gene or one of its numerous enhancers have been reported to be responsible for Léri-Weill dyschondrosteosis (LWD) and small portion of idiopathic short stature (ISS). Effect of reciprocal duplications is less distinct. The aim of our study was to determine and compare distribution of duplicated *SHOX* gene associated areas between the LWD/ISS patients and population sample.

Population sample of 250 unrelated probands was screened for PAR1 duplication/deletion using the MLPA method. Duplicated areas frequency distribution was compared to previously reported LWD/ISS cohort of 352 individuals.

There was not a significant difference in duplication frequency between the LWD/ISS cohort and our population sample. However, there is a single peak (*SHOX* gene) pattern of duplicated probes frequency distribution in population sample in contrast to a pronounced two peak (*SHOX* gene; CNE-9 enhancer) distribution pattern for LWD/ISS cohort.

We thus propose that only certain duplications of *SHOX* gene and related regions are associated with increased risk of LWD/ISS development.

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P04.75C

Sensitive NGS molecular diagnosis using a 49 gene custom panel for skeletal dysplasia

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Back-ground: Skeletal dysplasia represents an heterogeneous group of rare hereditary diseases. The most frequently mutated gene is COL2A1. To extend molecular diagnosis strategy to a greater number of genes, we implemented next generation (NGS) sequencing using a 49 genes custom panel. Methods : Agilent SureSelect^{QXT} target enriched library from 116 patients' genomic DNA was sequenced using Illumina v2 chemistry on MiSeq or Nextseq. Fastq files were aligned and reads were mapped against the human genome (hg19) with MiSeq Reporter (v2.2.31) or SeqNext module (v4.0, SeqPilot JSI). Variant calling was performed through SeqNext. **Results:** At least one pathogenic variant was found in 55 of 116 patients (47,4%). Most (31/55, 56,4%) carried an heterozygous mutation in one collagen gene (13 COL2A1, 5 COL11A1, 4 COL11A2, 5 COL1A1, 1 COL1A2, 1 COL10A1 and 2 COL9A3). Among 24 patients with mutations in non-collagen genes (43,6%), 5 displayed homozygous genotypes: 3 B3GAT3 p.(Gly223Ser) mutation, 1 SLC26A2 p.(Arg279Trp) and 1 PAPSS2 p.(Thr48Arg). Four patients displayed compound heterozygous genotypes: 1 SLC26A2, 2 PRG4 and 1 COL11A2. Three patients carried TRPS1 heterozygous mutations: p.(Gln293*), p.(Val793Glyfs*12) and p.(Arg921*). Finally, new pathogenic variants in unexpected genes such as in VCAN, COL11A2 and GDF5 were identified in 3 Czech dysplasia patients. Conclusion: We show here that our NGS approach, resolved molecular diagnosis for up to 47% of patients with various skeletal dysplasia phenotypes. Moreover, this approach not only revealed new pathogenic mutations in known causative genes but also in unreported genes.

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P04.76D

Prenatal diagnosis of a de novo TRPV4 associated skeletal dysplasia by targeted next generation sequencing

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We describe the first case of prenatal diagnosis of a de novo TRPV4 associated skeletal dysplasia. Detection of the fetal mutation was only possible due to application of a NGS panel including 170 with skeletal dysplasia associated genes.

Shortening of the long bones with abnormal flared metaphyseal regions was diagnosed in the 33rd week of the second pregnancy of a healthy woman. Chromosomal microarray analysis revealed normal results. A common heterozygous (hot spot) TRPV4 mutation could be detected by NGS panel diagnostic within two weeks (c.2396C>T, p.Pro799Leu). The TRPV4 gene encodes a cation channel that is thought to be involved in osmotic pressure regulation.

The woman was counselled regarding the different skeletal phenotypes which can be related to this mutation (e.g. metatropic dysplasia, spondyloepiphyseal dysplasia Koslowski and Maroteaux type) and other possible phenotypes associated mit TRPV4 gene mutations (neuromuscular diseases). The possibility of postnatal respiratory problems was adressed as well as postnatal examinations including MRI to exclude instability of the cervical spine which has been described in the literature.

The affected boy was born in the 38th week of pregnancy by cesarean section. No respiratory problems or instablility of the cervical spine occurred. He had multiple joint contractures, shortened humeri and femora, torticollis and asymmetric spine with kyphoscoliosis showing an overlap of skeletal and neuromuscular symptoms related to the TRPV4 gene mutation.

The application of NGS gene panel diagnostic enabled prenatal diagnosis of a rare skeletal disorder and made it possible to give advice for the expected postnatal situation.

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P04.77A

SPARC-related osteogenesis imperfecta with a myopathy-like presentation

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We report two sisters with healthy consanguineous parents, normal birth measurements, pes adductus, persistent hypotonia, small joint laxity, delayed motor development and progressive early thoracolumbar scoliosis. Pathological fractures started at age two years and loss of primary dentition was delayed. Molecular testing did not confirm the initial suspicion of Ullrich congenital muscular dystrophy.

Multiple fractures and low bone mineral density led to molecular testing for

osteogenesis imperfecta (OI). Exome sequencing showed no coverage of exon 4 in *SPARC* in either girl. Deletion breakpoints were determined by Sanger sequencing. The homozygous variant c.121-578_209-94del (NM_003118.3) present in both children introduces a change in reading frame likely resulting in nonsense-mediated decay.

Mendoza-Londono et al (1) demonstrated biallelic loss-of-function mutations in *SPARC* in two unrelated girls with severe bone fragility, progressive scoliosis and joint hyperlaxity. Ullrich muscular dystrophy was initially suspected in one. Subsequently, two siblings with marked joint laxity, progressive scoliosis and short stature with a homozygous splice site variant in *SPARC* were reported (2).

We describe the oldest known individual with *SPARC*-related OI and further delineate the phenotype. The resemblance to a congenital muscle disorder prior to the onset of fractures is in keeping with evidence that SPARC (secreted protein acidic and rich in cysteine) interacts with actin in muscle cells during development and remodeling (3).

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2. Alazami AM et al., Hum Genet,135:525–540, 2016
3. Jørgensen LH et al., Am J Pathol 187:457–474, 2017

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Mutated *DMRT2* Causes a Severe Type of Spondylocostal Dysostosis

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Spondylocostal Dysostosis (SCD) is a rare disorder characterized by vertebral segmentation defects and malformations of the ribs. In addition, SCD-patients have some degree of (kypho)scoliosis, short stature and suffer from respiratory impairment due to the reduced size of their thoracic cage. Mutations in *DLL3, MESP2, LFNG, HES7, TBX6* and *RIPPLY2* are known to cause different subtypes of SCD. Here, we present a male neonate with an apparent severe type of SCD only partly overlapping the previously described SCD-subtypes. Diagnostic radiological imaging demonstrated multiple costovertebral abnormalities. The proband presented with severe costal malformations (missing, fused, bifid, and hypoplastic ribs), vertebral malformations (including intervertebral fusions of the laminae and irregular ossification of the vertebral bodies) and a mild scoliosis. Other dysmorphic features included: cleft palate (soft palate), posteriorly rotated ears, short neck, asymmetry of the thorax, extra nipple, transverse palmar creases, sandal gap (bilateral), and sacrum acutum. Severe respiratory insufficiency was present from birth. Whole Exome Sequencing (WES) identified a homozygous start-loss variant in *DMRT2* (NM_006557.6: c.1A>T p.(Met1?)). Mutations in *DMRT2* (OMIM#604935) have not been described in relation to SCD before. It has been demonstrated that *DMRT2* knock-out mice exhibit severe rib and vertebral defects overlapping with the radiological phenotype of our patient. Therefore, it seems plausible that mutations in *DMRT2* can cause a severe type of SCD characterized by a striking skeletal phenotype in combination with extra-skeletal features. This is the first report of a human proband with a severe SCD-like phenotype most probably caused by a homozygous mutation in *DMRT2*.

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P04.80D

Recognizing the tenascin-X deficient type of Ehlers-Danlos syndrome: a cross-sectional study in 17 patients

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The tenascin-X (TNX) deficient type Ehlers-Danlos syndrome (EDS) is similar to the classical type of EDS. Because of the limited awareness among geneticists and the challenge of the molecular analysis of the TNXB gene, the TNX-deficient type EDS is probably to be under diagnosed. We therefore performed an observational, cross-sectional study. History and physical examination were performed. Results of serum TNX measurements were collected and mutation analysis was performed by a combination of next-generation sequencing (NGS), Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). Included were 17 patients of 11 families with autosomal recessive inheritance and childhood onset. All patients had hyperextensible skin without atrophic scarring. Hypermobility of the joints was observed in 16 of 17 patients. Deformities of the hands and feet were observed frequently. TNX serum level was tested and absent in 11 patients (seven families). Genetic testing was performed in all families; 12 different mutations were detected, most of which are suspected to lead to non-sense mRNA mediated decay. In short, patients with the TNX-deficient type EDS typically have generalized joint hypermobility, skin hyperextensibility and easy bruising. In contrast to the classical type, the inheritance pattern is autosomal recessive and atrophic scarring is absent. Molecular analysis of TNXB in a diagnostic setting is challenging.

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P04.81A

TNF-alpha gene variants influence the risk of Spondyloarthropathies in HLA-B27-negative patients

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Introduction: The group of spondyloarthropathies (SpA) includes ankylosing spondylitis (AS), psoriatic arthritis (PsA) and other rheumatic inflammatory disorders. A common genetic feature of spondyloarthropathies is the association with HLA-B27, but a variable percent of the patients are HLA-B27-negative. Despite the clinical efficacy of TNF-alpha-inhibitors, the manner in which TNF-alpha contributes to SpA remains unresolved. Due to TNF-alpha gene location in the region of HLA loci on chromosome 6p21.3, it is difficult to evaluate the contribution of TNF-alpha gene separate of HLA-B27 marker. The aim of this study was to analyze three single nucleotide polymorphisms (SNPs) of TNF-alpha gene in HLA-B27-negative SpA patients and controls of Romanian origin. Materials and methods. After HLA-B27 typing of 266 SpA patients and 159 controls (B27-SSP low resolution kit Olerup, Sweden), HLA-B27-negative subjects were selected. TNF-alpha gene SNPs rs1799724/-857C/T, rs1800629/-308G/A and rs361525/-238G/A were genotyped in 79 SpA patients (27AS/52PsA) and 141 controls (all HLA-B27-) using TaqMan Allelic Discrimination Assays (Applied Biosystems, USA). Association tests were performed with the software package PLINK v 1.9. Results. Controls and patients were in Hardy-Weinberg equilibrium for the three SNPs. The frequency of the minor 857*T allele was significantly higher in patients than in controls (32.3% vs. 18.6%, $p_{uncorr.} = 0.001$, OR 2.09, CI 1.333–3.276). The carriers of this allele (CT+TT genotypes) were more frequent among patients (53% vs. 32.8%, $p = 0.003$, OR 2.32). The haplotype TGG was over-represented in patients (32.3%) versus controls (18.6%, $p = 0.001$). Conclusion. TNF-alpha gene SNP rs1799724 influences the risk of SpA in HLA-B27-negative Romanians.

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P04.82B

Follow-up mutational analysis in uncombable hair sydrome

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Uncombable hair syndrome (UHS), also known as "spun glass hair syndrome", or "pili trianguli et canaliculi" is a rare anomaly of the hair shaft which occurs in early childhood and improves with age. UHS is characterized by frizzy, spangly and often fair hair that is resistant to being combed flat. Currently, about 100 cases have been reported worldwide. Recently, we identified homozygous and/or compound heterozygous mutations in 11 children in the three genes *PADI3*, *TGM3*, and *TCHH*, which are all involved in hair shaft formation. After publishing our novel data, numerous of people got interested in our study; and we collected DNA samples from more than 20 families with UHS. We performed Sanger sequencing on *PADI3*, *TGM3*, and *TCHH* to identify the underlying mutations. We identified the most common mutations c.881C>T;p.Ala294Val and c.335T>A;p.Leu112His in 10 affected individuals. In addition, we identified the novel nonsense mutation c.545C>T;p.Q169* in two patients. Some individuals did not carry any mutations in *PADI3*. Sequencing of the remaining genes in individuals where no mutations have been detected are ongoing. According to gnomeAD, the allele frequency of the most common mutations c.881C>T and c.335T>A is much more frequent than expected (e.g. we calculated that in the German population, there should be around 12.000 UHS cases). We confirmed that most mutations in patients with UHS can be identified in *PADI3*, however, we speculate that there is also a gene for a dominant form which is not known yet.

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P04.83C

A systematic screening for a monogenic cause in a cohort of 25 patients with vertebral segmentation defects

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Introduction: During embryogenesis, the process of somitogenesis is orchestrated by a wide range of genes for the precise vertebral column development. The disruption of this process results in either syndromic or non-syndromic vertebral segmentation defects. In this study, we investigated the genetic basis of syndromic and non-syndromic vertebral segmentation defects in a cohort of 25 patients. Materials and methods: We aimed at identifying pathogenic variants in the genes (*DLL3*, *MESP2*, *HES7*, *LFNG*, *TBX6* and *RIPPLY2*) known to cause non-syndromic vertebral segmentation defects by Sanger sequencing. Exome-sequencing was performed in five families. **Results:** We recruited 22 families with sporadic cases and one family with three affected members. After clinical and radiological evaluation, 19 families were categorised as non-syndromic and four families as syndromic. We performed Sanger sequencing in 18 sporadic cases with non-syndromic vertebral segmentation defects and detected three novel pathogenic variants: c.193G>T (p.E65*) in *MESP2*, c.661C>T (p.R221*) and c.1069T>G (p.C357G) in *DLL3*. Exome sequencing was performed in five families (four syndromic and the other familial non-syndromic vertebral segmentation defects) and identified five novel pathogenic variants: c.314G>A (p.C105Y) in *BMPER* and c.1243C>T (p.R415*), c.6407del (p.P2137Rfs*12), c.1204del (p.V402Wfs*88) and c.1892C>T (p.P631L) in *FLNB*. **Conclusions:** We identified a monogenic aetiology in eight families with vertebral segmentation defects (32%). Further evaluation is underway. The study has been funded by Indian Council of Medical Research.

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P04.84D

Williams syndrome and hereditary diffuse palmoplantar keratoderma

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Introduction: Williams syndrome(WS) is a multi-system disorder characterized by distinctive facial features, growth delay, mental retardation with typical neurobehavioral profile, cardiovascular anomalies, endocrine anomalies,and hypercalcemia. Dermatologic findings can include soft skin, forehead/peri-ocular/peri-oral wrinkling, skin picking, keratosis pilaris, atrophic areas of skin. Dry skin/mild hyperkeratosis can be present, but palmoplantar keratoderma (PPK) is not a typical finding in WS. Our case provides a very rare example of WS with severe hereditary diffuse palmoplantar keratoderma.

Case Report: We presented a 4-years-old girl born as the second child of first degree cousin marriage. On her physical examination; course facies, periorbital fullness, bulbous nasal tip, full lips and cheeks, long philtrum, horse voice, and severe hyperkeratosis on hands and feet detected. Subaortic ventricular septal defect, mitral valve prolapsus, and hypothyroidism detected. FISH result was 46.XX, ish del(7q11.2)(ELN X1)(7q22 X2)ELN deletion compatible with WS. A skin biopsy from the sole showed hyperparakeratotic stratum corneum, spongiosis, acanthosis, mononuclear inflammatory cell infiltration around the dermis veins compatible with palmoplantar keratoderma.

Conclusion: PPK is a heritable or acquired disorder characterized by abnormal hyperkeratotic thickening of the palm and sole skin. Hereditary PPK are divided morphologically into four types; diffuse, focal, striate,and punctate. To our knowledge, this is the first report of WS with hereditary diffusePKK in English literature. We want to draw attention to the fact that there may be a coincidence between WS and the developing PKK in this patient, as well as a finding that may accompany the WS. Gene studies are planned to clarify this situation.

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P04.86B

Identificationof idiopathic scoliosis-associated regulatory elements

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Adolescent idiopathic scoliosis (AIS) affects ~3% of the population worldwide, yet its genetic basis and tissues of origin remain largely unknown. Several genome-wide association studies (GWAS) and functional follow up studies have implicated nucleotide changes in gene regulatory sequences such as enhancers as potential drivers of AIS susceptibility. In addition, various assays using animal models have suggested that chondrocytes could be an important cell type for AIS susceptibility. In this study, we set out to characterize AIS-associated regulatory elements in chondrocytes by carrying out chromatin immunoprecipitation followed by deep sequencing (ChIP-seq). Chondrocytes were isolated from the axial skeleton of newborn mice (P2-P4) and ChIP-seq was performed using an H3K27 antibody that marks active genomic regions (enhancers and promoters). Our analysis identified a catalog of novel putative chondrocyte specific enhancers and promoters and highlights gene regulatory pathways involved in chondrogenesis. This catalog provides prime candidate sequences that can be analyzed for AIS-associated nucleotide variation. In addition, we plan to use a similar approach for additional AIS-associated tissues and functionally characterize AIS-associated regulatory elements using transgenic enhancer assays and CRISPR/Cas9 genome editing in mice. Grants: NIH 1P01HD084387-01A1. NM. is supported by Jane Coffin Childs Postdoctoral Fellowship.

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P05
Cardiovascular disorders

P05.01A

Focus on Risk of Familial Abdominal Aortic Aneurysm in Women

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Gender affects the presentation, treatment, and outcomes of abdominal aortic aneurysm (AAA). Although AAA is less prevalent in women, at least in the general population, female AAA patients have poorer prognosis than men. Gender differences in genetic predisposition for aneurysm disease remains to be established. Here we investigated familial risk of aortic aneurysm for women compared to men. AAA patients were routinely referred to the multidisciplinary vascular/genetics outpatient clinic for assessment of family history using detailed questionnaires. Abdominal aortic aneurysm risk for male and female relatives was calculated separately and stratified by gender of the AAA patients. Families of 568 AAA patients were investigated and 22.5% of the patients had at least one affected relative. Relatives of female AAA patients had a higher aneurysm risk than relatives of male patients (9.0 vs. 5.9%, P = .022). Compared to estimated gender specific population risks, female relatives had a 2.8-fold and male relatives a 1.7-fold higher AAA risk. Risk for female and male relatives of female patients was 5.5- and 2-fold increased, compared to a 2.4-fold higher risk for female and 1.6-fold higher risk for male relatives of male patients. The risk for aortic aneurysm in family members of AAA patients is higher than expected from population risk. The excess risk is largest for the female relatives of AAA patients and for the relatives of female AAA patients. These findings indicate expanding targeted AAA family screening to female and male relatives of all AAA patients, particularly of female AAA patients.

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P05.02B

The effect of adiposity on and cardiovascular risk factors and bone mineral density: a Mendelian randomization study

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Introduction: The incidence of overweight and obesity have increased rapidly and has become a major worldwide health problem. Adiposity constitutes an important risk factor for type 2 diabetes, dyslipidemia, hypertension, osteoarthritis, cardiovascular disease and is the major cause mortality, however, its causes and consequences are often difficult to determine. The study was aimed to use a Mendelian randomization design to assess whether adiposity causally affects known cardiovascular risk factors and bone mineral density. Methods: A total of 1100 adults were recruited from the Matsu community-based integrated health screening project. Three *FTO* SNPs (rs9939609, rs9930506, rs14211085) were used as instrument variables to estimate the causal effect of adiposity on blood pressure, fasting glucose, lipid levels, and bone marrow density in the Mendelian randomization analysis using two-stage least squares instrumental variable regression. **Results:** We found that all three SNPs of the *FTO* gene were significantly associated with body-mass index (BMI), and two SNPs (rs9939609, rs14211085) associated with fat percentage ($p<0.05$). The results of linear regression showed that both BMI and fat percentage are significantly associated with blood pressure, fasting glucose, lipid levels ($p<0.001$), and bone marrow density ($p<0.01$). However, Mendelian randomization analyses did not show that both BMI and fat percentage, instrumented by the three *FTO* SNPs, were associated with any cardiovascular risk factors and bone mineral density ($p > 0.05$). Conclusion: Our study could not replicate previous knowledge of the causal effects of adiposity on cardiovascular risk factors and bone marrow density in our Chinese population. Grant No: MOST 103-2314-B-010-009 & MOST 105-2314-B-010-017

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P05.03C

Genetic variants in familial abdominal aortic aneurysms identified by Whole Genome and Exome sequencing

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Introduction: Abdominal aortic aneurysm (AAA) is a frequent disorder with a prevalence of 5% in the elderly population. 20% of AAA patients are familial. In non-familial AAA predisposition also plays a role since known cardiovascular risk factors alone do not explain the

localized weakening of the aortic wall. Our goal is to identify genes that play a role in the formation of abdominal aneurysms.

Methods: The study includes approximately 1050 AAA patients, 350 patients with (fAAA)- and 700 patients without a family history of AAA (non fAAA). So far we sequenced the DNA of 470 patients (350 fAAA and 120 non fAAA). Whole genome sequencing (WGS) was performed in 3 families (15 individuals) and whole exome sequencing (WES) was performed in 365 families with familial disease.

Prioritization of resulting variants was performed according to the following gene sets:

1. Genes in diagnostics panel as applied in the Erasmus MC in thoracic or syndromic aneurysms (n = 25)
2. Genes involved in vascular function or disease (n = 4209)
3. All genes in the genome

Results: We present the detailed workflow of the analysis of the genomics data, including the results so far. In 73 out of 365 families a variant in one of the set 1 genes was found. Further analysis of the set 2 and 3 genes led to the identification of several candidate genes that show variants in more than one AAA family and that have not been linked to AAA before.

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P05.04D

Utility of targeted next generation sequencing in a representative Czech cohort of paediatric and adult patients with arrhytmogenic cardiomyopathy (AC). High prevalence of variants in DSP in left ventricular form of AC

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Introduction: Arrhytmogenic cardiomyopathy (AC) is associated with an increased risk of sudden cardiac death.

Patients and Methods: A total of 67 Czech AC cases (7 pediatric) were recruited. All patients/families had undergone genetic counselling, including cardiologic examination in their 1st degree relatives. Targeted NGS, comprising exonic regions with pathogenic variation (PV, using TruSight One- Illumina or SeqCap MedExome - Roche) was performed in 56/67 patients, while 11/67 were analysed by a custom panel (NimbleGen; Roche).

Results: Family history was positive in 18/67 (27%) of cases. Likely PV was found in 54/67 (80%) cases: *PKP2* (28/67; 28%) and *DSP* (13/67; 19%). Majority of *DSP*-positive cases (11/13) have predominant left ventricular involvement and underwent catheter ablation (7/13). Variants in *DSC2* were found in 4/67 (6%) and in *DES*, *TGFB3, DSG2* and SCN5A in 2/67 (3% each). Likely PV in *TMEM43, CTNN2, JUP, RYR2* occurred once (1.5% each). In 13/67 patients (19%) the underlying genetic aetiology remained unknown (6/13 did not bear any PV, while in 7/13 cases PV were found in genes associated with other types of hereditary cardiovascular diseases such as *KCNE3/TRPM4, SCN10A/TRDN, TRPM2, TNNT2, FBN1, MYH7, ANK3*, *LDB3, FLNC*).

Conclusion: The spectrum of PV in our AC patients corresponds to that observed in other European cohorts. Patients bearing *DSP* PV have predominantly left ventricular involvement. Family segregation studies identified additional "carriers" of PV, who were enlisted into long-term cardiologic follow up and discouraged from strenuous sports activities.

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P05.05A

miRNA profiling in a transgenic model of arrhythmogenic cardiomyopathy

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Arrhythmogenic cardiomyopathy (ACM) is one of the most common inherited cardiomyopathies, characterized by the

fibro-fatty replacement in the myocardium. Among the 13 known genes, those encoding for the desmosomal proteins plakophilin-2 (PKP2), desmoplakin (DSP), and desmoglein-2 (DSG2) are most commonly mutated. Clinically, ACM manifests with ventricular arrhythmias, syncope, and sudden death and shows wide inter- and intra-familial variability, which might be determined by epigenetic factors, such as microRNAs (miRNAs).

AIM: Defining the miRNA signature in a transgenic mouse model for ACM overexpressing the p.Q558X mutation in desmoglein-2.

METHODS AND RESULTS: We generated transgenic mice with cardiac-specific overexpression of the FLAG-tagged mutated (Tg-hQ) desmoglein-2, resembling the features of ACM. In Tg-hQ mice, desmosome density and length were significantly decreased and Wnt/β-catenin signalling suppressed.

RNA-Seq performed in three Tg-hQ hearts and three non-transgenic hearts revealed that 24 miRNAs were deregulated in transgenic animals. MiRWalk and GO analyses for selected miRNAs suggested that miR-217-5p, miR-216a-5p, miR-499-5p, and miR-708-5p might be involved in the regulation of the Wnt/β-catenin signaling.

CONCLUSIONS: We identified the miRNA signature in ACM hearts, with miR-708-5p, miR-216a-5p, miR-217-5p being the most upregulated and miR-499-5p the most downregulated miRNAs. All of them were predicted to be involved in the regulation of the Wnt/β-catenin pathway and might reveal the potential pathophysiology mechanisms of ACM, as well as be useful as therapeutic targets for the disease.

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P05.06B

DNA methylation within microRNA gene *MIR10B* in atherosclerosis

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DNA methylation and microRNAs are epigenetic mechanisms which can act in a tissue-specific manner, and their alterations are associated with atherosclerosis. To investigate DNA methylation patterns within microRNA genes we performed DNA methylation screening of 71 CpG sites in advanced atherosclerotic lesions of coronary arteries (CNA), intact internal thoracic arteries (ITA), saphenous veins (SV) and peripheral blood leukocytes (PBL) of six patients with coronary atherosclerosis using HumanMethylation27 BeadChip (Illumina). The results were validated and extended with DNA from CNA, ITA, SV ($n = 22$ each), atherosclerotic plaques of carotid arteries (CTA, $n = 120$) and PBL ($n = 142$) of patients with atherosclerosis, and PBL of healthy persons ($n = 90$) via bisulfite pyrosequencing. We found 50 CpG sites having low methylation level (< 0.1) and variability (range < 0.1) in all analyzed samples. Methylation profile of highly variable CpG sites (within 9 microRNA genes) demonstrated CNA having similarity with PBL. The most variable CpG sites were attributed to *MIR10B*, which was hypomethylated in atherosclerotic plaques and PBL compared to ITA and SV. Weak but significant positive correlation were shown between methylation levels in CTA and PBL. Methylation level within *MIR10B* promoter was slightly elevated ($p<0.05$) both in CTA and PBL of patients compared to PBL of healthy individuals. Disturbances in methylation of certain CpG sites were associated with cases of myocardial infarction, stroke, chronic lower limb ischemia, smoking etc. Methylation of *MIR10B* has tissue-specific pattern, associated with susceptibility to atherosclerosis, its clinical manifestations and risk factors. The study was supported by the Russian Science Foundation (no. 16-15-10150).

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P05.07C

Genomic alterations in the recruited macrophages in human atherosclerotic plaques

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Introduction: It is suggested that somatic genomic alterations may occur in key cells of atherogenesis, for instance, macrophages, and therefore have been implicated in atherosclerotic plaque formation. Thus, our purpose was an assessment of genomic alterations in macrophages obtained from atherosclerotic plaque using single-cell microarray technique. Materials and methods: Using Mouse monoclonal Anti-Human CD68 Antibody and Laser capture microdissection we obtained macrophages (n = 30–40 cells per sample) from atherosclerotic plaques (IV-V types) from the coronary arteries (n = 8) with subsequent whole genome amplification (GenomePlex® WGA Kit). The amplified DNA samples of macrophages and white blood cells from the same persons were hybridized on SurePrint G3 Human CGH 8×60K Microarray (Agilent Technologies). **Results:** We found several aneuploidies (4, 7, 12, 14, 15, and 22 chromosomes) in macrophages (≈1 per sample). Comparative analysis of genomic rearrangements in macrophages demonstrated that certain structural variations were detected in the similar genomic regions, whereas most of them were differently dispersed. The most common (50%) structural variation was located in 9q34.13-q34.2 (arr[hg19] 9q34.13 (9:134337452-135931774)x3) that contains about 20 genes. Conclusion: Our study indicates that genomic alterations are diverse in their structure and are widely represented in the recruited macrophages in atherosclerotic plaques. Furthermore, we identified common structural variation in 9q34.13 that contains genes potentially relevant to the development of atherosclerosis. The study was supported by the Russian Science Foundation (No. 14-15-00305).

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P05.08D

A novel missense variant in the gene *PLEC* increases risk of atrial fibrillation

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Introduction: Recently, rare mutations in the sarcomere genes *MYH6* and *MYL4* have been linked to risk of atrial fibrillation (AF). Here, we search for sequence variants that associate with AF using whole-genome sequence data and explore their effects on electrocardiogram (ECG) measurements.

Materials and Methods: We tested for association between sequence variants and AF among 14,255 Icelandic cases and 374,393 controls and ECG measures in 289,297 ECGs from 63,000 individuals. Genotype information was based on whole-genome sequencing of 15,220 individuals, imputation in 151,678 individuals and calculated genotype probabilities for untyped relatives.

Results: We discovered a novel missense mutation, p. Gly3988Ser, in the gene *PLEC* that associates with increased risk of AF, minor allele frequency 1.17%, odds ratio 1.55, *P*-value 8.0×10^{-10}. *PLEC* encodes plectin, a multidomain cytoskeleton linking protein which has a role in maintaining tissue integrity in the heart. P.Gly3988Ser significantly affects ECG measurements in the absence of AF. It affects P-wave morphology, lowers the R-amplitude and prolongs the PR-segment. Other AF associated variants have variable effects on the conduction system, ranging from none to extensive.

Conclusions: Through genome-wide analysis we discovered a coding variant in a gene encoding a structural component of cardiomyocytes that substantially increases AF risk. The novel mutation in *PLEC* affects conduction in both atria and ventricles. The diverse associations of AF variants with ECG measurements suggests a variable pathological mechanism behind their respective link to AF. The discovery implies an important role of structural components of cardiomyocytes and cardiac remodelling in the pathogenesis of AF.

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P05.09A

Candidate gene resequencing in a large BAV/TAA cohort: *SMAD6* as an important contributor

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Bicuspid aortic valve (BAV) is the most common congenital heart defect. Although most BAV patients remain asymptomatic, at least 20% develop thoracic aortic aneurysms (TAA). Historically, these BAV-related TAAs were considered hemodynamic consequences, but multiple lines of evidence currently suggest that genetic determinants also contribute to the pathogenesis of BAV and TAA. While BAV has a high heritability, few genes have been strongly linked to the BAV phenotype. *NOTCH1* is often considered the sole established human BAV(/TAA) gene, although several other genes have occasionally been linked to BAV, including *SMAD6* and *Nkx2.5*. Other candidate genes based on the presence of BAV in knockout mouse models (eg *GATA5, NOS3*) or in syndromic (eg. *TGFBR1/2, TGFB2/3*) or non-syndromic (eg. *ACTA2*) TAA forms have been suggested. We hypothesized that causal genetic variants in these genes would be enriched in patients presenting with both BAV and TAA. We performed targeted resequencing of 25 candidate genes using Haloplex enrichment in a strictly defined BAV/TAA cohort (n = 441; at least diameter of 4.0 cm at the sinuses of Valsalva or the ascending aorta) and in a collection of healthy controls with normal echocardiographic evaluation (n = 183). After additional burden analysis against ExAC, the strongest candidate susceptibility gene was *SMAD6*, with 2.72% of BAV/TAA patients harboring causal variants, including two nonsense, one in-frame deletion and two frameshift mutations. All six missense mutations were located in the functional MH1 and MH2 domains. In conclusion, *SMAD6* is the first gene with more than 1% contribution to the BAV/TAA phenotype in humans.

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P05.10B

Mutation load of multiple ion channel gene mutations in Brugada Syndrome

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Introduction: Brugada syndrome (BrS) is a primary arrhythmic syndrome which accounts for 20% of all sudden cardiac death cases in individuals with structurally normal heart. Pathogenic variants associated with BrS have been identified in over 20 different genes.

Methods: A new custom NGS panel exploring 66 cardiac genes was used to search for potential disease-causing variants in BrS patients.

Results: We identified in a patient a double heterozygosity for missense mutations in two genes related to BrS. The c.4501C>G nucleotide substitution in exon 26 of the *SCN5A* gene resulted in the amino acid change p. Leu1501Val. This variant was previously reported in BrS and in LQTS patients. The second change consists of a c.2531G>A nucleotide substitution in exon 17 of the *TRPM4* gene that leads to the amino acid change p. Gly844Asp. This variant is a known pathogenic mutation and it was reported in various cardiac arrhythmic disorders including BrS. Parents were heterozygous for each variation and the family history was negative for major cardiac events and sudden death, suggesting that single mutation is not sufficient *per se* to determine a clinical picture.

Conclusion: Differently from other arrhythmogenic channelopathies (as long-QT), digenic inheritance has never been reported in BrS. Our novel finding highlights the role of mutation load in this pathology and strongly suggests the adoption of gene panel to get an accurate genetic diagnosis,

which is mandatory for risk stratification, prevention and therapy.

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P05.11C

An *ABCC9* Mutation in a Family with Severe Brugada Syndrome and Diabetes

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Introduction: Brugada syndrome (BrS) is a rare inherited arrhythmia disorder that disrupts the flow of sodium ions, increasing the risk of ventricular fibrillation, although most affected individuals have a structurally normal heart. BrS contributes up to 12% of cases with Sudden Death Syndrome in the general population. It is a heterogeneous disorder and several families have been reported with an autosomal dominant mode of inheritance. Aim: Here we report an autosomal dominant family consisting of unaffected mother, affected father and two affected sons with severe BrS. The father and the eldest son, with long QT syndrome and diabetes, were also heterozygous for a variant in the sodium channel gene *SCN5A*. The youngest son was asymptomatic for BrS until tested; and is negative for the *SCN5A* variant. Our objective was to determine the genetic cause of severe BrS in this family. Materials and Methods: We performed whole exome sequencing (WES) on the family with severe BrS. Variants were called with GATK pipeline and were filtered using an autosomal dominant inheritance model to identify a causative mutation. **Results:** WES analysis successfully identified a novel missense mutation (c.3575C>A; p.T1192N) in exon 29 of the *ABCC9* gene in all affected individuals. This amino acid residue is highly conserved amongst species. Conclusion: We have identified a novel mutation within the *ABCC9* gene previously associated with BrS and early repolarization syndrome. Our finding provides a new target for molecular diagnosis and clinical management for BrS cases. Grant: We are funded by the British Research Council (BRC)

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P05.12D

Relation of cardiac dysfunction to thyroid stimulating hormone in children with Down syndrome

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Introduction: Down syndrome (DS) is the most common chromosomal abnormality and frequently associated with congenital heart disease. Recently, cardiac dysfunction was found in these patients even with anatomically normal hearts. The cause of which is still being investigated. At the same time, subclinical hypothyroidism (which is particularly common in DS) was suggested to decrease left ventricular diastolic function. The aim of this study was to evaluate cardiac functions in relation to the thyroid profile in children with DS.

Patients and methods*:* 100 consecutive children with DS were enrolled in this study with age range from 9 months to 264 months. Free T4, TSH levels and thyroid antibodies (anti-thyroglobulin and anti-peroxidase) were measured. Tissue Doppler imaging and two-dimensional speckle tracking echocardiography was used to evaluate cardiac functions.

Results: Diastolic dysfunction of left ventricle and right ventricle was found in 59% and 18% of children consequently. There was statistically significant difference between patients with and without left ventricle diastolic dysfunction regarding TSH level and a significant positive correlation between TSH level and E wave at tricuspid annulus

Conclusion*:* Biventricular diastolic dysfunction is a significant finding in children with DS that could be related to subclinical hypothyroidism. Prospective studies on a larger number of children and adults is need to confirm or exclude this hypothesis.

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P05.13A

The first genome wide association study in Iran; The Tehran Cardiometabolic Genetic Study (TCGS)

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Introduction: Cardiometabolic risk factors comprise cardiovascular diseases and/or diabetes, and need to be evaluated in different fields. **Objectives:** The primary aim of the Tehran Cardiometabolic Genetic Study (TCGS) is to create a comprehensive genome-wide database of at least 16,000 Tehranians, who are participants of the ongoing Tehran Lipid and Glucose Study (TLGS) cohort. **Methods:** TCGS was designed in collaboration with the Research Institute for Endocrine Sciences and the genetic company deCODE. Participants had already been followed for over a 20-year period for major cardiometabolic-related health events including myocardial infarction, stroke, diabetes mellitus, hypertension, obesity, hyperlipidemia, and familial hypercholesterolemia. **Results:** The TCGS cohort described here comprises 17,186 (86.3%) of the 19,905 TLGS participants who provided a baseline blood sample that was adequate for plasma and deoxyribonucleic acid analysis. This study is comprised of 849 individuals and 3109 families with at least one member having genotype information. Finally, 5977 males and 7422 females with the total genotyping rate of 0.9854 were genotyped with HumanOmniExpress-24-v1-0 bead chips (containing 649,932 single-nucleotide polymorphism loci with an average mean distance of 4 kilobases). **Conclusions:** Investigations conducted within the TCGS will seek to identify relevant patterns of genetic polymorphisms that could be related to cardiometabolic risk factors in participants from Tehran. By linking genome-wide data to the existing databank of TLGS participants, which includes comprehensive behavioral, biochemical, and clinical data on each participant since cohort inception in 1999, the TCGS will also allow exploration of gene-gene and gene-environment interactions as they relate to disease status.

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P05.14B

Next generation sequencing in the diagnosis of inherited cardiac disorders

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Introduction: Inherited cardiac disorders are characterized by complex genotype and phenotype picture, with many genes overlapping arrhythmogenic and structural diseases. The use of next-generation sequencing (NGS) allows to perform fast sequencing of wide regions. We evaluated and integrated a NGS panel and set up bioinformatic pipelines for diagnostic application. Materials and Methods: NGS analysis of genomic DNA was performed using Illumina Trusight Cardio, allowing analysis of 174 genes involved in several cardiac disorders on MiSeq platform. Moreover we developed a protocol for the enrichment of *SCN10A* gene, which has been recently associated to Brugada syndrome (BrS) and not present in the panel. **Results:** To validate this NGS approach we performed two runs including 18 patients with known genotype. NGS yielded a coverage >20X in 99.4% targeted regions, with 100% sensitivity and ≥94% specificity. We then performed analysis of 95 patients with different cardiac phenotypes. The higher diagnostic yield

was observed for cardiomyopathies and catecholaminergic ventricular tachycardia (80–100%), while for BrS and long QT syndrome we detected a variant in 33–52% patients, reflecting limitations in clinical definition. 64% identified variants were classified as of unknown significance, requiring further investigation to assign a pathogenetic role. In addition, 15% patients carried multiple variants, suggesting a more complex inheritance. **Conclusions:** These results underline potential and limits of NGS for heterogeneous conditions: NGS allows fast and efficient variant detection to ameliorate clinical management and family counseling, however it highlights the need of precise clinical definition and better strategies for the determination of variant pathogenetic role.

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P05.15C

NGS as a useful strategy in the molecular diagnosis of heterogeneous cardiomyopathies: a 327 case review

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Cardiomyopathy represents a heterogeneous group of disorders in which myocardium is structurally and functionally abnormal. The existence of overlapping signs between cardiomyopathies requires the simultaneous study of all known genes currently associated with the disease.

The objectives of this study were: 1. Establishing a molecular characterization of the studied patients. 2. Developing a diagnostic algorithm that could improve the management of these patients.

327 cases were included. Samples were analyzed by NGS targeted resequencing of a 238-gene panel, using SureSelect (Agilent) and the HiSeq platform (Illumina). Bioinformatic analysis was carried out using a state-of-the-art in-house annotation pipeline.

88 patients of cardiomyopathy were genetically diagnosed (27%) (Table 1), whereas the genetic cause of the pathology was not found in 190 patients who only were carriers of VUS (58%). On the other hand, 49 patients did not present any variant (15%).

A rate of 27% positive genetic diagnoses highlights the effectiveness of NGS for the genetic diagnosis of cardiomyopathy. Extending the genetic analysis to additional disease-related genes or genes included in the differential diagnosis could help to increase this rate and contribute to patient's clinical diagnosis disambiguation. Cosegregation and functional studies could help to re-classify VUS, achieving a higher diagnostic rate.

Table1. Results from genetic diagnoses, classified according to the type of cardiomyopathy (n = 327)

Clinical suspicion	Patients diagnosed		Detected variant effect			
	n	%	NS	MS	FS	SP
Familial cardiomyopathy (n = 12)	1	8.3%	–	1	–	–
Hypertrophic cardiomyopathy (n = 219)	61	27.9%	4	40	8	12
Dilated cardiomyopathy (n = 39)	14	35.9%	2	6	4	2
Restrictive cardiomyopathy (n = 6)	2	33.3%	–	1	1	–
Arrhythmogenic right ventricular dysplasia (n = 32)	6	18.8%	1	2	2	1
Left ventricular non-compaction (n = 18)	4	22.2%	–	2	1	1

NS: Nonsense. **MS**: Missense. **FS**: Frameshift. **SP**: splicing

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P05.16D

Diagnostic yield of three years next generation sequencing in clinical genetic diagnostics of cardiomyopathies

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Next generation sequencing (NGS) is successfully applied to find causal mutations and thus confirm the clinical diagnosis in patients suspected of hereditary cardiomyopathies. In 2012 NGS was introduced in our genome diagnostics laboratory using an enrichment kit targeting 55 genes associated with cardiomyopathies. We analysed DNA of 1126 patients with different cardiomyopathy subtypes. Classification of mutations was based on the ACMG guidelines. Additional haplotype and co-segregation analyses were performed to further support pathogenicity of potentially causal mutations. In 23% of the patients the clinical phenotype was (likely) explained by either a pathogenic or a likely pathogenic mutation, *viz.* in 14,4% (162/1126) of the patients a pathogenic mutation was identified, and in 9% (101/1126) one or more likely pathogenic mutations were identified. In addition, 52% of the patients carried at least one variant of unknown clinical significance (VUS), including patients who also carried a (likely) pathogenic mutation. The identified likely pathogenic and pathogenic mutations were found in half of the genes targeted with our NGS analysis. Yield in the different cardiomyopathy subtypes varied from 12% in patients with noncompaction cardiomyopathy to 23% in those with dilated cardiomyopathy. The contribution of VUSs to the clinical phenotype remains to be elucidated. To characterize these variants, data mining and sharing and further genetic and functional studies are implemented. In conclusion, NGS provides a genetic diagnosis in a significant proportion of cardiomyopathy cases and additional analyses are needed to further classify variants of unknown significance.

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P05.17A

Major contributors identified for increase in diagnostic yield in our 10 years of experience in genetic testing for cardiomyopathies; data sharing, titin (TTN) mutations and stricter clinical inclusion criteria

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Introduction: Inherited cardiomyopathies are defined by structural and functional abnormalities of the myocardium, characterized by extreme genetic and clinical heterogeneity. In the Erasmus Medical Center genetic testing is offered to patients with hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and noncompaction cardiomyopathy (NCCM). **Materials and Methods:** From 2006 till 2012 we performed Sanger sequencing of eight sarcomere genes. In the past four years, we applied a targeted next generation sequencing (NGS) 52 gene panel approach for ~900 patients. For the targeted approach we included the Titin gene (*TTN*), encoding the largest known human protein that plays a central role in sarcomere organization. We evaluated the diagnostic yield by comparing NGS gene panels with Sanger sequencing. **Results:** Sanger sequencing of eight genes resulted in a pathogenic mutation in 40% of the HCM patients. Using NGS, the majority of mutations in HCM were detected in genes, previously tested with Sanger sequencing. The increase in diagnostic yield from 40% to 50% is mainly achieved by data sharing and by re-evaluating patients with stricter clinical inclusion criteria. NGS in DCM and NCCM patients led to a doubling of the diagnostic yield, from 15% to 30%, mainly explained by truncating mutations in TTN in exons 259–359, encoding the A-band of Titin. **Conclusions:** Targeted NGS has proven most beneficial for NCCM and DCM patients, resulting in doubling the diagnostic yield. The increased yield for HCM patients was mainly achieved by stricter clinical inclusion criteria together with data sharing, resulting in reclassification of many missense variants.

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P05.18B

Cardiac and genetic features of noncompaction cardiomyopathy and it's relation to late outcome: A Dutch multicenter study of 327 pediatric and adult patients

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Noncompaction cardiomyopathy (NCCM) with excessive trabecularisation of the left ventricle presents as asymptomatic, with dyspnea, arrhythmias, or sudden death. This large multicenter study outlined the genetics and cardiac features of NCCM in adults and children.

Genetic examination allowed to classify 327 Dutch NCCM patients as 1) genetic, with a mutation in a cardiomyopathy gene (n = 111, 21 children), 2) probably genetic, familial cardiomyopathy without a mutation (n = 50, 8 children) and 3) sporadic, without a family history or mutation (n = 165, 22 children), and record clinical features and adverse events (i.e. death, heart transplant) in these groups. Most patients were diagnosed after age 40 years. The older patients were more likely to be sporadic and hypertensive. In 35% NCCM was genetic, with 68% involving *MYH7*, *MYBPC3* or - only in adults- *TTN*. In children *MYBPC3* defects occurred only as complex genotypes. Children with sporadic NCCM had better outcome than the (probably) genetic children. In adults the severity of NCCM was not linked to genetic status. A reduced LV-EF was associated with poor outcome in children and adults (p= 0,033). Genetic adults had more frequently a reduced right ventricular function. Four patients with a *MYH7* mutation had Ebstein's anomaly. Structural heart defects were not associated with adverse events.

Mutations in *MYH7, MYBPC3* and *TTN* were the most common causes for NCCM. Children had more complex genotypes associated with adverse events. Genetic status did not affect outcome in adults. Reduced left ventricular function was the most important predictor for adverse events in all patients.

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P05.19C

Cardiologic family screening for noncompaction cardiomyopathy shows variable phenotypes within and between families

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Introduction: In 50% of noncompaction cardiomyopathy (NCCM) there is a genetic cause of familial cardiomyopathy and/or a mutation in a cardiomyopathy gene. We investigate in a cohort of familial NCCM patients. Phenotypical expression of NCCM, or dilated- (DCM) and/or hypertrophic cardiomyopathy (HCM) in relation to the genotype. **Methods and results:** In 61(44%) families 108 relatives with a cardiomyopathy were identified by cardiologic family screening of 152 NCCM index cases; 72 relatives had NCCM, 6 overlapping features of NCCM/DCM, 17 DCM and 13 HCM. Of the 61 NCCM index patients 12(20%) also met the diagnostic criteria for DCM. NGS analysis of cardiomyopathy panel showed mutations in 42(69%) families. In 23 families (38%) with NCCM cases without features of DCM or HCM was found, with 15 (65%) mutations including 7 novel mutations, 5 reported previously in NCCM, 2 reported in DCM, and 1 in NCCM/DCM. DCM occurred in 30(49%) families of NCCM patients with 22 mutations: 10 were novel. 4 mutations were previously associated with in NCCM (18%), 3 with DCM, 5 with DCM/NCCM, In 5 (8%) families, family members were meeting the diagnostic criteria for HCM, 3 had a MYBPC3 (2 Dutch HCM founder) mutation. In addition, 2 index cases with NCCM/DCM and relatives with HCM had MYBPC3 mutations previously linked to HCM. **Conclusion:** In familial NCCM 45% of the specific mutations are novel, 24% specific for NCCM and an additional 19% specific for NCCM with overlapping features of DCM, suggesting NCCM involves distinct mutations in genes associated with HCM and DCM.

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P05.20D

A diagnostic odyssey in paediatric dilated cardiomyopathy: WES yields a diagnosis in more than one-third of patients

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Introduction: Although NGS-panels targeting dilated cardiomyopathy (DCM) are quite large (>45 genes), they do not include genes associated with common neuromuscular, syndromic, and metabolic causes of paediatric DCM. Therefore, combined whole exome sequencing (WES) and SNP-array analysis seems the preferred diagnostic approach. However, its yield in paediatric DCM has not been determined.

Materials and methods: We identified a cohort of 50 patients with onset of DCM <18 years. After excluding 17 patients with a genetic diagnosis that explained their phenotype *and* young age of onset, we performed SNP-array and trio-WES in 29 probands and four affected siblings. We used an HPO-based filter strategy for data analysis. All patients were carefully phenotyped for extra-cardiac manifestations.

Results: A genetic diagnosis was made in ten families (13/33 patients; 39%). Likely pathogenic or pathogenic heterozygous mutations were identified in *TNNT2*, *SCN5A*, *TTN*, *MYH7* (6), *TPM1*, as well as homozygous mutations in *GLB1* (GM1-gangliosidosis) and *SPEG* (centronuclear myopathy), and an 1p36.33p36.32 deletion. One child with syndromic DCM had compound heterozygous mutations in *CEP135* (primary microcephaly). Five patients carried autosomal recessive disease mutations that did not explain their phenotypes.

Conclusion: WES and SNP-array yielded diagnoses for 39% of our cohort, with most causal variants located in well-known cardiomyopathy genes. Variant filtering using additional HPO-terms in syndromic cases led to one additional diagnosis, but also to more VUS and incidental findings. Our study indicates that analysing a limited selection of well-known cardiomyopathy genes should be the preferred first diagnostic step during WES of isolated paediatric cardiomyopathy cases.

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P05.21A

High risk variants for cardiovascular diseases in population: analysis of Slovenian genomic database

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Introduction: Cardiovascular diseases are one of the largest public health problems worldwide. Analysis of population genomic data can reveal a mutation burden in cardiovascular diseases and consequently reveal the potential of genome analysis for precision medicine. The aim of the present study was to estimate the burden of rare pathogenic variants in cardiovascular genes in Slovenian population.

Materials and Methods: We designed a custom list of 136 genes associated with cardiovascular conditions, including cardiomyopathies, arrhythmias and vasculopathies. Exome sequencing data from 1324 individuals collected in our institution was de-identified and aggregate frequencies of variants in these genes were analysed. We divided variants into two main categories: (1) known pathogenic variants (2) rare variants with predicted pathologic effects.

Results: Altogether, we detected 47.831 rare variants in 136 cardiac disease-associated genes. Among these, 16 were known pathogenic dominant variants. The prevalence of known pathogenic variants in our patients was thus estimated at about 1.2%. We found out that additional 0.98% of patients harbor dominant variants with ClinVar conflicting interpretations of pathogenicity (13 additional variants). In addition, we identified 66 (5.0%) rare variants with theoretically predicted pathogenic effect (6 truncating and 60 novel predicted pathogenic missense variants).

Conclusion: In the present study, we estimated the burden of known cardiovascular disease associated pathogenic variants in the general population. Furthermore, we identified a considerable proportion of novel variants with predicted pathologic effect, opening further challenges and opportunities for exploration and individualized prediction of risk of cardiovascular diseases.

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P05.22B

Detailed investigation of congenital heart diseases frequency and types with clinical and genetic data in children with dysmorphology

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468 pediatric patients who applied or consulted to the dysmorphology policlinic were investigated(0–18 years old, 224 girls and 244 boys).Pedigree analyzes of all of the patients were performed and kinship associations were identified.Cardiologic evaluations and echocardiography of all patients were performed by pediatric cardiologists All patients had a standard anamnesis form and hospital records were scanned. Patients were first investigated whether there was any complication detected during the prenatal period (oligo/polyhydramnios,preeclampsia…etc).Natal period investigated birth week, type of delivery, birth weight and birth length. APGAR scores, presence of perinatal asphyxia were questioned in the postnatal period. In addition, the stages of motor development, social development and language development of the patients were evaluated. Patients were questioned about the presence of a surgical operation, seizure, chronic illness.Paternal kinship cases were searched for family histories. Because consanguineous marriage is high rate in our region. Autosomal recessive syndromes are therefore more common. In addition, the presence of individuals with other congenital heart disease in the family was questioned. Standard karyotype analyzes of the patients were performed. We have done FISH and molecular genetics studies to prove the syndromes that accompany congenital heart disease to the extent that our center has possibilities. The resulting data were analyzed statistically in the SPSS program.As a result, factors such as gender, intrauterine growth retardation, low APGAR score, low birth weight, motor, social, language developmental delay, past surgical operation story, seizure story, chronic disease story were found to be related to congenital heart disease in dysmorphology patients.

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P05.23C

Characterisation of the CYP11B2 intron 2 conversion using MinION Oxford Nanopore sequencing device

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Introduction: Polymorphisms affecting the synthesis of aldosterone or its regulation may have effects on blood pressure. An intron conversion replacing a section of CYP11B2 intron 2 with the corresponding region of the 94% identical CYP11B1 has been linked to hypertension risk. However, that polymorphism had never been described precisely yet. In 2014, Oxford Nanopore released a 3rd generation sequencer the MinION® device, well suited for sequencing of complex genomes with large repetitive elements or high similarities. The aim of this study was to use this new technology to characterise precisely the conversion. Materials and Methods: Forward and reverse primers were specifically designed to amplify CYP11B2 intron 2 region. Amplicons of 100 "wild-type" (WT) and 100 individuals carrying the intron conversion (CONV) from the Medical Research Council (MRC) British Genetics of HyperTension (BRIGHT) Study (http://www.brightstudy.ac.uk) were sequenced and processed through an in-house pipeline. **Results:** With a mean coverage of 460x per individual we assembled a CONV consensus sequence of 5.5kb that presents 85% identity with CYP11B2 gene sequence (GenBank: D13752.1). 95% and 97% identity were found between CONV consensus and respectively CYP11B1 and CYP11B2 intron 2 sequences. We defined 274bp of CYP11B2 intron 2 (from 7,052bp to 7,326bp) replaced by 264bp of CYP11B1 corresponding sequence (from 7,062bp to 7,326bp, GenBank: AH002666.2). Conclusion: This study presents a precise characterisation of the CYP11B2 intron 2 conversion that will lead to the assignation of a tagger SNP and encourages further investigation of that polymorphism into its role in the physiology of hypertension.

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Functional analysis of nexilin in hiPSC-derived cardiomyocytes

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The protein Nexilin was identified to be a structural protein in the Z-disk in the sarcomere of cardiomyocytes. It is

known to play an important role in sarcomere integrity as it was shown that loss of Nexilin leads to blurry Z-disks in zebrafish. Mutations in Nexilin were found to lead to dilated cardiomyopathy (DCM) in humans, zebrafish and mice. The underlying pathomechanisms are still unkown. The main aim of this study is to generate hiPSCs lacking Nexilin and functionally characterize derived cardiomyocytes to get a better understanding of the molecular mechanisms leading to DCM in humans.

In a first step of the project we aimed to establish the protocol of differentiation and characterize cardiomyocytes derived from human iPSCs. Therefore, modulation of canonical Wnt signaling is performed, leading to efficient differentiation of hiPSC to cardiomyocytes. Characterization of cells was performed before differentiation at RNA level using the markers Oct4, Klf4 and Nanog to show pluripotency of hiPSCs. After differentiation, characterization is performed using heart specific expression markers such as MYL2, Troponin T, Desmin, GATA4 and HCN4 at RNA level.

The next step was the generation of Nexilin knockout iPSCs using the CRISPR/Cas system. The resulted cell lines will then be differentiated into cardiomyocytes and the same aforementioned markers will be used for detailed molecular characterization of Nexn-KO iPSC-derived cardiomyocytes and compared to controls. Additionally, phenotypic characterization, sarcomere integrity and expression levels of cardiac transcription factors such as Smad proteins and Tbx5 will be analysed to understand the function of Nexilin.

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P05.25A

Role of CD68 gene expression in atherosclerosis

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Introduction: Oxidized LDL (oLDL) play a central role in the progression of atherosclerosis due to their cytotoxic effects in endothelial vessels. It is assumed that oLDL utilization occurs through the entire group of scavenger receptors (SR) located on monocytes/macrophages cells membranes. It's proposed that SR gene activity may serve as a sensitive marker for excessive accumulation of foam cells in the early stages of lipid spots, and as a result of an atherosclerotic plaque. So, the aim of this study was to determine the relative gene expression level of scavenger receptor CD68 in atherosclerotic patients with varying severity.

Materials and methods: Gene expression analysis was carried out on venous blood samples from 48 patients with confirmed multifocal atherosclerosis (IPA), 46 patients with acute coronary syndrome (ACS), 47 patients with risk factors (RF) for cardiovascular disease and 16 healthy donors. The relative gene expression level (RQ) evaluated by the 2deltaCt method and Student t-test ($p = 0.05$).

Results: In our study patients with chronic IPA characterized with non-significantly mild RQ decreased in blood and increased in carotid atherosclerotic plaques (0.84 and 1.04 respectively), however patients with ACS showed almost significantly 1.82-fold increased RQ ($p=0.061$). At the same time, individuals with RF have showed significantly low RQ (0.15, $p=0.050$).

Conclusion: Our results suggest that increasing level of CD68 in ACS is due to severe acute inflammation processes, which are not so actual for IPA patients as well as for FR individuals.

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Diagnostic yield of sequencing lipoprotein lipase genes pathway in patients with severe hypercholesterolemia

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Background: Elevated concentrations of LDL cholesterol and lipoproteins are correlated with risk for coronary artery disease (CAD) and can be caused by a wide range of genetic, lifestyle, and environmental factors. Gene sequencing allows for the identification of causative mutations in Familial Hypercholesterolemia (FH) and lipoprotein lipase genes, all belonging to the LPL pathway. *Methods:* We sequenced the *LDLR, PCSK9, APOB* and *LPL* genes in 88 probands presenting with hypercholesterolemia (LDL cholesterol ≥190 mg/dl), with previous CAD history ($N = 67$; 76.13%) or without ($N = 21$; 23.24%). By means of a custom-based IonAmpliSeq panel, we meant to annotate

rare (allele frequency <1%) damaging mutations included loss of function (i.e., nonsense, canonical splice site, and frameshift) and missense variants annotated as pathogenic or predicted to be damaging by computer algorithms. *Findings:* Across all participants, 41 damaging mutations in the *LDLR, PCSK9, APOB* and *LPL* genes were identified in 40 probands (one was a carrier of a double heterozygosity). Compared to non-carriers, heterozygous carriers displayed higher LDL cholesterol (22% higher, 95%CI 11 - 23; $p = 3 \times 10^{-12}$) and plasma triglycerides (19% higher, 95%CI 12 - 25; $p = 3 \times 10^{-12}$) as well as increased risk for CAD (Odds Ratio 2.84; 95%CI 1.35 - 2.51; $p = 0.0001$). Beyond rare mutations, FH mutation carriers had higher cumulative exposure to LDL cholesterol than noncarriers. *Interpretation:* In our series, about 46.6% carry a damaging mutation in the *LPL* pathway genes that are associated with higher plasma triglycerides as well as increased risk for CAD.

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P05.27C

Proactive Identification and Genetic Testing for Familial Hypercholesterolaemia (FH) in Primary Care: Experiences of Patients and General Practitioners

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Introduction

Familial hypercholesterolaemia (FH) is a common autosomal dominant disorder characterised by elevated cholesterol. However, around 80% of affected individuals remain undiagnosed. This study explored the experiences of GPs and patients participating in an intervention to identify and test individuals at high risk of FH in primary care.

Methods and materials

Semi-structured audiotaped qualitative interviews with patients and GPs from six UK family practices. They had participated in an intervention involving case finding and assessment of those at high risk of FH in primary care (database search of GP records for patients with cholesterol >7.5mmol/L, with computerised reminders to GPs and mailed invitation to patients). Experiences of, and attitudes towards this approach to identification, assessment and use of genetic testing for FH were explored, with descriptive thematic analysis of data.

Results

A sample of 23 (21.85%) of 95 patients assessed, and all 14 GPs (100%) participated. Patients and practitioners were generally receptive to proactive identification of risk of FH in primary care, experiencing the approaches used as acceptable and feasible. Challenges identified included: enhancing communication and information for patients; and accurate family history assessment and decision making on specialist referral for GPs. Both patients and practitioners accommodated genetic testing for FH in the general practice setting without significant problems, but shared concerns about time for effective communication and appropriate skills for interpretation of test results.

Conclusion

This study suggests the feasibility and acceptability of proactive identification of risk of FH, including genetic testing, in primary care.

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Familial Ebstein's anomaly: whole exome sequencing identifies novel phenotype associated with *FLNA*

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Mutations in Filamin A are associated with a diverse range of phenotypes with loss of function mutations typically causing periventricular nodular heterotopia and missense mutations associated with a range of skeletal abnormalities including Fronto-Metaphyseal Dysplasia (FMD), Otopalatodigital (OPD) and Melnick Needles Syndromes (MNS). Congenital heart defects have been reported in patients with skeletal phenotypes but diagnostically bony abnormalities have been the most striking feature of these conditions. We report a family in which 7 individuals over 2 generations had Ebstein's anomaly of the heart. Presentation in the 3 males in the family was with heart failure in the neonatal period, which was in one case fatal. The 4 females were all diagnosed with milder Ebstein's anomaly, requiring surgery in 1 case. Affected individuals had camptodactyly, reduced elbow extension and keloid scarring; males also described their joints as stiff and had more marked skeletal findings. A

molecular genetic diagnosis was made using DNA from two first cousins and whole exome sequencing. Affected family members were found to share a novel nonsynonymous G>A highly conserved (Phylop 0.998857) and deleterious (Gerp 5.23) variant in Filamin A on the X chromosome, leading to the substitution of glycine to arginine at position 1554. The mutation lies in the 14th filamin repeat region, mutations in which have been associated with OPD, FMD and MNS but not Ebstein's anomaly. The report of this family both significantly extends the phenotype of Filamin A and identifies this gene as important in the development of right sided congenital heart disease.

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A new case of Generalized Arterial Calcification of Infancy with compound heterozygous mutations in the ATP-binding cassette, subfamily C, member 6 gene

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Introduction: Generalized Arterial Calcification of Infancy (GACI) is a rare autosomal-recessive disorder characterized by calcification of the internal elastic lamina, fibrotic myointimal proliferation and subsequent stenosis of large and medium size arteries. Biallelic inactivating mutations in ENPP1 have been identified in most cases, while mutations in ABCC6 have been identified in 10% of GACI cases only. Methods: The 6-years-old proband died suddenly at school. Past medical history revealed recurrent intestinal angina and retrosternal pain with normal electrocardiography and echocardiogram findings at 2 and 3 years of age. A chronic ischemic heart disease with post myocardial infarction aneurysm, associated with widespread arterial calcification was identified at autopsy, leading to the diagnosis of GACI. Polymerase-chain-reaction products of ENPP1 (NM.006208.2) and ABCC6 (NM.001171.5) were directly sequenced bidirectionally on a ABI 3500Dx platform and analysed with LaserGene SeqMan. **Results:** No mutations were identified in ENPP1. Analysis of the ABCC6 gene identified two rare variants, both defined as "pathogenic" by *in silico* tools: a c.2018 T>C (Leu673Pro) in exon 16, previously associated with Pseudoxanthoma Elasticum (PXE), and a c.3836 C>T (Pro1279Leu) in exon 27 (both inherited from the parents). **Conclusions:** It is currently a matter of intense debate whether genetic and phenotypic overlap between GACI and PXE might reflect two ends of the clinical spectrum of ectopic calcification rather than two distinct disorders. Intriguingly, our patient, who had been symptomatic throughout his whole life, died at the age of 6 years, while affected GACI individuals usually die within the first 6 months of life.

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P05.30B

Compound heterozygous *GATA5* mutations in a girl with hydrops fetalis, congenital heart defects and genital anomalies

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GATA5 belongs to the GATA family of transcription factors characterized by highly evolutionary conserved zinc-finger DNA-binding domains. Mouse models have implicated a role of GATA5 during mammalian embryogenesis, including proper heart development and gender-specific regulation of female genitourinary tract formation. Candidate gene studies have associated *GATA5* heterozygous missense alterations with a broad variety of heart diseases. However, these studies did not include parental testing and none of these alterations were scrutinized in an *in vivo* model. Thus, the clinical relevance of the identified heterozygous alterations remains unclear. Recently, a first male patient bearing biallelic mutations in *GATA5* and complex congenital heart defects (CHD) was described. Here we report on a girl affected by hydrops fetalis, CHDs, clitoromegaly and postnatally increased 17-hydroxyprogesterone levels. Trio whole-exome sequencing identified compound heterozygous missense mutations in *GATA5*, p.Ser19Trp and p.Arg202Gln, as the only putative disease-causing alterations. Notably, extensive clinical follow-up of both parents and her twin-sister, all heterozygous for a *GATA5* mutation, was unremarkable. Extensive functional analyses revealed that the identified *GATA5* mutations fail to rescue the cardia bifida phenotype in a zebrafish model, mislocalize to subnuclear foci when transiently transfected in HEK293 cells and possess less transcriptional activity. In summary, our findings show that biallelic *GATA5* mutations are highly penetrant. In addition to heart diseases they can result in congenital abnormalities of the female

genitourinary tract, highlighting the gender-specific effect of GATA5 in humans. Furthermore, our data suggest low-penetrance of the heterozygous alterations previously associated with various heart diseases.

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Genomic variation associated with mortality in European ancestry among individuals with heart failure in Brazilian population

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Introduction: Heart failure (HF) is a relatively common chronic condition characterized by the inability of the heart to efficiently pump blood. The survival causes significant concern, because after HF is diagnosed, 20% of patients die within one year. To screen for genetic variants modulating mortality in patients with HF in Brazilian population, we used admixture mapping, a technique that scans the genomes of recently admixed populations and searches for genomic regions in individuals with disease where there is substantial deviation in one of the parental ancestries compared with the global genome average.

Materials and Methods: Genomic ancestry was estimated using 182.090 SNPs, three continental reference populations (European, African and Amerindian) and PCAdmix software. For admixture analysis, the mortality was adjusted for age, sex, hypertension and diabetes, using multivariate linear regression analysis.

Results: Our study included 492 Brazilian patients with HF. The estimated average of ancestry proportions observed was 61.8±21.8% European, 24.9±22.3% African and 13.3 ±10.6% Amerindian. We performed admixture mapping and identified a locus on chromosome 6p22.3 in European ancestry ($P=6.8\times10^{-5}$) associated with mortality in patients with HF, where the guanosine monophosphate reductase gene (GMPR) is mapped.

Conclusions: This study identified a locus associated with mortality in European ancestry among individuals with HF. The fine mapping in this region may be possible to uncover causative variants that contribute to mortality in HF.

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P05.32D

Elucidating the mutation spectrum underlying severe hypercholesterolemia in Finland

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Severe hypercholesterolemia is a major risk factor for coronary artery disease (CAD). Patients suffering from familial inherited forms of severe hypercholesterolemia have a 20-fold increased CAD-risk. Although CAD may be prevented by lowering cholesterol levels, less than 20 % of hypercholesterolemia patients are identified or treated in Finland and only half of the patients carry hypercholesterolemia-associated mutations.

Our study aims to unravel the genetic architecture underlying severe hypercholesterolemia in Finland by studying individuals with high LDL-cholesterol (>5 mmol/l) identified in our ongoing population study GeneRISK, targeting 10,000 subjects aged 45–64. Hitherto 4,570 study participants have been recruited. 4.6% had severe hypercholesterolemia, of which only 3% received lipid-lowering therapy. 88 cases have been screened for the 5 most common known familial hypercholesterolemia-associated *LDLR*-mutations enriched in Finland. Surprisingly, all subjects were mutation negative. Whole-exome sequencing of 72 subjects didn't reveal hypercholesterolemia-causing mutations in the *LDLR*, *APOB* or *PCSK9* genes either. Nonetheless, six patients carried rare potentially pathogenic missense variants in putative candidate genes *MYLIP* and *SORT1*. Polygenic modeling (genomic risk score (GRS) encompassing 106 SNPs explaining 17% of the LDL-cholesterol variation in

the population) suggested clustering of polygenic risk in 15 (20,8%) of the sequenced patients (GRS within the top 10% of population distribution).

In conclusion, hypercholesterolemia-treatment appears gravely suboptimal and the contribution of known hypercholesterolemia-mutations may be smaller than previously anticipated in Southern Finland. We intend to further screen up to 200 hypercholesterolemia cases, and to use *in silico* and *in vitro* methods to assess the function of putative pathogenic variants.

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P05.33A

Multiple gene variants in hypertrophic cardiomyopathy

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Background: Multiple likely pathogenic/pathogenic (LP/P) variants in hypertrophic cardiomyopathy (HCM) patients were described 10years ago with a prevalence of 5% and more severe phenotype suggested. Given increased gene panel sizes and more stringent variant curation, we sought to re-examine the significance of multiple rare variants. **Methods:** We used a retrospective cohort study design including HCM probands from a specialised HCM centre from 2002–2016. Research-based genetic testing was performed including 45 cardiac genes. **Results:** 382 probands met inclusion criteria. There were 224 (59%) probands with at least one rare variant (allele frequency ≤0.02%). Variants were analyzed using varying sized gene panels to represent comprehensive or targeted testing. Based on a 45-gene panel, 127 (33%) had a LP/P variant, 139 (36%) had variants of uncertain significance (VUS) and 66 (17%) probands had multiple rare variants. A targeted 8-gene panel yielded 125 (32%) LP/P variants, 52 (14%) VUS and 14 (4%) probands had multiple rare variants. No proband had 2 LP/P classified variants. Including affected family members (total n = 412), cluster-adjusted analyses showed younger age (OR 0.95, 95%CI 0.92–0.98, p=0.004) and family history of sudden death (OR 3.5, 95%CI 1.3–9.9, p=0.02) to be significantly more likely in multiple versus single variant patients when considering an 8-gene panel, but not larger panels. Those with multiple variants had worse event-free survival from all cause death, cardiac transplantation and cardiac arrest (log-rank p=0.008). **Conclusions:** Multiple LP/P variants are rare in HCM, but multiple uncertain variants occur in 4% leading to earlier onset and increased risk of events.

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Identification of disease-causing genetic variants in a representative cohort of Czech patients with early onset hypertrophic cardiomyopathy

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Introduction: Hypertrophic cardiomyopathy (HCM) is a relatively common AD disorder with an estimated population frequency of 0.2 %. Since HCM usually manifests later in life, early-onset HCM is rare and could be caused by pathogenic variants (PV) different from sarcomere genes. The aim of our study was to test this hypothesis by identification of PV in early-onset HCM in a representative cohort of unrelated Czech cases.

Materials and Methods: Nineteen paediatric HCM cases were subjected to NGS using a targeted panel comprising 174 cardiac conditions-related genes (TruSight Cardio -Illumina on the MiSeq platform - Illumina). All variants were validated by Sanger DNA sequencing and ACMG.net variant classification was used.

Results: Pathogenic / likely pathogenic variants were identified in 14/19 (73.7 %) cases in *MYH7* (7/19, 36.8%), *MYBPC3* (4/19, 21.1%), and *CSRP3* (1/19, 5.2%) genes. One patient is a double-heterozygote for *MYBPC3* and *MYH7* (5.2%) PV, while one patient (5.2 %) carries a PV in the *PTPN11* gene.

Conclusions: In paediatric HCM the thick filament genes *MYBPC3* and *MYH7* are most commonly affected, similarly as in late-onset HCM. High mutation detection rate corresponds probably to the severe phenotype and early age of studied cases. We also confirmed the diagnosis of one patient with Noonan syndrome demonstrating the usefulness of broader panels in the diagnosis of cardiac disorders which may be part of other genetic syndromes. The

identification of PV is important for stratified patient management and cardiologic follow-up of apparently healthy PV-positive family members.

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P05.35C

The variants spectrum and genotype-related histopathological alterations in Chinese hypertrophic obstructive cardiomyopathy patients

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Introduction: Patients with hypertrophic obstructive cardiomyopathy (HOCM) have a higher risk of sudden death than non-obstructive hypertrophic cardiomyopathy (HCM). A large amount of variants, predominantly in sarcomeric genes, have been identified.

Methods and results: A 93-cardiomyopathy-related panel was used to screen 191 Chinese HOCM patients. The overall diagnosis rate was 51.3%, including more pathogenic variants in *MYH7* (46.5%) than *MYBPC3* (33.8%), which was similar to previous studies in Chinese population but distinctive from other ethnic studies around the world. Using Jian's method with dbscSNV database, 20 variants were predicted to affect splicing, and primers were successfully designed for 18 of them. With the extracted and reverse-transcribed RNA from patients' cardiac tissue, only 6 variants were confirmed to actually cause aberrant RNA splicing, including all 5 pathogenic *MYBPC3* ones and another likely benign synonymous variant in *ANKRD1*. Moreover, histopathological examinations on septal tissues with haematoxylin and eosin and Masson trichrome staining revealed characteristic histopathological alterations in HOCM patients with sarcomeric variants by vacuoles degeneration, disorganization and whorling of muscle bundles, irrespective of variants number.

Conclusion: The characteristic spectrum of more *MYH7* than *MYBPC3* variants was specific for Chinese HCM population but not HOCM. Interpreting splice variants based on *in silico* tools without validation should be extremely cautious due to their unexpected low prediction accuracy. The existence of sarcomeric variant was correlated with distinctive histopathological alterations in HOCM patients, indicating different pathogenic downstream pathways underlying genetic and non-genetic HCM.

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P05.36D

Mosaicism in inherited cardiac conditions detected by targeted NGS analysis

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Molecular genetic analysis of over 500 consecutively referred patients clinically diagnosed with various inherited cardiac conditions was undertaken by next generation sequencing (NGS) of a targeted gene panel comprising 170 genes associated with inherited cardiac conditions. We detected four families in which either the index patient or a parent was found to be mosaic for a pathogenic or likely pathogenic variant.

A male patient, diagnosed with left ventricular hypertrophy and no family history, was found to be mosaic for the pathogenic missense variant c.1387G>A (p.A463T) in *MYH7*. Testing of both blood and saliva showed varying levels of mosaicism. Following an incidental diagnosis of dilated cardiomyopathy (DCM) in a 12-year old boy, who was heterozygous for a pathogenic *LMNA* variant (c.1622G>A, p.R541H), his father was found to be mosaic for the same variant, with a much milder phenotype than his son. Testing of the asymptomatic mothers of two different patients with catecholaminergic polymorphic ventricular

tachycardia (CPVT) and pathogenic missense variants in the *RYR2* gene (p.H2486P; p.V4190L), detected the variants as low-level mosaics. Sanger sequencing failed to detect the mosaicism.

Detection of mosaicism is important to provide accurate counselling to families, especially around recurrence risk for future pregnancies. The higher sensitivity of NGS for detecting mosaicism is one of its many advantages over Sanger sequencing as a first line diagnostic test and will also help to elucidate the potentially under-reported prevalence of mosaicism in cardiac disease.

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P05.37A

Diagnostic yield of DNA-diagnostics and "signal to noise" ratio in Russian patients with left ventricular non-compaction

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Introduction. Left ventricular non-compaction cardiomyopathy (LVNC) is characterized by two-layer myocardium (compact and "spongy") and affects mostly left ventricular apex, lateral wall and ventricular septum. Although mutations in more than 20 genes have been detected in patients with LVNC, the effectiveness of the DNA testing remains unclear and mutation spectrum is unknown.

Materials and methods. We had observed 44 probands diagnosed with LVNC. Mutational testing was performed by NGS (Ion Torrent) sequencing of the panel containing 12 genes, followed by Sanger sequencing of detected variants. PolyPhen2, SIFT, and MutationTaster were used for *in silico* characterization of new missense variants.

Results. Fifteen pathogenic/probably pathogenic variants were detected in 15 (34%) probands. Most of the variants were found in the *MYH7* and *MYBPC3* genes (7 and 6, respectively). In a single index case the heterozygous deletion in the *DES* gene was detected. Mutation p.Q1233* *MYBPC3* was found in 4 probands. Three probands had carried two genetic variants in different genes. Remarkable that only 4 variants found in this study were previously described as mutations. This makes the "signal:noise" (or "mutation:VUCS") ratio of 7:10.

Conclusion. Diagnostic yield of simultaneous analysis of the 12 genes in patients with LVNC was 34%. According to our data, the "signal:noise" ratio was 0.7:1, showing the prevalence of VUCS in LVNC-patients despite the fact that the genes encoding sarcomeric proteins are thoroughly studied. We assume this may point to particular mutation spectrum causing LVNC, though additional studies are necessary. This work was supported by grant RNF № 16-15-10421

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P05.38B

A founder mutation of the potassium channel KCNQ1 in long QT syndrome

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Long QT syndrome (LQTS), the most often diagnosed inherited arrhythmic syndrome, is characterized by abnormal prolongation of QT interval associated with a high risk of life-threatening ventricular arrhythmias and sudden cardiac death, often in young individuals. The prevalence of this disease has been shown to be at least 2:2500. Mutations in 15 genes encoding cardiac ionic channels and related proteins have been associated with LQTS so far but mutations in only 3 genes (*KCNQ1, KCNH2 and SCN5A*) account for 70% of the genetically identified LQTS. In 30 LQTS families from our database, we found 17 mutations in *KCNQ1* (LQTS type 1) and 8 mutations in *KCNH2* (LQTS type 2). The same *KCNQ1* mutation c.926C>T; p.T309I was present in 6 putatively unrelated families - a rare event in LQTS where each family is usually characterized by its own mutation. We hypothesized that the mutation may be considered as the founder mutation in our region. Haplotype analysis, performed on probands and family members by using highly polymorphic microsatellite markers, revealed of a "founder" effect. We repeated mutational analysis in

T309I families with a wider set of LQTS-related genes using Next Generation Sequencing method to identify possible further genetic variants for a more detailed genotype - fenotype analysis. Obtained data allows us to provide genotype and phenotype guided therapeutic measures to prevent malignant arrhythmias even in asymptomatic mutation carriers. This work is sponsored by grant of the ministry of health of the Czech republic: NV16-30571A.

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P05.39C

Identification of novel long QT syndrome-associated mutations by targeted sequencing analyses

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Introduction: Long QT syndrome (LQTS) is characterized by lethal ventricular arrhythmia that might lead to sudden cardiac death. Although 15 causative genes for LQTS have been identified so far, genetic etiology remains unknown in approximately 40% of LQTS patients. This study aims to identify undiscovered variants associated with and/or mutations responsible for LQTS to improve diagnostic accuracy and prognosis. Methods: We designed a custom sequencing panel of 100 candidate genes implicated in the pathophysiology of LQTS including 15 causative genes based on the previous report by Shigemizu et al. (PLoS One, 2015). Targeted next-generation sequencing was performed using genomic DNA obtained from peripheral blood of 552 Japanese LQTS patients. Variants in the exon and promoter regions were extracted, followed by filtering steps that excluded synonymous ones and those detected in public databases. **Results:** 71 novel rare missense mutations in the exon regions of 45 genes were identified in 77 patients. These variants were predicted to be damaging by both SIFT and PolyPhen-2. We also detected 222 novel variants in the promoter region of 62 genes in 309 patients. Bioinformatic analyses predicted affinity change of transcription factors including SMAD3 and GATA4 that are associated with arrhythmia resulting from these promoter variations. Conclusion: The present study identified previously undiscovered variants in the exon and promoter regions. These variants may play an important role that increases susceptibility to arrhythmia. This study was supported by a grant program (ID:15663808) of the Japan Agency for Medical Research and Development.

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P05.40D

Familial association between eight chromosomal regions with metabolic syndrome in Tehran Cardio-Metabolic Genetic Study (TCGS)

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Introduction: High risk of mortality by cardiovascular disease(CVD) is a most controversial worldwide subject. MetS and its components as main risk factors for CVD play important role. This study aims to estimate heritability and aggregation of MetS in TCGS participants and to evaluate linkage association between eight chromosomal regions and HDL-C in MetS affected families. Methods: All families were selected based on ATPIII criteria in two steps: 1) 278 families (n = 2080; 396 parents 1116 siblings and 528 other relatives) with at least two MetS affected in two generation to analyze Familial Intra Class Correlation(ICC) and heritability of HDL_C, 2) 80 families with at least two MetS affected and low HDL-C to analyze conditional-logistic likelihood-ratio based on nonparametric linkage analysis. To investigate possible genes linked among HDL_C and MetS, 22 flag single nucleotide polymorphisms were genotyped by Illumina chip-typing. **Results:** Total populations were aged 41.29±19.1 years. The heritability of HDL-C after confounder adjustment was 36% (P=0.032,CI=19.2). However, ICC showed significant correlation among first relatives in first selected families group this significant correlation in the second group was limited to mother-son (r=22%,P=0.0296). Contrasting ASP and DSP showed higher LOD scores in two regions 6p21.33 (rs2248462 (LOD=13.3), rs28366155(LOD=5.9)) and 11q23.3 (rs17519079(LOD=9.5), rs17120029(LOD=6.7), rs1942478(LOD=6.4), rs2075294(LOD=4.5)). Conclusion: This study indicates that Tehranian families are genetically predisposed to low HDL_C level. Also that 6p21.33 and 11q23.3 regions are very likely to contain genes that control HDL-C level in Tehranian families with metabolic syndrome. However, for confirming the linkage results more markers should assess in further studies.

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Myocardial microRNAs associated with Arrhythmogenic Cardiomyopathy

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Introduction: Arrhythmogenic cardiomyopathy (AC) is a clinically and genetically heterogeneous myocardial disease. Half of AC patients harbour private desmosomal gene mutations. Although microRNAs (miRNAs) have been recently emerged as key regulator molecules in cardiovascular diseases (CVDs), their impact on AC onset and progression is largely unknown. The aim of the study was to identify one or more miRNA signatures in genotype-positive AC-patients with different gene mutations. Methods: Frozen myocardial tissue of 6 heart transplanted AC probands: 2 plakophilin-2 (PKP2), 2 desmoplakin (DSP) and 2 desmoglein-2 (DSG2) mutation carriers, as well as 2 healthy controls underwent miRNAs expression profiling. Analysis of 84 miRNAs was performed by using miScript microRNA PCR Array kit. Relative quantification was carried out by ΔΔCt method. **Results:** Among the 84 miRNAs, 23 miRNAs were either over- or under-expressed in PKP2 probands, 17 in DSP carriers and 12 in DSG2 carriers, when compared to healthy controls. A subsequent comparison of the miRNA expression profile among mutation carriers revealed 12 miRNA in common between PKP2 and DSP carriers whereas DSG2 probands showed a unique miRNA signature. **Conclusions:** In our study, the altered miRNAs expression in AC probands compared to healthy controls proves their potential role in AC. Furthermore, the shared miRNA expression profile between PKP2 and DSP probands suggests a common pathogenetic pathway, different from DSG2 mutation carriers as to reflect clinical variability in AC. As for other CVDs, miRNAs might represent early disease biomarkers for AC.

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P05.42B

The genetic determination of the differentiation between idiopathic dilated cardiomyopathy and ischemic dilated cardiomyopathy

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Absract

Introduction: Dilated cardiomyopathy that causes congestive heart failure and sudden death has have high mortality and morbidity. Mostly dilated cardiomyopathy are accepted ischemic dilated cardiomyopathy (IsDC) or idiopathic dilated cardiomyopathy (IdDC). Although the treatments of both of IsDC and IdDC are similar (as 90%), coronary revascularization is feasible only for IsDC. There are several methods to distinguish IsDC and IdDC currently and some difficulties while performing these methods.

Material and Methods: In our study, we investigated these miRNAs for that IsDC is to distinguish from IdDC, let-7b, let-7c, miR-1, miR-15, miR-17, miR-19a, miR-19b, miR-20a, miR-20b, miR-23a, miR-24, miR-27a, miR-28, miR-30, miR-99b, miR-100, miR-101, miR-103, miR-106a, miR-125b, miR-126, miR-140, miR-191, miR-195, miR-199a, miR-214, miR-222, miR-320, miR-342 and miR-378 in IsDC and IdDC to distinguish both of them. The patients with congestive heart failure having dilated left ventricle and ejection fraction less than 50% were accepted to the study. Patients were divided two groups as IsDC (25 patients) and IdDC(25 patients) up to coranary angiography reports and 10 healthy people accepted as a control group.

Results: We found that miR-24, miR-28, miR-100, miR-103, miR-125b, miR-214, let-7b and let-7c were overexpressed and increased more than two fold significantly in both ISDC and IdDC groups when compared with control groups. Whereas miR-15b and miR-106a were overexpressed and increased more than two fold significantly only in IsDC group, when compared with IdDC and control groups.

Conclusions: As a results, only miR-15b and miR-106a seem to be decisive biomarkers to distinguish IsDC and IdDC.

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P05.43C

Exome sequencing-based molecular autopsy of formalin-fixed paraffin-embedded tissue in sudden death

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Introduction: Sudden death in the young is a devastating complication of inherited heart disorders. Finding the precise cause of death is important, but is often unresolved after postmortem investigation. The addition of postmortem genetic testing, i.e. the molecular autopsy, can identify additional causes of death. We evaluated DNA extracted from formalin-fixed paraffin embedded postmortem tissue for exome sequencing-based molecular autopsy of sudden death in the young.

Materials and Methods: We collected clinical and postmortem information on patients with sudden death. Exome sequencing was performed on DNA extracted from fixed postmortem tissue. Variants relevant to the cause of death were sought.

Results: Five genetically unresolved patients with sudden death were recruited. DNA extracted from fixed postmortem tissue was degraded. Exome sequencing achieved 20-fold coverage of at least 82% of coding regions. A threefold excess of singleton variants was found in the exome sequencing data of one patient. We found a pathogenic *de-novo SCN1A* frameshift variant in a patient with sudden unexpected death in epilepsy and a pathogenic *LMNA* nonsense variant in a patient with dilated cardiomyopathy.

Conclusion: DNA extracted from fixed postmortem tissue is applicable to exome sequencing-based molecular autopsy. Fixed postmortem tissues are an untapped resource for exome-based studies of rare causes of sudden death.

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P05.44D

Effect of the macrophage migration inhibitory factor genotype on risk of myocardial infarction is modulated by other risk factors

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Aim: To investigate the effect of the macrophage migration inhibitory factor (MIF) genotype on the risk of myocardial

infarction (MI), alone and in combination with other risk factor of MI.

Method: A total of 423 cases with a first MI and 465 controls from the Maltese Acute Myocardial Infarction (MAMI) Study were analysed. Data was collected through an interviewer-led questionnaire and various biochemical measurements. The MIF G-173C, C656G, and C624T polymorphisms were genotyped in all participants. Odds ratios (AdjORs) adjusted for conventional risk factors of MI were calculated as an estimate of the relative risk of MI.

Results: The allele frequency for the MIF G-173C, C656G and C624T were 18.0%, 19.1% and 3.7% respectively, amongst population controls. No risk of MI was associated with these genotypes when analysed individually. A haplotype analysis was conducted. Different haplotypes alone had no or minimal effect on risk of MI, however in the presence of smoking and/or HbA1c levels >6.5% and/or LDL-cholesterol >3.6mmol/L, the MIF -173GC/656CG/624CC haplotype was associated with a 5.8-fold (95%CI 2.8–12.2) increased risk of MI, relative to the wildtype haplotype in the absence of risk factors. This risk was higher than that associated with the risk factors and wildtype haplotype [AdjOR 4.2 (95%CI 2.2–8.1)]. An AdjOR of 1.3 (95%CI 0.5–3.6) was observed when the MIF -173GC/656CG/624CC haplotype was found in the absence of risk factors.

Conclusion: The MIF -173GC/656CG/624CC haplotype conveys a strong deleterious effect on the risk of MI only when found with at least another risk factor of MI.

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P05.45A

A combined analysis of common and rare variants in miRNA loci reveals miR-659 as a potential gene involved in myocardial infarction predisposition

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BACKGROUND: Coronary artery disease and its main complication, myocardial infarction (MI), are the major cause of death worldwide. Genome-wide association studies (GWAS) and whole-exome sequencing (WES) identified ~50 loci associated with the disease, which, however, explain <10% of the heritability. MicroRNAs (miRNAs) are important regulators of metabolic pathways, nevertheless their implication in cardiovascular diseases is still poorly understood. The aim of this project was thus the identification of miRNAs potentially implicated in MI susceptibility. **METHODS**: GWAS and WES data, derived from an Italian cohort of ~1,600 juvenile MI cases and 1,600 controls, were analyzed to identify miRNA loci potentially associated with the disease and rare variants directly affecting the miRNA precursor hairpin. Functional studies were performed to evaluate the effects of best candidate variants on miRNA stability and target recognition. **RESULTS**: The combined analysis of common and rare variants evidenced a rare nucleotide substitution located in the mature miR-659-5p and associated with a protective effect (p=0.033, OR=0.34). In-vitro experiments revealed that the variation affects the stability of the hairpin, decreasing miR-659 expression. Among predicted miR-659 targets, we were able to demonstrate that miR-659 directly targets the *IRS2* gene, a fundamental component of the insulin signaling pathway. Moreover, the identified variant partially releases the *IRS2* transcript from the miR-659-5p regulation. Since decreased expression of *IRS2* has been associated in mice with diabetes, a known MI risk factor, our results support the functional role of this rare variant, possibly exerting a protective effect against the development of diabetes, and, ultimately, of MI.

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P05.46B

Next Generation Sequencing as a powerful tool in genetic diagnosis of multiple cardiovascular disorders

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Cardiovascular diseases are the main cause of death worldwide and constitute a heterogeneous group of disorders that involve heart and blood vessels. Inheritable thoracic aortic disease (TAAD) and other monogenic cardiovascular diseases manifest as a separate entity or as a syndromic form. A NGS 238-gene panel for sequencing and CNV detection was used to diagnose a cohort of patients with clinical suspicion of inheritable TAAD. MLPA technique was performed to confirm the preliminary CNV results. 122 samples were analyzed using SureSelect-Kit (Agilent) through HiSeq platform (Illumina). Bioinformatic analysis was performed using an in-house pipeline for variant calling and CNV identification. Confirmation through MLPA was carried out following manufacturer's instructions (MRC-Holland) in the 19 candidate samples. The diagnostic algorithm was based on the data filtering for the variant effect, inheritance pattern, allele frequency and databases. In 33 patients (27%) pathogenic/probably pathogenic variants were found: 45.5% missense, 24.2% frameshift, 24.2% nonsense and 6.1% splicing. The CNV analysis and later MLPA confirmation revealed a partial deletion in 1 out of 19 patients. In 35 patients (28.7%) the variants were classified as VUS. For the remaining 54 patients (44.3%) no candidate variants were found. The overall genetic diagnosis ratio was 27.9%. The diagnostic ratio suggests that the use of both NGS sequencing and identifying CNVs, could be an effective strategy in the diagnosis of cardiovascular disorders to determine the genes involved in these pathologies. Functional and cosegregation analyses of VUS or/and extension of NGS analysis in negative patients would help to increase diagnosis ratio.

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P05.47C

Effect of the p.Arg357His mutation of PCSK9 on basal and postprandial lipoprotein metabolism

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PCSK9 (proprotein convertase subtilysin kexin-like 9) is a bivalent gene with gain of function (GOF) mutations responsible of high LDL-cholesterol levels characterizing Autosomal Dominant Hypercholesterolemia (ADH) and loss of function (LOF) mutations compatible with hypocholesterolemia. The p.Arg357His-PCSK9 mutant identified in a French ADH family suggested that it is a GOF mutation. This study aimed at evaluating the in vivo impact of this mutation on lipoprotein metabolism.

Two transgenic mice were created: i) Knock-In (KI) for the p.Arg357His mutant expressed ubiquitously; ii) transgenic mice (N) expressing the human p.Arg357His mutant only in the liver. To mimic the high LDL levels in humans that is absent in mice, we crossed KI and N mice to the double transgenic mice expressing human apo B100 and CETP (BC).

Unexpectedly, KI mice showed a significant but small decrease of total cholesterol (TC) basal level, as well as 3h after gavage with olive oil, but not under high-fat or high-sucrose diet. For all mice, basal triglyceride (TG) levels did not change, while the postprandial TG peak of KI and N mice was significantly lower (as observed in *Pcsk9*-KO mice), suggesting a role of PCSK9 in TG metabolism. Surprisingly, BCKI and BCN males were significantly leaner than BC males, weight of the perigonadal adipose tissue and percentage of fat mass were significantly reduced in BCKI males, indicating a possible role of PCSK9 in fat mass regulation.

Identification of a role of PCSK9 in fat mass regulation could open the door to new opportunities for treatments by PCSK9-antagonists.

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P05.48D

Genome-wide association meta-analysis of coronary artery disease and periodontitis reveals a novel shared risk locus

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Strong evidence of associations between the presence of coronary artery disease (CAD) and the widespread oral inflammatory disease periodontitis (PD) is derived from randomized clinical trials and shows that the epidemiological association between both diseases is independent of the shared risk factor smoking. Earlier, we gave evidence that the CAD associations of GWAS-lead SNPs at the genes ANRIL and PLASMINOGEN are shared with PD, and showed a rare variant at VAMP3 to be associated with PD and CAD. To elucidate the genetic basis of PD and CAD further, we performed a meta-analysis. In the discovery stage, we used an aggressive periodontitis sample (AgP; 680 cases, 4,130 controls) and the CARDIoGRAMplusC4D CAD meta-analysis (60,801 cases, 123,504 controls). Two SNPs at the known CAD loci ADAMTS7 (rs4468572) and VAMP8 (rs6547621) met pre-assigned selection criteria (P_AgP<0.05; P_CAD<10-5; P_META<P_CAD,P_PD) and were replicated in a sample of chronic periodontitis (3,286 cases, 3,328 controls) and the CARDIoGRAMplusC4D replication studies. rs6547621 showed shared association with CAD and PD (AgP:P=0.007, OR=1.5[95%CI=1.02–1.29]; CP:P=0.009[OR=1.10, 95% CI=1.02–1.29]). For this SNP, allele specific effects on VAMP8 expression are reported. Interestingly, both VAMP8 and VAMP3 form complexes with platelet syntaxin 4, indicating a shared disease relevant mechanism involving platelet granule secretion. Our data indicate that the epidemiological association of CAD and PD cannot be solely explained by shared environmental risk factors and thus contribute significantly to the current discussion on the relationship between CAD and PD. We conclude that both diseases share a molecular pathway involving platelet function, which increases the susceptibility for both diseases.

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P05.49A

Selenoprotein gene variants are risk factors for peripheral arterial disease in type 2 diabetes

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Introduction. In this study the role of common genetic variants in selenoprotein genes (*SEPP1 rs3877899, rs7579*, *SELS rs34713741 TXNRD1 rs35009941, TXNRD2 rs9605031, GPX4 rs713041)* and *SOD2* gene *(rs4880)* and concentration of selenoprotein P (SeP) and thioredoxin (Trx) in the development of peripheral arterial disease (PAD) were assessed. Materials and methods. A group of 450 patients and 522 controls was evaluated. Genotyping was performed using the TaqMan-based assays. Selenoprotein P (SeP) and thioredoxin (Trx) levels in plasma were assessed by ELISA. Results. The *SELENOS rs34713741T* allele was associated with PAD (recessive model: OR=1.58, P=0.031), with stronger effect in type 2 diabetes (T2DM, OR=2.54). Coexistence of the *SEPP1 rs7579A* and *GPX4 rs713041T* alleles with T2DM was related to 2.49-fold (P<0.0001) and 2.07-fold (P=0.001) increase in PAD risk, respectively. The interactions between the *SELENOS rs34713741T* and *SEPP1 rs7579A* alleles and T2DM were observed, that improved the prediction of PAD in relation to predictions from conventional cardiovascular risk factors alone. These alleles were also correlated with more advanced leg ischemia assessed by ankle-brachial pressure index (allele dose: r = - 0.399, P<0.0001 and r = - 0.205, P=0.048 respectively). The SeP concentration was decreased in T2DM, while positively correlated with blood cholesterol levels, especially HDLC. Conclusions. These results confirmed the protective role of SeP for cardiovascular system and suggested the impact of *SELENOS* and *SEPP1* variants on PAD risk in T2DM. Supported by the NSC in Poland under NN403250440 grant, and PUMS under 502-01-02214335-05962 and 502-14-02214335-10268 grants.

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P05.50B

Recessive hypoplastic left heart syndrome due to truncating mutation in *PKP2*

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Background Plakophilin-2 is the primary cardiac plakophilin, an essential component of desmosomes. Heterozygous mutations in *PKP2* encoding plakophilin-2 have been reported to cause autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC). ARVC is a genetically heterogeneous disorder characterized by progressive fibrofatty replacement of the myocardium, predominantly affecting the right ventricle. Recently, a homozygous deletion of the entire *PKP2* gene was described in two siblings with severe noncompaction cardiomyopathy of both ventricles.

Method and results We describe prenatally diagnosed left ventricular hypoplasia in two siblings that died shortly after birth. Autopsy showed extensive biventricular noncompaction, septal defects and subendothelial fibroelastosis, predominantly affecting the small left ventricle. One of the siblings also had an underdeveloped mitral and aortic valve, and a hypoplastic aortic arch. Next-generation sequencing of a panel of 52 cardiomyopathy-related genes was performed in DNA from peripheral blood samples from both parents. Both were found to carry a heterozygous truncating mutation c.1211dup in *PKP2*. This mutation has previously been described in heterozygous state in ARVC patients. Sanger sequencing confirmed that this mutation was present in homozygous state in their two affected daughters. Immunohistochemical staining showed strongly reduced expression of desmosomal proteins as well as the gap junction connexin-43 in the intercalated discs.

Conclusion Our findings confirm previous observations that plakophillin-2 is essential for myocardial formation and that lack of plakophillin-2 gives rise not only to noncompaction cardiomyopathy, but also to hypoplasia of the left ventricle and other congenital cardiac defects.

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P05.51C

Application of an LDL-C Genetic Risk Score for diagnosis of polygenic hypercholesterolemia in Northern Irish population: Preliminary trial to evaluate a Randox 6SNP Polygenic Hypercholesterolemia Array

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Introduction: Familial Hypercholesterolemia (FH) is a common genetic disorder characterised by elevated LDL-C and early symptoms of coronary heart disease, primarily caused by a mutation in genes LDLR, APOB or PCSK9. Despite clinical diagnosis of possible FH, 60% of possible FH patients are mutation-negative, likely due to an accumulation of common small-effect LDL-C raising alleles. The effectiveness of the Randox 6SNP Polygenic Hypercholesterolemia Array to identify polygenic FH was tested. Materials and Methods: 414 FH cases were selected for 6SNP genotyping by Sanger sequencing of five genes (rs7412, rs429358, rs1367117, rs6544713, rs629301 and rs6511720) and calculation of the weighted LDL-C genetic risk score (GRS). Genotyping was performed (10% cases) using the 6SNP Polygenic Hypercholesterolemia Array and the Evidence Investigator Analyser. **Results:** Definite FH patients (mutation positive - 196 cases) were shown to have a mean GRS of 0.705, whereas possible FH patients (mutation negative - 218 cases) had a mean GRS of 0.777 ($p<0.001$). Mutation negative patients have 2.3 times the odds of having a GRS exceeding 0.81 than mutation positive patients, thus increasing the chance of polygenic hypercholesterolemia. Accurate genotyping was confirmed in 100% of cases tested with the 6SNP Polygenic Hypercholesterolemia Array. Conclusion: The Randox 6SNP Polygenic Hypercholesterolemia Array successfully genotyped a subset of the Northern Irish possible FH population. This cost effective method could identify patients potentially affected with polygenic hypercholesterolemia, exclude additional testing for a monogenic cause and negate inefficient cholesterol based family cascade screening. This will improve management and treatment of clinically diagnosed FH patients.

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P05.52D

Patients with pulmonary arterial hypertension associated to several mutations

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Pulmonary arterial hypertension (PAH; OMIM #178600, ORPHA 422) is a rare disease that typically causes the obstruction of precapillary pulmonary arteries. Much of what is known about the genetic basis of PAH is related to *BMPR2* gene. However, some other candidate genes have been advocated. The aim of this study was to analyse patients with combined mutations in *BMPR2*, *ACVRL1*, *ENG* and *KCNA5* genes and to establish a genotype-phenotype correlation. We included 57 PAH patients and 50 controls. Using specific primers, we amplified and sequenced the genes. Genotype-phenotype correlation was performed using SPSS v.19 software. After mutation screening of *BMPR2*, *ACVRL1*, *ENG* and *KCNA5* genes in our cohort, we identified pathogenic mutations in 40 patients. We found a high percentage of patients with several mutations classified as pathogenic by *in silico* analysis. We found combined mutations in the *BMPR2* gene in 25% of the patients with several mutations included in this study. In addition, we detected one patient that shows two pathogenic mutations in ENG gene. We found statistically significant differences, comparing patients with several mutations and patients with one pathogenic mutation, for gender (p=0.045), age at diagnosis (p=0.040), PVR (p=0.030), CI (p=0.042) and no response to therapy (p=0.011). We show a series of patients with IPAH and APAH with a high percentage of them being carriers of more than one pathogenic mutation in several genes. We wonder whether these additional mutations act as a second event in the development of the disease, increasing the penetrance, modifying the patients phenotype.

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Design and application of a custom panel of NGS for Pulmonary Arterial Hypertension: HAP v1.2

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Introduction

Pulmonary Arterial Hypertension (PAH) is an infrequent cardiac disease characterized by an increase of pulmonary vascular resistance associated with a high morbidity and mortality. PAH can be classified in several groups depending on the etiology, including idiopathic (IPAH), hereditary (HPAH) and a more severe form PVOD (Pulmonary Venooclusive Disease).

Material and methods

We have selected a cohort of 168 patients with IPAH, HPAH and PVOD from the Spanish REHAP registry, which includes patients from all over the country. A panel of 21 genes related to PAH have been designed. A-in-house script for bioinformatic and variant analysis was performed and variant validation was done through Sanger sequencing.

Results

After variants filtering, we have found 18,45% (31/168) patients with pathogenic mutations, 5,8% with variants of unknown significance. Eight% of the samples were rejected after quality filtering. Additionally, two mutations in two different genes have been found in one patient from one family with multiple affected members.

Conclusions

PAH has an extremely wide clinical and molecular spectrum including penetrance, pattern of expression and

age of onset. Thus, NGS in combination with a well clinical characterization seems to be the best approach to study PAH. Interestingly, in one patient we were able to found two different mutations in two genes, one of them a nonsense well known mutation (*BMPR2*, c.961C>T; p. Arg321*) and a second hit in a potassium channel. As far as we know, this is the first time that a digenic inheritance has been found in a patient with PAH.

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P05.54B

Study of the contribution of SCN10A mutations to the Brugada syndrome genetic architecture

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The Brugada Syndrome (BrS) is an inherited primary electrical disorder of the heart characterized by ventricular arrhythmias and significant risk for sudden cardiac death. Mutations in the *SCN5A* gene, encoding a voltage-gated cardiac sodium channel, constitute the major genetic cause of BrS, accounting for ~20% of all clinically diagnosed cases. More than 20 other genes have been associated with BrS, but together only explain an additional ~5% of patients. Recently *SCN10A*, encoding another voltage-gated sodium channel, gained significant interest because GWAS studies associated *SCN10A* single nucleotide polymorphisms with BrS risk, QRS duration and cardiac conduction, and more recent studies detected rare potentially pathogenic variants in BrS patients. We screened the coding sequence of *SCN10A* in a cohort of 108 *SCN5A* mutation negative BrS patients using Sanger sequencing. One patient carried both a novel and very rare missense mutation, p.Leu309Pro (absent from ExAC) and p.Lys1247Glu (ExAC MAF = 0.000008). Another patient harboured the rare variant p. Lys417del (ExAC MAF = 0.0009) and two unrelated patients presented p.Arg1268Gln (ExAC MAF = 0.001). All variants altered conserved amino acid residues and were *in silico* predicted to be disease causing by Mutationtaster and SIFT. The p.Arg1268Gln variant was previously shown to reduce sodium current *in vitro*.

In conclusion, mutations in *SCN10A* potentially contribute to 3.7% of BrS patients in our cohort, further highlighting the role of *SCN10A* in BrS causation.

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P05.55C

The second report of early somatic mosaicism as cause of life threatening cardiac arrythmias

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Background: Brugada syndrome (BrS) was recognized as a disease responsible for sudden cardiac death, characterized by a right bundle-branch block with ST segment elevation in the leads V1 and V2. Extensive research has allowed better understanding of the genetic background and clinical management even though controversies still exist being the sodium channel NaV1.5, encoded by the *SCN5A* gene, associated with BrS in only a few proportion of cases. Somatic mosaicism, is increasingly recognized to play a causal role in a variety of mendelian diseases included Long QT syndrome due to *SCN5A* gene mutations (LQT3). We investigate the case of a 14-year old girl with clinical diagnosis of BrS and uncertain genetic causative mutation of the *SCN5A* gene. *Methods and Findings:* Firstly Sanger Sequencing identified a heterozygous variant of the *SCN5A* gene (NM_000335:c.6010T>C predicting p.Phe2004Leu). Because of discordant diagnosis with a second testing laboratory we then applied: 1) high sensitivity NGS with a cardiac arrhythmias custom-based gene panel on the Ion PGM platform with a minimum base coverage of 200×(ThermoFisher); 2) microsatellites profiling for the search of mosaicism at circulating leukocytes level and 3) q-PCR with mutation-specific TaqMan probes. We confirmed that the identified mutation is mosaic in origin, detectable in 2.2–67.8% of sequencing reads in constitutional DNA. *Conclusion:* Mosaic *SCN5A* mutations can cause of Brugada Syndrome in patients with overt phenotype. Sensitivity provided by classic Sanger sequencing might fail in detecting such conditions that are rapidly and securely detected by NGS thus enabling the detection of low-level mosaic mutations

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P05.56D

Sexual dimorphism in *SMAD3* mutation carriers: implications for management derived from a large family

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SMAD3 mutation carriers are at-risk for aortic dissection and for arterial aneurysms/dissections from brain to pelvis. Based on prior suggestions in the literature on gender differences in thoracic aortic aneurysm/dissection, we investigated whether there is a sexual dimorphism regarding risk of vascular events in *SMAD3* mutation carriers. In a large *SMAD3* pedigree, we included mutation carrying individuals who were either (1) >40 years or (2) had aneurysm/vascular dissection/intervention. We identified 22 mutation carriers and categorized them as unaffected or affected. We subcategorized affected individuals, between (1) aneurysm/dissection excluding the aortic root/ascending aorta versus (2) aneurysm/dissection with disease including the aortic root/ascending aorta (TAAD). In females, we identified 7 as unaffected whereas 6 were affected. Of these 6 affected females, 5 had arterial aneurysms without TAAD. All 9 males were affected. Eight of the males had TAAD and one had arterial aneurysm without TAAD. In addition, four other males in the family presented with sudden death and/or aortic dissection, but were not included because the precise nature and location of their aneurysm was undocumented. Our findings are in line with previous human SMAD3 family observations (VandeLaar et al, JMG, 2012) and mouse data (van der Pluijm I et al, EBioMedicine, 2016).

In summary, clinicians caring for individuals with a personal/family history of *SMAD3* mutations need to know that (1) the absolute risk of aneurysms is higher in males and (2) the risk of vascular events in the presence of a normal echocardiogram is higher in females.

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P05.57A

Should all individuals with Sotos syndrome be screened for thoracic aortic aneurysms?

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Sotos syndrome is an autosomal dominant overgrowth disorder caused by mutations in NSD1. Clinical findings typically include larger stature, macrocephaly, characteristic facial features and variable learning disability. Evidence of mild connective tissue dysfunction has been described in a minority of affected individuals, and only recently were four patients reported to have severe connective tissue laxity and aortic dilatation (Hood et al., 2015). We describe 3 additional patients manifesting enlargement of the thoracic aorta and milder evidence of connective tissue involvement. Patient 1 is a 9 year old male with a partial deletion of NSD1 exon 5 and typical features of Sotos syndrome, along with mild hypermobility, velvety skin, periumbilical wrinkling, pes planus, and a 3.2cm aneurysm of the ascending aorta (Z-score +5.2). Patient 2 came to medical attention at age 59 with a 6.1cm aortic root aneurysm and minimal connective tissue involvement. His 26 year old daughter (Patient 3) was subsequently screened and found to have a 4cm aortic root (Z-score +3.11), along with moderately elastic, doughy skin and elbow hyperextensibility. The father-daughter pair are proportionately tall and macrocephalic, with facial features of Sotos syndrome and a shared, novel nonsense mutation in NSD1. This report broadens our understanding of the growing association between Sotos syndrome and predisposition to aortopathy, and suggests that routine screening of the thoracic aorta in affected individuals may be warranted.

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P05.58B

Genetic investigations of sudden unexplained death victims using targeted capture and next-generation sequening

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Introduction: Sudden cardiac death (SCD) is responsible for a considerable proportion of natural deaths in young individuals. Some cases remain unexplained after medico-legal autopsy, termed sudden unexplained deaths (SUD). A major proportion of these are suspected to be caused by inherited cardiac diseases, especially channelopathies. Sudden death can in some cases be the first manifestation of the disease. It is generally expected that implementation of genetic investigations in forensic medicine may increase the diagnostic rate in SUD cases. The purpose of the study was to explore the utility of genetic testing using next-generation sequencing (NGS) by investigating the frequency of variants with likely functional effects in 100 genes associated with cardiac diseases in a cohort of suspected SUD victims. Methods: Genetic investigation of 61 unrelated, SUD cases <50 years was performed. Using the Haloplex Target Enrichment System from Agilent, all coding regions of 100 genes associated with inherited cardiomyopathies and cardiac channelopathies were sequenced on an Illumina MiSeq platform. **Results:** In 34% of the cases, variants with likely functional effects were identified. A total of 40% of these variants were located in genes associated with cardiomyopathies, and 60% of the variants in genes associated with cardiac channelopathies. **Conclusions:** We found that broad genetic screening of SUD victims can increase the diagnostic outcome, and that the investigation should comprise genes involved in both cardiomyopathies and cardiac channelopathies. These findings are of importance for clinical and genetic screening for those left behind.

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P05.59C

Targeted exome sequencing for mendelian cardiac disorders within the Genome Clinic in Geneva

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Introduction: Mendelian cardiomyopathies and arrhythmias represent heterogeneous disorders for which current guidelines recommend molecular analyses to identify a causative mutation(s) and subsequently provide predictive testing of relatives at risk. High-throughput sequencing (HTS) platforms allow to analyse simultaneously large numbers of cardiac disease genes.

Methods: In order to optimally integrate HTS in our clinic, we have created at the University Hospitals of Geneva a multidisciplinary "Genome Clinic Task Force", composed of clinical and molecular geneticists, bioinformaticians, bioethicists and a coordinator. This task force represents also a powerful tool for the diagnosis of cardiac disorders. We perform whole-exome sequencing (WES) followed by targeted bioinformatic analysis of a selection of genes (65 for cardiomyopathies, 47 for arrhythmias).

Results: So far, we have validated the HTS results of 36 different cardiac cases (11 with arrhythmias, 25 with cardiomyopathies). We found a pathogenic variant in 11 patients with cardiomyopathy (44%) and in 2 patients with arrhythmia (18%). The highest detection rate (58 %) was obtained for patients with hypertrophic cardiomyopathy (7/12). A variant of uncertain clinical significance (VUS) was found in 5 cases with arrhythmia (45 %) and in 3 cases with cardiomyopathy (12%).

Conclusions : Targeted exome analysis offers an efficient approach for the fast developing field of inherited cardiac diseases. However, cardio-genetic services face several challenges implementing this technology, especially variant interpretation, management of VUS or reduced penetrance. So far, we disclosed the VUS. To ensure optimal counselling and management of the concerned families it is essential to work as a multidisciplinary team.

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P05.60D

Integrated copy number and single nucleotide variant analysis in thoracic aortic aneurysms

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Thoracic aortic aneurysm (TAA) is a life-threating condition. It is usually considered an inherited disorder as approximately 20% of patients have at least one affected first-degree family member. Mutations in the transforming growth factor-β signaling pathway and vascular smooth muscle cell contractility apparatus have emerged as key players in the pathogenesis of TAA. The clinical variability might be explained by environmental factors and additional contributing genetic factors. One of these so-called modifiers can be copy number variations (CNVs). Genes affected by CNVs are good candidates for further research, as these genes may contain deleterious single nucleotide variants (SNVs) in other patients. Therefore, we determined the copy number profile of 167 TAA patients using SNP array and prioritized the CNVs based on gene content and frequency in control populations. Next, we determined the frequency of rare SNVs in genes encompassed by these CNVs in 121 TAA patients using whole exome sequencing (WES) data. We identified 71 unique CNVs that were absent or rare in controls, or that contained known or candidate TAA genes. Pathway analysis showed that these CNVs were enriched for genes involved in fibroblast growth factor signaling and extracellular matrix organization. Screening of our TAA cohort using the CNV-associated gene panel (n = 289) resulted in the identification of 63 putative deleterious SNVs. We are currently evaluating the presumed consequence of these variants and their hypothesized relation to TAA formation. In addition, we are assessing copy number status from WES data using ExomeDepth and CoNVex. Funded by: Dutch Heart Foundation (2014T007).

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P06 Metabolic and mitochondrial disorders

P06.01A

MLPA analysis of the *HGD* gene identifies novel genomic deletions in alkaptonuria

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Alkaptonuria (AKU) is characterised by ochronosis and ochronotic artropathy due to the accumulation of melanin-like pigment. This rare autosomal recessive metabolic disorder is caused by homozygous or compound heterozygous mutations of the *HGD* gene (3q13.33) coding for homogentisate dioxygenase (HGD). Until present, 142 pathogenic variants have been reported in about 400 AKU patients worldwide, as summarized in the *HGD* mutation database (http://hgddatabase.cvtisr.sk/). In some cases, only one or no *HGD* mutation could be identified by sequencing. Interestingly, only two larger genomic deletions within the *HGD* gene were seen so far: one of exon 2, including short flanking intronic sequences, and one of an almost entire intron 2, most likely leading into exon skipping. We designed MLPA probes for all 14 exons and intron 2 of the *HGD* gene and performed MLPA analysis in 22 AKU patients, in whom only one (14 cases) or no disease-causing mutation (4 cases) was found, as well as in those with only silent or intronic variants identified (4 cases). MLPA uncovered a heterozygous deletion of exon 13 in four cases from Italy and a heterozygous deletion of both exons 5 and 6 in one patient from Netherland. Deletion breakpoints of novel deletions will be defined. MLPA assay successfully identifies also previously described deletions of exon 2 and intron 2 of the *HGD* gene, in homozygous as well as in heterozygous state. No copy number changes were seen in remaining 17 cases and a mechanism causing AKU in them needs to be discovered. (7FP- DevelopAKUre 304985)

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P06.02B

Beta-mannosidosis caused by a homozygous intragenic inverted duplication in the *MANBA* gene precisely characterized by Whole Genome Sequencing

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Introduction: WGS is still not universally used as first step in the genetic investigation of Inborn Errors of Metabolism (IEM), mainly due to allegedly high economic cost. We present a case in which early use of WGS would have been beneficial both in temporal and economic terms.

Patient and methods: 13 year old girl, consanguineous parents, presenting with recurrent infections, mild intellectual disability, ADHD, behavioural disturbances, sensorineural hearing loss, alacrima and high pain threshold. Physical examination shows scalp flushing, upslanting, narrow palpebral fissures, broad nasal tip, thin lips, angiokeratomas, varicose veins and dilated conjunctival vessels. Numerous genetic analyses were undertaken over the years. SNP-array detected six regions of homozygosity, including one in 4q24, where *MANBA* gene is located. Present investigation included exome sequencing panel for lysosomal/peroxisomal diseases, β-mannosidase activity in leukocytes, cDNA analysis of *MANBA* transcripts and WGS.

Results: Exome sequencing panel showed increased coverage (+1.73) in exons 8 to 11 of *MANBA*. Abnormal distance and orientation of paired reads indicated the presence of an inverted duplication and the location of one breakpoint. β-mannosidase analysis showed 3% residual activity, which confirmed the diagnosis of β-mannosidosis. cDNA analysis revealed two transcripts lacking exons 8–9 and 8-9-10, respectively. The patient´s mother showed normal transcript in addition to the two truncated variants. WGS defined both breakpoints, which were confirmed by Sanger sequencing.

Conclusion: WGS is superior to exome sequencing in the identification and characterization of Copy Number Variants. Early use of WGS in the diagnostic flow of IEM should be considered.

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P06.03C

Improving the diagnosis of cobalamin and related defects by genomic analysis and functional and structural assessment of the variants identified

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Detection of cobalamin disorders involves biochemical analyses after the presentation of symptoms or by newborn screening. To date the gold standard method includes further, time-consuming biochemical and/or cellular analyses to reduce the number of subsequent gene-by-gene analyses. This paper reports the accurate and rapid genetic diagnosis by exome analysis via the massive parallel sequencing of DNA samples from a cohort of patients with cobalamin and related defects. The method was first validated simulating a real-world diagnostic scenario. Mendelian segregation, population frequency and a comprehensive structural and functional analysis of the variants have been able to identify disease-causing mutations in 13 genes involved in the absorption and synthesis of active cofactors of vitamin B_{12} (23 cases) as well as in the non-cobalamin metabolism-related genes *ACSF3* (four biochemically misdiagnosed cases) and in *SUCLA2* (one case) with an unusual presentation. Additionally, pathogenic mutations were detected in two pathway genes in three cases, suggesting a digenic inheritance. The present findings suggest that the technology used is sufficiently sensitive and specific, and the results obtained sufficiently reproducible, to recommend its use as second-tier test after the biochemical detection of cobalamin disorder markers in the first days of life. Grants: PI13/01239, the *Fundación Isabel Gemio*.

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P06.04D

A DOLK gene-associated Congenital Disorder of Glycosylation should be considered in the presence of ichthyosis and dilated cardiomyopathy

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A male child of non consanguineous 40 year old Irish parents was delivered by emergency Caesarean Section at 38+5 weeks, for non-reassuring CTG and decreased fetal movements. His birth-weight was 2.65kg (2nd-9th centile). He immediately required cardiopulmonary resuscitation, ventilation and transfer to the neonatal ICU. He developed hypotension, hypoglycaemia, cardiac failure, thrombocytopenia, coagulopathy, and had one tonic/clonic seizure. He was stabilised and transferred to a tertiary centre. On examination, several dysmorphic features, undescended testes, partial collodion membrane, keratosis, severe fissures, contractures and erythema were noted; suggesting a unifying diagnosis of congenital ichthyosiform erythroderma. An echocardiogram confirmed severe biventricular dilated cardiomyopathy with possible non-compaction of the right ventriculo-apical region. Investigations included cyto- and molecular genetics (karyotype, microarray, 10-gene ichthyosis panel) and metabolic workup. His transferrin isoforms were abnormal, suggesting a congenital disorder of glycosylation (CDG). Ultimately, bi-allelic mutations in the *DOLK* (*Dolichol Kinase*) gene provided molecular confirmation of this diagnosis. Despite maximal therapy, the patient continued to deteriorate and died on day 2 of life. Few disorders are associated with both ichthyosis and dilated cardiomyopathy, most of which are glycosylation disorders. To date, the predominant phenotype in patients affected by dolichol kinase deficiency has been dilated cardiomyopathy. Mild ichthyosiform dermatitis has been noted in some affected individuals, but we believe this to be the first reported case of a severe *DOLK*-related ichthyosis. Congenital disorders of glycosylation are rare, and variable, but should be considered in a child with early multi-systemic issues, particularly those involving heart, brain and skin.

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P06.05A

The introduction of NGS for the diagnosis of Congenital Disorders of Glycosylation

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Introduction: Congenital Disorders of Glycosylation (CDG) are a group of disorders caused by the alteration in synthesis and structure of protein and lipid glycosylation. Diagnosis of CDG is challenging because of its clinical and genetic heterogeneity. Until recently, CDG diagnosis has been perfomed by Sanger sequencing of one or few genes tentatively selected based on a combination of biochemical, cell biological and glycobiological investigations.

Material and Methods: We designed a capture assay for a panel of 79 genes associated with CDG and other genes associated to disorders whose phenotype resembles CDG. A total of 88 CDG cases were captured and Pair-End sequenced. The reportable range of our NGS assay was evaluated as well as CDG diagnostic routing.

Results: A genotype was called for more than 97% of the targeted bases and more than 99% of all coding bases plus 20 flanking bases of the transcripts used for analysis. A diagnosis was confirmed in 44 of 88 CDG patients, of which 27 had been previously tested negative by Sanger sequencing. Four PMM2-deficient patients were identified although a PMM2 enzymatic assay had been performed prior to NGS.

Conclusions: Our NGS assay allowed a diagnostic yield of 50% and the genotyping of the coding bases of all CDG genes, including *ALG1* that could not be assayed by genomic Sanger sequencing due to the abundance of pseudogenes. Given the prevalence of PMM2-deficient patients, enzymatic assays are replaced by PMM2 Sanger sequencing prior to NGS in our new diagnostic routing.

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P06.06B

Molecular genetic study of children in St. Petersburg, included in the Federal register of patients with pituitary dwarfism

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Clinical manifestations of heterogeneity of growth hormone deficiency when definitive results of hormonal stimulus test determines the need to search for molecular genetic markers of the disease to form personalized therapeutic algorithms. The study of the nature of the genetic changes in a cohort of patients with congenital hypopituitarism, manifested multiple anterior pituitary hormone deficiency or isolated growth hormone deficiency. Molecular genetic analysis in patients with congenital hypopituitarism was carried out by NGS using "Amplisec" technology. All patients with congenital hypopituitarism, who are in a special registry of Saint Petersburg, were included in this study. Differences in the frequency of detection of mutations in patients with multiple anterior pituitary hormone deficiency and in patients with isolated growth hormone deficiency were found. The mutation frequency of diagnosis in genes responsible for congenital hypopituitarism in patients of St. Petersburg were studied. Mutations in genes associated with congenital hypopituitarism were identified in 16.3% of patients with pituitary dwarfism (16 of 98). The most commonly diagnosed mutations are changes in gene *PROP1*. In carrying out the molecular genetic studies of patients with congenital hypopituitarism is necessary to consider the likelihood of the presence of these rare pathologies such as loss of genes *GHSR*, *ARNT2, BTK*. Currently conducting molecular genetic studies in patients with congenital hypopituitarism further predicts development of the disease and, if necessary, adjust the ongoing replacement therapy. The study was implemented under the Alfa-Endo Charity Program and the Russian Science Foundation grant №14-50-00069

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P06.07C

Diversity of reactions catalyzed by human and nematode cystathionine gamma-lyase

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Cystathionine gamma-lyase (CGL) is a multifunctional enzyme that plays a pivotal role in conversion of cystathionine to cysteine and is also known to catalyze production of hydrogen sulfide. In our study, we used purified human CGL and its orthologs CTH-1 and CTH-2 from model organism *C.elegans* to analyze the substrate specificity and to determine kinetic properties of selected reactions using HPLC and LC-MS/MS analysis of aminothiols and thioethers, respectively. The method of RNA interference was used to inhibit expression of nematode enzymes CTH-1 and CTH-2 *in vivo*. The biochemical analysis in *C. elegans* extracts shows that *cth-2* knockouts have elevated cystathionine and decreased cysteine levels compared to wild-type and *cth-1* knockouts. Consistently, concentration of the glutathione precursor cysteinylglycine was decreased in *cth-2* mutants. These data together with enzymatic activities indicate transsulfuration role of the CTH-2. Similarly to human patients with CGL deficiency, inhibition of cth-2 was not associated with a consistent pathological phenotype.Besides previously define reactions, we found that both human and nematode CTH-1 and CTH-2 are able to catalyze reaction which uses cysteine as the sulfur donor and homoserine or O-succinylhomoserine to yield cystathionine. Up to now, this reverse transsulfuration pathway was described only in bacteria, fungi and plants but not in humans or nematodes; its physiological role is at present unknown. Our data confirms C. *elegans* as a suitable model of human CGL deficiency which results in impaired transsulfuration and H2S-producing pathway.*This work was supported by the grant No. 16-30384A from the Czech Health Research Council.*

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P06.08D

MtDNA meta-analysis reveals both phenotype specificity and allele heterogeneity: a model for differential association

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Human mtDNA genetic variants have traditionally been considered markers for ancient population migrations. However, during the past three decades, these variants have been associated with altered susceptibility to various phenotypes, thus supporting their importance for human health. Nevertheless, mtDNA disease association has frequently been supported only in certain populations, due either to population stratification or differential epistatic compensations among populations. To partially overcome these obstacles, we performed meta-analysis of the multiple mtDNA association studies conducted until 2016, encompassing 53,975 patients and 63,323 controls. Our findings support the association of mtDNA haplogroups and recurrent variants with specific phenotypes such as Parkinson's disease, type 2 diabetes, longevity, and breast cancer. Strikingly, our assessment of mtDNA variants' involvement with multiple phenotypes revealed significant impact for Caucasian haplogroups H, J, and K. Therefore, ancient mtDNA variants could be divided into those that affect specific phenotypes, versus others with a general impact on phenotype combinations. We suggest that the mtDNA could serve as a model for phenotype specificity versus allele heterogeneity.

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P06.09A

Pitfalls in the diagnosis of Fabry disease: further evidence that p.Asp313Tyr is a non-pathogenic polymorphism

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Introduction: Fabry disease (FD, OMIM#301500) is a rare X-linked inborn error of metabolism caused by mutations in *GLA*. Over 700 unique DNA changes have been identified.

Case presentation: A 50-year-old male was referred to us with a diagnosis of FD on the basis of familial hypertrophic cardiomyopathy (father and three sisters were also affected) and identification of a c.937G>T (p.Asp313Tyr) change in *GLA*. Medical history could not document any symptoms of FD. In particular, there was no acroparesthesia, gastrointestinal symptoms, or hypohidrosis. Physical examination and comprehensive imaging and laboratory investigations showed absence of neurological or renal involvement. Enzymatic assay found normal alpha-galactosidase A activity in leukocytes. On the basis of the aforementioned findings and father-to-son transmission of the cardiomyopathy, NGS sequencing of sarcomeric genes was performed. A missense mutation, previously associated with sarcomeric HCM, was found in *TNNI3* (p.Arg186Gln). The diagnosis of FD was ruled out and costly enzyme replacement therapy was consequently not initiated, but the proband and his family members benefited from rigorous cardiac follow-up and genetic counselling.

Discussion: Our case highlights the importance of a proper knowledge and recognition of variants of unknown significance and non-disease-causing polymorphisms in FD. The presence of a *GLA* mutation alone does not constitute a diagnosis. Hence, expert knowledge of the gene and its mutations is essential for accurate diagnosis. The initiation of ERT in a patient misdiagnosed as having FD is wasteful from a health economic perspective and potentially harmful because efforts to diagnose the patient's disease will have ceased.

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P06.10B

Mitochondrial complex I deficiency in patient with FOXG1 mutation

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Introduction: FOXG1 syndrome presents as a severe neurodevelopmental multisystemic disease with a complex and quite multifaceted phenotype, as well as mitochondrial disorders. In this study, we report on the biochemical and genetic basis of a severe neurodevelopmental disorder in two Czech pediatric patients, who both manifest encephalopathy, epilepsy, developmental disability, microcephaly, dyskinesia, spasticity and failure to thrive.

Materials and Methods: Genetic causes of patients disorder were determined with the use of whole exome sequencing and MLPA assay. Biochemical study of oxidative phosporylation (OXPHOS) was performed in affected patient tissues.

Results: In both patients, heterozygous mutations were identified in the *FOXG1* gene. A girl carried large deletion encompassing exon 1. In a boy, additionally manifesting lactic acidosis and hearing impairment, novel pathological variant c.758delA was found. Furthermore, tissue specific decrease of the mitochondrial respiratory complex I activity was detected in his muscle.

Conclusions: Results give an extra supportive evidence on phenotypic overlap of signs seen in primary mitochondrial disorders and features of the FOXG1 syndrome in humans. Hence, we suppose that disturbances in OXPHOS system may also play a significant role in the etiology of the FOXG1 syndrome.

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P06.11C

L444P :A Founder mutation of GBA gene in patients affected with Gaucher disease from India

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Introduction: Gaucher disease (GD) is the most common autosomal recessive inherited lysosomal storage disorder in India. Present study was to identify the most common disease causing mutant allele and elucidate the molecular characterization of novel disease causing mutation in patients from India. Materials and Methods**:** Present study consists of 36 patients in the age of 1 month to 32 years with hepatosplenomegaly and thrombocytopenia. Six millilitre peripheral blood was collected from all the patients for plasma chitotriosidase, confirmative enzyme analysis and bi-directional Sanger sequencing of coding region covering exon-intron boundaries of the *GBA* gene. Novel mutations were further identified by *insilico* analysis using bioinformatics tools like SIFT, Polyphen2 and Mutation Taster. Structural study of four novel missense mutant allele was done using the Phyre2 software. Result and Discussion**:** Study has identified 19 mutation in 33 patients. %). The most common one was L444P (c.1448T>C) in 23 patients (63.88%) with 16 homozygous and 7 compound heterozygous mutant allele. Four novel mutations V17G (c.167T>G)/L444P (c.1448T>C), S97L (c.407C>T)/L444P (c.1448T>C), G360R (c.1195G>C)/R496C (c.1603C>T), A448T (c.1459G>A)/A448T (c.1459G>A), and A448T (c.1459G>A)/S125R (c.492C>G) in 6 (16.66%) patients were also identified. Conclusion: L444P is the most common mutant allele in Indian patients with Gaucher disease. While novel mutations are mostly affecting protein backbone leading to its misfolding. Acknowledgement: Present study was carried out as a part of National Task Force Multicentric project on Lysosomal storage disorders with a grant from Department of Health and Indian Council of Medical Research (Grant No: GIA/31(ii)/2014-DHR).

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P06.12D

Glucosylsphingosine concentration in the blood of Gaucher patients reflects the severity of GBA mutations - data from a large global cohort

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Gaucher disease (GD) is an autosomal recessive, rare genetic disorder characterized by the deposition of glucocerebroside in cells of the macrophage-monocyte system. Standardized, simple and highly reproducible workflows for the diagnosis are crucial, especially since more than 15 years a highly effective treatment is available. We have developed and systematically validated a high-throughput workflow for the simple testing of GD patients: beta-glucocerebrosidase enzymatic activity, glycosylsphingosine (lyso-Gb1) quantification in DBS followed by GBA gene sequencing from the same blood sample. Here, we report data from over 1,000 GBA variants in over 640 Gaucher individuals. Determination of lyso-Gb1 is performed by LC/MRM-MS. Lyso-Gb1 was investigated in three GBA cohorts comprising: (A) 133 GBA homozygotes ; (B) 226 GBA compound heterozygotes and (C) 201 Gaucher carriers. The lyso-Gb1 levels found Gaucher patients were: very mild (12 - 25.0 ng/mL), mild (25.1 - 50 ng/mL), moderate (50.1 - 200 ng/mL) and severe (>200 ng/mL). Lyso-Gb1 prouved a sensitivity and specificity of 100%. The clinical severity of different mutations could be correlated with the lyso-Gb1 in the homozygous cases, e.g. c.1295G>T have mild lyso-Gb1 values while c.1060G>A and c.518C>A have extremely high lyso-Gb1 values (>600 ng/mL). The most common mutations were c.1226A>G (30.1%) and c.1448T>C (24.7 %). While c.1448T>C is a severe mutation correlating to a massive increase of lyso-Gb1 up to 1,250 ng/mL. lyso-Gb1 for c.1226A>G can vary from very mild to moderate. Lyso-Gb1 concentrations in blood can be used for the easy and early diagnosis of Gaucher patients and treatment monitoring.

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P06.13A

A family of HHH syndrome with a novel missense mutation in SLC25A15 gene

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Introduction: Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is a rare genetic disorder of the urea cycle caused by mutations in the SLC25A15 gene. It represents a heterogeneous disease with clinical variability, ranging from a mild form with slight neurological impairment to a more severe form with coma, lethargy, hepatic signs and seizures. Only about 50 cases have been reported. We here report a HHH family with a novel missense mutation in SLC25A15 gene.

Case presentation: 24 year-old mother with a medical story of a deceased infant was referred to our genetic center because of a demand for a new baby. The index case, a female baby was born at term. Soon after term the baby died. Metabolic screening showed signs of HHH syndrome: hyperornithinemia 410 µmol/L (48–211 µmol/L), hyperammonemia 3999µmol/L (18,7–86,9µmol/L) and increased orotic aciduria. Blood liver enzymes were elevated (AST;236, ALT;301) and there were parameters of coagulopathy. The DNA of the baby was not available thus DNA of the parents were sequenced for mutations in the SCL25A15 gene. Analysis revealed a novel heterozygous missense mutation in exon 6 (p.D231V;c.692A>T) in each parent. The family did not accept the prenatal diagnosis and a male baby with HHH was born from second pregnancy. He had the same homozygous mutation. A protein-restricted diet was administered and no life threating complications (lethargy, coma and hepatic failure) developed.

Discussion/Conclusion:Early identification of HHH syndrome is extremely important, because hepatic failure can be prevented with treatment. Mutational screening of the SLC25A15 gene is crucial for an early and accurate diagnosis of the disease.

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P06.14B

A novel *HNF4A* pathogenic variant in a case of congenital hyperinsulinism with glycogenosis - like phenotype

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Introduction: Congenital hyperinsulinism (CHI) and glycogen storage disease (glycogenosis) are both causing hypoglycemia during infancy, but with different additional clinical features and therapeutic approach.

Patients and Methods: We present a child with an ambiguous phenotype - hyperinsulinemic hypoglycemia, physiological 3-OH butyrate, increased triglyceride serum levels, increased level of glycogen in erythrocytes, increased liver transaminases, and increased echogenicity on liver ultrasonography. As both parents of the proband were referred as healthy, we raised a clinical suspicion on glycogenosis with recessive inheritance.

Results: The whole exome sequencing revealed no mutation in genes causing glycogenosis, but a novel heterozygous variant LRG_483t1: c.427-1G>A in the *HNF4A* gene was identified. Aberrant splicing resulting in in-frame deletion c.429_476del, p.(T144_I159del) was confirmed by sequencing of *HNF4A* transcripts reverse-transcribed from whole blood RNA. Haploinsufficiency in HNF4A can cause CHI and results in maturity-onset diabetes mellitus in young adults. The same variant was found in five of eight tested family relatives (one of them already had diabetes, two had prediabetes). With regard to the results of DNA analysis, the insulin secretion inhibitor diazoxide was added to the therapy. Consequently, the frequency and severity of hypoglycemia in the proband decreased.

Conclusion: We have identified a novel pathogenic variant in the *HNF4A* gene in our patient with CHI and glycogenosis-like phenotype. The proband and her family members benefited from the genetic testing by WES method and consequently personalized therapy. Nevertheless, the *HNF4A* gene testing may be considered in selected CHI cases with glycogenosis-like phenotype prior WES analysis.

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P06.15C

Better identification of patients with pediatric hypophosphatasia. Utility of the laboratory screening for recurrent low levels of alkaline phosphatase adjusted for gender and age

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Introduction: Hypophosphatasia (HPP) is a rare hereditary disorder, which is characterized mainly by poor mineralization of bone and teeth. It is caused by mutations in the *ALPL* gene leading to deficiency of the tissue-nonspecific isoenzyme of alkaline phosphatase, and accumulation of its substrates: phosphoethanolamine, pyridoxal-5′phosphate and inorganic pyrophosphate. In Europe the prevalence of moderate and severe hypophosphatasia is estimated at 1/6,370 and 1/300,000, respectively.The most severe forms of hypophosphatasia present an autosomal recessive inheritance, whereas the moderate form can be both recessive and dominant. Screening for mutations in the *ALPL* gene allows diagnosis of approximately 95% of severe cases.Alkaline phosphatase levels depend on sex and age, mainly due to bone growth. The Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) study established reference lower-threshold levels of the enzyme. Materials and Methods: We evaluated the utility of screening for persistent low alkaline phosphatase without fluctuations in pediatric population, taking into account the reference values described by the CALIPER study. Sequencing of the coding exons and intron/exon boundaries of *ALPL* was performed in the selected patients. **Results:** We selected 38 children, of which 25 participated in the study. We found 3 patients with mutation in gene *ALPL* (12%). Increased levels of serum pyridoxal-5′ phosphate was detected in all of them. **Conclusions:** We observed higher mutation prevalence than previously reported. Thus, this study supports the efficacy of the screening with persistent low levels of alkaline phosphatase, using reference values obtained by the CALIPER study, to detect new cases of HPP.

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P06.16D

Mutational spectrum of CYP24A1 and SLC34A1 genes in a group of Polish patients with idiopathic infantile hypercalcemia

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Idiopathic infantile hypercalcemia (IIH) is a mineral metabolism disorder characterized by severe hypercalcemia, failure to thrive, vomiting, dehydration and nephrocalcinosis. Molecular analysis of *CYP24A1* and *SLC34A1* was conducted using Sanger sequencing and next-generation sequencing to identify the molecular basis of the disease in 41 Polish patients. A genetic etiology of IIH was established in 14 patients. Overall, eleven different pathogenic variants were identified. Five known mutations (c.428_430del, c.443T>C, c.964G>A, c.1186C>T, c.1226T>C) and three novel variants (c.107del, c.475C>T, c.1157+1G>A) in *CYP24A1* and three known mutations (c.272_292del, c.464T>C, c.1425_1426del) in *SLC34A3* were revealed. Biallelic occurrence of changes was observed in all patients, except for one case in which only one mutated allele of *SLC34A1* was found. The novel changes expand the list of pathogenic mutations in *CYP24A1*. The study identified the most frequent mutation c.1186C>T (p.R396W), which was found in 50% of *CYP24A1* alleles (12/24). Based on this (and general population) data, the overall carrier frequency for all mutations in *CYP24A1* was calculated as 1.36%. Thus, the incidence of IIH in the Polish population was estimated as 1:21,626 births. This indicates that at least 1,500 homozygotes and compound heterozygotes with IIH risk may be living in the country. Differences in the mechanism of developing hypercalcemia indicate that its prevention is different in each of the IIH defects. We suggest that molecular testing for *CYP24A1* and *SLC34A1* mutations be performed in each case of idiopathic hypercalcemia/hypercalciuria and high vitamin D levels to determine the appropriate acute treatment and prevention of complications. Grants: NSC-2014/15/B/NZ5/03541;CMHI-180/09.

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P06.17A

Genome-wide association study of infantile hypertrophic pyloric stenosis identifies four new loci

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Introduction: Infantile hypertrophic pyloric stenosis (IHPS) is the most common cause of intestinal obstruction in infancy. IHPS is caused by hypertrophy of the smooth sphincter muscle that surrounds the pylorus, which connects the stomach to the duodenum. Materials and Methods: We performed a genome-wide association study of 1,395 Danish surgery-confirmed cases and 4,438 controls in order to detect novel loci associated with IHPS. The analysis was based on 6.6 million variants imputed from the Haplotype Reference Consortium panel, using logistic regression on imputed SNP dosages for association testing. **Results:** In addition to variants at the known MBNL1, ARHGEF26, NKX2-5 and APOA1 loci, we found four new loci with variants reaching genome-wide significance (P<5×10-8). The most strongly associated variant (minor allele frequency; MAF=2%) had an odds ratio (OR) =2.5 (P=5.0×10-14) and is intronic to MTA3, a gene that when knocked out in mice leads to decreased levels of circulating LDL cholesterol. The second signal came from a common variant (MAF=26%, OR=1.3, P=2.7×10-9), which is an eQTL for SLMAP, a gene with highest expression in smooth muscle. The third variant (MAF=9%, OR=1.5, P=4.6×10-9) is an eQTL for PTPDC1, while the fourth variant (MAF=12%, OR=1.4, P=3.2×10-8) is in a gene desert at 13q21.31. Using the GCTA method on all genotyped SNPs, we estimated the heritability of IHPS to be 52%. Conclusion: Our findings identify novel genetic

associations with IHPS, and highlight their putative functional target genes.

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P06.18B

A proper definition of the target phenotype greatly improves the identification of rare causative mutations: lessons from the study of 280 suspected *HFE* hemochromatosis patients treated by phlebotomy

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Introduction: The best-known and prevalent form of hemochromatosis (HC) is a an adult-onset autosomal recessive condition usually associated with the *HFE* p.[Cys282Tyr];[Cys282Tyr] genotype. A second genotype, p.[Cys282Tyr];[His63Asp], is frequently observed. However, compound heterozygotes patients have a distinct iron overload phenotype (mild to moderate), usually associated with the presence of comorbid factors. Rare *HFE* variants exist but they are not screened in routine. **Materials and Methods:** We selected 35 patients from a cohort of 280 p.[Cys282Tyr];[=] or p.[Cys282Tyr];[His63Asp] patients treated by phlebotomy. All these patients presented with hyperferritinemia and elevated transferrin saturation (≥60% in males; ≥50% in females), which is a hallmark of hemochromatosis. We excluded patients with secondary causes of hyperferritinemia: excessive alcohol intake (n = 9), confirmed metabolic syndrome (n = 134), or both (n = 13). Next-Generation Sequencing was used to investigate the five known hemochromatosis genes (HFE, HFE2, HAMP, TFR2, SLC40A1). **Results:** Mutations were only detected in *HFE*. The p.Trp155* and c.76+2 T>C mutations, which cause a truncating protein, were identified in two p.[Cys282Tyr];[=] patients. They were not detected in 1460 control chromosomes. The splicing defect of the intronic mutation was confirmed using the *POLR2G* minigene system. A complex allele [His63Asp; Glu168Gln] was found *in trans* with the p.[Cys282Tyr] mutation in one patient. Haplotype inference and cloning experiments confirmed this finding. The [His63Asp; Glu168Gln] complex allele is not very rare in our local population (western extremity of Brittany; MAF: 4/1460). **Conclusion:** A complete *HFE* sequencing is mandatory in p.Cys282Tyr heterozygous patients with a typical hemochromatosis phenotype.

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P06.20D

Identification of a human D-lactate dehydrogenase

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Introduction: Human knockouts may provide direct insight into gene function. We identified two patients homozygous for loss-of-function variants in *LDHD*, lactate dehydrogenase D. Lactate dehydrogenases catalyze the interconversion of pyruvate and lactate during anaerobic glycolysis, with L-lactate the form utilized in eukaryotic metabolism. D-lactate, the stereoisomer, is normally present

physiologically at much lower levels. D-lactate in the human body has received recent attention due to D-lactic acidosis incidence, a complication of short bowel syndrome following bariatric surgery.

Materials and Methods:

Mass spectrometry performed on two patients identified increased urine excretion and elevated plasma concentration of D-lactate, D-2-hydroxyisovaleric acid and D-2-hydroxyisocaproic acid. Sanger sequencing was performed on *LDHD*, a candidate gene in a region of homozygosity identified by SNParray. Functional studies were performed in the zebrafish model organism using a *LDHD* knockout line and patient variant expression. Metabolic levels of D-lactate were evaluated by mass spectrometry.

Results:

Sanger sequencing identified two novel, homozygous *LDHD* missense variants. *LDHD* loss-of-function in zebrafish resulted in increased D-lactate concentration. Expressing human wildtype *LDHD* rescued D-lactate metabolism resulting in D-lactate concentration decrease. In contrast, patient variant *LDHD* expression was unable to restore LDHD function and resulted in elevated D-lactate levels. Together our results confirmed a role of LDHD in D-lactate metabolism and the loss-of-function effect of our patient's variant.

Conclusion:

LDHD has been identified as a putative metabolizer of D-lactate but its function had not been shown *in vivo*. Our work provides the first evidence that LDHD is essential for D-lactate metabolism in humans.

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P06.21A

Molecular basis of autosomal dominant hypercholesterolemia in the Czech Republic

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Introduction: Autosomal dominant hypercholesterolemia (ADH) is a lipid metabolism disorder characterized by an elevation in low-density lipoprotein cholesterol and increased risk for cardiovascular disease. Predominantly, the clinical phenotype of ADH is caused by mutations in the LDLR or APOB genes.

Materials and Methods: PCR-RFLP of the APOB mutation p.(Arg3527Gln); sequencing of the LDLR gene coding sequence (Sanger sequencing till 2014, actually next generation sequencing using ADH Master kit (Multiplicom, Belgium)); MLPA of the LDLR gene. Confocal laser scanning microscopy was used to analyze the LDLR expression and binding of the LDL particles to LDLR in stably transfected Chinese hamster ovary cells. DNA samples have been collected within the framework of the MedPed project.

Results: We assessed a spectrum of mutations in 3914 unrelated patients with clinical diagnosis of autosomal dominant hypercholesterolemia. We have found 432 patients (11.0%) with the APOB gene mutation p.(Arg3527Gln) and 864 patients (22.1%) with the LDLR gene mutation. 182 unique allelic variants in LDLR gene were detected. Using MLPA, 22 different large rearrangements were found. Functional analyses of LDL receptor protein revealed that the controversial sequence variant p.(Gly20Arg) is able to reach the cell membrane and to bind the LDL particle in a similar way as the wt LDLR protein.

Conclusions: The results achieved in the number of diagnosed patients are comparable with other countries worldwide. Confocal laser scanning microscopy was introduced to analyse the LDLR protein expression and function.

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P06.22B

Genetic study of Familial Hypercholesterolemia in a hospital population of Buenos Aires, Argentina

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Introduction

Familial hypercholesterolemia (FH) is a health problem that affects a significant number of people in Argentina and

it is one of the factors that determines the early establishment of cardiovascular disease. It is inherited in an autosomal dominant manner with 60–80% percent of the mutations located in the *LDLR* gene.

Material and Method

The LDLR gene coding regions, adjacent splice sites and promoter regions were Sanger sequenced in a cohort of 38 unrelated patients with clinical diagnosis of FH and 10 healthy normolipemic controls at the Metabolic Unit Service of Favaloro Foundation University Hospital.

Results and Conclusions

In this study we found 24 different pathogenic variants in the *LDLR* gene in 23 patients (60,5%), most of them located in exons 4 and 12. Of these 24 variants, 16 were previously described and 8 were novel variants (6 located in coding regions and 2 in the *LDLR* promotor) predicted to be pathogenic according to different *in silico* prediction tools. Four out of the 7 patients who carry the novel variants presented subclinical carotid disease, aortic and lower limb disease; 3 of them required myocardial revascularization surgery. In addition, 4 of these patients had family history of hypercholesterolemia.

These results show the importance of the genetic testing in index cases with the aim of achieving a better adherence to treatment, re-stratification of the risk of early cardiovascular disease onset, and a prompt genetic counseling in a familial context.

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P06.23C

Functional genomics of stimulated human hepatocytes revealed long non-coding RNAs involved in liver inflammation

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Introduction: In recent years functional genomics has become a powerful tool to understand complex cellular processes by studying gene transcription, translation, interactions and epigenetic regulation. This approach often involves high-throughput methods such as RNA-sequencing technology, a technique which allows scientists to detect a large proportion of functional RNA molecules known as long non-coding RNAs (lncRNAs). Here, we aim to investigate the involvement of lncRNAs in non-alcoholic steatohepatitis (NASH) by combining hepatocyte and liver transcriptome data.

Materials and Methods: We generated a cellular model to mimic NASH by stimulating human hepatocytes with free fatty acids (FFA) to mimic steatosis and tumor necrosis factor alpha (TNFα) to mimic inflammation for 30 min, 3 and 5 hours. Gene expression profiles were determined by performing whole genome RNA-sequencing.

Results: We detected 4373 differentially expressed genes in all conditions from which 109 were lncRNAs (FDR<0.1). Pathway analysis showed gene enrichment in crucial processes including oxidation-reduction, cell proliferation, lipid metabolism (FFA stimulation) and transcriptional and translational initiation, NF-kB signaling and Wnt signaling (TNF stimulation) as top enriched pathways. We have selected two lncRNA for functional studies: lncRNA1 showing 3 fold down-regulation and lncRNA2 showing 15 fold up-regulation upon TNFα stimulation. The response to inflammatory stimuli was in line with our human data where the lncRNA was negatively (lncRNA1) or positively (lncRNA2) correlated with NASH phenotypes.

Conclusion: Using functional genomic approach we identified lncRNAs which may be involved in regulation of liver inflammation. This knowledge may offer new avenues for the development of novel therapeutic strategies.

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P06.24D

Biochemical and genetic data in the largest global Fabry cohort

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Fabry disease is an X-linked inherited lysosomal storage disease characterized by a deficient alpha-galactosidase caused by mutations in GLA gene. Fabry diagnosis is performed in a high-throughput stepwise manner: (a.) males-

enzymatic activity, lyso-Gb3 quantification, followed by GLA gene sequencing and, (b.) females- GLA gene sequencing followed by lyso-Gb3. In the development and validation of the enzymatic assay we observed that alpha-galactosidase determination in DBS alone is insufficient for a precise diagnosis of Fabry disease, both in females (ppv 78%) and males (ppv 94.2%) due to multiple variables: leukocyte count; hematocrit level; sample handling, lyonisation effect in females. To eliminate the differences between samples several optimizations were introduced: chemical blank for each sample, a standard curve measured in the presence of blood extract and the ratio alpha-galactosidase to another lysosomal enzyme (beta-glucuronidase). Lyso-Gb3 was measured using mass spectrometry (LC/MRM-MS). Mild mutations or late onset patients present levels of lyso-Gb3 in normal range (21.59 % of all Fabry male cases). However, by combining the data from three different biochemical parameters (Lyso-Gb3, alpha-galactosidase and ratio alpha-galactosidase / beta-glucuronidase) we can simply distinguish between the cohorts of normal controls, mild (or late onset) Fabry cases and affected Fabry cases. The biochemical diagnosis was confirmed in all cases by genetic analysis. We report here the identification of over 390 unique GLA genetic variants in over 3,330 Fabry individuals. From the ca 3,400 allele sequenced in this study, the most abundant were: c. 937G>T (17.5%); c. 376A>G (20.3%); c. 352C>T (7.2%); c. 427G>A (5.6%).

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P06.25A

Mutation analysis of LIPA through NGS in a cohort of 731 patients with nonalcoholic hepatic steatosis and familial hypercholesterolemia

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Introduction Lysosomal Acid Lipase Deficiency (LAL-D) (*LIPA*, MIM613497) is a rare disease related to two different phenotypes: Wolman disease (WD) and cholesteryl ester storage disease (CESD). WD is a severe disease that causes disseminated organ foam cell infiltration of liver, spleen and other organs due to the accumulation of macrophages filled with cholesterol-esters and triglycerides. The allele frequency of the most common variant in CESD (c.894G>A;p.E8SJM) ranged from 0,05% in Asians to 0,17% in Caucasian. The aim of this study was to perform a molecular analysis of *LIPA* in a cohort of patients with nonalcoholic hepatic steatosis (NHS) and familial hypercholesterolemia (FH), looking for mutations and changes in *LIPA* and estimates the allele frequency of the c.894G>A mutation.

Material y methods Two different NGS panels were applied in 328 patients with FH and 403 patients with NHS. Design and capture were carried out with Nimbledesign and sequencing was done with both NextSeq500 and MiSeq platforms.

Results We have found a compound heterozygous mutation in a patient with NHS. Seven additional carriers of *LIPA* mutations have been found (five was the c.894G>A;p.

E8SJM mutation). Two of these patients with the recurrent mutation belongs to the NHS group (0,49%) and five to the FH group (1,1%).

Conclusions

Allele frequency of *LIPA* mutations in patients with NHS and FH are significatively higher compared to the general population. These results suggested that mutations in this gene could modulate in some way the phenotype in patients with nonalcoholic hepatic steatosis and familiar hypercholesterolemia.

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P06.26B

Clinical and molecular findings in Turkish patients with MPS IV

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Introduction: Mucopolysaccharidosis IVA (MPS IVA) is a lysosomal storage disease caused by the mutation in the GALNS gene, encoding galactosamine 6 sulfate sulfatase enzyme and characterized by progressive skeletal dysplasia with short stature and severe dysostosis multiplex. We aimed to investigate clinical and molecular features of the patients with MPS IVA. Material and Method: 26 clinically diagnosed and enzymatically confirmed MPS IVA patients from 19 families were enrolled in this study. All exons and exon-intron boundries of GALNS gene was studied by Sanger sequencing method in 18 families. **Results:** The median age of patients at diagnosis was 3.0 years (0,1–18,5 years). 23 patients were diagnosed with severe and three with intermediate form of disease. Initial finding was skeletal deformities. Short stature was apparent by early childhood in most of the patients; the median of height SDS at 2, 5, 10 and 15 years of age were -0.6, -3.4, -7.0, -8.1 respectively. Walking was impaired in 7 patients at the mean age of twelve. Corneal clouding, hearing loss and heart involvement were detected in 60–70% of the patients. Molecular analysis revealed ten known mutations (one deletion, nine missense) and one novel mutation (p.G139D) in the patients with severe form. Most common mutation was p.W141R. Known mutations were reported in severe form of the diseases previously. We determined homozygous known mutation (p.R251Q and p.F346L) in three patients which were presented as intermediate phenotype. Conclusion: This study is the first large cohort showed clinical characteristic and mutation spectrum of Turkish patients with MPSIV.

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P06.27C

Nucleotide analysis of a gene panel involved in Lysosomal Storage Disease (LSD) in patients with a strong clinical suspicion

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The diagnostic workup in patients clinically suspected of Lysosomal Storage Diseases (LSD) is often challenging due to the variability in the clinical phenotype and inconsistent genotype-phenotype correlations. The standard sequential workflow of a urine screening, followed by enzymatic testing and a final confirmation by iterated Sanger sequencing is time consuming, not widely available and poorly standardized. To counter these issues, we developed a next generation sequencing (NGS) gene panel for mutations in 50 LSD-causing genes . Over a period of ten months, we analysed 80 samples from patients with a strong clinical suspicion of an LSD. Our panel was able to determine the molecular cause of the disease in 14 cases (17%), including diseases such as ceroid lipofuscinosis type 6 and 7, Niemann Pick C, Wolmann disease, MPSI, II and IV (Morquio B), cystinosis, mannosidosis type I and II and finally mucolipidosis type II and III. Most of these patients were missed by multiple rounds of conventional biochemical testing. The NGS-based approach was also able to redirect certain diagnoses: e.g., a patient initially suspected of galactosialidosis was found to be compound heterozygous for pathogenic mutations in the *GNPTAB* gene, causing

mucolipidosis type II or III. Additionally, we identified several patients as carriers of pathogenic mutations in LSD genes, including *GBA*. Carriers of *GBA* mutations have an increased chance of developing early onset Parkinsonism, making early genetic counselling possible. In view of these results, we propose that panel-based NGS should be considered as first-line test in the LSD diagnostic workflow.

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P06.28D

Mitochondrial DNA sequence context in the penetrance of mutations: a study across multiple lineages results with diagnostic significance

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Introduction: Mitochondrial DNA (mtDNA) mutations are an important cause of inherited disease. Diseases caused by mtDNA mutations exhibit a high degree of clinical heterogeneity with a complex genotype-phenotype relationship, with many mutations exhibiting incomplete penetrance. There is evidence that the spectrum of mutations causing mitochondrial disease might differ between different mitochondrial lineages (haplogroups) seen in different global populations. This would point to the importance of sequence context in the expression of mutations. Methods: To explore this possibility, we looked for mutations, which cause disease in humans, in animals of other species unaffected by mtDNA disease. **Results:** The m.3243A>G mutation on the mt-tRNA-Leu(UUR) being the most frequently seen mutation in humans. This study looked for the presence of m.3243A>G in 2784 sequences from 33 species, as well other mutations reported in association with disease located on mt-tRNA-Leu(UUR). Our initial report showed a number of disease associated variations found on mt-tRNA-Leu(UUR) in other chordates, as the major population variant, with m.3243A>G being seen in 6 species. In these, we also found a number of mutations that could be compensatory that is could mask the pathogenicity associated with this change in humans. In follow-up work just completed, we can report finding other disease-causing mutations in other mt-tRNA's supporting our early observations and assertions. **Conclusions:** This work has important implications for the discovery and diagnosis of mtDNA mutations in non-European populations. It might provide a partial explanation for the conflicting results in the literature that examines the role of mtDNA variants in complex traits.

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P06.29A

Challenges in clarifying the at-risk status for malignant hyperthermia

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Malignant hyperthermia susceptibility (MHS) is an autosomal dominant disorder of calcium regulation in skeletal muscle; a molecular cause can be identified in 70% to 80% of affected individuals. While failure to recognize an at-risk patient can have serious consequences, inaccurately labelling a patient incurs costs to the health care system. Clarifying at risk status for MHS begins with testing a clearly affected individual. In a retrospective review of the 52 individuals referred from 2011–2016 for a personal or family history of MHS, 11 referrals were for affected individuals. Mutations in the RYR1 gene were found in 4/11 (36%). The mutation was known for 4/41 of the individuals referred based on family history. In the remaining 37 cases, the family history was confirmed in 30 but the affected relative was available to be tested in only 4 families; a mutation was found in 1/4. Our review revealed that 29% of the 52 referrals received for a personal or family history of MHS underwent genetic testing; a mutation was identified in 33%, a rate significantly lower than that reported in the literature. 87% of individuals with a confirmed family history of a MHS event were unable to undergo genetic testing; improvements in the sensitivity and specificity of MHS testing will allow genetic testing for individuals where the index case is unavailable.

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P06.30B

Mendelian randomization approach infers causality links between gut microbiome and glucose/insulin metabolism and type 2 diabetes

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Accumulating evidence suggests that the gut microbiome influences the development of obesity and metabolic diseases. Studies in mice have proposed a causal effect of gut microbiome on the development of obesity and type 2 diabetes (T2D). Due to the complex host-microbe interaction, we hypothesized that a bi-directional causal effect exists between gut microbiome, T2D and T2D intermediary phenotypes. We investigated the effects of impairments in glucose/insulin metabolism on microbiome in the LifeLinesDeep population cohort (1123 individuals with metagenomics and genomic profiles) using a Mendelian Randomization (MR) approach. We extracted summary statistics from 12 published genome-wide association studies on obesity, fasting insulin and glucose, insulin secretion, proinsulin and T2D, and constructed 40 genetic risk scores (GRS). Of the 410 taxonomies and 782 bacterial pathways assessed, 67 and 138, respectively, were significantly correlated with at least one GRS (FDR <0.1); evidence for causality was observed only for T2D, insulin secretion and HOMA-B. In particular, MR approach suggests that high levels of insulin secretion and HOMA-B cause a reduction in abundance of specific bacteria from the Firmicutes phylum, whereas a high load of T2D risk alleles causes an increased abundance; in mice, the reverse directional causal link, improved insulin sensitivity upon reduction of Firmicutes, was observed. Bacterial pathways associated to GRS of metabolic changes were predominantly related to biosynthesis of fatty acids, lipids and vitamins, as well as carbohydrates degradation. Our results highlight a causal relation of insulin/glucose metabolism in shaping the microbiome composition and function, bringing new insights into host-microbe interaction.

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P06.31C

Effectiveness of whole exome sequencing in unsolved patients with a clinical suspicion of a mitochondrial disease in Estonia

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Reaching a genetic diagnosis of mitochondrial diseases is challenging due to broad phenotypic and genotypic heterogeneity. However, there is growing evidence that the use of whole exome sequencing (WES) in patients with clinical suspicion of a mitochondrial disease is effective (39–60% by Wortmann et al 2015, Pronicka et al 2016). We aimed to study the effectiveness of WES used in Estonia in patients with an unsolved suspected mitochondrial disease.

A total of 21 cases were selected from patients whose fibroblast cell cultures have been stored since 2003. We classified the cases by Nijmegen mitochondrial disease scoring (MDS) system (Morava et al 2006) after re-evaluation of clinical data, and performed WES analysis. Sanger sequencing was used to confirm the findings and analyse familial segregation.

A phenotype-associated mutation was found in 14 patients (67%):

MDS	All/ solved cases	Phenotype-associated mutations	
		Mitochondrial function related genes	Other genes
Definite (score 8–11)	3/3	*SLC25A4, POLG*	*SMN*
Probable (score 5–7)	11/8	*SPATA5*, *MT-ATP6*	*MYH2, EPG5, BZRAP1, NKX2-1, CACNA1A, LMNA*
Possible (score 3–4)	7/3	*NDUFB11*	*ALS2, ATRX*

The diagnostic yield of WES in our cohort was even higher than previously reported proving a very good effectiveness. We also provide more evidence that the features characteristic for mitochondrial diseases overlap with other neurological disorders showing the need to use WES

in clinical genetic practice. This work was supported by Estonian Science Foundation grant PUT0355.

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P06.32D

A novel approach for investigating mitochondrial DNA variation in disease

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Introduction: As mitochondrial dysfunction has been implicated in the pathology of several common complex and late onset diseases, mitochondrial DNA (mtDNA) has been targeted in the last decade in the search for genetic markers to better predict disease onset and progression. Just as genome wide association studies have been utilised to scour the nuclear genome for disease associated variants, so too have haplogroup association studies done for mtDNA. As the limitations of these approaches become more apparent, the need for new ways to investigate the role of mtDNA variants in diseases is highlighted. Here we present a novel approach, the MutPred mutational load hypothesis, for investigating mtDNA variation in disease. This approach utilises MutPred pathogenicity scores, to then obtain a single metric which serves as an estimate of the likely impact of a person's non-synonymous mtDNA substitutions on the functioning of their mtDNA encoded proteins.

Materials and methods: We applied this novel approach in two common complex disease cohorts: a bi-ethnic cardiometabolic disease cohort, and a myalgic encephalomyelitis or chronic fatigue syndrome (CFS) cohort with participants from South Africa and the UK.

Results: While no role was found for non-synonymous mtDNA variants in hypertension or diabetes, preliminary results indicate significant differences between CFS patients and control groups.

Conclusions: The MutPred mutational load hypothesis offers an alternative and well powered approach for investigation mtDNA variation in disease.

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P06.33A

Mitochondrial dysfunction as a pathobiochemical mechanism of a wide range of neurodegenerative disorders

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Introduction: Mitochondrial dysfunction is the cause of many pathological disorders that are different their variety and complexity. Intensive study of the mitochondrial (mtDNA) polymorphisms made define it as typical pathological process, for which there is no nosology and etiological specificity. The frequency of mitochondrial dysfunction in population is 1:3000. Description: Clinical and genetic characteristics of mtDNA polymorphisms (203 patients) involve disorders of nervous (82.16% of patients), muscle (43.24%), ophthalmic (62.16%), cardiovascular (35.14%), skeletal (38.0%) and digestive system (40.54%). 75 patients (36.5%) had characteristic clinical features of classic mitochondrial syndromes MERRF, MELAS, NARP, Leigh, Kearns-Sayre, Leber. In 91 patients (45.31%) we found elements of syntropy, in which each of the "conglomerate diseases" retained its specificity. The more frequent of mtDNA polymorphisms in patient with neurological symptoms were tRNA polymorphisms-lysine: 8697G/A; 8860G; 8701G/A; 8856G/A; 8860 (CRS); 8251G/A; 8472S/T; 8448T/C; 8994G/A; 8337T/C; 8794S/T; 8584G/A; 8701A/G and amino acid substitutions tRNA-lysine (syn, thr/ala, pro/leu, met/val, met/thr, his/tyr, ala/thr). The causes of encephalopathy were associated with polymorphisms tRNA-lysine and new mutations (leucine tRNA) (3624 A/G; 3594S/T, 3705G/A, 3505/G, 3552T/A). **Conclusions:** In the process of studying the spectrum of

rare diseases have been identified high proportion of mitochondrial dysfunction. This makes possible to understand the pathogenetic mechanisms of the development of clinical symptoms and to develop pathogenetically substantiated therapy.

O. Grechanina: None.

P06.34B

A molecular diagnostic rate of >80% in a 10-year birth cohort of paediatric patients with mitochondrial disease

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Mitochondrial oxidative phosphorylation (OXPHOS) disorders are clinically heterogeneous and comprise over 200 different genetic disorders. We previously published a cohort of 86 children born over a 10-year period (between Jan 1st 1987 and Dec 31st 1996) with definite diagnosis of a mitochondrial OXPHOS disorder and onset of symptoms by 16 years.

These patients were referred to our testing centre for investigation from the 3 South-Eastern states of Australia and, when published, were regarded as representing all children with a definite diagnosis of OXPHOS disease in this area and time period. A definite diagnosis was based on defined clinical, pathological, enzyme and molecular criteria. This cohort now comprises 105 patients, mainly due to children who were investigated after the original study. All but two patients without a molecular diagnosis received whole exome sequencing with ethics approval to analyse a 'virtual panel' of ~2300 genes related to mitochondrial function. This resulted in a molecular diagnosis in 81.9% (86 patients) up from 23.3% (20 patients) in 2003. Mutations were identified in 34 different genes (31 genes known to cause mitochondrial disease, 2 non-mitochondrial disease genes with clinical overlap and 1 novel mitochondria-related gene *MRPS34*). Fifty-three patients showed an autosomal recessive inheritance pattern, 6 ×-linked and 27 had mtDNA-related disorders (including 6 patients with mtDNA deletions). Our study illustrates the increased ability to obtain molecular diagnoses in well defined cohorts, especially with the advent of massively parallel sequencing, and the ever increasing genetic complexity of mitochondrial disorders. Supported by Australian NHMRC project grant 1068409.

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P06.35C

Searching for the Maturity Onset Daibetes of the Young in Turkey by genetic diagnosis

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Introduction: Maturity-Onset Diabetes of the Young (MODY) is a monogenic insulin secretion defect. MODY2, associated with GCK mutations, MODY1, MODY3, MODY5 associated with hepatocyte nuclear factor transcription factors HNF4A, HNF1A, HNF1B mutations are the most common types. The aim of the study is to screen *HNF1A, GCK, HNF1B*, and *HNF4A* genes of MODY suspected patients to establish genetic profile of MODY patients in Turkey.

Materials and Methods: 81 MODY suspected patients were screened for the *HNF1A, GCK, HNF4A, HNF1B* gene mutations by next generation sequencing analysis (NGS). All patients were ICA/GAD negative. Their average age, age of diagnosis, weight, BMI, HbA1C, C-peptide is 32.13, 24.83, 63,62 kg, 22.54, 7.4,1.48 ng/ml, respectively.

Results: NGS analysis revealed variations in 11 patients. Two patients had novel GCK variations, p.R192Q, c.689delG. One patient had a novel HNF1B variation, p. His424Arg, two patients had HFN4A p.Tyr139Ile variation. Seven patients had HNF1A variations p.Val246Leu (novel), p.Arg263Cys, c.864-c.865InsGC, c.1130InsC, c.1136DelC. All the patients were heterozygous. One patient had both HNF4A p.Thr139Ile and HNF1A c.865InsGC variations.

Conclusion: Polyphen2 analysis revealed novel missense substitutions in GCK, HNF1B and HNF1A as probably pathogenic variations but HNF4A substitution as a benign variation although it is a change from polar to

nonpolar. The MODY frequency in Turkey is found as 13.58 % in diabetic cases with two MODY2, one MODY5 and 7 MODY3 patients. It is also revealed that MODY3 is the most common form of MODY in Turkey with 7.68% heterozygous frequency.

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P06.36D

A putative hypomorphic recessive variant disrupting the start-codon of Mitochondrial Pyruvate Carrier 2 (*MPC2*) in a child with suspected mitochondrial disease

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Introduction: The MPC1/MPC2 complex mediates pyruvate transport across the inner membrane of the mitochondrion. The genes encoding the mitochondrial pyruvate carriers were described in 2012 and *MPC1* missense mutations have been identified in three families with offspring exhibiting a devastating defect in mitochondrial pyruvate oxidation. *MPC2* variants have not yet been linked to human disease, and ubiquitous disruption of Mpc2 expression in mice confers embryonic lethality

Material and methods: A three year old boy presented in the clinic with developmental delay, hypotonia and lactate acidosis. The parents are healthy and unrelated and he has three unaffected brothers. Mitochondrial disease was suspected and exome sequencing was carried out on affected child, parents and one unaffected brother.

Results: This led to the identification of a homozygous variant disrupting the translational start-site of *MPC2* (NM_015415.3:c.3G>A p. (?)) in the affected child. Both parents are heterozygous carriers of this variant which has not previously been described. Sanger sequencing confirmed the genotype. None of the three unaffected brothers carry the variant.

Conclusion: The affected off-spring displays a similar phenotype to what has been described in patients with homozygous missense mutations in *MPC1*. Mice studies disrupting the start-site of *Mpc2* lead to the use of an alternative start-site and a truncated hypomorphic protein. We propose that this mechanism is responsible for the phenotype in the case presented here. Upon confirmation by functional analysis, this would to our knowledge be the first case identifying a MPC2 variant as the cause of mitochondrial disease.

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P06.38B

All exons are not created equal

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We speculated that *ACADM* exon 5 is vulnerable, and investigated presumed silent and missense variants identified in different parts of exon 5 in newborns with MCADD. Minigene studies revealed that several of the variants cause exon skipping by abolishing different ESEs. Employing siRNA/overexpression of splicing regulatory factors (SRFs) and RNA pull-down, we characterized new ESEs and ESSs and the binding SRFs. We show that the ESEs and ESSs need to be in a finely tuned balance to allow exon 5 inclusion. This balance is not only dependent on steric competition of SRFs for binding to juxtaposed ESE/ESSs, as previously demonstrated for the c.362ESE and c.351ESS. Instead the overall balance between ESEs/ESSs located throughout the exon needs to be maintained. In line with this we show that a c.331G>A missense variation abolishes an ESE to cause complete exon skipping. Remarkably, the polymorphic c.351A>C, which inactivates the distant c.351ESS, also makes splicing immune to the deleterious effect of c.331G>A. Moreover, inactivation of an ESS at pos. c.325, abolishes the splicing defect of the distant c.362C>T variant as well as that of the juxtaposed c.331G>A. We conclude that splicing of weak exons can be disrupted by several different missense/silent variations. Moreover, weak exons are critically dependent on the overall balance between ESE/ESSs located throughout the exon. It is thus essential to evaluate effects on splicing in the context of the haplotype.

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P06.39C

Discerning the "identical": unexpected mitogenome diversity behind the most common European mtDNA control region (D-loop) haplotype

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Introduction: Mitochondrial (mt)DNA is a vital tool in forensic genetics when nuclear markers do not provide results or maternal relatedness is investigated. The ~1.1 kbp non-coding mtDNA control region (CR) displays highly condensed variation and is therefore routinely typed. In this restricted range, matching haplotypes do not necessarily imply that the entire mitogenomes are identical or even belong to the same phylogenetic lineage. This is especially true for the most frequent West Eurasian mtDNA CR haplotype that occurs at a frequency of 3–4% in European populations and is observed in numerous clades within haplogroup H ("Helena") and some relatives. In a seminal study, we investigated the power of massively parallel complete mitogenome sequencing in 29 Italian samples displaying the most common West Eurasian CR haplotype. This allowed the detection of an unexpected high diversity with 28 distinct haplotypes clustering into 19 clades of haplogroup H and raised the power of discrimination from 0 to >99% [1].

Materials and Methods: Here we present novel results from the ongoing investigation of an expanded pan-Italian sample of almost 300 individuals carrying the most common CR haplotype.

Results and **Conclusions:** Even with the currently ~100 samples examined so far, no saturation is reached. This study demonstrates the benefit of complete mitogenome sequencing for forensic applications to enforce maximum discrimination, highest phylogenetic resolution and more comprehensive heteroplasmy detection.

[1] Bodner M et al.: Helena, the hidden beauty: Resolving the most common West Eurasian mtDNA control region haplotype by massively parallel sequencing. FSI:Gen 2015; 15:21.

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P06.40D

next generation sequencing identifies mutations in *GNPTG* gene as a cause of familial form of scleroderma-like disease

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Introduction: Scleroderma is a multisystem disease, characterized by fibrosis of skin and internal organs, immune dysregulation, and vasculopathy. The etiology of the disease remains unknown, but it is likely multifactorial. However, the genetic basis for this condition is defined by multiple genes that have only modest effect on disease susceptibility. **Material and Methods:** Three Moroccan siblings, born from non-consanguineous Moroccan healthy parents were referred for genetic evaluation of familial scleroderma. Whole Exome Sequencing (WES) was performed in the proband and his parents, in addition to Sanger sequencing that was carried out to confirm the results obtained by WES. **Results:** Mutation analysis showed two compound heterozygous mutations c.196C >T in exon 4 and c.635_636del TT in exon 9 of *GNPTG* gene. Sanger sequencing confirmed these mutations in the affected patient and demonstrated that their parents are heterozygous carriers. Conclusion: Our findings expand the mutation spectrum of the *GNPTG* gene and extend the knowledge of the phenotype-genotype correlation of Mucolipidosis Type III gamma (MLIII gamma). This report also highlights the diagnostic utility of Next Generation Sequencing (NGS) particularly when the clinical presentation did not point to specific genes. Keywords: Mucolipidosis III gamma; *GNPTG*; Whole Exome Sequencing.

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P06.41A

Glycosaminoglycans by tandem mass spectrometry in dried blood spots as a tool to reduce false positive results by enzyme analysis

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MPS-I has already been added to the US Recommended Uniform (Newborn) Screening Panel, other mucopolysaccharidoses (MPSs) will likely follow. Pseudodeficiency is a common issue to several, if not all, MPSs, leading to an increasing number of false positive results. To improve this situation, we developed and validated a test to measure dermatan (DS), heparan (HS), keratan (KS) and chondroitin sulfate (CS) in dried blood spots (DBS) when low enzymatic activities are found by either newborn screening or diagnostic assays.

DS, HS, KS and CS (data not shown) are extracted from DBS and enzymatically digested to disaccharides prior to LC-MS/MS analysis. Run time is 7 minutes/sample.

Newborn DBS (N = 129), pediatric DBS (age > 2 weeks - 18 years) (N = 97) and adult DBS (N = 125) controls were analyzed.

		DS (nmol/L)		
	N	Median	Min	Max
MPS-I	7	770.1	242.3	1222.2
MPS-II	6	466.7	297.7	764.7
MPS-III	6	46.5	30.2	83.9
MPS-IVA	4	54.9	31.2	80.9
Controls	97	48.2	19.4	127.2
		HS (nmol/L)		
	N	**Median**	**Min**	**Max**
MPS-I	7	172.1	100.7	230.6
MPS-II	6	260.7	134.5	321.7
MPS-III	6	166.7	115.4	294.1
MPS-IVA	4	59.6	35.7	69.5
Controls	97	41.2	10.1	94.3
		KS (nmol/L)		
	N	**Median**	**Min**	**Max**
MPS-I	7	1900.2	701.8	3858.2
MPS-II	6	1976.8	1495.1	3605.3
MPS-III	6	1254.7	567.5	1899.4
MPS-IVA	4	2163.9	1880.6	2348.1
Controls	97	948.2	236.9	2466.6

Preliminary data show that our test is a rapid and specific method for timely diagnosis and treatment of patients with MPSs, allowing identification of cases with low enzymatic activity due to pseudodeficiency.

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P06.42B

Second report of a patient with mutations in *FLAD1*, a new gene associated to multiple acyl-CoA dehydrogenase deficiency

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Case report: We present a 6 months old female with bilateral cataracts. She developed scoliosis and myopathy at four years of age. Metabolic investigations showed high excretion of ethylmalonic acid, and high medium- and -long chain acylcarnitines in plasma. These results were suggestive of multiple acyl-CoA dehydrogenase deficiency (MADD), and palmitate oxidation in fibroblasts confirmed the suspicion. Consequently, the patient was treated with riboflavin and low fat diet. However, after 3 years of treatment clinical response was lacking.

Methods: In house designed Haloplex panel (Agilent technology), containing all seven genes associated with MADD was used.

Results: The patient was found to be compound heterozygous for two new mutations in *FLAD1*: c.1555-3C>G and c.797_798delAGinsT.

Discussion: *FLAD1* encodes for FADS, that is a protein implicated in the biosynthesis of flavin adenine dinucleotide (FAD), which is a redox cofactor involved in several important reactions in the metabolism. Until now, only a single article (Olsen et al. Am J Hum Genet, 2016) recruiting nine patients with mutations in *FLAD1* have been

published. Half of them were shown to have single aminoacid changes in the FADS domain and were riboflavin-responsive; our patient was not responsive which can be explained by the more severe mutations. Clinically, she presents myopathy, and high ethylmalonic acid excretion, as most of the published cases, but this is the first time that cataracts are associated with *FLAD1* mutations

Conclusion: *FLAD1* should be studied in patients with MADD and high excretion of ethylmalonic acid and cataracts, wether or not they were riboflavin-responsive.

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P06.43C

Neuronal Ceroid-Lipofuscinoses in Portugal: the reference center casuistry

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Introduction

Neuronal Ceroid-Lipofuscinoses (NCLs) is a group of lysosomal storage disorders. Altogether NCLs are the most common neurogenetic storage diseases in pediatric age. NCLs are genetically heterogeneous as thirteen genes are known to be involved, usually with some correlation with age of onset, clinical features and course. Symptoms present in a progressive way and usually include intellectual and motor deterioration, seizures and visual loss.

Patients and methods

This work includes 43 patients, belonging to 33 families, with clinical suspicion of NCL with laboratory confirmation in our center.

For NCL1 and NCL2 testing enzyme activity assays were used. For the diagnosis of the other types of NCLs, PCR amplification and sequencing were the standard procedures and NGS was used in some unusual situations.

Results

Patient's clinical data and laboratory results are presented.

Fourteen out of the 15 variants present in the NCLs families were first described in Portuguese patients, 12 of them by our centre.

The most prevalent type of NCLs in Portugal is NCL3 and the "1kb deletion" allele is present in 85% of these 13 families. NCL2 is the disease in 8 families and NCL6 has an unusual high prevalence, affecting also 8 families and with the p.I154del variant accounting for 75% of the families affected by this type of NCL.

Conclusions

These results lead us to modify the diagnostic algorithm proposed for NCLs as to include the screening for the frequent NCL6 variant in the first tier approach, increasing the diagnostic yield expectancy at this stage for 78%.

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P06.44D

Functional characterization of eight missense variants of the phenylalanine hydroxylase

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Phenylketonuria (PKU) and hyperphenylalaninemia (HPA) are a group of genetic disorders predominantly caused by reduced or absent enzymatic activity of the phenylalanine hydroxylase (PAH) which is responsible for phenylalanine metabolism. PAH is active only in its tetrameric form and it requires 6(R)-L-erytro-5,6,7,8-tetahydrobiopterin (BH4) as a cofactor, molecular oxygen and iron. To date, 970 variants have been identified in the *PAH* gene but only a small number of missense mutations has been functionally characterized *in vitro* to elucidate their impact on the PAH protein. We used *in vitro* PAH functional assay to determine the residual enzymatic activity of eight PAH variants (p.N167Y, p.T200N, p.D229G, p.G239A, p.V245I, p.F263S, p.A342P, and p.I406M) previously identified in Czech HPA patients. All mutants were expressed in *E. coli*; also in the presence of GroEL and GroES bacterial chaperones to determine whether the formation of tetramers would be restored. The pathogenic effect on PAH stability was tested using expression in HepG2 cells, also in the presence of BH4 precursor sepiapterin which has chaperone-like and stabilizing effect on PAH protein. Our *in vitro* PAH functional assay showed that four PAH variants retained residual enzymatic activity. While p.T200N, p.G239A, p.F263S,

and p.A342P variants expressed in HepG2 exerted increased protein levels when cultivated with sepiapterin, the protein levels of remaining variants remained unchanged. Our findings contribute to better understanding of the function of PAH mutated proteins and optimal PKU treatment using BH4 supplementation. This project was realized with the financial support of grant APVV-0240-12.

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P06.45A

Cognitive outcomes in phenylketonuria: report of a 23 years follow-up of 72 patients diagnosed in the newborn period

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Introduction: phenylketonuria is a metabolic genetic disorder inherited in a recessive manner, which use to be efficiently treated thanks to appropriate diet in childhood. Recently, cognitive suboptimal outcome in adults who underwent a well conduct treatment pointed out the necessity of maintaining diet rules in adulthood.

Materials and Methods: 72 phenylketonuria patients diagnosed, followed and treated with hypoprotidic diet and sapropterin supplementation since the newborn period underwent four neuropsychological evaluations until adulthood, performed by the same skilled neuropsychologist. Evolution of cognitive functions are described and compared between typical and atypical phenylketonuria patients.

Results: cognitive functions showed a subtle impairment in performance IQ compared to verbal IQ in phenylketonuria patients irrespectively of the clinical form of the disease. When compared, atypical and typical phenylketonuria patients displayed significant different evolution and prognosis. Atypical phenylketonuria patient are likely to keep a good global IQ, verbal IQ and performance IQ independently of their observance whereas typical phenylketonuria patients presented cognitive function impairment even with a well done treatment.

Conclusions: one genetic disorder but different diseases? The two groups with very different prognosis highlight the need for prediction tool which can help to position the treatment, its goal and the strictness of the follow-up. Genetic council should also be adapt to the clinical form of the disease.

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BH4-response prediction and genotype-phenotype correlations in phenylketonuria patients

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Phenylketonuria (PKU) caused by *PAH* gene mutations is a common metabolic disease that leads to mental retardation. The phenomenon of BH4-sensitive PAH (phenylalanine-hydrohylase) deficiency is confirmed by number of studies. The severity of PKU correlates with the *PAH* genotype. Severe *PAH* variants provide the reduced PAH activity <10%. Patients with two severe mutations usually have moderate or classic PKU and do not respond BH4 therapy. DNA samples of 165 unrelated PKU patients from Russia were analyzed for the presence of 25 common *PAH* gene mutations using allele-specific MLPA method. Disease-causing mutations on 87.6% of chromosomes were identified. In 126 patients (76.4%) both pathogenic variants were detected, in 37 patients (22.4%) - only one mutation, in 2 patients no *PAH* mutations were detected. At least one mutation was detected in 98.8% of PKU patients, although only frequent *PAH* mutations have been studied. Severe mutations were detected on 72.7% of chromosomes studied, mild mutations - on 15.2%. Two severe *PAH* mutations were identified in 55.2% of examinees (91 pers.), 95% of them (86 pers.) have moderate (PA=15–20 mg/dL) or classic (PA>20mg/dL) PKU. We suppose, that these patients will not respond BH4-therapy. R408W/R408W represents 50% of all non-responsive genotypes. In 28.5% of patients (47 pers.) at least 1 mild mutation was identified. Among them 38.3% have mild HPA (PA=2–10 mg/dL), 23.4% mild PKU (PA=10–15mg/dL), 8.5% moderate, 23.4% classic PKU. Severe PKU forms in these cases still may be responsible to the BH4-therapy. DNA-testing is a useful tool for the therapy prediction in PKU patients.

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P06.47C

Mendeliome massive-parallel sequencing reveals a Primary CoQ10-deficiency in two patients with severe encephalopathy and lactic acidosis

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Mutations in eight of the at least 13 genes involved in CoQ10-biosynthesis in mammals have been associated with Primary Coenzyme Q10-deficiency in humans, which is one of the much-reduced numbers of autosomal-recessive mitochondrial disorders with effective treatment. Fatal multisystem disorders or Steroid Resistant Nephrotic Syndrome with neurological symptoms are frequently related to Primary CoQ10-deficiencies. Here we described two new patients with severe encephalopathy and lactic acidosis, harbouring bi-allelic mutations in *COQ2* and *PDSS1* genes.

Candidate variants were identified by massive-parallel sequencing using TruSightOne panel and bioinformatics analysis with VariantStudio software. To demonstrate the pathogenicity of the nucleotide-changes we quantified CoQ10 (LC-MS/MS) in patient's-cells and analysed the downstream effects of CoQ10-deficiency on mitochondrial function and dynamics. Because CoQ10 is a mobile lipophilic electron carrier critical for electron-transfer in the respiratory chain and an important ROS scavenger for preserving mitochondrial cristae structure, we have evaluated the real-time oxygen consumption rate in intact fibroblasts, and mitochondrial ultrastructure by electronic microscopy.

Patient's cells with *COQ2* and *PDSS1* mutations displayed decreased variable levels of CoQ10 (26% *COQ2*-patient and 6% for *PDSS1* of control values) which were apparently related with respiratory activity parameters. Ultrastructure analysis of mitochondrial morphology showed an enlargement of COQ2-deficient's mitochondria and perturbed cristae morphology for PDSS1-deficient cells.

Reverse-phenotyping in these patients confirmed the utility of genomic testing as a first option for a prompt diagnosis of specific patients with clinical suspicion of mitochondrial disease, in which a rapid treatment can stop the progression of the symptoms.

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P06.48D

Blocking of *PCCA* pseudoexon inclusion as a tool to increase *PCCA* gene expression

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Introduction: loss-of-function mutations in the propionyl CoA carboxylase A (*PCCA*) gene cause Propionic acidemia (PA). The intronic mutation c.1285–1416A>G increases inclusion of a pseudoexon located in intron 14, thereby targeting the mRNA for nonsense-mediated decay, causing enzyme deficiency. The mutation is located in a splicing regulatory element (SRE) outside the splice sites. We investigate the mechanisms of *PCCA* pseudoexon inclusion and we assess the effect of splice-shifting oligonucleotides (SSO) blocking the activating SRE.

Methods: by RNA pull-down we identified the splicing regulatory proteins (SRPs) involved in the regulation of the *PCCA* pseudoexon. To further define their roles, analysis of minigenes and endogenous *PCCA* was performed in different cell types including patient cells, in knock-down and SSO transfection experiments.

Results: several members of the hnRNP family of SRPs inhibit inclusion of the *PCCA* pseudoexon; however, the strengthening of a splicing enhancer is the main cause of the pathogenic missplicing. Treatment with SSOs reduces inclusion of the pseudoexon, and, interestingly, produces a significant increase of the total levels of *PCCA* mRNA in both patient and normal cells.

Conclusions: PA patients could benefit from treatments that increase the activity of the PCCA enzyme. Blocking the inclusion of the *PCCA* pseudoexon with SSOs can strongly increase the levels of normal mRNA in patient cells with the activating mutation. Interestingly, PCCA levels were also significantly increased in normal cells indicating that this

treatment may also be relevant in patients harbouring missense mutations with some residual enzyme activity.

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P06.49A

Large-scale genetic meta-analysis and correlation analysis in up to 61,457 Europeans show large genetic overlap between fasting and random plasma glucose levels

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Introduction: Fasting plasma glucose (FG) and 2-hour post-prandial plasma glucose (2hGlu) are used as the gold standard tests for diagnosing type 2 diabetes (T2D). High non-fasting i.e. random plasma glucose (RG) levels may also indicate abnormal glucose homeostasis. We sought to characterise the genetic architecture of RG and its genetic relationships with other glycaemic traits and T2D.

Materials and Methods: We conducted a fixed-effects inverse-variance meta-analysis of 15 genome-wide association studies on RG adjusted for age, sex and time since last meal, when available. We analysed ~2.5M HapMap2-imputed and CardioMetabochip SNPs in up to 61,457 non-diabetic Europeans. The LD score regression was used to evaluate the genetic correlation between RG, FG, 2hGlu, fasting insulin, glycated hemoglobin (HbA1c), homeostasis model assessment of beta cell function/ insulin resistance (HOMA-B/-IR), and T2D.

Results: We identified four loci associated with RG at genome-wide significance ($P<5\times10^{-8}$): *G6PC2* (rs573225, $P=2.4\times10^{-40}$), *GCK* (rs6975024, $P=4.8\times10^{-30}$), *ADCY5* (rs7613951, $P=9.1\times10^{-11}$), and *SLC2A2* (rs16847990, $P=3.6\times10^{-8}$). These FG and HbA1c established loci variants show directionally consistent effects on RG. We observed strong ($r>0.6$) genetic relationships between RG, FG (r[SE]=0.89[0.13], $P=1.03\times10^{-11}$) and HOMA-IR (r[SE]=0.61[0.16], P=0.0001), and modest ($0.4\le r\le0.6$) with T2D (r[SE]=0.51[0.12], $P=1.52\times10^{-5}$) and HbA1c (r[SE]=0.40[0.15], P=0.009).

Conclusions: Our meta-analysis on RG has detected FG-associated variants, and the genetic correlation analysis also shows large genetic overlap between RG and FG. The smaller genetic correlation between RG and the other non-fasting measure HbA1c is likely due to the nature of many HbA1c loci being erythrocytic.

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P06.50B

A multi exons deletion in the *TANGO2* gene region is a recurrent cause of its functional defect

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Introduction: Rhabdomyolysis and energy deficiency is a clinically and genetically heterogeneous disorder. *TANGO2* (transport and Golgi organization 2) encodes a protein with a putative function in redistribution of Golgi membranes into the endoplasmic reticulum in Drosophila, while a mitochondrial localization has been demonstrated in mice. Mutations in this gene have recently been associated with rhabdomyolysis, epilepsy, hypothyroidism, metabolic crises with encephalopathy, and cardiac arrhythmia. *Materials and Methods*: Sanger sequencing of the *TANGO2* gene was performed in a 27-year-old woman from Belgian origin. She presented with seizures and developmental delay in the first years of life. Later she developed hypothyroidism and a dilated cardiomyopathy. She suffered from two severe episodes of rhabdomyolysis with cardiac arrhythmias. *Results*: Sanger sequencing showed a homozygous multi exons deletion (exon 3 - 9) of the *TANGO2* gene in our patient. LR-PCR confirmation for the presence of this heterozygous allele deletion in both her parents, to validate the independent segregation of the deletion, is in progress. A previously reported 34 kb partial gene deletion is suspected. *Conclusions*: Sanger sequencing showed a homozygous deletion encompassing the larger coding part of the *TANGO2* gene in a patient suffering from a complex phenotype. *TANGO2* deleterious mutations have recently expanded the genetic compendium as an important cause of rhabdomyolysis in combination with mitochondrial metabolism energy defects. The multiple exons deletion has been observed frequently in the small pool of published patients. It has been suggested that this pathogenic allele variant

might represent a more common disease allele in European populations.

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P06.51C

TANGO2 loss of function mutations: an emerging complex neurometabolic disorder

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Introduction: bi-allelic loss of function mutations in TANGO2 gene have been recently described in patients with a variable phenotype characterized by acute episodes of rhabdomyolysis, hypoglycemia, intellectual disability, hypothyroidism, seizures and cardiac arrhythmias. Today 15 patients have been reported in the literature. Materials and Methods: we report a family with two novel mutation in TANGO2 gene co-segregating with the disease. The proband is a male from first pregnancy of unrelated, apparently healthy parents. Family history, pregnancy and neonatal history were unremarkable. He was diagnosed with developmental delay at 6 months and with primary hypothyroidism at 12 months. He had 2 acute episodes of rhabdomyolysis at 13 and 19 months. The second episode of rhabdomyolysis was complicated with cardiac arrest. We noticed that the acute crisis were triggered by fasting and viral infections whereas bacterial infections had no triggering effect. Muscle biopsy showed normal respiratory chain studies. CGH-array and NGS analysis of the most common genes associated to HyperCKemia did not identify clinically relevant variants. **Results:** exome sequencing revealed the presence of two mutation in compound heterozygosis in TANGO2 gene (maternal p.Arg88* and paternal p.Glu112fs mutations). **Conclusions:** we described two novel pathogenic mutation in TANGO2 gene and a new patient with TANGO2 associated disease. Our clinical observation indicate that TANGO2 associated disease is recognizable on the basis of clinical signs: in this case psychomotor delay associated with recurrent rhabdomyolysis' crisis and hypothyroidism. Exome sequencing allowed a rapid confirmation of the diagnosis preventing additional invasive diagnostic tests.

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P06.52D

Effect of management of thiamine responsive megaloblastic anemia syndrome with sulbutiamine

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Introduction: thiamine responsive megaloblastic anemia syndrome (TRMAS) is a rare autosomal recessive metabolic disorder. Management of TRMAS focuses on lifelong use of pharmacologic doses of thiamine (Th). High-dose Th may ameliorate diabetes, anemia and, conjecture, hearing defect if started from birth or prenatally. TRMAS management with Th hydrochloride and Benfotiamine, a synthetic Th analog, has been described. Effect of lipophilic synthetic thiamine derivate, sulbutiamine, effectively crossing blood-brain barrier and possessing good bioavailability, has never been published. Materials and methods: we report a 6y old boy clinically and molecularly diagnosed with TRMAS at the age of 3y. Thiamine 100mg/day was administered. The condition markedly improved several days after the initiation of treatment - therapy had positive effect on anemia and glycaemia, also psychological status of the child slightly improved. After several months the patient developed allergic reaction. Moreover, the effect of thiamine was not sufficient for timely mental development and psychological balance. After careful consideration thiamine was replaced with sulbutiamine 100mg/day. **Results:** we observed transitory decrease of hemoglobin to 110g/l which resolved within 1st month of treatment. Blood glycaemia remained stable, with rare elevations due to stress. Unfortunately, 1 year of treatment with sulbutiamine did not improve the patient's mental health: emotional outbursts, aggressive behavior, speech delay, and inability to address hygiene tasks remain. **Conclusions:** our experience demonstrate that sulbutiamine benefits in maintenance of blood glycaemia and hematopoiesis in TRMAS patient. The expected improvement of mental performance was not attained supposedly due to the late diagnosis or possible additional underlying condition.

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P06.53A

An *RNASEH1* gene variant interacts with HLA tagSNPs in Colombian families with type 1 diabetes

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Introduction: We have recently found association of type 1 diabetes (T1D) with *RNASEH1* gene variants. Here we tested whether *RNASEH1* gene variants interact with classic HLA alleles, in a set of Colombian families with T1D Methods: We studied 200 familial trios from Medellin-Colombia, with T1D. Cases were tested for anti-GAD65 and anti-IA2 auto-antibodies in patients sera, using an ELISA-based kit. DNA was isolated from blood cells by phenol-chloroform method. *RNASEH1* gene variability was identified in 96 cases by Sanger sequencing of the coding region. Forty-five tagSNPs were then typed by the KASP method (LGC genomics) in the 200 families. TagSNPs for classic HLA alleles were identified for the population where the sample comes from (CLM) using information available at www.internationalgenomes.org. Thus, another 45 SNPs set was tested in both class-I and class-II HLA, using the same genotyping approach as above. Statistical analyses included association (TDT) and interaction (M-TDT) tests. **Results:** We found that SNP *rs7607888* at *RNASEH1* gene associates in a protective manner to develop T1D (P-value= 2e-3). Also, tagSNPs for classic class-I HLA alleles (P-value= 3.9e-9) as welll as for class-II HLA (P-value= 9.7e-28) were associated with the disease. Interaction of *rs7607888* with two class-I classic HLA alleles (p-value= 3e-9) and three classic class-II HLA alleles (P-value= 5.3e-10) in addition to four risk class-II haplotypes (2.47e-8) was found. **Conclusions:** Our results show that *rs7607888* at *RNASEH1* in the context of certain *HLA* alleles increases its contribution to the risk for developing T1D in Colombian families.

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P07
Immunology and hematopoietic system

P07.01A

Application of next generation sequencing in the conundrum of primary antibody deficiency

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Introduction: Primary antibody deficiency (PAD) is the most common form of primary immunodeficiency, with a prevalence of about 1 in 600 in the general population. A correct molecular diagnosis in patients with PAD is crucial for the appropriate therapeutic management. However, 90% of patients with late B cell development disorders and 10% of cases with the defect in early B cell development have no identified causative gene mutation. Methods: Clinical and immunological phenotyping of a cohort of PAD patients with unknown genetic defects after having been subjected to the classical targeted sequencing underwent whole-exome sequencing analysis. **Results:** Exome sequencing analysis was performed on 110 PAD probands mostly with childhood onset (94.5%) born to predominantly consanguineous parents (82.2%). This method and subsequent multiomics functional assays identified the disease-causing variants in 88% of the patients. The findings of this study also confirmed 4 new PAD causing genes and also suggested 14 novel candidate-genes. Medical implications of the definite genetic diagnosis were reported in 50% of the patients, including hematopoietic stem cell transplantation, tertiary preventive screening tests, targeted medication and prenatal diagnosis. **Conclusions:** Due to misclassification of the conventional clinical and immunological phenotyping for targeted sequencing, employing next generation sequencing as a preliminary step of molecular diagnosis approach to patients with PAD is essential and could help in many facets of management and treatment of the patients and their family members. This study also illustrates the power of exome sequencing in the identification of novel and candidate genes underlying primary antibody deficiency.

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P07.02B

Natural history and treatment outcome of patients with adenosine deaminase (ADA) 2 deficiency: Twenty years of the Israeli experience

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We present 30 Israeli patients with PAN-like vasculitis or leg ulcers and ADA2 deficiency, followed for the last 2 decades. 28 were of Georgian -Jewish origin, and 2 of Arab descent. 2/3 had familial disease. There was no consanguinity, no sex predilection. In most the disease started before age 10 years, 5 in infancy. The variability and extent of the clinical spectrum from life threatening, multisystem vasculitis including strokes to mere livedo reticularis, made diagnosis difficult.

Before genetic diagnosis could be applied, the hallmark of the group was "hereditary Georgian-Jewish PAN of childhood", encompassing the milder form of "Georgian Ulcers". In 2011, the G47R mutation in *CECR1*, encoding ADA2, was detected in 20 of the 22 examined patients the biological role of ADA2 was confirmed. Homozygosity of the same mutation was detected in all patients, regardless of the severity of the disease.

Our greatest challenge was treatment of the severely ill. 10 patients (30%) with moderate to severe disease received anti-TNF agents, with good response. Some patients homozygous to the G47R mutation in ADA2 had milder phenotype and received azathiorpine, methotrexate, prednisone and local treatment only. Four patients died of severe vasculitis, not receiving biologic treatment.

In summary, we report a long follow-up of the largest series of ADA2-deficiency patients, most homozygous for the same *CECR1* mutation. Clinical manifestations and severity of ADA2-deficiency are variable. Therapy of anti-TNF agents is effective and warrants genetic screening of siblings for proper diagnosis, follow-up and timely treatment.

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P07.03C

The search for missing heritability: combining rare and common variants to reveal novel disease association

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Genome-wide association studies (GWAS), utilising common variants, have implicated more than 40 genes in the development of ankylosing spondylitis (AS). However, as with other complex diseases, a large part of the genetic heritability remains missing. The aim of this study was to evaluate the effect of rare variants on AS, and so assess their role in this genetic puzzle.

A targeted NimbleGen array was created to cover 1% of the human genome; focusing on immunological pathways including key genes and their regulatory elements. Leveraging the expectation of finding rare disease associated alleles in a homogenous population, we sequenced 691 Swedish samples (310 cases) to a mean coverage of 35.5 ×. GATK best practices and additional QC pipeline resulted in 236,014 high quality variants (11% novel) in 4,030 genes. A multistage approach was taken, with single variant and aggregate (SKAT) tests performed for all combinations of minor allele frequencies.

Whilst the majority of association resided within the MHC, a novel finding outside this region implicates the *IL-36* pathway in AS. This significant signal was not found in univariate tests, but in aggregate association with rare and common variants ($p = 4.4\times10^{-6}$). This demonstrated how even a moderately sized population can identify signals of

missing heritability if all alleles, not only common, are evaluated for their role in disease.

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P07.04D

MAGI2 gene variant association and expression alterations in celiac disease

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In celiac disease (CD), there is a loss of intestinal barrier function due to structural alterations in the tight junction (TJ) network, the most apical unions between epithelial cells. The association of TJ related gene variants points to an implication of this network in disease susceptibility. In the current study, we replicated the association of rs6969266*G ($P = 0.0029$; OR =1.88 [95%1.24–2.87]), located in an intron of TJ-related *MAGI2* gene and upstream of RP4-587D13.2 long non-coding RNA (lncRNA). We found that both, *MAGI2* and RP4-587D13.2, are significantly downregulated in duodenal biopsies of active and treated CD patients. Moreover, both genes showed a significant coexpression along tissues and in intestinal biopsies. However, associated rs6969266 did not seem to regulate the observed altered expression. To mimic the situation in the CD gut, we silenced the expression of *MAGI2* in human intestinal C2BBe1 cell line and observed the alteration of several related TJ genes. Moreover, gliadin challenge in *MAGI2* silenced cells provokes more pronounced gene expression alterations. The nuclear localization of RP4-587D13.2 together with its coexpression with *MAGI2* suggests that this lncRNA could be affecting *MAGI2*-related pathway. These findings show that the associated region is functionally altered in disease and suggest that RP4-58D13.2 and *MAGI2* may be regulating the celiac disease related TJ alterations.

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P07.05A

Gain-of-function *SAMD9L* mutations cause a syndrome with cytopenia, myelodysplastic syndrome, immunodeficiency and neurological disease

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We studied two families with cytopenia, myelodysplastic syndrome (MDS), immunodeficiency, and progressive cerebellar dysfunction. In both families we identified heterozygous germline missense mutations in *SAMD9L*, a tumor suppressor gene located on 7q21. Both mutations, p. Ile871Thr and p.Arg986Cys, decreased cell proliferation relative to wild-type protein, suggesting a gain-of-function effect. Out of ten carriers of either *SAMD9L* mutation, three developed MDS with monosomy 7 or del(7q), of which two in childhood. Interestingly, in MDS-derived DNA, the mutated *SAMD9L* allele was located on the deleted chromosome 7. Five carriers, three with spontaneously resolved cytopenia in infancy, harbored somatic revertant mosaicism in blood cells in the form of uniparental disomy (UPD) of 7q with loss of the mutated allele or *in cis SAMD9L* truncating mutations. Clinically, revertant mosaicism was associated with milder disease, yet neurological manifestations persisted in three individuals. Examination of DNA derived from neonatal dried blood spot in one individual indicated that somatic reversions occurred postnatally in $CD34^+$ hematopoietic stem cells (HSC). Two carriers also harbored a rare, *in trans* germline *SAMD9L* missense loss-of-function variant, potentially counteracting the gain-of-function *SAMD9L* mutation. Our results demonstrate that gain-of-function mutations in the tumor suppressor gene *SAMD9L* cause cytopenia, immunodeficiency, cerebellar

dysfunction, and predispose to MDS with -7/del(7q). HSC that eliminate *SAMD9L* gain-of-function mutations through -7/del(7q) gain a competitive advantage and expand clonally, simultaneously predisposing to MDS. Hematopoietic revertant mosaicism through UPD(7q) or *in cis SAMD9L* mutations is common in carriers of *SAMD9L* mutations and improves hematological manifestations, though leading to clonal hematopoeisis.

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P07.06B

Generalized pustular psoriasis as an oligogenic disease and further evidence for significant younger age at manifestation in carriers of *IL36RN* mutations

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Recent research has identified pustular psoriasis as either Mendelian traits or major genetic risk factors contrasting numerous associated SNPs in classical plaque psoriasis. Autosomal-recessive mutations in *IL36RN* have been identified in ~25–40% of patients with generalized pustular psoriasis (GPP), a rare, severe pustular psoriatic variant. Also, heterozygous missense variants in *CARD14* and *AP1S3* have been associated to pustular psoriasis and shown to be functionally relevant.

61 GPP patients were screened for qualitative and quantitative coding variants in *IL36RN, CARD14* and *AP1S3*. We identified two *IL36RN* mutations in 15 GPP patients, and single heterozygous mutations in 5 patients. We did not observe a rare non-coding variant in any carrier of a single *IL36RN* mutations by sequencing introns as well as the *IL36RN* promotor. Three of the 20 carriers of *IL36RN* mutations (15.0%) carried a heterozygous functional variant in either *AP1S3* or *CARD14*, while only two carriers of heterozygous *CARD14* variants were observed in the group of non-carriers (4.9%). Also when assuming that a single *IL36RN* mutation contributes to disease, our findings point to a rather oligogenic than monogenic inheritance in those GPP patients.

Our genotype-phenotype correlation revealed a strong association between bi-allelic mutations in *IL36RN* and earlier age at manifestation (p=7.4×*10-04).

Overall, our genetic studies suggest a lower impact of *AP1S3* and *CARD14* in GPP than of *IL36RN*. Moreover, its oligogenic basis might currently be underestimated, as genetic risk factors apart from the three known genes remain to be identified in the majority of GPP patients.
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P07.07C

Uncovering the etiology of the neglected autoimmune disease pemphigus foliaceus: the first genome-wide association study

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Introduction: Pemphigus foliaceus (PF) is an autoimmune blistering skin disease characterized by autoantibodies against desmoglein 1. It occurs sporadically all over the world but is endemic in Brazil and a few other countries, including Tunisia, Peru and Colombia. In Brazil, PF represents a local health problem, with the highest incidence (up to 0.83 cases per 10,000) and prevalence (3.2% in the Terena population) ever reported for an autoimmune disease.

Material and Methods: We performed a genome-wide association study (GWAS) with 235 patients and 5,658 controls, which have been genotyped with an Illumina platform. After standard quality control procedures, 204,967 single nucleotide polymorphisms (SNP) remained. We performed logistic regression analysis using principal components to correct for possible population stratification.

Results: As expected on basis of our previous studies, most of the SNPs that reached genome-wide significance are in the major histocompatibility complex (MHC) class II region. However, our results spotted new markers in MHC and non-MHC genes and intergenic regions that were not seen in previous studies (e.g. an intergenic MHC SNP, OR = 5.3, $p = 2 \times 10^{-18}$; a *NOTCH4* variant, OR = 4.2, $p = 4 \times 10^{-7}$, a long non-coding RNA variant at chromosome 7, OR = 0.4, $p = 10^{-5}$).

Conclusion: Our findings help to dissect the associations between MHC genotypes and PF susceptibility. In addition, we identified new genes and non-coding elements that might be involved in PF etiology and suggest new pathways for this complex and poorly understood disease.

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P07.08D

Identification of novel surface markers and regulatory networks governing specific hematopoietic progenitor populations

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Introduction : Hematopoietic stem cells (HSCs) specify their lineage fate in non-self-renewing multipotent progenitors (MPPs). MPPs are a compartment defined by three subsets: MPP2, MPP3 and MPP4. A more restricted progenitor is the common lymphoid progenitor (CLP) which has lost the ability to produce myeloid cells in vivo. MPP and CLP are the best candidates to sustain rapidly and efficiently hematopoiesis of differentiated lineages and compensate for complications of anti-cancer therapies. Our objective is to identify novel surface marker genes to better isolate specific progenitor populations and to reveal gene networks involved in their regulation.

Materials and methods : We performed whole transcriptome strand-specific sequencing from three mouse progenitors: MPP2, MPP3 and CLP. Three biological replicates, per cell population, were sequenced at high coverage (2 ×120 millions reads).

Results : We performed differential expression analysis and identified, by gene ontology and by unsupervised classification, cell surface genes specifically co-expressed per progenitor cell. Correctly classified reference surface markers, currently used to purify progenitors, validate our bioinformatic methodology. Transcriptional regulation of these markers was further assessed, by searching for co-expressed transcription factors (TFs) and enriched TF binding sites in their promoters, to establish regulatory networks specific to each progenitor population.

Conclusions : Our transctiptome profiling analysis allowed us to expand the current repertoire of surface markers for better isolation of hematopoietic progenitor cells and to determine gene networks involved in their regulation. Experimental validation of our bioinformatic results is ongoing.

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P07.10B

HLA genotyping by Next Generation Sequencing in the clinical laboratory

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Introduction: The major histocompatibility complex (MHC) gene family in humans is termed the human leukocyte antigen (HLA) locus, and is one of the most polymorphic loci in human genome. HLA and related genes play important role in the immune system. A close HLA match between donor and recipient is critical for the success of organ transplantation. Design of highly specific primers for the most reliable and accurate HLA typing was in major of our research. Materials and Methods: HLA primers were designed to identify genes of MHC class I and class II. By using bioinformatics tools we designed 14 primer pairs targeting specific genes: HLA-A, HLA-B, HLA-C, DQA1, DQB1, DPA1, DPA2, DPB1, DPB2, DRA, DRB1, DRB3, DRB4, DRB5. Majority of genes were covered as a whole and all were checked for the specificity. We compared the results of standard HLA-typing method, commercially available HLA-NGS typing kits and our protocol on our clinical samples. For data analysis we checked commercially available pipelines. **Results:** The highest HLA resolution was observed in our test. Our newly designed primers can be used for HLA typing, giving more accurate information than using currently available methods. **Conclusions:** HLA typing using our newly designed primers and NGS technology generates high-resolution allelic information for donor and recipient allowing for the higher level of HLA allele matching. "Presented work was financially supported by The National Centre for Research and Development from the Innovative Economy Operational Programme founds, in the framework of the European Regional Development Fund."

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P07.11C

Considerations when using IGH clonality assay by high-throughput sequencing for minimal residual disease monitoring of acute lymphoblastic leukemia in the clinical testing laboratory

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Introduction : Assessment of minimal residual disease (MRD) is used for most B-acute lymphoblastic leukemia(B-ALL) as a powerful prognostic factor which impact on treatment protocols. Although several tools have been devised for MRD monitoring, immunoglobulin heavy chain (IGH) clonality assay using high-throughput sequencing (HTS) is pending to be applied in clinical testing laboratory. Before application in clinical settings, we tried to draw up a practical guideline of MRD monitoring with IGH clonality assay by HTS.

Materials and Methods : We screened clonal *IGH* rearrangements at diagnosis using both fragment analysis and HTS using IdentiClone IGH Gene Clonality Assay(InVivoScribe Technologies, San Diego, USA) and LymphoTrack Dx Assay–PGM(InVivoScribe Technologies), respectively. Longitudinal samples ($n = 20$) obtained over the course of therapy were analyzed for the presence of MRD by IGH HTS as well as morphology, fluorescence in situ hybridization(FISH) and RT-qPCR of fusion transcripts. LymphoTrack Visualization and Vidjil algorithm were selected for analysis platform.

Results : Among 22 initial samples, four showed discrepant results between fragment analysis and HTS. Three patients showed clonality only by HTS and 1 patient did only by fragment analysis. Median number and size of IGH clone at diagnosis were 2(0–4) and 33.15%(1.96%-78.08%), respectively. IGH HTS data were well correlated with morphology, FISH and RT-qPCR of fusion transcripts. Furthermore, samples with profound hematogones and chromosome 14 hyperploidy can be successfully monitored by the HTS.

Conclusion : These results demonstrated that IGH clonality assay by HTS is an effective method for MRD monitoring in most of B-ALL as alternatives of IGH RT-qPCR.

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P07.12D

Deciphering Juvenile Idiopathic Arthritis by exome sequencing: toward a precision medicine and therapeutic targeting

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Objective: Juvenile idiopathic arthritis (JIA) is the main cause of inflammatory rheumatisms in childhood. JIA is a heterogeneous condition subdivided in seven subgroups and is thought to be a multifactorial disorder due to combination of environmental and genetic factors leading to a deregulation of immunity.

Recently, next-generation sequencing has helped uncover some of the genes responsible for Mendelian types of JIA namely *LACC1*, *COPA* and *STAT3*. This discovery pinpointedt that some JIA could be Mendelian disorders instead of multifactorial diseases.

Methods: We performed trios exome sequencing in 46 individuals addressed for JIA. Trios were spread into 5 of the 7 subgroups of JIA. Immuno-monitoring was assessed using flow cytometry analysis on blood samples from affected individuals.

Results: We have identified pathogenic variants in 10.8% (5/46) of patients and likely pathogenic variations in 13% (6/46). Identified pathogenic variations included variants already reported in previously known genes (*CECR1*), novel variants in known genes (*SLC29A3*). In addition, we report a novel syndrome with JIA (NAIAD syndrome by mutations in *NLRP1*). All patients with pathogenic variations had, among other anomalies, blockade in B cells maturation.

Conclusion: Exome sequencing confirms the genetic heterogeneity of JIA. We also highlight the fact that arthritis in young patients might be a symptom of Mendelian diseases. In addition, we highlight the power of precision diagnosis in some patients leading to accurate genetic counselling and the discovery of new physiopathological bases of JIA that could lead in a precise therapeutic orientation and a targeted therapy.

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P07.13A

HRM analysis of KLF1 and Aγ/Gγ genes in cases with elevated fetal haemoglobin in the Greek population

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Introduction: KLF1 is an erythroid transcription factor acting both as direct activator of β-globin and indirect repressor of γ-globin gene expression. An increasing number of KLF1 gene variations have been shown to lead in elevated expression of Fetal Hemoglobin (HbF), thus considering KLF1 as a potential therapeutic target for thalassaemia patients.

Materials and Methods: A total of 76 cases a) 35 β-thalassaemia heterozygotes and b) 41 normals presenting high levels of HbF>3% were studied for KLF1 and Aγ/Gγ gene mutations, by HRMA followed by direct sequencing where appropriate. A matched control of 32 normal samples presenting HbF<3% was also analyzed for the above genes and served for comparison.

Results: i) KLF1: Four already characterized alterations (-148G>A, c.304T>C, c.544T>C, c.115A>C) were detected for the first time in the Greek population. Although -148G>A mutation is considered to down-regulate KLF1

expression and thus increasing HbF, it was identified in heterozygosity in three normal samples. Two new alternations, not yet recorded (-251C>G, c.178 delGGGA/-), were also identified. While -251C>G variation is considered as a SNP, c.178 delGGGA/- is probably altering KLF1 function as it was detected in a normal sample with HbF 10%. ii) Aγ two new alterations (-221; insAGCA/-, -176A>T) were detected in normal samples.

Conclusions: KLF1 and Aγ/Gγ genes analysis in Greek population samples revealed variations that could possibly contribute in differential HbF expression. For the first time, HRM analysis served as a rapid, cheap and accurate approach for the screening/scanning for both KLF1 and Aγ/Gγ genes.

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P07.14B

Screening of the 19q13 region revealed lncRNA variants associated with susceptibility to pemphigus foliaceus

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Pemphigus foliaceus (PF) is a blistering autoimmune skin disease endemic in Brazil characterized by the detachment of epidermal cells and the presence of autoantibodies against desmoglein-1. We have demonstrated strong associations of both polymorphisms and differential expression levels for several genes within the extended leukocyte receptor complex (LRC) with susceptibility to PF. The extended LRC genes within the 19q13 genomic region encode diverse immune-associated molecules. Here we aimed to search for new genes and single nucleotide polymorphisms (SNP) that may contribute to PF susceptibility in the 19q13 region.

We analyzed 8,669 SNPs genotyped by Illumina plataform of 235 patients and 194 controls. After standard quality control, 2,503 SNPs were used to perform a logistic regression analysis, using principal components as covariant to correct for possible population stratification.

The strongly associated SNP (*rs8103298*, OR=2.47, $p<10^{-4}$) was within a lncRNA (TCONS_l2_00013175). Two other associated SNPs, *rs9304655* and *rs453394*, are located at the protein coding region of the genes *IGFL2* and *CCDC8* (OR=1.61, OR=0.64, $p<10^{-3}$). We also performed a haplotype logistic regression. Two haplotypes (i) the *AGAAG* haplotype (*rs9304655*, *rs6509255* (*IGFL2* gene upstream variants), rs10439109 (intergenic variant), rs10412596 (*lnc-IGFL2-1*), *rs2253940* (*lnc-IGFL1-1*)) and (ii) the *GG* haplotype (*rs453394*, *rs878609*; *CCD8* gene upstream variants) were associated with PF susceptibility (OR= 1.78, OR=1.60, p=0.0006). These results are now being validated in a PF different cohort.

This screening of the 19q13 region showed new genes and lncRNA variability might influences PF pathogenesis, revealing different candidates for further functional studies.

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P07.15C

Comprehensive targeted next-generation sequencing panel: A rapid diagnostic tool for unraveling primary immunodeficiencies in pediatric patients

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Primary immunodeficiency (PID) disorders are a heterogeneous group of inherited diseases caused by a variety of monogenic immune defects. Thus far, mutations in more than 250 different genes causing PIDs have been described. Diagnosis can be costly and time consuming because of genetic and phenotypic heterogeneity of these disorders. Next Generation Sequencing (NGS) has recently become a popular tool for gene identification and molecular diagnosis of human diseases. Targeted NGS, in which a selected number of genes are sequenced, allows rapidly genetic testing across a large number of diseases in clinical practice. Here we present the utility of targeted next generation sequencing (TNGS) of a comprehensive primary immunodeficiency panel. TNGS workflow based on an Ion AmpliSeq™ Primary Immune Deficiency Research Panel was designed for sequencing 266 PID genes on Ion S5™ Sequencer. Twenty pediatric patients with PIDs were analyzed and identified probable disease-causing mutations. Pathogenic variants were detected in 9 patients in which 6 of them were novel (45%). These variations included hemizygous mutations in *BTK, heterozygous mutations in CTLA4, PIK3CD* and homozygous mutations in CD40LG, *LRBA*, PNP, *TTC37* and *ZAP70*. In conclusion, these results suggested that TNGS approach based on a comprehensive gene panel allow fast identification of PID mutations that will have further implications for therapeutics.

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P07.16D

A high-performance analysis pipeline to find disease-causing mutations in patients with Primary Immunodeficiencies

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The emergence of Next Generation Sequencing (NGS) has greatly modified the landscape of human genetics especially with Whole Exome Sequencing (WES) which focuses on the exonic regions of human genomes. As the protein coding region of the genome, exomes often harbor disease causing genetic mutations that can be linked to a phenotype in Mendelian diseases.

Based on this knowledge we present an ongoing genetic study and give an overview of results obtained so far. The designated aim of our project on Immunodeficiencies is to create a workflow, initiated by WES results. This should allow the identification of patients with unusual or novel genetic forms of Primary Immunodeficiency diseases (PID) and is aided by a thorough clinical characterisation.

Patients included in our studies present with symptoms of immunodeficiency or -dysregulation of unknown origin. High throughput genetics including WES and transcriptomics are initially performed on patients and guide the choice of functional immunological assays conducted afterwards. These choices are hinged on the prior data analysis which involves several steps.

So far, we have included 25 PID patients in our pipeline. We have found four patients with well-known PIDs, who were not diagnosed previously due to their unusual clinical and immunological presentation, three further patients were found to have mutations associated with recently described PIDs.

These initial results are very encouraging, yet as there are very few patients in our cohort with the same disease WES results can be difficult to interpret this especially if a mutation has not yet been described yet.

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P07.17A

Genome-wide association analysis of axial involvement in psoriatic arthritis identifies suggestive evidence for association to four susceptibility loci in 845 patients of European origin

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Psoriatic arthritis (PsA) is a chronic inflammatory joint disease occurring in ≥30% of patients with psoriasis vulgaris (PsV). PsA is considered a T-cell mediated disease with a complex genetic basis. To date, genome-wide association studies (GWAS) identified 15 susceptibility loci. Most of these loci overlap with those identified for PsV and some of them e.g. with ankylosing spondylitis. To identify genetic risk factors relevant in axial involvement, we analyzed genome-wide association data of 845 PsA patients of European origin for presence (26%) or absence (74%) of axial involvement. Axial involvement was diagnosed as sacroiliitis or spondylitis.

None of the loci reached genome-wide significance, but we identified four with suggestive significance characterized by ≥5 SNPs with a p-value between 5.0E-05<p<5.0E-08. Odds ratios were higher than for most PsA susceptibility factors identified so far, ranging from 1.70 - 2.16. Two of the four loci were located in intergenic regions while the other two were in introns of two genes. One of these genes, *VAV3*, has been described to be involved in bone density in mice and to be expressed in skin and immune cells. We selected the most significantly associated SNP (rs4462178, p=3.3E-06, OR=1.74) at *VAV3* for replication studies of an independent cohort of 619 German PsA patients, but failed

to identify evidence for association, potentially due to lack of power. Larger cohorts with detailed phenotypic data will eventually allow dissecting symptom-specific disease subgroups with diverse genetic etiology.

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P07.18B

Multiple rare variants in immune genes predict common respiratory infections burden in isolated populations

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Introduction: Common respiratory infections are causing a substantial burden of disease, despite numerous infection control measures. The aim of this study was to elucidate the role of host genetic in infections susceptibility. **Materials and methods**: A follow-up, postal survey was sent to participants from the 10,001 Dalmatians cohort, focusing on the annual frequency of common cold, influenza and pneumonia, with 1,082 responses. HRC imputed linear regression GWAS was adjusted for age, gender, years of schooling and socioeconomic status. Participants originated from two different isolated islands, requiring a weighted fixed effects meta-analysis, with Bonferroni adjusted P-value of 3.7E-09. **Results**: We found 45 significant hits in meta-analysis for the life-time risk of pneumonia, majority of them being rare intron variants. Some of the most interesting results belong to CXCL1, MARCH1, PAX5, and RASA3 genes, which were previously implied in inflammation and infection response processes. Five other genes involved in immunity, including IL12RB1 and C5, were found to be just under the formal significance threshold. We identified 4 marginally suggestive SNPs for cold and influenza frequency, also implied in immunity and infection response. **Conclusions**: Despite small sample size, genetic architecture underlying host susceptibility to common respiratory infections suggest the role of numerous rare variants. This result could explain high levels of diversity, observed in an individual susceptibility risk and infectious disease outcomes across population. **Funding**: Medical Research Council UK, Croatian Science Foundation grants 8875 and 8445, FP7 project PREPARE (602525). We gratefully acknowledge contribution from the Institute for Anthropological Research in Zagreb, Croatia.

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P07.19C

High throughput single-cell gene expression analysis of blood cells from healthy individuals and patients with rheumatoid arthritis with highly multiplexed amplification

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Introduction: Bulk RNA studies used to examine gene expression patterns in patients are limiting due to bulk RNA masking the contribution from different cell types within a sample. Recent techniques in high throughput single-cell RNA sequencing, with the ability to analyze the transcriptomes of thousands of cells simultaneously, are promising approaches to discern the contribution of these patterns in specific cell types. However, the cost of sequencing the entire transcriptome of every cell limits the number of biological samples that can be studied, especially if functionally important genes are expressed at relatively low levels. To circumvent this issue, we used a targeted approach to study the digital gene expression of a selected set of gene markers (454), to study the gene expression between healthy and Rheumatoid Arthritis (RA) patients.

Materials and Methods: Using the BD™ Resolve system, we captured and barcoded mRNA transcripts from ~10,000 single-cells from three sets of cryopreserved peripheral blood mononuclear cells from healthy and RA patients. We then performed targeted multiplex PCR to amplify the cDNA of the 454 genes, followed by sequencing.

Results and Conclusions: We found that by focusing on selected genes, we used a relatively small amount of sequencing while identifying differences in immune populations between individuals. Additionally, we observed activated and distinct monocyte populations in RA patients as compared to healthy. These results show how high-resolution single-cell gene expression analysis using targeted amplification can be used to routinely examine peripheral blood cells, and may open up the development of new diagnostic approaches.

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P07.20D

Slc7a7$^{-/-}$ mouse model develops Lysinuric Protein Intolerance immune related abnormalities

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Lysinuric protein intolerance (LPI, MIM 222700) is an inherited aminoaciduria caused by defective cationic amino acid (CAA) transport mainly, at the basolateral membrane of epithelial cells in kidney, and also intestine. LPI is caused by mutations in the SLC7A7 gene, which encodes y+LAT1 transporter. Hallmarks of LPI are intestinal and renal impairment of CAA that cause a urea cycle dysfunction. Moreover, major and life threatening complications are immune-related disorders in which y+LAT1 contribution is still unknown. Because *null Slc7a7* mice are lethal, a ubiquitous and tamoxifen-inducible ablation of *Slc7a7* in mouse has been generated. LPI patients treatment, low-protein diet and citrulline supplementation, is needed to maintain *Slc7a7-/-* mice alive. As in humans, mouse CAA plasma concentration is reduced. This lower availability of urea cycle substrates, the hyperglutaminemia, hyperammonemia and orotic acid hyperexcretion denotes a urea cycle dysfunction that could be the cause of brain edema in *Slc7a7-/-*. As in humans, hyperferritinemia, hematological imbalance and clonal expansion defect, are present in the model. Finally, a live threatening immunity alteration in LPI patients is pulmonar alveolar proteinosis (PAP). Approximately 1/3 of *Slc7a7-/-* develops PAP 90 days after induction. At the basis of the disease, alveolar macrophages (AM) play a main role and appear foamy and full of surfactant. The loss of y+LAT1 expression in macrophages could contribute to its malfunction. In summary, the first LPI animal model is an excellent tool to study the unknown mechanisms of pathophysiology of LPI and improve treatment and health care of patients.

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P07.21A

Whole genome linkage and exome sequencing analyses in an autosomal recessive Takayasu arteritis family

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Objective: Takayasu arteritis (TA) is an inflammatory large vessel vasculitis affecting mainly aorta and its branches. Inflammation in vessels causes thickening of wall, fibrosis, dilatation and nonspecific symptoms such as fever, hypertension and arthralgia. It is a rare disorder with unknown aetiology and the worldwide incidence is 0.4 to 2.6 per million. We studied a consanguineous family consisting of two affected and one unaffected sibs and their healthy parents in order to identify the causative mutation or linked loci.

Methods: Whole genome single nucleotide polymorphism (SNP) genotyping was performed for five family members using Illumina OmniExpress-24 BeadChip targeting ~700,000 SNP markers. Using genotyping data, we performed multipoint parametric linkage analysis assuming recessive inheritance and complete penetrance. Also exome sequencing was performed for index patient to search for a rare, homozygous deleterious variant in the possibly linking regions.

Results: Whole genome linkage analysis resulted 25 genomic regions with LOD score above 1.50. Within the family members, all candidate regions shared homozygosity by only affected individuals. Causative variant search in linkage regions identified seven homozygous candidate variants in which five of them were located in 19q13.33. Candidate non-synonymous variants were found in *ANXA8L*, *EHBP1L1*, *MYH14*, *KCNJ14*, *SYNGR4*, *TULP2* and *SHANK1* genes.

Conclusion: This is the first whole genome linkage analysis in a TA family with recessive inheritance. Linkage and following exome sequencing analyses revealed seven possible variants that may be causative to disease. Further variant and candidate gene investigations are still in process.

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P07.22B

whole exome sequencing revealed a novel mutation in TMPRSS6 gene in an iron-refractory iron deficiency anemia patient

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Identification of two common types of anemia, beta thalassemia (BT) and iron deficiency anemia (IDA) have great importance to prevent misdiagnosis followed by proper treatment. BT and IDA are autosomal recessive disorders characterized by hypochromic microcytic anemia which can be differentiated by hematological indices. Defects in *TMPRSS6* gene, which encodes a trans-membrane serine protease protein (Matriptase-2), result in Iron Refractory Iron deficiency anemia (IRIDA). Matriptase-2 regulates systemic iron homeostasis by down-regulation of the hepcidin protein. Here we report an Iraqi kindred with 2 affected siblings in which the 6 years old boy suffered from severe anemia with high dose of iron supplement. Considering the high level of hemoglobin A2, the beta globin gene sequencing was performed and a heterozygote C41–42 mutation was detected which does not justify the severity of the proband phenotype. We then performed Whole exome sequencing for proband which revealed a novel homozygote 8 bp deletion (c.1647-1654 delGCAGTGTG) in *TMPRSS6* gene. The deletion located in LDLR-class A domain of Matriptase-2 leads to a premature stop codon at position 11 of the new reading frame (p.Gln550Trp fs*11). In silico prediction programs (SIFT, polyphen 2, Mutation taster) are in support of its probable pathogenecity and predicted this variant as disease causing. The co-segregation analysis identified that the parents were both carrier while the genotype of the affected sister was homozygote. Our findings indicated that next generation sequencing could be a promising method for molecular detection in mendelian disorders such as genetic forms of hypochromic deficiency in high prevalence countries.

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P08 Intellectual Disability

P08.01A

Genetic and phenotypic dissection of 1q43q44 microdeletion syndrome and neurodevelopmental phenotypes associated with mutations in ZBTB18 and HNRNPU

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Subtelomeric 1q43q44 microdeletions cause a syndrome associating intellectual disability, microcephaly, seizures and anomalies of the corpus callosum. Despite several

previous studies assessing genotype-phenotype correlations, the contribution of genes located in this region to the specific features of this syndrome remains uncertain. Among those, three genes, *AKT3*, *HNRNPU* and *ZBTB18* are highly expressed in the brain and point mutations in these genes have been recently identified in children with neurodevelopmental phenotypes.

In this study, we report the clinical and molecular data from 17 patients with 1q43q44 microdeletions, four with *ZBTB18* mutations and seven with *HNRNPU* mutations, and review additional data from 37 previously published patients with 1q43q44 microdeletions. We compare clinical data of patients with 1q43q44 microdeletions with those of patients with point mutations in *HNRNPU* and *ZBTB18* to assess the contribution of each gene as well as the possibility of epistasis between genes.

Our study demonstrates that *AKT3* haploinsufficiency is the main driver for microcephaly, whereas *HNRNPU* alteration mostly drives epilepsy and determines the degree of intellectual disability. *ZBTB18* deletions or mutations are associated with variable corpus callosum anomalies with an incomplete penetrance. *ZBTB18* may also contribute to microcephaly and *HNRNPU* to thin corpus callosum, but with a lower penetrance. Co-deletion of contiguous genes has additive effects.

Our results confirm and refine the complex genotype-phenotype correlations existing in the 1qter microdeletion syndrome and define more precisely the neurodevelopmental phenotypes associated with genetic alterations of *AKT3*, *ZBTB18* and *HNRNPU* in humans.

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P08.02B

Intellectual and psychological functioning in probands and non-proband carriers of 22q11.2 duplication

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Introduction Microduplication of the LCR-a to LCR-d region on chromosome 22q11.2 is a recurrent copy number variant (CNV) found in clinical samples with neurodevelopmental disorders. It is also found at lower frequency in controls, and is often inherited. Case reports of the clinical features of probands is subject to ascertainment bias, and there is limited data on the intellectual and psychological functioning of non-proband adults.

Subjects and Methods We recruited 22 people with this CNV for psychological assessment. Seven were parents of probands, and 5 were siblings or cousins. Standardized psychological assessment measures and questionnaires were administered, including measures of intellectual (IQ), academic, adaptive, psychiatric, behavioral and social functioning.

Results Intellectual and academic skills were within the average range, with a trend towards slightly lower scores in probands versus non-probands. In probands, adaptive skills were within age expectations. Higher rates of attention deficit (probands only) and anxiety (both groups) were identified. The rate of autism spectrum disorder (5% of total sample), was much lower than previously reported.

Conclusions Intellectual and academic abilities were intact in probands and non-probands with 22q11.2 duplication, and symptoms of attention deficit and anxiety were identified.

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P08.03C

Adolescents and adults with 22q11.2 deletion syndrome: Our experience

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22q11.2 deletion syndrome is the most common microdeletion syndrome. This multisystem disorder has age related presentation needing age related screening. Congenital structural malformations of heart (74 %), palate (69%), renal (32%), immune deficiency (70%) endodocrinology complications (hypocalcemia, hypothyroidism), learning difficulties and psychiatric issues predominate the clinical phenotype. Behavioural and psychiatric issues are seen in 70–90 % . More than 40 % individuals have autistic spectrum disorder, ADHD or both. Bipolar disorder, schizophrenia and schizoaffective disorders are reported in 10–30 % of teenagers and adults with 22q. The risk of severe psychiatric illness is 25 times higher than general population in these individuals. We started a specialised 22q transition clinic in Northern Ireland in April 2016 as there was no system or arrangement in place to recognise, anticipate or proactively deal with the issues commonly associated with this disorder. . There are currently 115 individuals with 22q11.2 deletion on our data base including 55 adolescents and adults. The analysis of our patient cohort showed that the physical issues related to congenital structural malformations are usually addressed post diagnosis in early childhood. However issues related to learning difficulties and psychiatric illnesses evolve with age and are often under recognised. The main purpose of this clinic was to address these issues in adolescents and adults. We present our data describing the clinical phenotype in adolescent and adult individuals of this cohort. We also present three interesting cases with unusual central nervous system phenotype leading to delayed diagnosis of 22q11.2 deletion syndrome.

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P08.04D

Further delineation of a novel 2q11.1q11.2 microduplication syndrome

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Riley et al. Am J Med Genet 2015 recently reported an approximately 1.5 Mb recurrent duplication in 2q11.2 with variable outcome including a normal carrier mother and 3 patients with mild to moderate developmental delay, short stature and variable other features. We now observed 9 novel carriers from 6 families including two patients with de novo duplication. While 8 duplication carriers had low normal IQ or mild intellectual disability, one patient with de novo duplication suffered from profound developmental delay, microcephaly, craniosynostosis, strabism and congenital heart defect. Under the suspicion of a second hit in the latter patient we performed trio exome sequencing which indeed revealed an additional disease causing mutation in a known ID gene (*KAT6A*). The symptoms in our patient not described in the patients with 2q11.2 microduplications can be explained by the de novo *KAT6A* mutation. Our observation therefore indicates that the recurrent duplication 2q11.2 causes mild intellectual deficits with short stature, gastroesophageal reflux with vomiting/feeding difficulties and dysmorphic features as frontal bossing and other minor facial features, while a more severe phenotype may be attributed to second hits.

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P08.05A

Evaluation of array comparative genomic hybridisation for patients with developmental dissabilities or congenital anomalies

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Introduction: Chromosomal microarrays (CMA) are widely used as first-tier test for patients with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA).

Objectives: Review the data after the implementation of an algorithm for array comparative genomic hybridisation (aCGH) testing in patients with unexplained DD/ID, ASD, or MCA and assess the diagnostic yield.

Materials and methods: We performed aCGH to 724 patients referred from the neuropediatrics department with unexplained DD/ID, ASD, or MCA. aCGH was performed mainly with the Signature Genomics CGX 8×160K arrays (Perkin Elmer), following ClinGen guidelines (ISCA). Results were reported following ISCN 2013 recommendations. If a CMA variant was observed, parental samples were analyzed. Copy number variations are assigned the following interpretations: abnormal (well established syndromes, de novo variants and large changes); VOUS (variants of unknown significance) and likely benign (not previously reported but inherited from a healthy parent). Diagnostic yield was defined as the number of patients with abnormal variants divided by the total number of patients tested.

Results. 894 patients and 277 parents were studied. 170 patients (19%) showed an abnormal result, and were classified as follows: 128 abnormal (14,3%), 30 likely benign (3,4%) and 12 VOUS (1,3%). The diagnostic yield is 14,3%.

Conclusions. The use of CMA as first-tier clinical diagnostic test for patients with unexplained DD/ID, ASD, or MCA has proven to surpass the classical approach with conventional cytogenetics. Karyotyping is still needed to address whether the imbalance is due to parental chromosomal rearrangements and for genetic counselling purposes.

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Angelman syndrome resulting from UBE3A mutations : variants update and experience of molecular diagnosis for genetic counselling

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Introduction: Angelman syndrome (AS) is a neurodevelopmental disorder caused by absence or inactivation of the maternally expressed *UBE3A* gene. The paternal allele of *UBE3A* gene is silenced through genomic imprinting in brain. Different molecular anomalies, involving the *UBE3A* gene, were previously described. Genetic counselling depends on molecular mechanism and parental inheritance. About 10% patients have loss of function mutations. In this subgroup, offspring of carrier mothers are at 50% risk of having AS.

Materials and Methods: Between 1990 and 2015, 548 patients have been referred to our laboratory, for *UBE3A* gene analysis. Different approaches, with evolution over time, were used to screen mutations. Results with instructions for family study were given back to prescriber by letters. Parental analysis was always recommended to determinate *de novo* or inherited mutation status.

Results: We reported 57 patients with a mutation in *UBE3A* gene. The diagnostic rate was 10,4%. Most of mutations were truncating. We identified 41 unpublished mutations with 12 missense variants that were predicted to be damaging in software. We observed a small number of polymorphisms. Parental analysis could be performed in 72% cases. Between these cases, mutations were *de novo* in 59% patients. In maternal inheritance cases (41%), analyses were extended to family members in 47%. We performed 11 prenatal testing with 3 positive diagnoses.

Conclusions: At the time of NGS, variants updating is crucial. Because there is no cure, molecular diagnosis, even if doesn't change the treatment, is particularly important to prevent recurrence risk.

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De novo ARX mutations in female patients with agenesis of the corpus callosum and intellectual disability

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Loss of function mutations in the Aristaless-related (ARX) gene are typically responsible for brain malformation phenotypes in males, including X-linked lissencephaly with abnormal genitalia (XLAG, also known as LISX2) (MIM# 300215) and Proud Syndrome (MIM# 300004). Within affected families, carrying females are usually asymptomatic but few symptomatic females were reported, with intellectual disability (ID) and/or agenesis of the corpus callosum (ACC). De novo ARX mutations in affected females have only been infrequently reported.We describe here four unrelated female patients with ACC, ID and seizures, related to de novo loss of function mutations in ARX. All patients presented with intellectual disability, from moderate to severe. Seizures occurred between 2 months to 3 years of life. Brain MRI showed complete ACC in ¾ patients and partial ACC with absent anterior commissure in ¼ patient. ACC was isolated in ¼ patient whereas other cerebral or extra-cerebral malformation was associated in ¾ patients (hippocampic hypoplasia (n = 1), bilateral lenticular hypoplasia (n = 1), bilateral optic atrophy (n = 1)).We report three truncating and one missense mutations, localized in the homeobox domain of the protein. All mutations arose de novo. The mutations were identified by next generation sequencing (panel of known genes involved in ACC or epilepsy, or whole exome sequencing).This report confirms the importance of screening ARX and female patients with ID, seizures and ACC.

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P08.08D

Multilocus genomic variation contributes to atypical Prader-Willi phenotype in monozygotic twins

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Introduction: We describe 15 yo monozygotic twin girls with dual molecular diagnoses of Prader-Willi syndrome (PWS) and Pitt-Hopkins syndrome (PTHS). They were diagnosed with PWS at nine months of age due to neonatal hypotonia and feeding difficulties. They later developed an atypical PWS phenotype, including severe intellectual deficiency, lack of ambulation and speech, and absence of hyperphagia.

Methods: A genetic investigation was carried out using array-CGH, microsatellite and methylation assays, WES (whole exome sequencing) and RNA studies.

Results: Genetic studies confirmed the PWS diagnosis due to the paternal deletion of 15q11-q13 and methylation of *SNRPN* promotor. WES revealed a second mutational event consisting of a novel variant c.145+1G>A affecting a *TCF4* canonical splicing site of exon 3. The unaffected mother is a mosaic carrier of the *TCF4* mutation. RNA studies confirm that this variant abolishes the donor splicing site at intron 3 and that an alternative non-canonical splicing site is utilized; a premature stop codon in exon 4 is predicted to truncate the protein. Re-evaluation of clinical symptoms indicate that both variants are likely contributing to the twins phenotype. The characteristics of PWS are accentuated in the neonatal period and those of PTHS predominate from childhood on.

Conclusion: This case illustrates the challenges of reaching a final diagnosis in complex clinical presentations and highlights the importance of WES in the investigation of such clinical cases.

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P08.09A

Characterization of functional consequences of three loss-of-function mutations affecting long and short *AUTS2* isoforms

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Intellectual disability (ID) is a common neurodevelopmental disorder, characterized by a high genetic heterogeneity, with more than 700 genes involved in monogenic forms of ID. One of these is *AUTS2* for which numerous intra- and inter-genic deletions have been reported in patients with a variable global developmental delay and ID, with or without autism and microcephaly. The severity of the phenotype seems to correlate to the location of the deletions and if they affect or not a short isoform transcript located in the 3′part of the gene, highly expressed in brain and encoding a nuclear isoform presumably involved in regulation of gene expression. Even if this evolved between the two last human genome versions hg19 and hg38, this short isoform remains still not fully characterized. By using a targeted and whole exome sequencing strategies we identified four novel loss-of-function (LoF) mutations affecting both isoforms and causing developmental delay, with or without autistic traits, in a total of 12 individuals (two de novo and two inherited from affected parents). Our data enlarged the AUTS2 syndrome phenotype, and showed that high phenotype variability can be observed among patients with the same LoF *AUTS2* mutation. We carried out functional studies on available patient's fibroblasts to investigate the consequences of these mutations and showed that short and long transcripts carrying the mutations at least partially escape to NMD. We also performed transcriptomic analyses to identify genes commonly dysregulated in patients and to better characterize the short AUTS2 isoform, identifying at least two novel exons.

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P08.10B

Cerebrofaciothoracic dysplasia - four new cases further delineating the phenotypic spectrum with a recurrent *TMCO1* mutation

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Introduction: Cerebrofaciothoracic dysplasia (CFTD; MIM #213980) is a rare, autosomal recessive, developmental disorder. It is characterised by distinctive craniofacial features, intellectual disability, and skeletal abnormalities, mostly affecting the ribs and vertebrae. CFTD is caused by homozygous loss of function mutations in the *TMCO1* gene. Nineteen molecularly confirmed patients have been reported previously. Here, we present the phenotypic and genotypic features of a further four patients, including three brothers from a consanguineous Pakistani family and an additional unrelated Caucasian patient with no family history of consanguinity.

Materials and methods: The patients were ascertained via routine referrals to Clinical Genetics services in the UK. Exome sequencing was undertaken as part of the Deciphering Developmental Disorders study.

Results: All patients had typical craniofacial dysmorphisms, including brachycephaly (1/4), synophrys (3/4), highly arched eyebrows (2/4), hypertelorism (1/4), microdontia (2/4), and gingival hyperplasia (1/4). Skeletal features included rib abnormalities (2/4), abnormal vertebrae (1/4), Sprengel deformity (1/4), long hyperextensible fingers (2/4), and talipes equinovarus (3/4). 2/4 patients had an intention tremor. One patient had atypical café au lait patches and an anteriorly placed anus, which are novel features. All patients had severe learning difficulties.

Interestingly, molecular testing demonstrated all had the same homozygous c.292_293del (p.Ser98*) *TMCO1* mutation (NM_019026.4 transcript), despite one patient being unrelated.

Conclusion: This report allows further delineation of the CFTD phenotypic spectrum. Some novel clinical features are presented. The *TMCO1* mutation found is a recurrent mutation, previously only seen in an isolated Amish population, revealing a likely mutation hotspot in this gene.

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P08.11C

Identification of copy number variations from whole-exome sequencing using eXome Hidden Markov Model (XHMM): A FRENCH experience

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Whole-Exome Sequencing (WES) is becoming a standard application for the detection of gene mutations responsible for human disease, especially SNVs and indels. To date, most studies of CNVs were diagnosed by array-CGH or array-SNP with a lower detection limit of about 30kb. The challenge is now to detect both SNVs and CNVs using an exome-wide approach with a single test. Several algorithms have been developed to call CNV from WES data (Exome CNV, CONTRA, ExomeCopy, ExomeDepth, ConiFer, XHMM). The XHMM program is optimized for the identification of rare variants in large exome data sets, in particular exonic CNVs smaller than 30kb. We used XHMM to screen for exome CNVs in data from 350 patients with developmental disorders for whom exome sequencing was performed in a research or diagnostic setting with normal array-CGH 4×180K Agilent design used in our lab. We identified pathogenic CNVs, ranging in size from 685pb to 11kb (all confirmed by qPCR), in five patients (1.4%). Among these, four patients showed clinically relevant deletions in neurodevelopmental disorders (4.5 kb homozygous deletions of *PPT1*; 5kb for *PIGN*; 637pb for *TCF4;* 2.2kb homozygous *CLCN2* deletion), one patient had Cohen syndrome (11kb homozygous *VPS13b* deletions). This study shows that the XHMM program detected five CNVs from whole-exome data. This study underlines the interest of whole-exome sequencing in genetic disorders to identify SNVs and CNVs especially those smaller than 30kb not detected by array-CGH.

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P08.12D

Clinical spectrum of four patients with Coffin-Siris syndrome and novel mutations in *ARID1B* gene

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Coffin-Siris syndrome (CSS) is a multiple malformation / intellectual developmental disorder (IDD) syndrome caused by mutations in genes of the BAF complex, *ARID1B* being the most frequent (40% of affected individuals). Mutations in *ARID1B* also cause non-syndromic IDD, without other specific clinical features. It is recognized as one of the most frequently mutated genes in patients with developmental disorders caused by *de novo* dominant mutations.

We reviewed clinical and molecular data of individuals from our genetics service with a clinical diagnosis of CSS and a variant in *ARID1B*. Phenotypic features were compared to CSS and *ARID1B*-related IDD cohorts reported in the literature and publicly available databases.

Four female patients were identified with a clinical diagnosis of CSS and an *ARID1B* potentially pathogenic variant. Clinical features include classical features of CSS, including IDD, characteristic facial features and hirsutism; hypoplasia of the fifth digits was a variable feature. All patients have different putative mutations, including two truncating and two splice donor variants; the variant

occurred *de novo* in three cases; in one case parental samples were unavailable.

CSS is a clinically recognizable DD syndrome, with mutations in *ARID1B* found in approximately 40% of affected patients reported in the literature. Mutations are most frequently loss of function, while those affecting splicing account for approximately 7% of pathogenic and likely pathogenic variants in *ARID1B*-related IDD in public databases. We report 2 *novel* splice variants, discuss the broader phenotypic spectrum of *ARID1B*-related IDD and provide case reports of how molecular diagnosis affects parental reproductive decisions.

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P08.13A

Usefulness of multiplex ligation-dependent probe amplification assay for genetic screening of Korean patients with developmental delay/mental retardation/autism spectrum disorder

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Background: Approximately 20–50% developmental delay (DD)/mental retardation (MR) and 10% of autism spectrum disorder (ASD) cases can be explained by genetic causes. Clinical genetic testing is needed for patients with unexplained DD/MR/ASD and multiple congenital anomalies. Herein, the genetic spectrum of Korean patients with unexplained neurodevelopmental disorders was retrospectively analyzed. The diagnostic yield of various genetic tests was also evaluated in Korean patients with DD/MR/ASD.

Methods: Retrospective chart and genetic test result review of MLPA, CMA, and karyotyping testing were conducted during a 55-month period on patients with unexplained neurodevelopmental disorders with or without dysmorphism or seizures. MLPA analysis was used for screening the 294 patients were included in this study, out of which 224 patient tested karyotyping. 97 patients referred for CMA test were included.

Results: A total of 30 pathogenic cases were identified in 10.2% patients, out of which 20 pathogenic results were detected by MLPA seven were detected by both MLPA and CMA. Nine pathogenic results were detected only by CMA. Pathogenic results were identified in 93.3% of patients with DD and/or MR. Microdeletion/microduplication syndrome was detected in 20 patients using MLPA with a diagnostic yield of 6.8%, whereas the diagnostic yield of CMA was 16.5%.

Conclusions: The present findings highlight the clinical utility of MLPA as a routine diagnostic test for the diagnosis of unexplained DD/MR/ASD cases in Korea. MLPA is recommended as a screening for identifying the genetic causes of disease in DD/MR/ASD patients.

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Next-generation sequencing allows a diagnostic yield of 24% in monogenic epilepsies

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Epilepsy is a group of neurological disorders affecting 0.7% of the population. Etiologies are widely heterogeneous, including monogenic causes. Most of these disorders affect children, which stresses the need for early molecular diagnosis. We developed a 90-gene panel for the diagnosis of Mendelian epilepsies. Target exon libraries were generated using SeqCapEZ capture technology (Roche®). Sequencing was carried out on a Miseq or a NextSeq500 (Illumina®). A total of 329 DNAs from patients without diagnosis have

been analyzed. Patients had epileptic encephalopathy (301), focal epilepsy (10), benign familial neonatal or infantile epilepsy (5), or other types of epilepsy. We identified pathogenic variants in 79 out of 329 patients, including 3 mosaic mutations with a rate between 15 and 20% in blood and 3 deletion or duplications of whole exons. Pathogenic variants were *de novo* in the genes *KCNQ2*(6), *KCNT1*(5), *SCN2A*(5), *SCN8A*(4), *GNAO1*(3), *CHD2*(3), *SYNGAP1*(3), *PCDH19*(3), *ATP1A3*(3), *FOXG1*(2), *PRRT2*(2), *DEPDC5* (2), *GRIN2A*, *GABRB3*, *SCN1A*, *STXBP1, SLC6A1*, *MBD5* ; homozygous or compound heterozygous in *PIGN*(2), *PNPO*(2), *WWOX*(2), *PLCB1*, *ST3GAL3*, *POLG*, *SCARB2*, *QARS*, *TBC1D24*; and X-linked in *CDKL5*(4), *PIGA*(3), *SLC9A6*(3), *ALG13*(2), *SLC35A2*(2), *MECP2* and *CASK*. This gene panel increased the diagnostic rate of monogenic epilepsies to 24%. The identification of the disease-causing mutation(s) will provide accurate genetic counseling and might have therapeutic consequences in a growing number of cases. For example, the identification of a homozygous mutation in *PNPO* in a patient with neonatal drug-resistant epilepsy led to treatment adaptation (increase and fragmentation of Pyridoxal Phosphate doses), which resulted in satisfactory control of seizures.

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P08.15C

Large gene panel and exome sequencing for the etiological diagnosis of intellectual disability

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Introduction Clinical and genetic heterogeneity in intellectual disability (ID) represent a major challenge for etiological diagnosis. Classically, an etiologic diagnosis was obtained in ~40% of cases with a combination of targeted genes or chromosomal analysis and microarrays. Next Generation Sequencing (NGS) greatly improved the diagnosis yield, up to 40% of unresolved cases.

We evaluated successively two NGS strategies as the last-tier test for patients with ID referred to our genetic unit: large gene panel and whole exome sequencing (WES).

Materials and Methods:

We performed trio based NGS (proband + parents) using TruSightOne (TS1) gene panel (including 4813 OMIM genes) for 263 patients with ID and WES for the next 127 patients. 22 patients were studied with both tools.

Results: Our diagnostic rate was 21% with TS1 and 42% with WES. 55% of the disease-causing mutations identified with WES were found in genes not included in TS1, because they were too recently described (29/53). Seven diagnoses with WES were performed in 22 patients without diagnosis after TS1 studies (32%). Moreover, 9 additional likely pathogenic variants were found with WES in yet unpublished genes (ongoing international collaborations). All diagnoses were validated thanks to a strong collaboration between clinical and biological geneticists.

Conclusion:

NGS is a powerful tool for etiological diagnosis in ID. WES has a better diagnostic yield than a large panel because of the rapid advancement of knowledge and the regular identification of new genes of ID. Clinical validation of the sequenced variants is an indispensable step in the diagnosis process.

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P08.16D

Diagnostic yield of exome trio analysis to identify the genetic etiology in 260 undiagnosed cases

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Introduction: Whole exome sequencing (WES) has entered the medical practice as an effective diagnostic test transforming the molecular diagnosis and clinical management of undiagnosed genetic diseases. Exome trio analysis is an effective strategy to identify *de novo*, hemizygous, homozygous and in compound heterozygous potentially causal variants of rare genetic disorders.

Materials and Methods: We performed exome sequencing using Ion AmpliSeq™ Exome RDY technology (Life Technologies) with Ion Proton™ and Ion S5-XL™. Sequencing reads were analysed using Torrent Suite software. Trio annotated variants using ION Reporter were prioritized with an in-house analytical pipeline to identify causative genetic variants.

Results: We present the analysis of 260 trios referred to a single institution. Patients were mainly children with syndromic intellectual disability (57%). The genetic etiology was potentially elucidated in 94 probands harboring 56 causal variants and 38 likely causative variants, achieving a 36% molecular diagnostic rate. Among these patients, 57 harbored *de novo* variants, 7 hemizygous maternally inherited variants, 7 in compound heterozygous variants, 18 newly homozygous variants and 5 variants inherited from parents. Patients with syndromic intellectual disability (42%, 62/149) and specific neurological disorders (39%, 13/33) showed higher molecular diagnostics rates than patients with non-neurologic disorders (29%, 8/27) and non-syndromic intellectual disability (21%, 11/51).

Conclusions: In our cohort exome trio analysis provide a diagnostic yield of 36% in patients whom traditional molecular diagnostics strategies were uninformative. The implementation of WES as a first-tier diagnostic approach will provide a higher diagnostic yield and a cost-efficient option particularly in rare syndromic intellectual disabled patients.

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P08.17A

De novo STXBP1 splice donor mutation identified by whole-exome sequencing in a familial apparently balanced translocation carrier with intellectual disability and non-syndromic epilepsy

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Familial apparently balanced translocations (ABTs) segregating with discordant phenotypes are extremely challenging for interpretation and counseling due to the scarcity of publications and lack of routine techniques for quick investigation. We report a familial ABT shared between a

proband with intellectual disability (ID) and epilepsy, and his non-affected mother. All possible mechanisms underlying the differential phenotypes were thoroughly investigated using FISH, array-CGH, and whole-genome mate-pair sequencing; however, no associations were determined.

In the current study, the same family was revisited using whole-exome sequencing (WES) (patient-unaffected parents trio) in an attempt to identify patient-specific mutations underlying phenotypic differences between the two carriers of the same ABT. WES data analysis was initially focused on a list of ~700 genes identified across previous ID-associated studies.

WES revealed a novel, patient-specific heterozygous T>G splice donor mutation in intron 13 of syntaxin-binding protein 1 (*STXBP1*) (OMIM-602926), which was also confirmed by Sanger sequencing. *STXBP1* is an autosomal dominant gene essential for neurotransmitter release through syntaxin regulation, and *STXBP1* disruptions have been previously reported in ID patients. The *STXBP1* splice donor mutation identified here is predicted to cause exon 13 skipping thus affecting domain 3a, which together with domain 1 form the central cavity of STXBP1 protein for syntaxin binding.

In conclusion, this study supports our previous published findings demonstrating that in the majority of familial ABTs, translocations are unrelated to the phenotype. In the current familial ABT, an *STXBP1* splice donor mutation might explain the proband's phenotype; however future expression studies will further support this.

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P08.18B

Paradoxical effect of baclofen in fragile X syndrome mouse model

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Introduction: Fragile X syndrome (FXS) is the most common monogenetic cause of intellectual disability and autism. Effective disease modifying therapy is lacking. However, expanding knowledge on the pathophysiology and involved pathways has opened possibilities for targeted treatment. FXS is caused by a CGG repeat expansion in the 5′ UTR of the *FMR1* gene, resulting in lack of expression of the *FMR1* protein (FMRP). FMRP is a major transporter and translational regulator of specific mRNAs at the post-synaptic compartment in neurons. Absence of FMRP in FXS leads to an imbalance in excitatory and inhibitory network functioning, resulting in aberrant synaptic plasticity. Evidence from studies in mice demonstrate that the major inhibitory neurotransmitter system in the brain, the GABAergic pathway, is reduced in FXS and autism. We tested the effect of chronic baclofen treatment, a $GABA_B$ agonist, on social behavior in *Fmr1* KO mice.

Materials and methods: young adult male *Fmr1* KO mice and WT littermates received baclofen or control drinking water from weaning. We tested two different doses in two social behavior paradigms, the automated tube test and the three chamber sociability test.

Results: Unexpectedly, chronic baclofen treatment worsened the social behavioral phenotype in the automated tube test and induced a social behavioral phenotype similar to FXS in wildtype littermates in both tests. In the highest dose, hyperactivity was measured.

Conclusion: Altogether, the disappointing results of recent clinical trials and our current results indicate that baclofen treatment for FXS should be reconsidered and further evaluated before its application as targeted therapy.

S. Zeidler: None.

P08.19C

Glass syndrome caused by a novel mutation in the *SATB2* gene - is there something specific for cases with point mutations?

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Introduction: Glass syndrome (OMIM 612313) is a disorder caused by heterozygous interstitial aberrations on chromosome 2q32-q33 or a heterozygous mutation in the *SATB2* gene located within the Glass syndrome chromosome region. It is characterized by variable degrees of psychomotor delay/intellectual disability and craniofacial dysmorphism (like downslanting palpebral fissures, cleft palate, crowded teeth, and micrognathia). Other features may include joint laxity, arachnodactyly, skeletal anomalies and behavioral problems. Given the variety of mechanisms leading to alterations of the *SATB2* gene, the term "*SATB2*-associated syndrome" (SAS) has also been proposed. Materials and Methods: Targeted next-generation sequencing (NGS) was performed in a 16-year-old girl and her consanguineous parents of Polish origin. She presented with intellectual disability (IQ = 35), thin, marfanoid habitus with arachnodactyly and unrecognizable facial dysmorphism (posteriorly rotated ears, synophrys, long and

prominent nose, short philtrum, abnormal position/widely spaced teeth). **Results:** NGS revealed the presence of a novel heterozygous mutation in the *SATB2* gene c.716del, p.(Arg239fs) in the proband, not present in either parent. **Conclusions:** Mutations in *SATB2* are associated with variation in the severity of developmental defects, including even Rett-like phenotypes. However, to date only 11 cases with point mutations have been reported. The aim of the study is to present another case of Glass syndrome, referred to our Department at the age of 14 years. In particular, we want to draw attention to the specificity of phenotypic anomalies, which may improve the clinical diagnosis of Glass/SATB2-associated syndrome. The study was financed by NSC Project Harmonia 4 No. UMO-2013/08/M/NZ5/00978.

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P08.20D

Characterization of Glycosylphosphatidylinositol Biosynthesis Defects on biomarkers, phenotypic data and automated image analysis

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Glycosylphosphatidylinositol (GPI) biosynthesis defects (GPIBDs) are a group of phenotypically overlapping syndromes with intellectual disabilities that are caused by recessive mutations in currently 14 genes of the molecular pathway. The serum activity of alkaline phosphatase (AP), a GPI-linked enzyme, has been used to divide GPIBD patients into Hyperphosphatasia with Mental Retardation syndrome (HPRMS) and other subtypes, and link these phenotypic series to certain subsets of genes. However, with the increasing number of identified cases we now know that also AP is a variable feature in GPIBDs. We therefore studied the discriminatory power of flow cytometry that is based on multiple GPI-linked substrates. In addition we evaluated computer-assisted classification from FDNA that is based on all clinical features and as well as on the facial gestalt of patients with a GPIBD.**Results:** We found certain malformations more likely to be associated with particular gene defects. However, especially at the severe end of the clinical spectrum of HPMRS, there is a high phenotypic overlap with another subset of GPIBDs, termed Multiple Congenital Anomalies Hypotonia Seizures syndrome (MCAHS). The cell surface reduction of GPI-linked markers correlates with the severity of the phenotype, but no gene-specific profile could be identified. Interestingly, it was facial recognition software that achieved the highest accuracy in clustering GPIBDs. The effectiveness of gestalt analysis in the correct gene inference in a GPIBD is remarkable and illustrates how the information contained in human faces is still pivotal in the delineation of genetic entities.

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P08.21A

Novel compound heterozygous variants in GPT2 in a family with microcephaly and intellectual disability

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We here describe an index male patient diagnosed with microcephaly, intellectual disability (IQ:39), developmental delay, spastic paraplegia, normal serum biochemical and metabolic test results that harbors compound heterozygous missense variants, NM_133443:c.[400C>T] and NM_133443: [1435G>A], in the glutamic pyruvate transaminase (*GPT2*) gene. Both of these variants, c.400C>T (p.R134C) and c.1435G>A (p.V479M), resides in the pyridoxal phosphate-dependent aminotransferase domain. The missense variants affect highly conserved amino acids and are classified to be disease-causing by both SIFT and PolyPhen2. The candidate variants were not found in the Exome Aggregation Consortium (ExAC) dataset or in dbSNP. Index patient has an affected sister and 4 affected paternal aunts who were also diagnosed with microcephaly, intellectual disability and developmental delay. Affected sister has a better IQ(63), but has ADHD and seizures. Index patient's parents are first degree cousins and healthy. Paternal aunts' parents are nonconsanguineous and healthy. Upon Sanger sequencing, we were able to confirm these mutations in all affected family members and confirmed that index patient and his affected sister inherited one mutant allele from each unaffected parent. This was rather an interesting finding since autosomal recessive, homozygous mutation would be a more expected finding in a consanguineous family. To the best of our knowledge, this is the fourth family in the literature (Ouyang et al, Celis et al) with *GPT2* mutation and is the first family in which a novel compound heterozygous variant in *GPT2* gene was identified.

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P08.22B

A recurrent, *de novo* nonsense mutation in the *GRIN2B* gene, comparison of the clinical phenotypes

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The *GRIN2B* gene encodes the N-methyl-D-aspartate (NDMA) receptor that is found at the excitatory synapses throughout the brain and is implicated in learning and memory. Mutations in the *GRIN2B* gene are associated with autosomal dominant mental retardation 6 and early infantile epileptic encephalopathy 27.

Patients with intellectual disability and or developmental delay were referred to our clinic for whole exome sequencing. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including an intellectual disability/ developmental delay (ID/DD) gene panel of ~711 genes for filtering of the data. Analysis of whole exomes was only performed when informed consent was available and gene panel analysis did not reveal a candidate mutation. In total, 709 index patients, trios or singles, have been included in this study. Here, we describe three unrelated patients with a recurrent de novo nonsense mutation, p.Arg847*, in the *GRIN2B* gene. In the same gene, four other mutations were found, another nonsense, a frameshift, a missense mutation and a whole gene deletion, each in one patient. The clinical phenotype of patients carrying the recurrent mutation and the other mutations are compared and will be presented.

Overall, the patients with the recurrent mutation in *GRIN2B* showed a more severe clinical phenotype with severe developmental delay, little or no speech, facial dysmorphology, hypermobility of the joints, and behavioural problems.

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P08.23C

Phenotypic spectrum of patients with GRIN2B encephalopathy and setup of a database of NMDA-receptor disorders

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Introduction: N-methyl-D-aspartate receptors (NMDAR) are ligand-gated cation channels which mediate excitatory neurotransmission in the brain. NMDAR are obligatory heterotetramers composed of two Glycine-binding GluN1-(GRIN1) and usually two Glutamate-binding GluN2-subunits (GRIN2A, GRIN2B, GRIN2C and GRIN2D). Heterozygous *de novo* variants in GRIN2B have previously been reported in patients with intellectual disability (ID), autism and epilepsy.

Results: Overall, *de novo* variants in 60 patients were classified as pathogenic/likely pathogenic, with 21 patients having been reported in the literature. Patients presented with neurodevelopmental disorders (100 %) and a spectrum of autism (27 %), epilepsy (52 %), dystonic, dyskinetic or choreatiform movement disorder (10 %), cortical visual impairment (8 %) and cerebral volume loss (9 %). Six patients presented with a consistent malformation of cortical development intermediate between tubulinopathies and polymicrogyria revealing novel phenotypic consequences of channelopathies.

Outlook: There are only a few recurrent (likely) pathogenic variants in *GRIN2B*, comparable to all other NMDAR subunits, as most patients constitute single cases. Over time, the phenotype of patients with (likely) pathogenic variants in known disease genes tend to be reported more scarcely in the scientific literature. Therefore, we are in the process of setting up a database to enlist patients with variants in any NMDAR subunit. Data can be submitted online and will be displayed in the database after review. As all subunits share significant sequence-homology, combining genetic and phenotypic data of patients from all subunits will hopefully deepen our understanding of phenotypic consequences and functional aspects of different segments of the NMDAR.

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P08.24D

HUWE1 mutations cause dominant XLID: a clinical and genetic study of 22 patients

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Whole-gene duplications and missense mutations in the *HUWE1* gene (NM_031407.6) are a known cause of X-linked intellectual disability (ID). Increased gene dose causes non-syndromic mild to moderate ID with speech delay in affected males. Various missense mutations may cause syndromic and more severe ID in males with female sparing, the latter linked to skewed X-inactivation.

Here, we report the largest *HUWE1*-cohort consisting of 14 females and 8 males, with 16 missense mutations and one splice site mutation. Nine patients were ascertained through the DDD-project. *HUWE1* variants were interpreted according to the ACMG 2015 Guidelines. The X-inactivation pattern was examined, and the current literature has been reviewed. We highlight common clinical features consisting of moderate to profound ID, delayed or absent speech, short stature and facial dysmorphism such as a broad nose, deep set eyes, epicanthus, short palpebral fissures, a short philtrum and small hands and feet. We describe for the first time that females can be severely affected, despite X-inactivation of the mutant X chromosome. A notable exception to this are two additional females with the R110Q mutation in *HUWE1*, presenting with a specific phenotype with mild ID, different facial features, scoliosis and craniosynostosis, confirming Taylor et al's previous report. In these females the X chromosome was skewed in favour of the mutant transcript.

In summary, *HUWE1* missense mutations may cause syndromic ID in both males and females, and the latter appears to be more common and more severe than in the classical X-linked recessive families.

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P08.25A

Attempts to elucidate role of *ZBTB11* gene in intellectual disability

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Exploring genes and pathways underlying Intellectual disability (ID) can clarify the complex puzzle of how cognition develops. As part of ongoing systematic studies to identify candidate ID genes, linkage analysis and whole exome sequencing revealed *ZBTB11* in two consanguineous families. *ZBTB11* encodes an understudied transcription repressor and its zebrafish mutant showed brain and spinal

cord degeneration with prominent apoptosis of central nervous system.

To evaluate possible disrupting effects of two missense variants identified in *ZBTB11*, HEK293 cells were transfected with wild-type/mutant ZBTB11-GFP constructs and protein localization was investigated by confocal fluorescence microscopy. To explore target genes and pathways, ChIP-sequencing was performed on transfected cells. We also investigated role of *ZBTB11*-ortholog in Drosophila brain by targeting RNAi using UAS/Gal4 system.

These variants (p.H729Y, p.H880Q) disrupt canonical Zn^{2+}-binding residues of C2H2 zinc finger domains, leading to possible altered DNA binding. We found abolished localization for mutants being excluded from the nucleolus where the wild-type recombinant protein is localized (confirmed by B23/NPM1marker). Although ChIP-sequencing results did not fulfill quality criteria, some relevant targets can be observed with the help of recent ENCODE data of HEK293 stably expressing eGFP-ZBTB11 and will be compared with mutants. The drosophila F1 offspring with no expression of *ZBTB11*-ortholog in brain were generated and subjected to behavioral and structural investigations.

This study provides information on ZBTB11 localization in nucleolus and clearly shows how these variants disrupt protein localization and therefore its function. The upcoming results from ChIP-sequencing and Fly models will elucidate the way it is involved in ID.

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P08.26B

ArrayCGH analysis in patients with intellectual disability/developmental delay in Turkish Children living in Trakya Region

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Introduction: İntellectual Disability is a neurodevelopmental disorder identified at the beginning of the developmental period as the lack of conceptual, social, and practice areas of both mental and adaptive functions. [1]. The diagnostic approach to these patients should include classical karyotyping, Fragile- X analysis, neurological imaging, flourescent in situ hybridization and array based comparative genomic hybridization (arrayCGH) after taking a detailed physical examination and medical history [2,3]. The aim of this study is to investigate the copy number variations (CNVs) by using arrayCGH analysis in patients who had been found to have normal karyotype and does not have Fragile-X. **Materials and Methods:** 45 patients [male: 27 (9.6 years), female: 18 (7.8 years)] were included in the study who were directed to Genetic Diagnosis Center of Trakya University Faculty of Medicine between March 2015 and November 2016 with a diagnosis of intellectual disability/developmental delay Classical karyotyping, Fragile-X analysis and arrayCGH analysis (Agilent 4×180K ISCA CGH + SNP) have been applied to all patients. The pathogenicity of the CNVs found have been assesed by using the databases. **Results:** CNVs have been determined in 14 (9 male; 5 female) out of 45 patients (31.1%). The disease-related arrayCGH results and clinical characteristics are shown in Table 1. **Conclusions:** Our results support the importance of arrayCGH analysis in detecting submicroscopic chromosomal abnormalities in patients with intellectual disability/ developmental delay.

Table 1. Array-CGH results and clinical features of the 15 Turkish patients with pathogenic CNVs

Patient	Age	Gender	arrayCGH results	Size	Inheritance	Clinical features	Classification (ISCA, UCSC, DGV, ClinVar and DECIPHER)
2	9 year	M	arr cgh(hg 19) 2q37.2 (236,114,456–236,512,302)x3	397 kb	*de novo*	Intellectual disability	VOUS
5	1 year	F	arr cgh(hg 19) 22q11.21 (18,894,832 - 21,440,514)x1	2546 kb	*de novo*	Developmental delay, intellectual disability, speech delays	Pathogenic
7	1 year	F	arr cgh(hg 19) 6q23.3-q27 (138,180,512–170,906,796)x3	32726 kb	*de novo*	Developmental delay, intellectual disability, speech delays	Pathogenic
13	13 year	M	arr cgh(hg 19) Xq21.31-q21.32 (91,701,124–92,238,826)x3	538 kb	*de novo*	Intellectual disability	VOUS
15	8 year	M	arr cgh(hg 19) 4q32.3 (167,766,818–169,176,455)x3	1410 kb	*de novo*	Intellectual disability	VOUS
22	1 year	F	arr cgh(hg 19) 1q21.1-q21.2 (146,507,518–147,824,207)x3	1316 kb	*de novo*	Developmental delay, Intellectual disability, speech delays	Pathogenic
26	17 year	M	arr cgh(hg 19) 10q21.3 (68.359.435–68.486.777)x1	127 kb	*de novo*	Mild Intellectual disability	VOUS
27	13 year	M	arr cgh(hg 19) 2q37.3 (240.057.136–243.040.276)x1 arr cgh(hg 19) 20q13.32-q13 (56.546.112–62.908.674)x3	2983 kb6363 kb	*de novo*	Severe intellectual disability, hearing loss	Pathogenic
30	12 year	M	arr cgh(hg 19) 16p11.2 (29.656.684–30.190.568)x1	534 kb	*de novo*	Intellectual disability, Hyperactivity	Pathogenic
33		F			*de novo*		Pathogenic

Table (continued)

Patient	Age	Gender	arrayCGH results	Size	Inheritance	Clinical features	Classification (ISCA, UCSC, DGV, ClinVar and DECIPHER)
	9 year		arr cgh(hg 19) 20q11.21-q12 (29,842,786–41,548,389)x3 arr cgh(hg 19) 20q13.31 (55,846,129–56,177,485)x1	11706 kb331 kb		Developmental delay, Intellectual disability	
35	15 year	M	Chromosome 1 LOH	-	*de novo*	Intellectual disability, bilateral cryptorchidism	Pathogenic
38	11 year	M	arr cgh(hg 19) Xp.21.1 (32,791,931–33,641,487)x3	850 kb	*de novo*	Intellectual disability	VOUS
40	1 year	M	arr cgh(hg 19) 15q11.2 (22,698,522–23,300,287)x1 arr cgh(hg 19) 16q12.1 (48,304,344–48,549,404)x3	602 kb245 kb	*de novo*	Intellectual disability	VOUS
41	6 year	F	arr cgh(hg 19) 6p23-p22 (14,087,024–24,834,000)x1	10747 kb	*de novo*	Developmental delay, intellectual disability, speech delays, VSD	Pathogenic

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P08.27C

Parent of origin dependent expression of *CNTN6 in vitro* in neurons derived from induced pluripotent stem cells of patient with intellectual disability and 3p26.3 microduplication of paternal origin

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Introduction: Understanding the pathogenesis of chromosomal diseases is complicated by tissue-specific effects of chromosomal imbalances and the size of chromosome rearrangements, usually involving multiple genes with different functions. These problems can be overcoming using current technologies of somatic cell reprogramming as well as high resolution molecular karyotyping, allowing to identify submicroscopic chromosomal aberrations affecting a single gene. Here, we report about features of neuronal *in vitro* expression of *CNTN6*, which was recently associated with autistic spectrum disorders and intellectual disability.

Materials and Methods: Neuronal cells were obtained by *NGN2* differentiation of induced pluripotent stem cells derived from a patient with intellectual disability and 3p26.3 microduplication of paternal origin affecting *CNTN6* only (Kashevarova et al., 2014) as well as from healthy donor with normal karyotype.

Results: For the first time, the preferential expression of maternal allele of *CNTN6* was observed both in patients and donors neuronal cells. It was found also, that expression of duplicated allele of paternal origin was significantly reduced though the *CNTN6* copy number increasing due to chromosomal microduplication.

Conclusions: Obtained results can explain the reported in literature mode of inheritance of chromosomal rearrangements affecting *CNTN6* in several generations by parental origin of CNV. They provide evidence also, that some identical phenotypes in patients with reciprocal microdeletions and microduplications syndromes can be explained by haploinsufficiency in spite of opposite changes in the gene copy number due to suppression effect of chromosomal microduplication on the gene expression level.

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P08.28D

A de novo missense mutation affecting the KCNMA1 gene causes intellectual disability, seizures, facial dysmorphism and connective tissue disorders

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KCNMA1 gene encodes the pore-forming subunit of a large-conductance voltage- and Ca^{2+}-activated K^+ channel (BK), which is almost ubiquitously expressed in mammalian tissues. BK channels have been implicated in several functions including modulation of neurotransmission, control of smooth muscle tone, epithelial transport and, more recently, their involvement in the maintenance of mesenchymal stromal cell population, migration and differentiation has been also suggested. Gain-of-function mutations of KCNMA1 gene have been already associated with a human syndrome of coexistent generalized epilepsy and paroxysmal dyskinesia, yet the impact of loss-of-function mutations of this channel on human physiology and development remain poorly characterized.

Using Whole Exome Sequencing analysis, we recently identified two unrelated patients carrying the same heterozygous *de novo* missense mutation and presenting with an undiagnosed polymalformative association of intellectual disability, seizures, facial dysmorphism and connective tissue disorders. This variant affects a conserved nucleotide resulting in an amino acid substitution (p.Gly375Arg) associated with high-confidence predicted damaging impact on the structure and function of Kcnma1 protein. Indeed, p. Gly375Arg affects the transmembrane segment S6, which together with P and S5 segments makes up the pore-gate domain, supporting the hypothesis of its detrimental impact on K^+ conductance. Currently, a collaborative effort is being deployed in order to model *in vitro* the outcomes of such mutation on Kcnma1 function. Furthermore, using a combination of genotype-first strategy coupled with an international data sharing approach, three further unreported patients could be identified, which will contribute to shed light on the pleiotropic functions of KCNMA1 in human clinical phenotype.

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P08.29A

Genetics of Neurodevelopmental Disorders [GND] Consortium - International effort to elucidate the genes underlying autosomal recessive neurodevelopmental disorders

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Autosomal recessive neurodevelopmental disorders (NDD, including intellectual disability and autism spectrum disorders) are extremely heterogeneous, and more than half of NDD genes are unknown. Exome in suspected recessive NDD (i.e. consanguineous parents or more than one affected sibling) frequently leaves several potential disease-causing candidate variants. To confirm a candidate gene's involvement, typically overlapping cases with variants in the same gene are necessary. Under a conservative assumption that there are ~1800 recessive NDD genes with equally distributed burdens, ~10,000 cases are needed to identify at least two mutations in 90% of the genes. This means that deciphering the genetics of NDD can only be approached via intensive cooperation and data sharing worldwide. While gene-matching efforts have impacted this discovery, a phenotype-driven approach that does not require prior identification of candidates would be more powerful. We have established the GND (Genetics of Neurodevelopmental Disorders) Consortium to provide a free portal for sharing exomes with HPO-determined phenomes (www.gnd.academy). Each contributor maintains exclusive control over his/her data, sharing only candidate variants that meet pre-specified criteria. GND will process data through 3rd party HIPAA-compliant software to match genotype/phenotype with genome-wide significance as well as incorporate gene-networks to support nominally significant genes. GND will return results to groups with matching genes/phenotypes. More than 3,000 cases are already included in GND and we aim for 10,000 cases in 2017. We invite all colleagues worldwide to join GND to elucidate the genetics of neurodevelopmental disorders.

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P08.30B

Mendeliome sequencing increases the diagnostic yield in patients with unexplained intellectual disability by 30 %

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Introduction In the past, array-CGH technology has boosted the detection rate significantly; but up to 50% of children with developmental delay still remained undiagnosed. Since next generation sequencing (NGS) has the power to improve the diagnostic yield tremendously, we applied this method to 108 index patients with developmental delay or intellectual disability (ID) and pre-excluded genomic imbalances.

Method DNA samples of 80 parent-patient trios plus 28 individual patients were analyzed for mutations in 4813 genes, using the TruSight-One gene panel (Illumina). After sequencing with median target coverage of 80-fold, sequence variants were called with the CLC Biomedical Genomics Workbench (Qiagen). All variants with putative effect on amino acid level (i.e. frameshift, splice site disruption, missense) and with plausible mode of inheritance (*de novo*, recessive, X-linked) were evaluated according to the ACMG guidelines. The results were discussed in a team of clinicians and molecular geneticists; relevant variants were validated by Sanger-sequencing.

Results Using the mendeliome in a diagnostic setting, we established a diagnosis in 33 of the 108 index patients (30%). For 12 further patients, we found one or two possibly causative candidates (11%). Five patients (4.6%) showed incidental findings which either made treatment or surveillance necessary. (e.g. homozygous MUTYH-mutations, SDHA-mutation). Nine patients (8%) have been identified to be carriers for a recessive disease (PAH- or CFTR-mutations).

Conclusion Mendeliome next generation sequencing significantly increases the diagnostic yield in patients with ID unsolved with array-CGH by at least 30%. However, variant interpretation remains challenging and calls for national and international data exchange.

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P08.31C

Characterization of genotype and phenotype in 710 patients with developmental delay and intellectual disability based on chromosome microarray analysis

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Introduction: Developmental delay (DD) and intellectual disability (ID) commonly present with co-occurring conditions like epilepsy. Chromosome microarray analysis (CMA) has been recommended as a first-tier diagnostic test for DD/ID. Assessing the diagnostic yield of CMA in DD/ID with different co-occurring condition can offer clinicians the phenotypic clue of probable pathogenic finding. This study aimed to characterize pathogenic copy number variations (pCNVs) in a mixed cohort of DD/ID and identify phenotypic clues associated with these pCNVs.

Materials and Methods: The study was conducted retrospectively in 710 DD/ID patients with CMA performed. Clinical data were collected, and the interpretation of CMA results followed ACMG guidelines.

Results: A total of 247 pCNVs were identified in 201 patients (28%). A large portion of these pCNVs were copy number losses, and the size of copy number losses was generally smaller than gains. Pathogenic CNVs distributed over all chromosomes, and an enrichment was found in chr7, 15 and 22. The diagnostic yields were not statistically different among groups classified by ID severity ($p=0.084$, fisher's exact), while the likelyhood of a pathogenic finding increased when congenital heart defects, facial dysmorphism, microcephaly or hypotonia was present in DD/ID patients ($p<0.01$, fisher's exact).

Conclusion: Our results suggest varied yields of CMA among DD/ID patients with different phenotypic presentation.

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P08.32D

Exome pool-seq: large-scale exome sequencing of pooled DNA samples in intellectual disability

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Introduction: Despite falling costs of high throughput sequencing and great advances in identification and confirmation of novel disease genes, there is a need for genome-wide, simple, cheap and fast screening technologies in extremely heterogeneous disorders such as intellectual disability (ID). **Material and Methods**: We exome sequenced 96 individuals with sporadic ID in 8 pools of 12 equimolar concentrated samples each. Variants were called using a ploidy of 24 and filtered for loss-of-function variants or likely pathogenic (previously reported, deleterious prediction) missense variants in 923 known ID genes (SysID database). Additionally, 543 ID candidate and 1,694 haploinsufficiency intolerant genes were screened for loss-of-function variants. Confirmation within the pool and segregation was tested by Sanger sequencing. **Results**: In known ID genes we identified 19 (proven or assumed) *de novo*, (likely) pathogenic variants (*ACTB*, *AHDC1*, *ANKRD11*, *ATP6V1B2*, *CASK*, *CHD8*, *GNAS*, *KCNQ2*, *KMT2A*, *MED12*, *MED13L*, *RIT1*, *SETD5*, *SIN3A*, *TCF4*, *TUBA1A*, *WAC*, *ZBTB18*), two pathogenic variants that were inherited from a symptomatic or healthy parent each (*ZMYND11*, *IFIH1*), two likely pathogenic X-linked variants in boys (*ATRX*, *MAOA*), and a homozygous variant in the recessive *TRAPPC11*. Additionally, we identified 4 *de novo* variants in candidate genes. In our cohort this resulted in a high detection rate of ca. 25%, similar to other screening approaches, but allowing more flexibility for future data evaluation. **Conclusion**: Our high detection rate of at least 25% establishes exome pool-seq as a new screening approach for large-scale, cost-efficient and flexible sequencing in highly heterogeneous but well characterized disorders like ID.

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P08.33A

PRiSM, a functional genomics screen for relative quick assessment of neuronal pathogenicity of ND candidate genes/mutations

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Neurodevelopmental disorders (ND) affects 2% of the population of which the majority has a genetic cause, but in a considerable amount of cases the gene involved is still unknown. Next Generation Sequencing (NGS) technology has made tremendous advancements in gene sequencing technology, allowing it to become a diagnostic tool to identify mutations in patients with a neurodevelopmental disorder. However, in cases when missense mutations are identified, the pathogenicity is often unclear.

In our lab we have recently developed a functional genomics screen (**P**ipeline for **R**apid ***i****n vivo* and ***i****n vitro* **S**creening of **M**utations, **PRiSM**), to relatively quick assess the neuronal pathogenicity of ND candidate genes and/or mutations, without any *a priori* knowledge about the role of the gene. Central in the design of PRiSM are the following features: (1) It is sensitive to pathogenic mutations which have a dominant effect on down-stream signaling; (2) It features parallel assays which yield complementary data and allows scaling; (3) It allows easy implementation of specialized follow-up measurements (e.g. imaging, electrophysiology). We have started with the assessment of the pathogenicity of previously published intellectual disability (ID) candidate mutations on neuronal maturation *in vitro* and neuronal migration *in vivo*. We will show here a brief overview of the tested mutations and show one example to illustrate what PRiSM entails.

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P08.34B

Prevalence and origin of chromosomal microduplications in patients with intellectual disability

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Introduction: To date, more than 230 microdeletion and 80 microduplication syndromes were revealed in patients with intellectual disability and developmental delay. Pathogenic microduplications are founded rarely, probably because of softer and more variable manifestation of clinical signs or frequent inheritance from apparently healthy parents. However, they are more often detected by high-resolution molecular cytogenetic methods.

Materials and Methods: We examined 140 patients with autism, intellectual disability and developmental delay in age from 3 to 18 years by array-CGH (4×44K, 8×60K, Agilent Technologies). Identified microduplications were confirmed and their origin was defined by PCR-RT.

Results: Chromosomal microduplications with pathogenic or probably pathogenic significance were found in 14 patients (10%). Microduplications size ranged from 115 kb to 32.2 MB. Eleven patients have only one microduplication: 1q25.1-q25.2, 2p25.3-p25.2, 3p26.3, 5q33.1, 6p22.2, 10q26.3, 12q24.12 (two cases), 14q11.2, 18p11.31, 20q13.12. One patient was a carrier of microtriplication 4q21.21-q21.22 with 1,6 Mb in size. One patient has a combination of microduplication with microdeletion: dup2p25.3-p23.3 and del2p25.3; another one has a complex combination of CNVs: del9p24.3-p24.2, dup9p24.2-p13.3, trip9p21.1-p13.3, and delXq28. Identified microduplications had a *de novo* origin in 6 patients (46%), whereas in 7 patients (54%) they were inherited from a healthy parent. In one case the parental DNA samples were not available for examination.

Conclusions: We determined the prevalence of clinically significant chromosomal microduplications, which are amount to 10% in patients with intellectual disability, and confirmed the relatively high rate of their transmissions from healthy carriers. This study was supported by Russian Science Foundation, grant 16-15-10229.

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P08.35C

The clinical utility of array-CGH and targeted NGS in idiopathic intellectual disabilities and developmental delays: a case report of *SCN2A* p.Ala263Val variant

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Introduction: Next-generation sequencing (NGS) techniques have become a powerful tool for the identification of the genetic causes of the heterogeneous conditions such as intellectual disabilities, multiple congenital anomalies and autism spectrum disorders.

Material and methods: We present our first experience with targeted NGS approach using commercially available design SureSelect Inherited Disease (Agilent Technologies) containing more than 2700 genes known to cause inherited disorders. We report on a case of 9-year-old boy with a diagnosis of severe intellectual disability related to early myoclonic encephalopathy. This patient was examined according to our investigatory algorithm, from G-banding karyotype (46,XY) to array-CGH on oligonucleotide DNA microarrays (Agilent Technologies) followed by confirmative targeted quantitative PCR and FISH.

Results: We detected a *de novo* copy-number gain of 18q21.23 (539 kb) classified as probably benign. Consequently this patient was included in our pilot study using targeted NGS with pre-designed gene panel SureSelect Inherited disease and Illumina MiSeq. We detected *de novo* heterozygous missense genetic variant in *SCN2A* gene, resulting in the amino acid residue change from alanine to valine at position 263 (p.Ala263Val). This variant had been previously described as definitely pathogenic in patients with Otahara syndrome.

Conclusions: This case has proved the usefulness and effectivity of our molecular diagnostics algorithm enhanced by NGS approaches leading to higher diagnostic yield of heterogeneous genetic conditions.

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P08.36D

Next generation sequencing diagnostic yield in intellectual disability

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Introduction: Intellectual disability (ID) is characterized by significant limitations in intellectual functioning and adaptative behavior. It is estimated that ID affects approximately 60 million of people worldwide. However, around 60% of the cases remain without a genetic diagnosis, which can provide not only information on the etiology of ID but also allows genetic counseling. **Material and Methods:** 188 exomes corresponding to 65 families underwent next generation sequencing (NGS) using different groups of patients: (i) 8 familial cases with several affected generations and many affected members; (ii) 15 family nuclei (healthy parents and siblings); (iii) 27 trios; (iv) 15 index cases. On the other hand, targeted-resequencing was performed in 100 additional patients with ID. **Results:** Identification of causative alterations was achieved in 15% of families' studied by exome sequencing (10/65) and 1% of targeted resequencing. Moreover, 6 out of the 10 families' diagnoses belong to the group of large families with several affected generations and many affected members, reaching a diagnostic yield of 75% (6/8) within this group. In addition, candidate alterations were identified in 45% of cases. Conclusion: NGS increases the diagnostic yield in all the groups studied. However, the best results are obtained in families with more than one family member affected. Nevertheless, a good clinical description is essential to both avoid unnecessary studies and to validate the causality of a variant. Acknowledgements: ISCIII [(PI12/00879], FEDER CERCA Programme and AGAUR (2014 SGR603) CIBERER (ISCIII).

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P08.37A

Update on GeJo-ARID: a cooperation project between Germany and Jordan for the identification of new genes in autosomal recessive intellectual disability

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In a bilateral project between Germany and Jordan, we examined 40 consanguineous Jordanian families with intellectual disability (ID) of probably autosomal recessive inheritance. We conducted whole exome sequencing (WES) for one affected individual per family and filtered for rare bi-allelic variants.

With this approach we could identify homozygous pathogenic variants in the 6 previously established ID-genes *ALDH5A1*, *DNAJC6*, *GCDH, GPR56*, *NT5C2* and *WDR62*. In 3 further ID-genes (*GMPPB*, *PGAP2* and *PNKP*) we were able to identify likely pathogenic variants.

In a family with one affected individual with severe intellectual disability and epileptic encephalopathy we could identify a homozygous truncating mutation in *DENND5A* (c.2547delG, p.Lys850Serfs*11) and establish *DENND5A* as a new ID-gene.

Although, family pedigrees suggested autosomal recessive ID, we were able to identify autosomal dominant causes in 3 families: a de novo deletion on chromosome 4q21.22-21.23, a de novo frameshift variant in *SHANK2* and a de novo stop variant in *TCF4* in two affected siblings, most likely due to germline mosaicism.

In total, with our approach of WES of affected individuals with likely autosomal recessive ID, we identified the underlying causes of intellectual disability in 13 out of 40 families demonstrating that our approach is viable to identify causative variants for intellectual disability.

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P08.38B

Clinical delineation of the phenotype of five females carrying heterozygous mutation in KDM5C gene

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X-linked intellectual disability (XLID) is characterized by extreme genetic heterogeneity with implication of more than 100 genes on the X chromosome. To date, 28 mutations have been reported in the lysine Specific Demethylase 5C (*KDM5C*) gene, which encodes for a histone demethylase. *KDM5C* appears to be one of the major cause of moderate to severe XLID in males, and has been associated with short stature, facial dysmorphism, behavior disorders, epilepsy and other variable features. For most of these mutations, female carriers (mothers or sisters) have also been reported but with poor phenotypic descriptions. Females present a large phenotypic spectrum, ranging from unaffected to specific learning difficulties or ID, but never severe, contrary to males. Here, we present an accurate study of the clinical and molecular features of five affected females carrying unreported heterozygous *KDM5C* variants (three missense and two frameshift) detected by whole-exome sequencing. They all presented with ID, mild hypertrichosis and endocrine disorders, as well as subtle common facial dysmorphism. Strikingly, they had a very particular language disorder, predominant on the expressive part and contrasting with a correct comprehension for cognitive age. In conclusion, our findings extend the number of *KDM5C* mutations in affected females, and thus provide further evidences of its role in ID in females. This study, conducted thanks to whole-exome sequencing deployment in ID diagnosis, highlights the increasing implication in sporadic female cases of genes previously known to be responsible for XLID in males.

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P08.39C

Kleefstra syndrome in a trilingual patient showing preservation of language skills

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Background: Kleefsta Syndrome is caused by haploinsufficiency of EHMT1, usually caused by deletions at 9q34.3. The phenotype typically includes moderate to severe intellectual disability. Expressive language in patients with Kleefsta syndrome is usually most severely affected, with most patients limited to one to two word phrases, if they speak. Many adults show regression of learned language and motor skills. Case reports exist of occasional patients with milder than expected intellectual disability, including one child with a small deletion and a near normal IQ with verbal apraxia.

Case report: We report a case of a 22 year old patient with a deletion at 9q34.3, including EHMT1, CACNA1B, and TUBBP5, who has mild intellectual disability and for whom language is a strength. She speaks three languages and is able to read, write, spell and type. There have been no signs of behavioural regression, motor regression or apathy to this point.

Conclusions: Due to the now widespread use of chromosomal microarray and exome sequencing, traditional phenotypes for many syndromes are expanding. For Kleefstra syndrome, this has largely meant an expansion of the degree of associated intellectual disability to include more mildly affected patients. While some patients, especially those with quite small deletions or mutations, have some preservation of speech function, verbal apraxia has continued to be a fairly constant feature of the syndrome. This case further expands the known phenotype to include patients with strong expressive verbal skills in comparison with their level of functioning.

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P08.40D

A novel homozygous *ROGD1* mutation in two siblings with kohlschutter-tonz syndrome: a rare entity

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Introduction: Kohlschutter-Tonz syndrome is an autosomal recessive disorder characterized by global developmental delay, intractable seizures, and amelogenesis imperfecta. Majority of cases is caused by homozygous or compound heterozygous *ROGD1* mutations, however, biallelic *SLC13A5* mutations have also been reported recently.

The ROGDI gene encodes a leucine-zipper protein with high expression in the human brain and spinal cordMaterials and Methods: We here report on clinical and molecular findings of a 16-year-old and a 11-year-old two siblings with Kohlschutter-Tonz syndrome.

Results: The siblings were referred to our center with seizures and developmental delay at the ages of $2^{2/12}$ and $2^{9/12}$, respectively. Both siblings had normal motor and mental developmental milestones initially, however, with the onset of seizures, regression was observed. Physical examination revealed normal growth with facial features including hypertelorism, prominent columella, short philtrum, and yellow teeth. Cranial MRI in the elder sister revealed asymmetric thickening of the right temporal lobe while in the younger sister cerebral frontal atrophy was detected. Seizure control was achieved with levetiracetam and phenobarbital in both. During the clinical follow-up both siblings were diagnosed with Kohlschutter-Tonz syndrome. DNA sequence analysis revealed homozygous c.201-1G>T in intron 3 of *ROGD1* which is very likely to destroy the splice acceptor site of exon 4 of *ROGD1*. Both parents were heterozygous for the mutation.

Conclusion: Kohlschutter-Tonz syndrome is a rare entity with an almost consistent phenotype, however, the diagnosis may sometimes be overlooked. Co-occurrence of amelogenesis imperfecta with seizures and developmental delay should be suggestive of the diagnosis.

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P08.41A

De novo mutations in SETD2 cause Luscan-Lumish syndrome

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Introduction: Luscan-Lumish syndrome is an autosomal dominant hereditary disorder characterized by macrocephaly, intellectual disability, speech delay, low sociability, and behavioral problems. Other features include postnatal overgrowth, obesity, advanced carpal ossification, frequent respiratory infections, developmental delay, and seizures. Materials and Methods: We present clinical and genetic characteristics of female patient 3-year old with Luscan-Lumish syndrome. The girl was born at term following a normal pregnancy. She is the only child of healthy unrelated parents. Her birth weight was 3740 g, length 57 cm, and OFC 35 cm. Echocardiogram reveals patent ductus arteriosus. The patient was developmentally delayed and did not walk until 18 months of age. Speech is absent. By 2-year old girl appeared trichotillomania. Eruption of secondary teeth begun by 2,5-year old. Now her height is at the 75^{th} percentile (98 cm), weight is at the 97^{th} percentile (17.3 kg). She has macrocephaly with a head circumference greater than the 97^{th} percentile (55 cm). A MRI of the brain at 3-year old revealed ventriculomegaly. An EEG is normal. A phenotype features include macrocephaly, hypertelorism, wide nasal bridge, long nose. Behavioral manifestations are anxiety, obstinacy, impaired communication and limited eye contact. **Results:** Exome sequencing detected c.1102C>T (p.Arg368Ter, NM_014159.6) mutation in heterozygous state in SETD2 in the proband that was not present in either parent and was confirmed with dideoxy sequencing. **Conclusions:** Our clinical case describes the phenotype associated with mutation in SETD2 and contributes to the evidence base the role of this gene in intellectual disability.

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P08.43C

Description of *MED13L* haploinsufficiency syndrome in early adulthood and report of a recurrent missense mutation

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We previously described the recognizable *MED13L* haploinsufficiency syndrome which has been further characterized after reports of additional patients diagnosed by genome-wide testing. Here, we present two new patients, highlighting new aspects of the syndrome. The first patient was a 14-year-old girl with moderate intellectual disability (ID) and facial features of low set ears, horizontal eyebrows, upslanting palpebral fissures, midfacial hypoplasia, bulbous nose, and hypotonic mouth with protruding large tongue. Her features had led to the primary suspicion of Kleefstra syndrome at early adulthood but whole exome sequencing (WES) revealed a de novo frameshift mutation in *MED13L*. The second patient was a 6.5-year-old boy who presented with global developmental delay, absent speech, and some clumsiness as well as facial features of squared, low set ears, mild ptosis, flat malar region, and mild broadening of the nose. WES revealed a deleterious de novo missense mutation in *MED13L*, identical to the mutation previously reported in an ID patient. Notably, our in silico modelling predicted the missense mutation to decrease the stability of an alpha-helix and thereby affecting the MED13L secondary structure. These findings, therefore, highlight the importance of MED13L de novo missense mutations causing ID and suggest a potential mutational hotspot along the gene. However, further patients are needed for better genotype-phenotype correlation among missense mutations. Considering all the reported patients so far, *MED13L* haploinsufficiency syndrome appears to be a frequent cause of undiagnosed ID with facial dysmorphism which should be considered in the differential diagnosis of 22q11.2, 1p36 deletion and Kleefstra syndromes.

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P08.44D

Genotype and phenotype features of 22 patients with intellectual disability caused by *MED13L* variations

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Introduction: MED13L pathogenic variations have been reported in patients with moderate intellectual disability (ID), hypotonia, and dysmorphic facial features with or without congenital heart defects. Here, we report a series of 22 patients with MED13L variations or deletions and compare data to previously reported cases.

Patients and methods: An international cohort has been formed with point variations involving MED13L identified by next generation sequencing for 18 patients or intragenic deletions identified by CGH-array for 4 patients.

Results: We identified 4 intragenic deletions, 3 nonsense, 9 frameshift, 2 splice-site and 4 missense variations. Truncating mutations were distributed along the entire gene. Missense variations were grouped in exons 15, 16 and 30. The severity of the MED13L phenotype was similar between the missense variation group and the truncating variation group. All patients presented developmental delay (DD) or mild to severe ID. Speech delay was major with an extremely poor language. Absence of language was noticed

in 30% of patients. Neurological evaluation revealed global hypotonia and ataxia. We observed common facial presentation with a bulbous nasal tip, depressed nasal bridge and bulbous nasal bridge, hypotonic open-mouth, deep philtrum and cupid-bow upper lip, bitemporal narrowing, and high forehead. Among ten patients who had a cardiac echography, one had a patent foramen oval.

Conclusions: MED13L patients share a common phenotype with moderate to severe DD/ID, speech impairment and facial dimorphism. The type of mutation does not appear to interfere with the severity of the phenotype.

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P08.45A

Genetic studies of microcephaly

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Microcephaly is an abnormally reduced head size and estimated to affect 1:6250 to 1:8500 live births. It can be caused by non-genetic factors, but most cases are believed to have genetic etiologies. However, only a proportion is usually clarified by routine diagnostic evaluation, while the majority remain undiagnosed. In this study, we aimed to identify genetic causes for a cohort of 50 patients with microcephaly using chromosomal microarray analysis and trio whole exome sequencing. Until now, 44 patients have been analyzed. Pathogenic variants were identified for 4 patients with primary microcephaly affecting genes ASPM, CDK5RAP2 (2 patients) and CENPJ (9.1%), and for 10 patients with secondary microcephaly (22.7%) affecting genes CASK, PQBP1, SLC9A6, KMT2A, ERCC6, TRAPPC9, KARS, PTPN11, TRMT10A and TRIO. While mutations in CASK, KMT2A, TRIO and PTPN11 were de novo, those in the other genes were inherited in a recessive or X-linked pattern. Potentially pathogenic variants in novel candidate genes, which were predicted to be deleterious and affect genes with microcephaly-related functions, were determined for 10 patients (22.7%). To elucidate the cellular impact of these variants, subsequent molecular biological analyses will be performed. We expect that the findings will help improve disease management and genetic counseling, and will further expand our knowledge of human brain development.

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P08.46B

Phenotypes in siblings with homozygous mutations of TRAPPC9 and/or MCPH1 support a bifunctional model of MCPH1

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Autosomal recessive primary microcephaly (MCPH) is a genetically heterogeneous subgroup of autosomal recessive intellectual disability (ARID), where the size of the brain is very small since birth. *MCPH1* is an intriguing MCPH-causing gene, playing a role at the centrosome as well as in DNA Damage Repair (DDR), and it is not clear which of these two mechanisms causes MCPH in man. Complementation studies showed that the N-terminal BRCT1 domain was required for centrosomal localization while BRCT2 and BRCT3 were responsible for DDR. TRAPPC9 is involved in vesicular trafficking, and truncating *TRAPPC9* mutations have been reported in 5 ARID families.

We here report on a family where two siblings with MCPH were homozygous for *TRAPPC9* (p.L178P) and *MCPH1* (p.R741X) mutations. Brain MRI showed anomalies associated with the TRAPPC9 defect, supporting the implication of that gene, and this is the first report with a

missense mutation, furthermore with microcephaly of prenatal onset. Importantly, an asymptomatic sister with normal head size was homozygous for the *MCPH1* truncating mutation and did not carry the *TRAPPC9* mutation. The *MCPH1* mutation in both affected siblings might, at first, have been considered as causal, and this family illustrates that the clinical interpretation of genetic variants remains error-prone. More importantly, our observation in the normal sister is definite evidence that the lack of MCPH1 BRCT3 domain does not cause MCPH in man, and supports a bifunctional model of MCPH1 where the centrosomal function only is involved in brain volumic development. Supported by EraNet ERare.

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P08.47C

Is there a mirror phenotype for 2p15p16.1 microdeletion syndrome

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Introduction: The 2p15p16.1 microdeletion syndrome has been described in 33 cases (Bagheri et al., 2016). Its core phenotype includes intellectual disability, microcephaly, hypotonia, delayed growth, common craniofacial features, and digital anomalies. Patient cell line and zebrafish studies identified three candidate genes *XPO1*, *BCL11A*, and *REL*. Their knock-down in zebrafish caused microcephaly, dysmorphic body, hindered growth, small fins and structural brain abnormalities. It was recently suggested that 2p15p16.1 microduplication causes mirror phenotypes and macrocephaly in carriers (Loviglio et al., 2016). Our aim was to test this possibility in a larger number of patients with the duplication and by overexpression of the 3 candidate genes in zebrafish. **Methods**: Clinical and genomic information for patients with 2p15p16.1 microduplication was extracted from DECIPHER. Overexpression of *XPO1*, *REL* and *BCL11A* in zebrafish embryos was performed by human RNA injection in 1-cell stage embryos. Head size, growth and body morphology was examined at 2 days post-fertilization. **Results**: Twelve patients were reported in DECIPHER with 2p15p16.1 duplications and phenotypes and only 1 had macrocephaly. Patients with microcephaly were also noted in this cohort ruling out a clear association of duplication and macrocephaly. Overexpression of *XPO1* and *REL* in zebrafish did not cause phenotypic abnormalities, while *BCL11A* overexpression caused a hindered body growth and dysmorphic body trunk, but comparable head structure and size to controls. **Conclusion**: Our studies do not support the existence of mirror phenotypes in cases with 2p15p16.1 duplication. The overexpression of *BCL11A*, however, may be associated with body dysmorphology but not head size anomaly.

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P08.48D

Homozygous METTL23 mutations cause mild autosomal recessive intellectual disability with dysmorphic features : A new clinical entity

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Intellectual disability is a neurodevelopmental disorder that affects 1 to 3% of the population worldwide. If enviromental factors could explain the intellectual disability in some cases, the remaining majority (up to 60%) has no identifiable cause and about 25 to 50% are thought to have a genetic cause.

This impairement can be grouped into isolated (non-syndromic) or syndromic intellectual disability where patients present with other clinical features in addition to intellectual impairement. Most known non syndromic intellectual disability are X-linked, while the number of autosomal genes related to this disorder is growing rapidly. However, it is difficult to identify additional subtle clinical signs and describe a clear phenotype with the scarcity of similar cases with the same genetic etiology.

We report on the case of 2 moroccan siblings presenting mild intellectual disability with minimal dysmorphic features in which whole exome sequencing analysis revealed homozygous mutation in the *METTL23* gene. Mutations in this gene have been reported to cause mild intellectual disability but the association with dysmorphic features remains controversial.

Hereby, we highlight the similarity of the dysmorphic traits and the characteristic facial features in patients with *METTL2*3-related intellectual disability, suggesting a new

clinical entity associating mild intellectual deficiency with facial dysmorphy for an efficient diagnostic orientation and a better phenotype-genotype correlation in intellectual disability disorders.

W. Smaili: None. **S. Chafai Elalaoui:** None. **A. Zrhidri:** None. **J. Lyahyai:** None. **L. Raymond:** None. **G. Egéa:** None. **M. Taoudi:** None. **S. EL Mouatassim:** None. **A. Sefiani:** None.

P08.49A

De novo mutations of *MYT1L* in individuals with intellectual disability

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Microdeletions of chromosome band 2p25.3 have been reported in more than 20 patients. Common clinical features include intellectual disability (ID) /developmental delay, central obesity and behavioural difficulties. *MYT1L* is deleted or disrupted in all published patients and thus became the main candidate gene for these clinical features. However, only two patients with *de novo MYT1L* point mutations have been reported.

Here, we present two novel patients with *de novo MYT1L* sequence variants we identified by trio whole exome sequencing in a cohort of 311 individuals with ID of unknown aetiology. Patient 1 carried a nonsense mutation (c.1531G>T, NM_015025.2; Gly511*) whereas patient 2 carried a direct splice site mutation (c.2769-2A>G). According to prediction algorithms, both *MYT1L* variants are deleterious (patient 1: SIFT score 0, CADD score 42; patient 2: CADD score 24.6). Additionally, patient 2 carried a *de novo* variant in *SETD1B* that is predicted to be benign (CADD score 2.5) and a known frequent SNV (rs749218728, MAF 0.0000323).

A comprehensive clinical characterisation of the two patients yielded only mild to moderate ID, behavioural problems and muscular hypotonia as common clinical signs. Surprisingly, obesity was only present in patient 2. Tall stature and microcephaly were present in one patient each. This clinical picture is compared to the published phenotypes of patients with *MYT1L* point mutations, with microdeletions of *MYT1L* only and with larger 2p25.3 deletions. With the reduced penetrance regarding obesity shown here, the clinical picture of *MYT1L* is becoming more and more unspecific.

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P08.50B

Neurodevelopmental disorders linked to Aristaless homeobox gene: A "fault disease model"

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Studying molecular convergence in neurodevelopmental disorders caused by mutations in specific disease-related genes permits us to define druggable molecular pathways. The purpose of our study is to assess the degree of damage associated with the ARX-KDM5C and to establish a correlation between similar phenotypes and same cellular functions. Mutations in *ARX*, a homeotic transcription factor, have been found in a spectrum of X-chromosome phenotypes including cortical malformations, chronic Epilepsy and XLID. About *KDM5C*, its mutations have been reported as an important cause of XLID. Its protein is a histone demethylase acting as transcriptional repressor during brain development. Here we summarize *in vitro* and *in vivo* functional analysis of two classes of ARX mutations by studying the impact on the stimulation of *KDM5C*, already reported by us as an ARX disease-target gene. We have proven that PolyAlanine elongations are partial loss-of-function mutations that impair the activation of KDM5C transcription; while HD missense mutants are loss-of function alterations, which abolish the transcriptional activity attributable to the WT protein. Since H3K4me3 is the hallmark of open chromatin, ARX-dependent KDM5C defects could compromise chromatin remodelling. We propose a "fault disease model" showing that the degree of spectrum of KDM5C defects correlate with the severity of the neurophenotypes associated with ARX mutations. Modelling ARX and KDM5C defects in the identical stem cell line might allow us to assess the degree of molecular

convergence caused by altered dosage of these two disease-related genes and the *in vivo* validation of potential drugs that could compensate KDM5C-H3K4me3 deregulation.

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P08.51C

A *de novo* intragenic deletion in *TANC2* in a girl with clinical features of the Angelman-Rett continuum

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Background: Neurodevelopmental disorders are a group of clinically and etiologically heterogeneous conditions often characterized by comorbidity of intellectual disability (ID) and Autism Spectrum Disorders (ADSs). These conditions show a wide range of overlapping features that may represent a serious difficulty for diagnoses exclusively based on clinical grounds. Elucidating the etiology offers insights into the pathogenic mechanisms and allows a better understanding of the clinical expression of these diseases. **Methods**: We report the clinical features of a female subject affected with severe global developmental delay since early infancy; the clinical picture evolved into a profound intellectual disability and motor impairment, absent speech and autistic traits with hand stereotypies, constipation, sleep and autonomic disturbances and facial dysmorphic features.

Results: an intragenic deletion in the coding sequence of the *TANC2* gene (OMIM *615047) was identified by Array CGH. The alteration was confirmed by quantitative PCR analysis and its limits refined by transcript analysis. Deficiency of the TANC2 protein, a synaptic protein widely expressed throughout the brain has been found to be embryonic lethal in mice; a few structural alterations of *TANC2* are listed in genomic databases but specific phenotype correlation have not been reported.

Conclusions: we describe for the first time the clinical phenotype associated with a pathogenic mutation of the TANC2 gene. Our findings contribute to defining the pathogenic role of *TANC2* and underline the importance of considering this gene as possibly involved in otherwise unexplained neurodevelopmental disorders characterized by intellectual disability, autistic traits and clinical features of the Angelman-Rett spectrum.

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P08.52D

19p13 duplications including *NFIX* cause intellectual disability, growth retardation and microcephaly: a reversed Sotos syndrome-2?

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NFIX gene codes for a transcription factor implicated in neurogenesis and chondrocyte differentiation. Microdeletion and loss of function mutations of *NFIX* are responsible for the Sotos syndrome-2 (also described as Malan syndrome), a syndromic form of intellectual disability associated with overgrowth, advanced bone age and macrocephaly. We report here a cohort of 9 patients with 19p13 microduplications including *NFIX*. These patients exhibit variable intellectual disability, short stature with bone age delay, and microcephaly, which can be described as a reversed Sotos syndrome-2 phenotype. The opposite effects of *NFIX* haploinsufficiency and overexpression could explain these mirror phenotypes associated with deletions and duplications. There is evidence in the literature suggesting that NFIX is a negative regulator of endochondral ossification, and that tall stature of patients with

Sotos syndrome-2 could be related to a decrease of this repression. Conversely, *NFIX* overexpression in patients with duplication could lead to increased repression, explaining the short stature. It is striking that a similar reversed phenotype has already been described in patients with duplication encompassing *NSD1*, the gene which deletions and loss of function mutations are responsible for classical Sotos syndrome. Even though the *type/contre-type* concept is controversial, this model seems to give a plausible explanation for the pathogenicity of the 19p13 duplication, and the common phenotype observed in our cohort.

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P08.53A

De novo mutations in *NOVA2*, a RNA-binding protein, cause intellectual disability with growth retardation and epilepsy

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Intellectual disability (ID) is a common neurodevelopmental disorder, characterized by a high genetic heterogeneity, with more than 700 genes described to be involved in monogenic forms. We performed a whole exome sequencing on a patient affected by intellectual disability, growth retardation, microcephaly, epilepsy, subcortical atrophy and traits of pyramidal syndrome as main features. We identified a de novo loss-of-function (LoF) mutation in *NOVA2*, a gene which has never been implicated in ID before and highly intolerant to LoF variants (from ExAC data). NOVA2 is a neuron specific RNA-binding protein that regulates alternative-splicing events during brain development. Knockout mice for this gene showed an overall motor weakness and dysfunction, a failure to thrive and they died shortly after birth (*Saito* et al. *2016*). Through data exchange we have been able to identify a second patient with a de novo LoF mutation in *NOVA2* presenting with similar phenotype. To further confirm the role of *NOVA2* in ID, we have undertaken to knock-down the gene in zebrafish. In parallel, we are investigating the effect of *NOVA2* silencing on cell proliferation and splicing regulation in human neuronal stem cells, which has never been reported.

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P08.54B

Clinical delineation of the recurrent de novo c.607C>T mutation in PACS1

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We have previously described two unrelated boys with a strikingly similar facial appearance and intellectual disability in whom an identical de novo mutation in PACS1, c.607C>T, was detected by exome sequencing (NM_0180026.2, OMIM 615009) [1]. In vitro and in vivo studies showed that the mutant protein forms cytoplasmic aggregates with concomitant increased protein stability. Further, expression studies of mutant PACS1 mRNA in zebrafish embryos suggest that the mutant zebrafish phenotype is driven by aberrant specification and migration of SOX10-positive cranial neural crest cells, probably explaining the craniofacial phenotype in patients. Since this initial report, we have collected clinical information on 19 individuals with this identical mutation [2]. Social media (facebook) has shown its value in collecting additional patients with this rare genetic disorder. There is a distinctive facial appearance (19/19). Intellectual disability (mild-moderate) was present in all. Hypotonia is common in infancy (9/19). Seizures are frequent (12/19) and respond well to anticonvulsive medication. Structural malformations are common, including heart (10/19), brain (12/15), eye (9/19), kidney (3/19) and cryptorchidism (6/12 males). Feeding dysfunction is presenting in infancy (6/19). Six individuals had gastroesophageal reflux and five needed gastrostomy tube placement. There is persistence of oral motor dysfunction. In summary, this recurrent de novo c.607C>T mutation in PACS1 gives rise to a clinically recognizable syndrome. We provide suggestions for clinical work-up&management. Given the recognizable facial gestalt, we hope that the present study will facilitate clinical recognition of further cases.

1. Schuurs-Hoeijmakers et al. AJHG, 2012
2. Schuurs-Hoeijmakers et al. AJMG, 2016

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P08.55C

Two different additional mutations in TBL1XR1 identified in patients with Pierpont syndrome, as opposed ton only recurrent one previousely described

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Introduction: Pierpont syndrome is a rare and sporadic syndrome, including developmental delay, facial characteristics, and abnormal extremities. Recently, a recurrent neomutation c.1137A>C; p.Tyr446Cys in TBL1XR1 was identified in 6 patients by whole-exome sequencing. A dominant-negative effect of this mutation is strongly suspected, and patients with TBL1XR1 deletion and other doesn't share the same phenotype.

Material and methods: The first patient is a 14-year–old Caucasian boy with severe global developmental delay. He walked unaided until 3 years old, does not speak, has autistic features, severe sleep disturbance, facial and extremity features of Pierpont syndrome, and severe postnatal growth retardation (height at -3SD whereas OFC at +0.5SD). The second patient is a 24-year-old Caucasian female who has developmental delay, Arnold Chiari malformation, facial characteristics of Pierpont syndrome and severe behavioral disorders. Her height is at -1.5 SD.

Whole-exome sequencing was performed for both patients.

Results: We identified a heterozygous missense neomutation in TBL1XR1 in each patient different from that reported in literature, namely c.974 G>A; p. Cys325Tyr for patient 1 and c.1336T>C; p.Tyr446His for patient 2. The second mutation is localized in the same nucleotide as the recurrent mutation. The first one is localized in another

nucleotide but concerns the protein's same functional and physical domain. The software PolyPhen-2 predicts pathogen mutations.

Discussion: The localization of these mutations and clinical features of Pierpont syndrome suggest that their functional consequences are the same as the recurrent mutation previously described and provides additional data to understand molecular mechanisms of TBL1XR1 anomalies.

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P08.56D

The long and winding road to the diagnosis of 33 patients with intellectual disability/developmental delay by target/exome sequencing: the significance of re-evaluation of both phenotypic and genotypic data

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Introduction: It has been demonstrated that diagnosis of intellectual disability/developmental delay (ID/DD) benefits from clinical application of target/exome sequencing. The yield varies from 16%-50%. Materials and Methods: Target/exome sequencing was performed on 33 ID/DD patients. Excluded criteria: proband (i)with pathogenic CNVs found by microarray; (ii)with acquired brain damage; (iii)abnormal metabolic screening. First-round data analysis: SNV/indel was called by GATK. OMIM data was downloaded in May 2016. Second-round data analysis: CNV was calculated by XHMM. OMIM data was updated to January 2017 version. The candidate mutations were returned to the clinician. If necessary, the patients were invited to the clinic for a second examination. **Results:** Molecular diagnosis was obtained in 20/33 patients(60%). Notably, seven cases (21%) were solved during re-evaluation of geno-/phenotypic phase. Compound heterozygous mutations in *UNC80* gene were detected in two sporadic patients. The causal ID disorder was not added to the OMIM database in the first-round analysis. One patient carry 3.8 kb exonic deletion in *ARID1B* gene detected by XHMM. No probe locate in this region in microarray. Two patients, with *KMT2D* and *ATRX* mutation respectively, only showed ID/DD in their first visit to clinic. A followup evaluation at elder age revealed their distinct features. Two *CREBBP* mutations were found in two patients. They represent a unique entity caused by defects in exon 30/31 of *CREBBP* gene. **Conclusions:** Our study confirmed the utility of exome sequencing in the diagnosis of ID/DD. Furthermore, re-evaluation of patients' phenotype and genotype lead to a 21% improvement of yield.

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P08.57A

Genetic diagnosis of rett syndrome by *Next Generation Sequencing*

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Objectives: Rett syndrome (RTT) is an early-onset neurodevelopmental disorder that almost exclusively affects girls and is totally disabling. It had been described 3 genes that cause RTT. However, the etiology of the 15% of these patients still remains unknown.During the latest years the NGS has allowed to promote the genetic diagnosis because of the quickness and affordability of the method. To evaluate the usefulness of the NGS in the genetic diagnosis, we present the genetic study of Rett-like patients using different techniques based on this technology.

Material and Methods: It has been studied 1659 patients with Rett-like clinical diagnosis as well as it has been reviewed patients who was previously studied by Sanger Sequencing in the 3 Rett genes. It has been performed: Custom panel with 17 gens related to Rett-like clinic through *HaloPlex Target Enrichment System, for Illumina Sequencing;* Commercial panel, *TruSightOne Sequencing Panel* (*Illumina*); Whole Exome Sequencing (WES) in trios with *TruSeq Sample Preparation Kit (Illumina)*. **Results:** It has been diagnosed genetically 477/1659 patients with a Rett-like suspicion. It has been positive results in a 30% by Sanger (100% Rett genes), 23% by custom panel (58% Rett genes), 24% by TSO (25% Rett genes) and 32% by WES (25% Rett genes).**Conclusions:** The genetic study by NGS allows to study a larger number of genes associated with Rett-like clinic simultaneously,

providing a genetic study to a wider group of patients. Finally, the study of the progenitors remains essential for their characterization as well as functional studies.

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P08.58B

Rett syndrome genes - molecular screening methods changes the Danish RTT cohort and challenge the clinical criteria

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Introduction: The neurodevelopmental disorder Rett syndrome (RTT) was first described by Dr. Andreas Rett in 1966. Currently, the diagnosis of both typical and atypical RTT, is based on the clinical consensus criteria defined in 2010. A molecular finding is only considered confirmatory of the clinical diagnosis. Most patients with molecularly verified diagnoses have pathogenic variations in either *MECP2, CDKL5* or *FOXG1* but an increasing number of genes associated with RTT like conditions are reported. An optimal diagnostic strategy for patients with RTT, is important for both clinicians and families. **Materials and Methods**: We have reviewed clinical and molecular data of all the Danish patients with pathogenic variations in *MECP2*, *CDKL5* and *FOXG1*. **Results:** All 115 patients with *MECP2* variations were molecularly diagnosed with a single gene analysis. *CDKL5* variations were detected using a single gene analysis only in 4/11 of patients. The remaining *CDKL5* variations and all 4 *FOXG1* variations were identified by screening techniques. All patients who fullfilled the clinical criteria of typical RTT carried a *MECP2* variation, while patients with C*DKL5* and *FOXG1* variations had some RTT symptoms, but were not typical. **Conclusion:** The survey indicates that large scale screening methods will greatly increase the discovery of patients with *CDKL5* and *FOXG1* variations. In our cohort patients with *CDKL5* and *FOXG1* sequence variations do not all fit the current clinical criteria for atypical RTT. We propose to expand a RTT spectrum and include both the clinical and the molecular diagnosis.

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Detection of a *de novo* mosaic *MECP2* mutation in a patient with Rett syndrome phenotype

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Rett syndrome is a progressive neurodevelopmental disorder accounting for a large portion of intellectual disability (ID) in females. It is most frequently caused by mutations or deletions in MECP2 gene that codes for the methyl-CPG-binding protein 2 and is located on Xq28. Here we present an interesting case of a novel mosaic MECP2 point mutation found in a patient with a typical Rett phenotype.

The patient is a female with autistic features, psychomotor delay, speech regression and stereotypic hand movements, initially referred for copy number screening to investigate Angelman or Rett syndromes. The results of MLPA analysis were negative and subsequent Sanger sequencing of MECP2 gene revealed a mosaic C→T nonsense mutation at position 139 of exon 3. A second round of Sanger sequencing with region-specific primers confirmed the presence and the mosaic state of the mutation. The same primers were used to test the biological parents, who were found negative. Restriction enzyme analysis gave normal results for both parents and was consistent with a mosaic mutation in the patient. In order to estimate the level of mosaicism, Next-Generation Sequencing was performed, showing approximately 25% abnormal cells.

This is the first known female mosaic MECP2 mutation carrier, who is exhibiting the full spectrum of Rett

syndrome phenotype. The specific mutation was not previously detected in other patients.

Further studies including a buccal swap are currently ongoing and are expected to provide insights into tissue specificity, thereby allowing for a better understanding of the patient's phenotype and relevant mechanisms.

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P08.60D

New *MEF2C* mutations in Rett-like phenotype patients

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Objectives: Rett syndrome (RTT) is an early-onset neurodevelopmental disorder that almost exclusively affects girls and is totally disabling. It had been described 3 genes that cause RTT: *MECP2*, *CDKL5* and *FOXG1*. However, the etiology of the 15% of these patients still remains unknown. During the latest years the Next Generation Sequencing (NGS) have allowed to sequence a large number of genes at the same time. In this way, several genes have been implicated in RTT typical and variant forms. Here, we present 4 patients with 3 mutations in *MEF2C* gene that related to the *MEF2C* haploinsufficiency syndrome. **Material and Methods:** It has been studied 242 patients with Rett-like clinical diagnosis as well as it has been reviewed patients, who was previously studied by Sanger Sequencing in the 3 Rett genes, using the *TruSight One Sequencing Panel* (*TSO Illumina*). Moreover, it has been performed Sanger sequencing of the found mutations in patients and their progenitors. **Results:** Genetic diagnosis was achieved in 55 of 242 patients (23%). The 60% of these positive results have mutations in Rett genes (*MECP2*, *CDKL5* and *FOXG1*) and the other 40% mutations in Rett-like genes. We herein present 3 novel mutations and presume pathogenic in *MEF2C* gene. **Conclusions:** The genetic study by NGS allows studying a larger number of genes associated with Rett-like clinic, providing a genetic study to a wider group of patients. *MEF2C* haploinsufficiency could phenotypically overlaps with RTT, especially the early onset seizure variant.

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Phenotype and genotype of 72 patients with clinical diagnosis of Rubinstein-Taybi syndrome

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Introduction RSTS is a rare autosomal dominant neurodevelopmental disorder characterized by broad thumbs and halluces. RSTS is caused by mutations in *CREBBP* and *EP300* genes in 50–60% and 10% of cases, respectively. To date, about 230 causative mutations in *CREBBP* and 76 *EP300*-patients have been described. **Materials and Methods** A total of 72 individuals with the suspected diagnosis of RSTS were recruited. DNA was obtained from blood of patients and their parents, when possible. *CREBBP* and *EP300* MLPA, panel based-NGS of *CREBBP* and *EP300* genes and Sanger sequencing confirmation were carried out. Sequence changes were compared to the parents. **Results** We found *CREBBP* mutations in 35 patients (49%), *EP300* in 8 (11%), and nothing in 22 (30%). Other syndromes were confirmed in the rest (10%). Variants detected were (*CREBBP*/*EP300*): large deletions (11/1), nonsense (12/1), missense (4/1), frameshift (5/4) and splicing (3/1). Relating to phenotype, ID was more severe in *CREBBP* patients, as well as the psychomotor, language delay and the behavioral problems. Typical features such as, broad thumbs and downslanted palpebral fissures were

detected equally in both, and angulated thumbs were rare in *EP300*-patients. In contrast, the presence of microcephaly was higher in *EP300*-patients. **Conclusions** Phenotypic spectrum of RSTS patients is wide, although it is usually less marked in *EP300*. It is difficult to establish a correlation genotype-phenotype. The description of more RSTS patients may contribute to better understand the range of phenotypes in order to provide clinical pointers that would improve the detection and diagnosis of these patients.

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P08.62B

Phenotypic expansion of the autosomal recessive *KIF14* related disorder

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Kinesin proteins are critical for various cellular functions such as intracellular transportation and cell division, and have been linked to monogenic disorders and cancer. Filges et al. (2014) previously reported a *KIF14*-related lethal fetal ciliopathy phenotype in one single family (OMIM#616258): The affected fetuses, who were compound heterozygous for two *KIF14* frameshift mutations, had severe IUGR, microcephaly, cerebral and cerebellar hypoplasia, renal and uterine agenesis/hypoplasia, arthrogryposis and dysmorphic facial features. The corresponding mouse model, *laggard* (*lag*), carrying a spontaneous *Kif14* splice site mutation, has growth retardation, brain size reduction, and severe hypomyelination of the central nervous system including optic nerve hypoplasia (Fujikura et al. 2013). Both the *lag/lag* and the conditional *Kif14* knockout mice die before weaning.

We report here two consanguineous families with homozygous variants in *KIF14* (NM_014875.2): One with missense c.2522C>T, p.(Ser841Phe), and one with frameshift c.246delT, p.(Asn83Ilefs*3), both variants detected by WES and familial segregation with the phenotype confirmed. The four affected individuals presented with intellectual disability, hypotonia, similar dysmorphic facial features, proportionate short stature, but had normal birth weight. The missense mutation siblings have normal head circumference at age 5y and 13y, respectively. While the siblings with the frameshift mutation, presently 23y and 30y, have severe microcephaly with prenatal onset, optic nerve hypoplasia, blindness, and microphthalmia.

Our report demonstrates phenotypic expansion of the *KIF14*-linked autosomal recessive Mendelian disease, ranging from a lethal fetal disorder with severe brain growth restriction and IUGR to a congenital syndrome with developmental delay/intellectual disability, postnatal short stature, +/- microcephaly and blindness.

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Analysis of 31-year-old patient with *SYNGAP1* gene defect points to importance of variants in broader splice regions and reveals developmental trajectory of *SYNGAP1*-associated phenotype

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We report an adult female patient with severe intellectual disability, severe speech delay, epilepsy, autistic features,

aggressiveness, sleep problems, broad-based clumsy gait and constipation. Whole exome sequencing identified a *de novo* mutation in the *SYNGAP1* gene. The variant was located in the splice donor region of intron 10 and replaced G by A at position +5 of the splice site. The variant was predicted *in silico* and shown experimentally to abolish the regular splice site and to activate a cryptic donor site within exon 10, causing frameshift and premature termination. This observation underscores the importance of considering not only variants in the canonical splice dinucleotides GT and AG, but also in broader splice regions. Clinical picture of the patient corresponded well with the characteristic *SYNGAP1*-associated phenotype observed in previously reported patients. However, our patient was 31 years old which contrasted with most other published *SYNGAP1* cases who were much younger. She had a significant growth delay and microcephaly although both features normalised later. The cognitive and language performance remained at the level of a one-year-old child even in adulthood and showed a slow decline. Myopathic facial features and facial dysmorphism became more pronounced with age. Although the gait of the patient was unsteady in childhood, more severe gait problems developed in her teens. While the seizures remained well-controlled, her aggressive behaviour worsened with age and required extensive medication. These observations contribute to the knowledge of the developmental trajectory in individuals with *SYNGAP1* gene defects. Supported by 00064203, CZ.2.16/3.100/24022, NF-CZ11-PDP-3-003-2014, LM2015091.

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P08.64D

TARP syndrome - first reported adult patient

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Introduction: TARP syndrome is a rare X-linked syndrome with intellectual disability, **T**alipes equinovarus, **A**trial septal defect, Pierre **R**obin sequence, and **P**ersistent left superior vena cava. Other symptoms include pulmonary hypoplasia, syndactyly, polydactyly, and hypotonia. TARP syndrome was first described in 1970. In 2010, *RBM10* at Xp11.23 was identified as the responsible gene. 15 patients from 6 families have previously been reported. Most reported patients died in infancy or early childhood. We present the first reported adult with TARP syndrome.

Material and Methods: We report a 27-year-old male with atrial septal defect, Pierre Robin sequence, severe intellectual disability, and syndactyly of the second and third toe. Persistent left superior vena cava and talipes equinovarus were not reported in this patient. The index person also had posterior sloping of the forehead, prominent nasal bridge, long and prominent nose, downturned medial canthi, esotropia, severe myopia, small hypoplastic teeth, and severe scoliosis. These features have not previously been reported in this syndrome.

Results: Sequencing of a diagnostic exome panel containing 749 known intellectual disability genes revealed a hemizygous mutation in *RBM10*, NM_005676.4: c.273_283delinsA. This frameshift mutation has not previously been reported. The mutation was verified by Sanger sequencing and was not found in DNA from the mother of the index person.

Conclusion: This is the first report of an adult with TARP syndrome. The c.273_283delinsA mutation is the first reported in exon 4 of the *RBM10* gene. Exon 4 is skipped in some isoforms suggesting a possible cause for the milder phenotype.

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P08.65A

Most severe case of thyroid-alpha-receptor deficiency in a female patient with severe growth and mental retardation, macrocephaly, pubertas tarda and dysgerminoma

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We report on a 19-year old female; the second child of non-consanguineous German parents. She was born at term with normal measurements but macrocephaly. A meconium ileus, malrotation and umbilical hernia were present at birth. A small ASD closed spontaneously. She developed epileptic spasms at 4 months of age and at 8 years of age she developed atonic seizures with no reoccurrence under therapy. cMRI showed myelination disorder. She walked at age 6. A dysgerminoma was detected at age 14 and was treated with salpingo-ovarectomy and chemotherapy. The patient has hypothyroidism and is treated with L-Thyroxin. Her menstruation started with 18 years, she got no pubic hair yet.She is very small (17 cm < 3. centile), has a disproportionate stature and mild muscular hypotonia. Dysmorphic features include coarse face, macrocephaly, macroglossia, hypertelorism, smooth philtrum, thin upper lip vermillion, wide nasal base and ridge and downslanting palpebral fissures. She has short, broad thumbs, brachydactyly V and flat feet. She has severe mental retardation with minimal understanding and developed no expressive speech. She has a cheerful temper. Exome sequencing was performed, and led to the identification of a previously unreported deletion in the THRA gene (c.1150del, p. Arg384Alafs*5). There are several adjacent deletions known. Segregation analysis in the parents confirmed a de novo mutation and the diagnosis of Thyroid-alpha-receptor deficiency in the patient. The patient has a more severe phenotype (in particular mental retardation and dysmorphisms) then previously reported cases. It remains unclear whether the dysgerminoma is part of the disease.

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P08.66B

TRIO variants in individuals with variable intellectual deficits

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The TRIO gene has been recently associated to autosomal dominant mental retardation (MRD44), characterized by mild intellectual deficits (ID), distinctive facial features and digital abnormalities. Mutations in the RhoGEF1 domain alter GTPase activation and cause a distinct phenotype including microcephaly, ID, behavioral difficulties and specific digital features.

We analyzed the 57 TRIO exons with amplicon-based next-generation sequencing in 76 unrelated individuals with apparently non-syndromic ID and autism spectrum disorders. On average, 94% of the target regions have a read depth of at least 20×, with mean depth of coverage of 250x for each individual.

We identified eight *TRIO* rare single nucleotide variants (SNVs), of which seven are non-synonymous. A SNV resulting in a stop codon in the RhoGEF1 domain was identified in a boy with learning difficulties, mild ID, and subtle dysmorphic features, maternally inherited. A novel missense variant mapping on the RhoGEF1 domain, was found *de novo* in a boy with severe ID, speech failure, and corpus callosum agenesis, but neither microcephaly nor autistic features. Other SNVs map to Spectrin repeats or the C-terminal domain. Three were inherited from an unaffected parent, and predictions of their pathogenicity is discordant among different computational tools.

Our findings confirm that truncating *TRIO* gene mutations are associated to mild phenotypes. However, in contrast to previously reported cases, we identified a missense mutation in the RhoGEF1 domain associated to a severe phenotype in absence of microcephaly.

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P08.67C

Study of a Hispanic Wolf-Hirschhorn´s cohort: genotype-phenotype correlation analysis

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AIM: Wolf-Hirschhorn (WHS) is a poly-malformative syndrome associated with a delay of growth/development, epilepsy, and facial dysmorphias resulting from a loss of genetic material in the short arm of chromosome-4. Our main goals are to describe social-demographic characteristics and clinics of an Argentinian-Spaniard cohort of patients with WHS; to know the prevalence of epilepsy and its electro-clinical features; to describe the possible existence of EEG-patterns; to establish a possible genotype-phenotype relationship.

Patients and methods: A cohort of Argentinian-Spaniard patients (72) with clinical/genetic diagnosis of WHS was analyzed by SNP-arrays (cytoSNP850K-Illumina). We matched general epidemiological features, clinical and genetic information for each patient. All this was under a statistical analysis at different levels.

Results: The sample showed similar clinical manifestations to other previously described ones; epilepsy affects 90% of patients (it is polymorphic), with the first crisis before the 2 years of life and significant predisposition to develop status (55%). However, 64% of the patients displayed an acceptable level of control of crisis. Thus, the level of psychomotor-development in our cohort seems to be better than other ones.

Conclusions: Socio-demographic and clinical characteristics of a one of the largest series of WHS patients characterized by high-resolution arrays were described, including the degree of psychomotor development and patients´ level dependency, the possible existence of specific EEG-patterns in this syndrome, aspects of epilepsy and its prevalence. We are establishing for the first time, a relationship between degree of psychomotor-development and certain variables related to epilepsy, and the size of the deletion.

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The importance of Array-CGH in the identification and characterization of 4p16.3 microdeletions in patients with Wolf-Hirschhorn Syndrome: A genotype/phenotype correlation attempt

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Wolf-Hirschhorn syndrome (WHS: OMIM 194190) is a contiguous gene deletion disorder caused by partial deletion in the region 4p16.3 of the short arm of chromosome 4. WHS is most often caused by *de novo* terminal deletions; although, interstitial deletions, unbalanced translocations and complex genomic rearrangements are also frequent. Clinical features of WHS patients include severe growth delay, short stature and low body weight, intellectual disability, a typical and distinctive craniofacial appearance ("Greek warrior helmet profile") and seizures (or EEG anomalies). Two critical regions have been proposed: a 165 kb proximal region (WHSCR1) and a second new critical region (WHSCR2) distal to WHSCR1 including *LETM1* (a candidate gene for seizures) and the 5´ end of *WHSC1*.

In our cohort of 1500 patients, with ID and ASD, studied by Agilent 180K oligonucleotide array-CGH, we have identified 7 new patients with 4p16.3 deletions and revisited 3 other previously detected by FISH and/or standard cytogenetics, redefining the deleted region. All patients exhibited clinical findings compatible with WHS. In two patients with 4p16.3 small interstitial deletions of 287Kb and 611Kb the WHS phenotype was incomplete. Seizures in one patient, with a deletion not overlapping *LETM1* suggest the idea that this gene alone could not be the cause of seizures in WHS.

With the implementation of Array-Comparative Genomic Hybridization the diagnostic of WHS patients can now be better characterized at a molecular level with the establishment of exact breakpoints, allowing the identification of specific regions and candidate genes for specific features and a better genotype/phenotype correlation.

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P09

Neurogenetic and psychiatric disorders

P09.001A

Mutations in CoA Synthase cause pontocerebellar hypoplasia

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Introduction: Pontocerebellar Hypoplasia (PCH) is a heterogeneous neurodegenerative disorder with a prenatal onset. Genes involved in PCH often have a function in RNA metabolism; defects in mitochondrial function have also been described. We identified mutations in the *COASY*-gene, encoding Coenzyme A (CoA) synthase, an enzyme essential in CoA synthesis, in four patients from two families with prenatal onset microcephaly, PCH and arthrogryposis.

Methods: Mutations were identified by whole exome sequencing. Functional characterization of the mutations was done by means of RNA analysis, immunoblot and determination of CoA and acetyl-CoA levels in amniocytes.

Results: In family 1, compound heterozygous mutations were identified in *COASY*: c.1549_1550delAG (p.Ser517-Profs*61) and c.1486-3C>G. In family 2, all three affected siblings were homozygous for the c.1486-3C>G mutation. In both families, the mutations segregated with the phenotype. RNA analysis in family 2 showed that the intronic variant leads to skipping of exon 7 with partial retention of intron 7, disturbing the reading frame and resulting in a premature stopcodon. Immunoblot analysis detected no CoA synthase in fibroblasts of a patient from family 2. In amniocytes from the patient from family 1, reduced CoA and acetyl-CoA levels were detected compared to controls.

Conclusions: Partial CoA synthase defects were previously described as a cause of Neurodegeneration and Brain Iron Accumulation (NBIA), and here we demonstrate that loss of function mutations in *COASY* are associated with lethal PCH and arthrogryposis. Functional characterization confirmed virtually absent CoA synthase protein levels in fibroblasts and a decrease in CoA and acetyl-CoA levels in amniocytes.

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P09.002B

Global metabolomic profiling unravels metabolite perturbations in Rett syndrome

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Introduction: Rett syndrome is a severe neurodevelopmental disorder affecting females with an incidence of 1 in 10,000 caused by loss-of-function mutations in the *MECP2* gene encoding the methyl-CpG-binding protein 2. The pathogenetic mechanisms involved in neurodevelopmental symptoms and regression of Rett syndrome are not completely understood. Metabolic alterations are emerging as important features of Rett syndrome, as loss of function mutations in squalene epoxidase (*SQLE*), the rate-limiting step in cholesterol biosynthesis, were found to ameliorate symptoms in Mecp2 null mice. Materials and Methods: For the present study, we performed global metabolome profiling using a semi-quantitative tandem mass spectrometry-based technique that measures over 700 metabolites on 13 patients with a diagnosis of Rett syndrome confirmed by *MECP2* mutations. Targeted expression and biochemical analyses were performed on lymphoblast cell lines and plasma. **Results:** Metabolomic profiling unraveled significant biochemical perturbations in nicotinamide-derived metabolites, sphinganine, and tryptophan-derived molecules, all of which have relevant roles in brain function. Moreover, we detected higher levels of *SQLE* and lower levels lanosterol synthase (*LSS*), the subsequent step to *SQLE*. Interestingly, the expression of both *SQLE* and *LSS* is regulated by *HDAC3* which is known to interact with *MECP2*. **Conclusions:** In conclusion, a comprehensive metabolomic profiling of Rett syndrome samples unraveled perturbations in tryptophan, nicotinamide, and sphingolipid metabolism. In addition, we confirmed cholesterol biosynthesis disruption. Further investigations are required to

understand the role of such perturbation in the disease pathogenesis and to investigate whether these metabolites might be effective disease biomarkers.

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P09.003C

Role of *miR-146a* in the differentiation and neural lineage identity determination of human neural stem cells: relevance for autism spectrum disorders

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Autism spectrum disorders are a group of neurodevelopmental disorders caused by the interaction between genetic, epigenetic and environmental factors. One of such epigenetic factors is microRNA (miRNA), which play key roles in neuronal development and synapse formation. We and others recently identified the upregulation of *miR-146a* as a common event in various cell types from autistic patients: olfactory mucosa stem cells, skin fibroblast and lymphoblastoid cell lines. Here, we showed by Taqman RT-qPCR that *miR-146a* is upregulated specifically in the temporal lobe of autistic children (age 4–10) and not in adolescents and adults. To understand its roles in early development, we generated human H9 neural progenitor stem cells stably over expressing *miR-146a*. We demonstrate that, in undifferentiated condition, overexpression of *miR-146a* has no effect on the proliferation and apoptotic rate of the cells. By contrast, in differentiated condition, *miR-146a* significantly enhances neurite outgrowth and branching and favors differentiation into neuronal like TUBIII+ cells. Transcriptomic analyses by RNA-Seq demonstrated that 10% of the detected transcripts were significantly deregulated in differentiated cells (P<0,05, fold change > 1,5). More importantly, 16 of 44 detectable markers for different neuronal lineages and layers were affected (*DCX, GAD1, CALB2* and *FOXB2* etc), suggesting that *miR-146a* also plays a role in neural lineage determination. These results are consistent with the deregulation of layer formation and layer-specific neuronal differentiation observed in brain cortical samples from autistic patients and give important clues about the role of *miR-146a* upregulation in the etiology of the disease. Grant references: 16FRM002_9UMF1163, ANR_SAMENTA 0211_EPI_ASD

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P09.004D

Elucidating the spectrum of protein-altering *de novo* variants in neurodevelopmental disorders with epilepsy

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We conducted exome-wide enrichment analyses of protein-altering de novo variants (DNV) in 7088 published and unpublished parent-offspring trios of neurodevelopmental disorders (ND) comprising 2151 patients with epilepsy (epileptic encephalopathy (EE) or epilepsy comorbid with developmental delay / autism spectrum disorder / intellectual disability). In patients with ND and epilepsy, we revealed enrichment in known EE genes, indicating an overlapping genetic spectrum of EE and ND with epilepsy. When stratifying the ND cohort for patients with epilepsy, we identified significant enrichment of DNV in 33 genes, seven of which with previously limited evidence for disease association. In the combined cohort of 7088 cases, we identified 84 significant genes including eleven with previously no or limited evidence for disease association. We found that genes involved in synaptic transmission were more frequently mutated in individuals with epilepsy while chromatin modification was more frequently altered in individuals without epilepsy. Moreover, we investigated a potential clinical-diagnostic impact by evaluating 24 commercial and academic sequencing panels designed for ND with epilepsy. On average 54% of the 33 significant epilepsy genes were covered by an individual panel, while 10.5% of the panel genes lacked evidence for disease association (according to variant-intolerance scores, brain expression and DNV enrichment). 29.8% of DNV in the 33 significant genes were associated with therapeutic consequences with CEBM (Centre for Evidence-Based Medicine) level of evidence of IIb or higher. In summary, we provide novel insights into the genetic architecture of ND with epilepsy as well as clinical-diagnostic implications for this heterogeneous phenotypic spectrum.

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TBCD mutations cause autosomal recessive early childhood-onset neurodegenerative encephalopathy

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Microtubules are essential components of all eukaryotic cells. They form cylindrical structures composed of rows of α/β-tubulin heterodimers which are formed with the aid of tubulin specific chaperones comprised of five tubulin folding co-factors (TBC): TBCA, TBCB, TBCC, TBCD, and TBCE. Here, we report on four families (two Japanese families, a Chinese family, and an Israeli family) with affected siblings showing early onset, progressive diffuse brain atrophy with regression, postnatal microcephaly, postnatal growth retardation, muscle weakness/atrophy, and respiratory failure. Bases on autosomal recessive model, we identified biallelic *TBCD* mutations in eight affected individuals from the four families by whole exome sequencing. A total of seven mutations were found: five missense mutations, one nonsense, and one splice site mutation resulting in a frameshift. *In vitro* experiments revealed the impaired binding between most mutant TBCD proteins and ARL2, TBCE, and β-tubulin. The *in vivo* experiments using olfactory projection neurons in *Drosophila melanogaster* indicated that the *TBCD* mutations caused loss-of-function. Furthermore, the autopsied brain from one deceased individual showed characteristic neurodegenerative findings: cactus and somatic sprouts formation in the residual Purkinje cells in the cerebellum which are also seen in some diseases associated with mitochondrial impairment. These findings might reflect structural and metabolic abnormalities resulting from disrupted mitochondrial transport in neuronal cells caused by TBCD depletion. Defects of microtubule formation caused by *TBCD* mutations may underlie the pathomechanism of this neurodegenerative encephalopathy.

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P09.007C

Characterizing and quantifying the effect of the recurrent copy number variants between BP1-BP2 at chromosome 15q11.2

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6.2% (9 of 145; 95% CI: 3.2–11.3) and 2.3% (1 of 44; 95% CI: 0.4–11.8) respectively. The average frequencies of additional clinically significant CNVs were similar in carriers of the deletion (55 of 415, 13.3%) and the duplication (26 of 223,11.7%) respectively. This is equal to the rate of any clinically significant CNVs observed in probands referred for a CGH array (10.2%). Together with the low rate of de novo*, this suggests a very mild or negligible contribution of the 15q11.2 deletion and duplication to the developmental disorders observed in proband carriers. We are currently investigating weather the rate of malformations and medical problems is higher in carriers of the 15q11.2 CNVs than what one would expect for probands referred for CGH array.*

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Improved prediction of genetic predisposition to psychiatric disorders using genomic feature best linear unbiased prediction models

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Introduction: Accurate prediction of unobserved phenotypes from observed genotypes is essential for the success in predicting disease risk from genotypes. However, the performance is somewhat limited. Genomic feature best linear unbiased prediction (GFBLUP) models separate the total genomic variance into components capturing the variance by a genomic feature (e.g. GO term) and the remaining genomic variance by differential weighting of the genetic variants within the two groups. Previously we have demonstrated (on pigs and fruit flies) increased predictive ability when the genomic feature is enriched for causal variants. Here we apply the GFBLUP model to a small schizophrenia case-control study to test the promise of this model on psychiatric disorders, and hypothesize that the performance will be increased when applying the model to a larger ADHD case-control study if the genomic feature contains the causal variants.

Materials and Methods: The schizophrenia study consisted of 882 controls and 888 schizophrenia cases genotyped for 520,000 SNPs. The ADHD study contained 25,954 controls and 16,663 ADHD cases with 8,4 million imputed genotypes.

Results: The predictive ability for schizophrenia for the null model (all SNPs weighted equally) was low (0.07). Few GO terms did show a tendency of increased predictive ability; e.g. GO:0008645 had a predictive ability of 0.11 (unadjusted t-test p-value = 7.4×10–5), and explained 9% of the genomic variance, and 1.5% of the total phenotypic variance (0.6% for the null model).

Conclusion: The improvement in predictive ability for schizophrenia was marginal, however, greater improvement is expected for the larger ADHD data.

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Normal repeat tract of *ATXN2* and *DMPK* genes as potential modifiers of age-at onset in familial amyloid polyneuropathy

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Introduction: Familial amyloid polyneuropathy (FAP ATTRV30M) is a dominant neurological disease, caused most frequently by a V30M substitution in transthyretin (*TTR*) (chr18q12.1) in Portuguese families. Age-at-onset (AO) varies between 19–82 years, variability also exists between generations. Unstable oligonucleotide repeats have been associated with several neurodegenerative diseases. Our aim was to study the variation of normal repeat length of 10 such candidate-genes using a family-centred approach, as possible modifiers of AO in Portuguese FAP

ATTRV30M families (as shown with other diseases, as ALS).

Materials and Methods: We analysed 329 patients (128 families). Repeat length (at *ATXN1*, *ATXN2*, *ATXN3*, *ATXN7*, *HTT*, *JPH3*, *ATN1*, *TBP*, *DMPK* and *AR*) was assessed by single and multiplex PCR, using fluorescently-labeled primers, followed by capillary electrophoresis. Results were analysed with GeneMapper™ v.4.0 software.

Results: Repeat size at *ATXN2* and *DMPK* was significantly associated with AO variation in FAP ATTRV30M. For *ATXN2*, the presence of at least one allele longer than 22 CAGs associated to earlier onset, decreasing mean AO by 5 years (p=0.002); for *DMPK*, carrying at least one allele with longer than 13 CTGs lead to an increase of 4 years in mean AO (p=0.012). No association was found for the remaining repeat *loci*.

Conclusions: Normal repeat length at *ATXN2* and *DMPK* may modify AO in FAP ATTRV30M, functioning as a risk or a protective factor. This may be due to their role in RNA metabolism and as modulators of various cellular processes, including mitochondrial stress. This may prove to have implications for genetic counselling.

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P09.010B

Clinical, chromosomal and molecular characterization of patients with agenesis of the corpus callosum

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Agenesis of the corpus callosum (ACC) is one of the most frequent brain malformations. Its prevalence is approximately 0.05–0.7% in general population and 2–3% in patients with intellectual disability (ID). A genetic origin is suspected in most cases but the genetic diagnosis is made in only 30–45% of patients.

To identify genetic causes of ACC, we recruited 183 patients with ACC and ID and 90 patients with ACC but no ID. All patients were evaluated clinically, cytogenetically by chromosomal microarray analysis and molecularly by next generation sequencing (either panel of genes or whole exome sequencing [WES]).

Among patients with ACC and ID, clinical examination allowed a diagnosis for 18 patients (yield 10%), whereas chromosomal imbalances explained the phenotype in 26 of them (14%). Next, we sequenced 423 genes (associated with ACC in humans or candidate genes) in 99 patients. We identified a pathogenic mutation in 21 patients (21%) in 9 genes. We performed WES in 26 trios and established a diagnosis in 9 patients (37.5%). Thanks to this combined approach, we obtained a diagnosis in 40% of patients with ACC and ID.

In addition, we performed WES in several families with ACC without ID. This allowed us to report the first two genes responsible for ACC without ID.

Our results show that next generation sequencing approaches are powerful diagnostic tools for ACC and confirm the large genetic heterogeneity of this condition. The identification of genes involved in ACC without ID allows an innovative approach in the prenatal diagnosis of ACC.

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P09.011C

Modeling early neuropathology in Down syndrome and APP-associated Alzheimer's disease using Induced Pluripotent Stem Cells

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Introduction: Early-onset Alzheimer's disease (AD) may be caused by rare variants in the gene encoding amyloid precursor protein (APP) on chromosome 21. Down syndrome (DS), owing to trisomy 21 (T21), is associated with a neuropathology similar to that in AD. Thus, *APP* is associated with early onset dementia through either increased gene dosage in T21 or through rare structural gene variants in rare cases of AD. **Methods:** In search for shared neuropathological mechanisms we established induced pluripotent stem cells (iPSC) from patients with DS and T21 as well as from AD associated with the APP variant p.V717I. iPSC were differentiated into neuroepithelial stem cells and neuronal cells together with WT controls. After four weeks of differentiation, we generated transcriptomes using whole RNA-sequencing (Illumina HiSeq). **Results**: Pathway analysis of AD derived cells confirmed differentially expressed genes in pathways for glutamatergic synaptic function and insulin secretion. Analysis of DS cells revealed dysregulations in extracellular matrix-receptor interaction and proteoglycan pathways. Shared dysregulated pathways in AD and DS include focal adhesion, previously associated with Aβ signaling and cell death in AD. We identified 347 differentially expressed transcripts that were shared in neuronal cells derived from AD and DS patients, e.g. the AD associated genes *A2M* and *CYP26A1*. **Conclusion:** Our data indicate that several genes previously implicated in the etiology for AD is also dysregulated in neuronal cells generated from individuals with DS. Identification of shared pathways and mechanisms in these two disorders may provide further clues to the neuropathogenesis in early onset AD.

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Effect of sodium butyrate on some alternative splicing genes and bace1 isoforms in SH-SY5Y cell line

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Alzheimer Disease (AD) is caused by many risk factors. Alternative splicing of BACE1 pre-mRNA affects AD. We aimed to investigated effect of sodium butyrate (NaB) on BACE1 isoform such as BACE1/501, BACE1/457 and BACE1/432 and alternative splicing factors such as hnRNP H, U2AF35, U2AF65, SRSF1, SRSF2, SRSF5 and SRSF6. SH-SY5Y cells was treated 1 mM and 5 mM NaB concentration, histone deacetylase inhibitor. According to Mann Whitney U-test, it hasn't been found significant difference between two doses of NaB. (p>0,05) Both 1mM and 5mM NaB has increased BACE1/501 expression to control group. While 1mM NaB has decreased expression of BACE1/457, 5 mM NaB has increased expression of BACE1/457. 5 mM sodium butyrate has increased expression of BACE1/432 to control group. Although it wasn't obtained statistically significant results in expression of hnRNP H, U1AF35, U2AF65, SRSF1, SRSF2, SRSF5 and SRSF6 genes, it was observed that U2AF65 gene has a relative increase in expression of U2AF35 and SRSF6 in 1 mM NaB treatment. Both in 1 mM and 5 mM NaB, U2AF65 gene has a important decrease In 5 mM NaB, it was determined relative decrease from %35 to %80 in average expression profile of SRSF6, SRSF1, SRSF2, SRSF5 and U2AF65 genes, respectively. It has referred that contribution of U2AF65 gene in increase of BACE1/457 and BACE1/432 expression is more than the others and increase of NaB concentration cause U2AF65 gene to bind G-quadruplex motif in BACE1 exon 3 rather than hnRNP H. Supported by CUBAP/TSA-2015-5311.

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Genomic variants in obesity-related genes are associated with sporadic amyotrophic lateral sclerosis in Greek patients

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Amyotrophic lateral sclerosis (ALS) is one of the most common forms of motor neuron disease. ALS is a neurodegenerative disorder that affects the upper and lower motor neurons in the motor cortex, brain stem, and spinal cord and leads to death within 3–5 years. Approximately 90% of the ALS patients suffer from sporadic ALS, having both an environmental etiology and a strong genetic component. Today, there is no effective treatment or diagnostic means for ALS patients. We have performed whole-genome sequencing of 10 ALS patients and 7 healthy (non-ALS) individuals of Greek origin (110x sequencing depth). Extensive data analysis identified 174 genomic variants that were present in all 10 ALS patients but none of the 7 non-ALS ethnically matched controls. Replication of genotyping in 27 sporadic ALS patients and 50 ethnically matched control individuals showed that *FTO* (rs2892469, p=0.005; rs17217144, p<0.001; rs1861869, p=0.003) and *TBC1D1* (rs6850200, p=0.017) genomic variants are positively associated with the disease phenotype. These variants did not reach statistical significance, when comparing 148 and 124 sporadic ALS patients of Turkish and Italian descent, respectively, against 74 and 87 ethnically matched controls. Both proteins are associated with obesity. FTO is a fat mass and obesity-associated protein. TBC1D1 has been identified as a regulator of insulin-dependent glucose transport and variants in the *TBC1D1* gene were linked to obesity. This is the first study that reveals an association between obesity-related genes and ALS pathobiology, which, however, should be considered as a pilot study due to the small sample size.

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Differential expression of several miRNAs and host genes *AATK* and *DNM2* in leukocytes of sporadic ALS patients

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disorder. Multiple interactions among genetic components, environmental influences and epigenetic mechanisms may contribute to disease development. Materials and Methods: we evaluated differential expression of ten miRNAs, miR-9, miR-338, miR-638, miR-663a, miR-124a, miR-143, miR-451a, miR-132, miR-206 and let-7b and three miRNAs host genes *C1orf61* (miR-9), *AATK* (miR-338) and *DNM2* (miR-638) in leukocyte samples of 84 patients with ALS and 27 controls. **Results:** we observed most homogeneous and differential dysregulation across our patients' cohort for miR-206, miR-124a, miR-143-3p, miR-638 and miR-451. No significant differences were observed between ALS patients with or without C9orf72 expansion. We also detected significant down-regulation of *AAKT* and *DNM2* genes and thus for the first time connected them with ALS cases. Reduced expression of *AAKT* could be at least partly explained by observed methylation of its promoter. **Conclusions:** we have detected differential expression of 10 miRNAs involved in ALS pathology in leukocyte samples of patients with sporadic form of ALS. Seven of these miRNAs have not been previously investigated in peripheral blood leukocytes. We observed most homogeneous and differential dysregulation across our patients' cohort for miR-206, miR-124a, miR-143-3p, miR-638 and miR-451. Our finding places these five miRNAs among miRNAs that are worth of additional research in leukocytes of larger cohorts from different populations in order to prove their potential as ALS disease biomarkers and therapeutic targets. In addition in most recent studies reported the connection between *AATK* and frontotemporal dementia (FTD) and *DNM2* and hereditary spastic paraplegia (HSP).

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P09.015C

Effects of asenapine and paliperidone on cognition and locomotion: altered gen expression levels of BDNF and CREB in the hippocampus of mice

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Introduction: One of the major symptoms of schizophrenia is cognitive deficits and may be further aggravated by antipsychotic treatments. Asenapine and paliperidon two newer atypical antipsychotics are used frequently in clinics. This study aimed to investigate the effects of asenapine and paliperidone on emotional memory and locomotion in naive mice, using passive avoidance (PA) and open field tests. Brain-derived neurotrophic factor(BDNF) and cyclic adenosine monophosphate (cAMP) response element binding protein(CREB) regulates cell survival, neurogenesis, neuronal morphology and neuroplasticity. Increased levels of BDNF are associated with improved learning and memory.

Materials and methods: Mice were treated with asenapine (0.05, 0.075 mg/kg) and paliperidon (0.25, 0.50 mg/kg) for 15 days and drugs were also administered intraperitoneally 60 min before the tests. Effects of drugs on BDNF and CREB mRNA levels in the hippocampus of mice were determined using RT-PCR.

Results: In the PA test, there was no significant difference between the first day latency of the animals. Retention latency significantly decreased in asenapine 0.075 mg/kg ($p<0.01$) and paliperidon (0.25,0.50 mg/kg) ($p<0.01$) groups. In open field test, both asenapine (0.075 mg/kg) and paliperidon (0.5 mg/kg) did not significantly alter total distance moved and speed of the animals ($p>0.05$). Asenapine and paliperidon increased the expression of BDNF and CREB in the hippocampus of naive mice.

Conclusion: Asenapine and paliperidone disturb memory when chronically administered while do not alter the locomotion of the animals and had slightly enhancing effect on BDNF expression levels associated with enhanced expression of CREB.

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Autosomal recessively ataxia with oculomotor apraxia type 2 in two Bulgarian patients - case report

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The autosomal recessively ataxia with oculomotor apraxia, type 2 (AOA2) is a neurodegenerative disorder characterized by juvenile or adolescent age of onset, gait ataxia, cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia, and elevated serum AFP levels. It is caused by pathogenic genetic variants in the senataxin (*SETX)* gene.

Here we report two unrelated patients with mutations in the *SETX* gene. Both of them have very similar clinical features of AOA2 and common brain MRI pathological findings. Cerebellar atrophy, axonal sensorimotor neuropathy and oculomotor apraxia are presented.

The SETX gene was screened for the pathogenic disease causing variants by Sanger sequencing and MLPA analysis.

The molecular genetic testing revealed compound heterozygous *SETX* variants in both cases. The first patient was found to be compound heterozygous for novel splice site pathogenic variant c.6106+1G>T and missense one c.5825T>C, (p.Ile1942Thr). The second patient was a compound heterozygous carrier of the same missense variant c.5825T>C and deletion of exon 7: c.719-?_838+?del.

Repetitive genetic elements account for 47.5% of the entire *SETX* gene explaining the higher possibility for deletions/duplications. In the present study we detected one deletion and 3 point mutations, being more prevalent in our sample. The missense mutation p.Ile1942Thr was detected 2 times in unrelated patients. It is interesting to mention that one of the patients is from Turkish Gipsy origin, while the second one is from Bulgarian origin. This variant could be common in Balkan region.

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Whole exome sequencing identifies novel candidate genes involved in Asperger syndrome

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Autism Spectrum Disorders (ASD) are neurodevelopmental conditions characterized by difficulties in communication and social interaction, alongside unusually repetitive behaviours and narrow interests. Asperger Syndrome (AS) is one subgroup of ASD and differs from classic autism since AS

patients present with no language or general cognitive delay. Despite the clear genetic component of ASD and AS, extreme genetic heterogeneity has made difficult the identification of causative genes. We performed whole-exome sequencing in nine unrelated individuals (8 males and 1 female) with AS. Variant filtering for functional, damaging rare variants (MAF<0.1%) was performed. Parallel sequencing of their parents failed to identify potential *de novo* variants in AS probands. However, we found seven potential variants in X-chromosome genes that were previously associated with non-syndromic ASD and/or intellectual disability. Potential damaging variants identified in these AS male patients were: c.1117C>T (p.R373C) in *UPF3B* (12/87660 in ExAC), c.306T>C (p.C1023R) in *FRMPD4* (9/87549 in ExAC), c.5472G>T (p.M1824I) in *HCFC1* (not described in ExAC), c.145A>C (p.N49H) in *Cxorf36/DIA1R* (not described in ExAC), c.2270G>A (p.K757R) in *OGT* (not described in ExAC), c.1772C>A (p.T591N) in *ZNF182* (not described in ExAC) and c.4808C>T (p.T1603M) in *NHS* (5/87571 in ExAC). Functional analysis of these variants will be carried out to determine whether these novel variants have a deleterious effect on protein expression and function. This study identified novel candidate genes involved in Asperger syndrome.

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P09.018B

Phenotype characterization of the SCA28 knockin mouse model and derived embryonic fibroblasts

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SpinoCerebellar Ataxia type 28 (SCA28, OMIM#610246) is a rare form of autosomal dominant ataxia, accounting for ~1.5% of the European cases. The causative gene, *AFG3L2*, encodes for a mitochondrial protein that assembles into homo- or hetero-hexamers with paraplegin (*SPG7*) to compose the **m**atrix-**A**TPase **A**ssociated with various cellular **A**ctivities protease, a large proteolytic complex with crucial roles in mitochondria. We generated a knockin (KI) mouse model harboring the p.Met665Arg mutation in Afg3l2 peptidase domain, reported in patients with early onset of the disease. *Afg3l2*$^{KI/KI}$ (KI-ho) were embryonically lethal, whereas *Afg3l2*$^{KI/+}$ mice (KI-hz) were viable and developed a late-onset ataxia, starting at 18 months of age. Morphological analysis revealed preserved cytoarchitecture of cerebellar lobules, with unaltered thickness of the molecular and granular layers and a conserved number of Purkinje cells (PC). We evaluated mitochondrial morphology and function, studying both homo- and heterozygous Mouse Embryonic Fibroblasts (MEF). We showed mitochondrial dynamics impairment, detected as the complete loss of long Opa1 isoforms in KI-ho MEF, with a fragmented mitochondrial network. A similar imbalance of Opa1 isoforms was present in cerebellum and brain homogenates from KI-hz mice. Bioenergetics analysis revealed a 25% reduction of Complex III activity in KI-ho ($P<0.05$), detected by spectrophotometric analysis of the respiratory chain. KI-ho showed reduced mitochondrial translation efficiency, supporting decreased protein synthesis. These results suggest that mutations hitting the peptidase domain negatively impact on *m*-AAA complex function, probably acting as hypomorphic on AFG3L2 chaperone function or quality control function. This work is supported by Italian Telethon Foundation Grant GGP12217.

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Clinical features and genotype-phenotype correlations in variant Ataxia-Telangiectasia

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Background Ataxia-Telangiectasia (A-T) is characterised by neurological, immune and respiratory complications. Individuals with residual ATM kinase activity have a milder form of the disorder, but previously reported cohorts are small.

Methods Patients were identified through the UK Ataxia-Telangiectasia Service and the Nijmegen Medical Centre, Netherlands and were classified as having variant A-T based on genotype (mutations which allow retained ATM kinase activity, or mutations in the initiator methionine codon).

Results A total of 49 adults from 41 families (19 male/30 female, mean age 40 years, range 20–58) were studied. Symptom onset was before age five in 27/49 (55%) patients. Thirty-seven patients use a wheelchair with median age at first wheelchair use 26.5 years (range 8–51). Conjunctival telangiectasia were present in 29/46 assessed (63%). Alpha-fetoprotein levels were raised in 36/39 (92%).

Eleven patients had malignancies (4 breast cancers, dermatofibrosarcoma protuberans, neuroendocrine tumour, CML, CLL, ALL, ectopic pituitary tumour and pancreatic cancer). Four had monoclonal gammopathy of unknown significance. No individuals had severe respiratory or immunological complications. Seven women from five families had offspring.

Individuals with missense mutations producing mutant protein with retained kinase activity had milder disease compared to the rest of the cohort, in terms of overall severity ($p= 0.00167$), progression ($p= 0.00198$), A-T NEST score ($p = 0.0492$), age at first wheelchair use ($p= 4.05\times10^{-05}$) and eye movements ($p =0.000414$).

Conclusion This is the largest study to date of variant A-T. It demonstrates a clear genotype-phenotype correlation and provides new prognostic information for individuals with variant A-T.

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P09.020D

ATXN3L: may an assumed pseudogene compensate partial loss-of-function of normal ATXN3 in Machado-Joseph disease (MJD/SCA3)?

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The Machado-Joseph disease (MJD) subfamily of deubiquitinases comprises ataxin-3 (ATXN3), ataxin-3 like (ATXN3L) and Josephin domain-containing proteins 1 (JOSD1) and 2 (JOSD2). Much attention has been given to *ATXN3* after the identification of an exonic $(CAG)_n$, responsible for the autosomal dominant neurodegenerative MJD when encoding a polyQ tract above 61 glutamines. The other members of this protease family are poorly explored; in particular, *ATXN3L*, remained in the shadow under the assumption that it would be a non-functional sequence due to its lack of introns. The partial loss-of-function mechanism suggested for MJD pathogenesis, together with the proved deubiquitinating activity of all four MJD family members led us to hypothesize that they may exert a neuroprotective role in MJD.

We carried out an analysis of *ATXN3L*, *JOSD1* and *JOSD2* expression patterns in a panel of 23 human tissues from healthy individuals. The evolutionary conserved paralogue of *ATXN3* - *ATXN3L*, is transcribed in testis, placenta and brain. Interestingly, *ATXN3L* transcription seems to differ across brain regions, being highly expressed in the cortex, followed by substantia nigra and residually in the cerebellum. In contrast, *ATXN3*, *JOSD1* and *JOSD2* were found to be ubiquitously expressed. The observation of distinct *ATXN3L* expression levels in brain regions differently affected by MJD may be relevant for the observed large clinical variability not explained by the $(CAG)_n$ expansion size. Next, we will attempt to detect ATXN3L protein in brain extracts of healthy individuals and assess expression levels of *JOSD1* and *JOSD2* in patients with different age-at-onset using blood samples.

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P09.021A

Structural Variation of Chromosomes in Autism Spectrum Disorders

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Autism spectrum disorders (ASDs) are characterised by impaired socialization, reduced communication, and restricted, repetitive, or stereotyped activities and interests. The influence of genetic factors on the severity of the disorder is not well characterised - due to the considerable clinical and genetic heterogeneity of the ASD cases. The unbalanced copy number variations (CNVs) of chromosomal regions has been identified in some, but not all, individuals with autism spectrum disorders. Array comparative genomic hybridization (aCGH) is a high resolution molecular cytogenetic tool which enables large scale detection and characterisation of these variants.

The aim of this work was to summarize the positive results of aCGH tests of patients with clinical symptoms of ASD.

The presence of CNVs was tested in ADS patients (age 1–31 y.o.) using aCGH method and NimbleGene (2×135k) or Agilent (SurePrint G3 CGH ISCA v2 8×60K) microarray kits.

Deletions or duplication were found in 40 ADS cases, in most chromosome pairs with observed size of the CNVs affected region ranging from 102kb to 29Mb. Most of the changes encompassed genes with known pathogenic CNVs, with few regions containing alterations of unknown significance. In 2 cases more than one variation was uncovered.

Our results have confirmed both the genetic heterogeneity of the ASDs patients and the capability of aCGH microarrays in quickly characterising the underlying variation of chromosome structure.

The chromosome microarrays are now a first-tiered genetic investigation for many conditions, with scope and ease of use exceeding conventional cytogenetics or testing of the individual regions of the genes.

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P09.022B

Interest of searching dysmorphic features in Autism Spectrum Disorder: Comparison of clinical geneticists and Face2Gene photos analyses

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Background: Autism Spectrum Disorder (ASD) is defined according to DSM-5 and ICD-10 criteria as early social communication impairments and repetitive/restrictive behaviors or interests. Geneticists have advanced current knowledge on genetic syndromes associated with ASD. Clinical genetic examination searching for dysmorphic features and malformations is a very important step towards the identification of genetic disorders associated with ASD. *Objective and Methods:* to compare the etiological genetic hypotheses stated by clinical geneticists trained in dysmorphology to the ones resulting from the software program Face2Gene based on biometric analyses and algorithms. Clinical geneticists and Face2Gene analyses were both performed on the same facial photos of 79 children and adolescents with ASD and intellectual disability. ***Results:*** *The qualitative variable of "clinical dysmorphy" observed by the geneticists was significantly and*

*moderately correlated with the qualitative variable of "Face2Gene dysmorphy" (Phi coefficient = 0.35, p = 0.0039). The inter-judge agreement represented by the Cronbach's Alpha coefficient was 0.51. Furthermore, there was no significant correlations between dysmorphism scores and autism severity ratings based on the ADOS (current severity), ADI-R past time (period of life from 4 to 5 years old), or ADI-R present time.*Conclusion: *this study highlights the need to conduct systematically clinical genetic examination searching for known genetic disorders for all individuals with ASD. Biometric analysis software can provide a helpful additional method, either used after clinical genetic evaluation to complete the diagnostic strategy of the geneticist, or used before clinical evaluation to sensitize the families to the interest of clinical genetic examination in ASD.*

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P09.023C

Novel *PAK3* mutation causing X-linked autism and mental retardation

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A Bedouin Israeli kindred presented with a phenotype of autism and profound intellectual disability, affecting males of several generations. Linkage analysis (750K SNP arrays) of 8 family members identified two possible disease-associated loci, on chromosomes 4 and X. Whole exome sequencing (WES) data of an affected individual were analyzed and filtered for known benign variants within these two loci, using our in-house databases along with open access databases (1000 genomes, NHLBI ESP, ExAC etc.). Of the variants identified, only a single variant segregated as expected within the kindred and was not found in 100 ethnically-matched controls: a heterozygous c.212C>G missense mutation in *PAK3* within the Xq23 locus, resulting in a p.S71C substitution (LOD score 5.08). In-silico analysis of the novel variant showed that it is likely to have a deleterious effect on the mature protein. Mutations in *PAK3* were previously described as causative for X-linked intellectual disability. Our data implicate this novel *PAK3* mutation as the cause for X-linked intellectual disability with bona-fide autism. This finding correlates well with the known function of PAK3 as a key regulator of synapse formation.

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Ras-like without CAAX 2 *(RIT2)*: a susceptibility gene for autism spectrum disorder

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Ras-like without CAAX2 (RIT2) which encodes a GTP-binding protein has recently been reported as a new susceptibility gene for Autism Spectrum Disorders (ASD) in a genome-wide association study. Since the gene is suggested to be involved in the pathogenesis of different neurological diseases, we investigated the association of two single nucleotide polymorphisms (SNP) rs16976358 and rs4130047 of this gene with ASD in Iranian patients. A total of 1004 individuals, comprising 532 ASD cases and 472 healthy subjects participated in this study. Allele frequency analyses showed significant over-presentation of rs16976358-C allele in cases versus controls ($P<0.0001$). In addition, rs16976358 CC genotype (OR (95% CI) =3.57 (1.72–7.69) and $P<0.0001$) and rs4130047 CC genotype (OR (95% CI) =0.64(0.43–0.97) and $P= 0.035$) were associated with ASD in recessive inheritance model. Besides, haplotype analysis demonstrated an association between the C/T haplotype block (rs16976358/rs4130047) and ASD (OR (95%CI) = 0.44 (0.31 - 0.62), $P<0.0001$). Altogether, our findings provided additional confirmation for the *RIT2* gene participation in ASD risk and suggested the rs16976358 variant as a possible genetic risk factor for this disorder.

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Trancriptional regulation of the ASD gene *Rbfox1*

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Autism spectrum disorders (ASD) are neurodevelopmental, heterogeneous disorders that occur in 1 of 150 children. Although it is known that ASD have a strong genetic basis only few causative genes have been identified.

The *RBFOX1* gene encodes an RNA-binding protein that regulates pre-mRNA splicing events in specific cell types including neurons. The *RBFOX1* gene, which is located on chromosome 16p13.2, contains a large noncoding part at the 5'end with at least four alternative promoters driving expression of alternative *RBFOX1* transcript isoforms that differ in their 5'UTR exons. Rare copy number variants (CNVs) in the 5' noncoding part of the gene have been found in patients with several neurodevelopmental disorders including ASD. The detected CNVs likely interfere with the transcriptional regulation of specific *RBFOX1* transcript isoforms. However, as the transcriptional regulation of the *RBFOX1* gene has not been studied so far the pathogenic potential of these CNVs are still unclear.

In this project, we have been able to demonstrate that the expression of *Rbfox1* in the embryonic and adult brain of the mouse is mainly driven from two out of the four alternative promoter regions which are conserved in humans. We have further identified transcription factors that bind to sequences in either the first or the second of these two promoters and drive Rbfox1 expression in cortical neurons. At the moment we are carrying out RNAi experiments to elucidate how the knockdown of specific transcription factors influences expression of all Rbfox1 transcripts as well as of the specific Rbfox1 transcript isoforms.

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Resequencing study of five microRNAs suggests an involvement of *MIR2113* and *MIR499* in the development of bipolar disorder

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Bipolar disorder (BD) is a severe and highly heritable neuropsychiatric disorder with a lifetime prevalence of 1%. Molecular genetic studies have identified the first BD susceptibility genes. However, the disease pathways remain largely unknown. Accumulating evidence suggests that microRNAs, a class of small non-coding RNAs, contribute to basic mechanisms underlying brain development and plasticity, suggesting their possible involvement in the pathogenesis of several psychiatric disorders, including BD. The most promising candidate microRNAs comprise *MIR499a*, *MIR708*, *MIR1908* (Forstner et al., 2015), *MIR137* (Strazisar et al., 2014), and *MIR2113* at a genome-wide significant BD locus on chromosome 6 (Mühleisen et al., 2014).

The aim of the present study was to determine whether rare variants within these five candidate microRNAs contribute to the development of BD. For this purpose, we performed Sanger sequencing of 1,000 BD patients and 1,000 sex-matched healthy controls, all of German origin.

We identified seven rare variants (minor allele frequency<1%) within the premature microRNAs 2113 and 499a. These include a rare point mutation (rs140486571) in *MIR499a* which was detected in nine BD patients and five controls (P=0.297). No rare variants were detected in the premature microRNAs 137, 708 and 1908. Functional analyses in HEK293 cells indicated that rs140486571 impairs *pri-miR-499a* processing. The investigation of rs140486571 in additional samples of BD patients and controls is currently underway and results will be presented. Further research is warranted to elucidate the precise involvement of the implicated microRNAs and their downstream pathways in BD. AJ.Forstner and A.Verhaert contributed equally to this work.

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P09.028D

Exome sequencing of multiply affected bipolar disorder families and follow-up resequencing implicate rare variants in neuronal genes contributing to disease etiology

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Bipolar disorder (BD) is a severe and highly heritable psychiatric disorder affecting about 1% of the world's population. The disease is characterized by recurrent episodes of mania and depression.

As the cumulative impact of common alleles may only explain ~38% of the phenotypic variance for BD, rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In this study, we performed whole-exome sequencing in 226 individuals of 68 large multiplex BD families of European origin. We filtered for rare (minor allele frequency<0.1%), nonsynonymous, potentially functional and segregating variants.

We identified 1214 variants implicating 1122 different genes. Gene enrichment analysis of 294 genes that were among the 20% most "intolerant" genes showed a significant enrichment for 18 pathways ($p<0.001$) including neuron projection and cell-adhesion.

For follow up analyses, we prioritized genes that were either found in at least two unrelated families in the present study or previously reported in next-generation sequencing or GWAS studies of BD. In addition, we enclosed genes that were predominantly driving the significant pathways in the above-mentioned gene enrichment analysis.

The 42 most promising genes are currently being followed up by resequencing in larger cohorts of 2500 independent BD cases and 2500 controls of European ancestry using the single molecule molecular inversion probes technology. The candidate genes include *SYNE1*, which is a genome-wide significant risk gene for BD.

Our preliminary results suggest that rare and highly penetrant variants in neuronal and cell-adhesion genes contribute to BD etiology.

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Polygenic burden analysis of longitudinal clusters of psychological features in a cross-diagnostic group of individuals with severe mental illness

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Bipolar disorder (BD), schizophrenia (SZ) and schizoaffective disorder (SZA) are complex genetic disorders with largely polygenic architecture and severe and overlapping psychiatric symptoms. Stratification of cases into

homogeneous subgroups across diagnoses using both psychometric and genetic information could identify individuals with higher risk for severe illness.

Examined at four time points over 18-months, a subset of 198 participants (46.9±12.4 yrs; 46% female) with DSM-IV diagnoses of SZ, SZA or BD from an ongoing longitudinal cohort study (www.kfo241.de) were genotyped on Illumina's Infinium PsychArray and imputed using the 1000genomes. 67 variables from the Positive and Negative Syndrome Scale (PANSS), the Inventory of Depressive Symptoms (IDS) and the Young Mania Rating Scale (YMRS) entered cluster analyses. Longitudinal trajectories derived from abstract data dimensions computed by factor analysis for mixed data (FAMD) were used for clustering. SZ-polygenic risk scores (PRS) based on the Psychiatric Genetics Consortium 2 SZ results were tested for cluster association at 11 thresholds.

Two clusters were identified in the first two dimensions: (A) individuals with continuously low scores on PANSS and IDS (70.7%) and (B) individuals with consistently high scores on PANSS and IDS (29.3%). Clusters differed significantly with regard to Global Assessment of Functioning (higher in (A); FDR-adjusted p-value=2.23×10–10), while there were no significant differences regarding sex, age, diagnosis, center, age at onset, family history, duration of illness, or association with the SZ-PRS.

In this preliminary data set, longitudinal clustering identified cross-diagnostic homogeneous subgroups. Surprisingly, more severe psychopathological features were not associated with increased genetic risk burden.

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P09.030B

DNA damage, aneuploidy and chromosome instability in the Alzheimer's disease and autism brain

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Introduction It is consistently noted that brain diseases are associated with aneuploidy and chromosome instability (CIN) in the brain. Alzheimer's disease (AD) and autism spectrum disorders (ASD) seem to be the most likely disorders to exhibit such an association. Here, we have attempted to narrow the rates of aneuploidy and CIN in the AD and ASD brain and to elucidate pathways brain-specific CIN. **Materials and methods** Brain samples of controls (n = 25) as well as AD (n = 10) and ASD (n = 6) individuals was studied using multiprobe FISH, quantitative FISH and interphase chromosome-specific multicolor banding. Specificity of CIN suggested specific pathway alterations specific for this diseases. **Results** Aneuploidy rate affecting sex chromosomes in AD was two times higher than in control (median: 2.8% and 1.3%, respectively, p =0.004). Aneuploidy rate affecting autosomes and sex chromosomes in ASD was also increased as compared to controls (median: 1.9% and 0.5%, respectively, p =0.002). CIN was observed in AD and ASD exclusively. Specificity of CIN suggested DNA damage response pathway to be altered. **Conclusions** Increased aneuploidy and CIN (chromosome rearrangements and breaks) rates result in genomic instability (GIN) confined to the brain. Paradoxically, these phenomena are observed in brain cells regardless of their post-mitotic nature. According to our data, brain-specific GIN in neuropsychiatric disorders is likely to originate from improper DNA damage recovery similarly to oncogenic mechanisms. Supported by the Russian Science Foundation (project #14-35-00060) (autism studies) and ERA.Net RUS Plus Programme (AD studies).

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P09.031C

BRAT1-related myoclonic encephalopathy: a new cause of congenital stiffness

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Introduction: Neonatal myoclonic encephalopathies represent a diagnostic challenge that have been largely

circumvented by generalized use of gene panels or whole-exome sequencing approaches, often replacing an hitherto complex metabolic work-up. However, there is still room for first line Sanger-sequencing whenever clinical evidence points to a specific diagnosis, as illustrated by the history hereby presented.

Materials and methods: Two males born to Algerian first-cousins died at 6 and 3 weeks after a similar clinical course of myoclonic pharmaco-resistant seizures and generalized hypertonia leaving them in flexed position. Tongue myoclonic movements were present as well. Brain MRI, EMG, search for mutations in *GLRA1, GLRB, STXBP1, KCNQ2* and *SCN8A* were negative. Based on the similarity between this very unusual clinical picture and the usual presentation of *BRAT1*-related encephalopathy, *BRAT1* sequencing was undertaken and identified the homozygous mutation c.2068G>T in exon 14 in the two index patients, introducing a premature stop codon [p.(Glu690*)]. Both parents were heterozygous carriers of the *BRAT1* nonsense mutation.

Conclusion: *BRAT1* should be added to the growing list of genes related to early-onset severe lethal encephalopathy with epilepsy. Major hypertonia is a paramount feature and death occurs generally in the first weeks of life with severe deceleration of head circumference growth. Our data confirms the clinical presentation observed in the 12 previously reported *BRAT1* mutation-positive patients.

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P09.032D

C9ORF72 genetic screening in Serbian patients with neurodegenerative disorders

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Introduction: Recently discovered hexanucleotide repeat expansions in the non-coding region of *C9ORF72* gene seems to be most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This is the first genetic change found to be the link between ALS and FTD. However, this mutation was found with variable frequency in other neurodegenerative disorders also. The size of the GGGGCC hexanucleotide repeats in the *C9ORF72* alleles can range from 2 to more than 4000 and the precise cut-off for pathogenic repeat size is still under debate.

Materials and Methods: We have analyzed large cohort of Serbian patients diagnosed as: ALS (252), FTD (261), Alzheimer's disease (AD, 146), and Huntington disease like syndrome (HD like, 115). The region containing hexanucleotide repeats in *C9ORF72* was PCR amplified with fluorescently labeled primer; fragment analysis was performed on ABI 3500 genetic analyzer. Cut-off size for repeat expansion was 30 repeats. All apparently normal homozygous samples were screened for the expansion using repeat-primed PCR.

Results: *C9ORF72* hexanucleotide expansions were detected in 9 (3,57%) ALS and 5 (1,84%) FTD cases. In addition, 1 (0,87%) HD like patient showed *C9ORF72* expansion and 1 (0,68%) AD patient had borderline repeats number. All other patients showed normal alleles ranged from 2–27 repeats.

Conclusion: These are the first results of genetic screening for *C9ORF72* expansions in Serbian population. Our data confirms significance of *C9ORF72* analysis in neurodegenerative disorders. In order to improve this test, we intend to develop Southern blot for the exact estimation of the expanded repeat size.

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A novel variant in ELF2 gene in cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) upregulates ataxin-2 translation in BE(2)-M17 cells

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Introduction: To identify the gene segregating the cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) in British kindred and to investigate its pathogenic effect. **Material and Methods:** We present a family

with autosomal dominant CANVAS with incomplete penetrance and variable expressivity; the proband and his sister had a complete phenotype and a first cousin a partial syndrome. Exome sequencing was performed in 4 individuals and functional studies of the candidate mutation were completed in transduced BE(2)-M17 cells. **Results:** We identified a novel variant in the ELF2 gene at chr4: g.140058846 C>T, c.10G>A, p.A4T which segregated in all patients. We also showed that the mutated ELF2 (mt-ELF2) gene upregulates ATXN2 gene expression and increases ataxin-2 translation, supporting a pathogenic effect. Both, western blot and confocal microscopy confirmed increased ataxin-2 in BE(2)-M17 cells transduced with lentivirus expressing mt-ELF2 (CEE-mt-ELF2), which was not observed in cells transduced with lentivirus expressing wt-ELF2 (CEE-wt-ELF2). Moreover, we observed a significant decrease in the number and size of lipid droplets in the CEE-mt-ELF2-transduced BE(2)-M17 cells, but not in the CEE-wt-ELF2-transduced BE(2)-M17, a finding previously observed in neurodegenerative diseases. **Conclusions:** The transcription factor ELF2, which interacts with the ETS domain within the 5´-UTR in the ATXN2 gene could be a repressor of ATXN2 expression. Furthermore, mt-ELF2 could induce an ATXN2 gain-of-function involving lipid homeostasis with reduction of lipid droplets. This novel mechanism confirms that regulation of ATXN2 gene transcription may be crucial in the pathophysiology of cerebellar ataxias. Funded by an MRC Grant MR/J004685/1.

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P09.034B

Next-generation DNA sequencing to identify novel genetic risk factors underlying cerebral vein thrombosis

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Background: Cerebral vein thrombosis (CVT) is a rare, life-threatening disease affecting annually 4 adults/million. Genetic risk factors are deficiencies of the natural anticoagulant proteins antithrombin, protein C, protein S or factor V Leiden and prothrombin 20210A mutations. In 20% of patients, the cause of CVT remains unknown.

Aim: To identify novel genetic risk factors underlying CVT using targeted next-generation DNA sequencing (NGS).

Methods: We investigated 171 Italian CVT patients and 298 healthy controls. Patients were selected using the following criteria: objective diagnosis of CVT, Caucasian decent, no active cancer. We performed targeted NGS analysis of the protein-coding regions of 737 candidate genes related to hemostasis and inflammation, 150 ancestry informative markers and 28 thrombosis-associated variants.

Results: We obtained 34,357 variants that passed quality control, of which 4,591 variants were common and low-frequency with minor allele frequency (MAF) >1% in 618 genes. Single variant association testing using logistic regression analysis identified rs8176719 insertion/deletion (indel) variant in the *ABO* gene associated with CVT (age and sex adjusted $P=1.37\times10^{-6}$; OR 2.04; 95% CI 1.53–2.73; Bonferroni $P=0.006$). Gene-based association analysis of 13,161 rare variants (MAF ≤1%) using Burden test revealed a borderline association of variants located in intron 2 ($P=0.02$) and introns 15–20 ($P=0.01$) of the *F8* locus with CVT.

Conclusions: Targeted NGS identified a common indel variant rs8176719 in the *ABO* gene as a risk factor for CVT. Gene-based test of association using Burden test revealed non-significant association of rare intronic variants in the *F8* gene with CVT.

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P09.035C

A genome-wide association study of common SNPs and rare coding variants influencing risk for cluster headache

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Cluster Headache (CH) is a primary headache, characterized by unilateral pain with circadian and seasonal rhythmicity. The etiology of CH is poorly understood, and a complex genetic predisposition is likely to be involved. Smoking is the most frequent habit in CH patients, possibly acting as an environmental trigger; however, its link with CH is unclear. Previous genetic studies investigated a limited number of candidate genes, providing no confirmed associations. We performed a genome-wide association study (GWAS) in a clinically well-defined cohort of 99 Italian patients with CH and a control sample of 360 healthy Italian individuals matched for age and smoking status. We used the Infinium PsychArray (Illumina), which combines common highly-informative genome-wide tag SNPs and exonic SNPs. Single marker case-control association analysis using common SNPs lead to the identification of an interesting suggestive association ($P=9.1\times10^{-6}$) within the PACAP receptor gene (*ADCYAP1R1*). Furthermore, gene-based association analysis on rare exonic variants in 745 candidate genes provided a significant evidence of association for a rare variant of *MME* ($P=2.5\times10^{-5}$), encoding for neprilysin. Both gene products are known to have a pivotal function in pain mechanisms, thus making these associations particularly stimulating. To confirm our initial association results we are currently conducting a replication study in an independent sample, and fine mapping in the *ADCYAP1R1* locus is ongoing. This study is the first comprehensive association study of common SNPs and rare exonic variants influencing risk for CH, which implicates gene involved in pain processing in CH susceptibility.

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Genetic variation associated with the variability of cognitive functions in elderly

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Cognitive decline with age, including pathological manifestations in the form of dementia, is an important social and medical problem that leads to a significant reduction in the quality of life of the elderly. The aim of the study was to find genetic markers associated with the variability of the cognitive functions in the elderly in the Russian population. A random sample of 710 elderly subjects (mean 71.3 years) was collected from population of Tomsk, Russia. The cognitive functions were assessed using Montreal Cognitive Assessment (MoCA). 62 SNPs in 45 genes previously reported to be associated with cognitive performance and/or Alzheimers's disease in multiple GWAS or meta-analysis studies were genotyped using MALDI-TOF mass spectrometry. Parametric (ANOVA) and nonparametric (Kruskal-Wallis test and the median test) methods were used to estimate the difference in scores of cognitive tests among genotypes. 17 markers were detected associated with MoCA performance test. The strongest effect on cognitive functions was demonstrated for genetic markers of APOE, TOMM40, PVRL2 and APOC1 genes, located in the same locus on chromosome 19q13.32. Highly significant associations with MoCA were also found for gene markers of SORL and CSMD1 genes. Analysis of variance indicates that the proportion of variance in MoCA values, explained by the variability of genetic markers, is ranged from 0.5% to 1.3% for each of the associated SNP. The largest contribution (8.6%) to cognitive performance variability is attributable to genetic variation at PVRL-TOMM40-APOE-APOC1 linked gene cluster. This work was supported by the Russian Science Foundation (project # 16-14-00020).

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Colpocephaly in Chudley-McCullough syndrome

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Abstract

Background: The Chudley-McCullough syndrome[1] is a very rare autosomal recessive disorder characterized by partial corpus callosum agenesis, colpocephaly with a right-sided tendency and bilateral severe to profound sensorineural hearing loss. Other abnormalities include cortical dysplasia, frontal polymicrogyria, cerebellar dysgenesis, gray matter heterotopy, arachnoid cysts and sometimes mental retardation [2,3].

Until now, only forty cases have been described in literature [3]. Mutations in the G-protein signaling modulator 2 gene (*GPSM2*), which produces the Leu-Gly-Asn repeat-enriched protein (LGN), were found to be responsible for the syndrome [4].

Main findings: we present two adult sisters with asymmetrical enlarged ventricles who were treated surgically under the assumption of "hydrocephalus", yielding no clinical benefit. They were diagnosed subsequently with the Chudley-McCullough syndrome. The enlarged ventricles in this syndrome are secondary to a developmental malformation called colpocephaly, instead of a true hydrocephalus caused by a cerebrospinal fluid circulation disturbance.

Conclusion: The ventriculomegaly in Chudley-McCullough syndrome is secondary to colpocephaly and not caused by a true hydrocephalus and thus we recommend conservative follow-up without cerebrospinal fluid diversion, unless there is evidence of a raised intracranial pressure. We emphasize the importance of determining the right diagnosis for patients with this rare syndrome, to prevent unnecessary surgical interventions.

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Impact of defective protein N-glycosylation on the developing mouse cerebellum

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Introduction: Disruption of protein N-glycosylation causes Congenital Disorders of Glycosylation (CDG), which are multisystemic disorders with severe neurological symptoms. Early-onset cerebellar atrophy and/or hypoplasia are frequently observed, especially in CDG cases with mutations in the *SRD5A3* gene. Materials and Methods: In order to understand how an N-glycosylation defect could affect cerebellar development, we developed a cerebellum specific *Srd5a3* conditional KO mouse. **Results:** This model recapitulates the human defect with abnormal protein N-glycosylation and motor coordination impairment. Cerebellar development is abnormal at the histological level with the presence of granule cells ectopia in the molecular layer. Proteomic profiling allowed us to identify a family of deregulated adhesion N-glycoproteins that are likely to be responsible of the observed histological defect. Further work is currently on going to confirm the implication of those adhesion molecules in the disease and to test for therapeutic options, *in vitro*. **Conclusions:** We have generated a suitable model for SRD5A3-CDG, whose results may be extrapolated to other CDG. Second, we have shown that the cerebellar defect is likely the consequence of an impaired granule cells development. Investigation of the biochemical basis of the cerebellar defect suggests that the misregulation of a limited set of glycoproteins involved in neural cell adhesion may play a prevalent role in the cerebellar defect.

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P09.040D

CNV-risk: A database of published association results examining genetic copy number variants and their effect on psychiatric illness and cognition

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Rare genetic copy number variants (CNVs) comprise one of the most severe risk factors for psychiatric illness. SNP-microarray technology has helped to establish strong associations between specific CNV loci and psychiatric illness, cognitive and dysmorphic features. CNVs are highly pleiotropic, and cause impairment to specific cognitive domains (and brain structures) in both affected and healthy carriers.

The literature describing CNV effects is growing rapidly, but there is currently no central resource that compiles this complex knowledge in a manner that facilitates easy combined analysis. Here we present a solution, through the development of a structured database loaded with CNV association results from published literature.

We constructed a structured SQLite database schema to hold information on; loci positions, association results,

phenotypes, sample size, and inter study relations. The data source are tables from published articles which are loaded into the database via a function that integrates overlapping loci (prompting users with best database match through position and locus names comparisons). Along with the database we present a set of interactive graphical visualisations using the R Shiny package to help query CNV effects.

The database presented here allows researchers to perform efficient meta-analysis, cross querying, and annotation of local datasets. In addition we provide running database updates along with the tools needed to include additional data. This resource may be valuable for researchers as well as clinical geneticists who seek to compare their practice with the latest published literature. The database and applications are available for download from https://github.com/hilge/CNV-risk.

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Genome-wide characterization of copy number variants in epilepsy patients

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Epilepsy will affect nearly 3% of people at some point during their lifetime. Previous copy number variants (CNVs) studies of epilepsy have used array-based technology and were restricted to the detection of large or exonic events. In contrast, whole-genome sequencing (WGS) has the potential to more comprehensively profile CNVs but existing analytic methods suffer from limited sensitivity and specificity. To improve on this, we developed PopSV, an algorithm that uses multiple samples to control for technical variation and enables the robust detection of CNVs. Using WGS and PopSV, we performed a comprehensive characterization of CNVs in 198 epilepsy samples and 301 controls and found an enrichment of rare exonic events in patients. Notably, this genome-wide survey also revealed an enrichment of CNVs in proximal non-coding elements of previously known epilepsy genes. In total, coding and non-coding putative pathogenic events were found in 98 (46.4%) patients. This study was supported by finding from Genome Canada, Genome Québec, Canadian Foundation for Innovation, NSERC and CIHR.

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Severe cognitive impairment and early-onset epilepsy in six patients with the *de novo* p.Glu590Lys variant of *CUX2*

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Cut homeodomain transcription factor CUX2 plays an important role in dendrite branching, spine development, and synapse formation in layer II-III neurons of the cerebral cortex. Abnormal dendrites and synapses in Cux2(-/-) mice correlate with reduced synaptic function and defects in working memory. A *de novo CUX2* p.Glu590Lys variant was reported in two patients involved in large-scale whole-exome sequencing (WES) studies on intellectual disability and epileptic encephalopathies. We report on clinical data

of six patients carrying the *de novo* p.Glu590Lys variant. There were 4 males and 2 females. Mean age at inclusion was 13.6 years [8–21]. Epilepsy occurred in all patients. Age at onset of seizures ranged from 2 months to 1 year [mean = 6.6 months]. Seizure types at onset were myoclonic seizures, atypical absence with myoclonic component, and focal seizures. Seizures were drug-resistant in all patients but one. EEG initially showed generalized polyspikes and waves (4) or multifocal epileptiform discharges (2). Two patients are seizure-free under treatment whereas the others still have persistent seizures. Cognitive regression was noticed in childhood at least for two patients, at 8 and 12 years, respectively. All patients had severe cognitive impairment and autistic features were present in 4. Two patients had ataxic gait. Brain MRI only showed minor and non-specific anomalies.In conclusion, patients carrying the p.Glu590Lys variant of *CUX2* display a homogeneous clinical presentation with infantile-onset epilepsy frequently including myoclonic jerks with polyspikes and waves or multifocal epileptiform discharges. Patients have severe cognitive impairment sometimes associated with psychomotor regression in childhood.

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P09.043C

Biallelic mutations in Disabled-1 (DAB1) in a patient reminiscent for a RELN phenotype at brain MRI

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The Disabled-1 (*DAB1*) gene encodes a key regulator of Reelin signaling, which plays a critical role in the correct positioning of neurons within the developing brain in mouse and humans. From animal studies it is known that Reelin binds to the lipoprotein receptors VLDLR and APOER2 on the surface of neurons leading to the phosphorylation of DAB1 which serves as an intracellular adaptor protein (Huang et al., 2005). In mice, both Reln and Dab1 are required for the proper layering of neurons in the cortex. Disruption of murine *Dab1* generates a phenotype comparable to that of Reelin- deficient mice. Autosomal recessive mutations in Reelin in humans show a similar phenotype to the murine counterpart. Until now, no disease associated mutations in *DAB1* have been described in man.

Screening of patients with neurodevelopmental disorders and brain malformations by whole exome sequencing revealed compound heterozygosity for two splice-site mutations (c.307-2A>T, p.? and c.67+1G>T, p.?; NM_021080.3) in the coding region of the *DAB1* gene in an 11 year old girl who presented with oral motor difficulty, squint, dysdiadokokinesis, cerebellar ataxia, mild pyramidal signs, epilepsy well controlled by oxcarbazepine, mood changes treated with methylphenidate and a 55 TIQ score. The brain MRI showed mild cortical pachygyria, more prominent frontally, hypoplasia of cerebellar hemispheres and more pronounced vermis hypoplasia, enlarged perivascular spaces and lateral ventricles, suggestive of *RELN* mutation, which could not be found after Sanger sequencing. We suggest that mutations in *DAB1* should be considered in patients with a RELN-like phenotype at MRI.

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P09.044D

Results of a diagnostic NGS gene panel for patients with dementia

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In the Netherlands, diagnostic testing is offered to an increasing number of patients suffering from dementia. The identification of a causal mutation supports the clinical diagnosis, especially in patients with dementia of unknown etiology. Furthermore, when a causal mutation is found, presymptomatic testing becomes an option for their relatives. During the last decade, our laboratory offered diagnostic testing for the 5 most prevalent dementia genes by Sanger sequencing. Since September 2014, we also offer a virtual gene panel based on next generation sequencing (NGS). Our virtual panels consist of whole exome sequence (WES) data filtered for specific disease genes. The advantage of virtual panels is that they are flexible and can be immediately changed when new genes are discovered. Copy number variations (CNV) analysis on WES data is possible and will be implemented as well. For dementia we started with a panel comprising 41 genes that have been associated with Mendelian inherited dementia at an early age. Here we present the outcome of the results in the first

180 tested individuals and compare the diagnostic yield of the NGS gene panel with the Sanger gene panel. Furthermore, we show that the diagnostic result changed the clinical diagnosis in some of these patients.

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P09.045A

Non-random mating signal on major histocompatibility complex in GAIN ADHD study

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Introduction: Mate choice in human is under the influence of a complex interplay of socio-cultural, psychological and biological factors. Among biological factors, the role of the MHC has been discussed. A significant disassortative mating signal has been previously found in European HapMap samples Here, using genome-wide data, we investigate non-random mating at the MHC in a large European and Middle Eastern dataset.

Materials and Methods: We analyzed GAIN ADHD dataset, a trio study for Attention Deficit Hyperactivity Disorder comprising genotypes for 883 unrelated couples from 7 European countries and Israel. In order to quantify relatedness between spouses, we jointly used two measures: Rousset's distance and the genetic correlation based on standardized genotypes. Those were applied firstly to genome-wide data and secondly to the MHC.

Results: Our results show that, in most populations except Spain and the UK, there is a significant tendency to choose a partner more genetically related than if chosen at random in the population. These patterns may reflect socio-cultural factors influencing mate choice or may relate to the sampling strategy. Contrariwise, the MHC region displays significant signature of disassortative mating in the Netherlands, Ireland and the UK.

Conclusion: Our results support that non-random mating exists at MHC in human populations, a mating strategy possibly optimizing MHC diversity and immunity in the offspring. The MHC is associated with many multifactorial diseases and our findings highlight the need to better understand the influence of such mate choice related fine scale structures on association studies.

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P09.046B

Application of next generation sequencing for genetic diagnosis of dystonia

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Dystonia is a genetically heterogeneous disease that causes involuntary muscle contractions. These contractions result in repetitive movements or abnormal postures in the patients. Nowadays, more than 30 genes are known to be responsible of this pathology; however still there is high number of patients without a genetic diagnosis. Fifteen patients with clinical suspicion of dystonia have been studied using the TruSight One Sequencing Panel (Illumina). This panel contains exonic regions harboring disease-causing variants in approximately 4,800 clinically relevant genes. The study was focused in genes previously related to dystonia and we identified 7 genetic variants responsible for the phenotype in 5 patients. In particular, changes have been identified in *GCH1*, *PANK2*, *PARK2*, *SGCE* and *THAP1* genes. The variants detected in *SGCE* and *THAP1* genes had been never previously described. Confirmatory analysis and familial studies of these variants allowed establishing pathogenicity of these changes. The application of next generation sequencing allowed the identification of pathogenic mutations in almost 35% of patients with dystonia. These results encourage the use of massive sequencing for the diagnostic of dystonia as well as for other clinical and genetic rare diseases.

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Novel mode of inheritance of *ADCY5*-related generalized dystonia and myoclonus

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Introduction Following the association of familial dyskinesia and facial myokymia (FDFM) with *ADCY5* mutations in 2012, monoallelic *ADCY5* mutations have been associated with benign hereditary chorea and a mixed hyperkinetic syndrome of dystonia, chorea, and myoclonus. We report two siblings with generalized dystonia and myoclonus associated with two novel pathogenic variants of *ADCY5*. **Material and methods** A 27 year old woman and her 24 year old brother presented for evaluation of a movement disorder. Both had generalized dystonia and myoclonus. Due to familial juvenile-onset generalized dystonia with myoclonus and the previous negative evaluations, exome sequencing was pursued. Testing revealed the presence of a maternally inherited frameshift variant (c.409_428del20; p.G137Cfs*184) and a paternally inherited missense variant (c.3037C>T; p.R1013C) in *ADCY5* in both siblings. Both variants were interpreted as pathogenic, the maternally inherited mutation because it results in a frameshift alteration and the paternally inherited missense mutation because it is a previously unknown variant, is evolutionarily conserved across species, and is predicted to be deleterious by *in* silico analyses. **Conclusions** *ADCY5* mutations are believed to be inherited in an autosomal dominant fashion based on observations from the original FDFM pedigree. Here we report two siblings with a phenotype consistent with ADCY5-related dyskinesia and biallelic pathogenic variants in *ADCY5*. This family adds to the growing knowledge about ADCY5-related dyskinesia by providing an example of how certain pathogenic variants when inherited in an autosomal recessive manner may produce a movement disorder phenotype identical to that produced by autosomal dominant mutations.

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P09.048D

Pleiotropy of genes involved in early onset epileptic encephalopathies

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BACKGROUND: Epileptic Encephalopathies (EOEEs) are a group of clinically heterogeneous neurological disorders of early infancy, characterized by premature and often drug-resistant seizures typically resulting in altered neurodevelopment with motor and cognitive deficits. Indeed EOEEs represent a common clinical outcome in a broad spectrum of genetically heterogeneous conditions. As single gene testing is no longer a practical approach, the use of Next Generation Sequencing should be applied as a routine molecular diagnostic strategy in patients with early onset seizures.

MATERIALS and METHODS: We developed a customized targeted panel containing 31 genes which has been used for the analysis of 89 well-selected individuals, on Ion PGM™ Sequencer using Ion Reporter software 5.0.

RESULTS: We have identified and characterized 18 disease-causing variants that seem to further expand the phenotypic spectrum of early onset epilepsy genes. We have detected pathogenic variants in *SCN1A, SCN2A, SCN8A, SCN1B, KCNQ2, FOXG1, PCDH19*, *ALDH7A1* genes. A novel *ALDH7A1* homozygous mutation c.1256C>T was detected in a newborn, seventh child of consanguineous parents who previously lost three children affected with seemingly different phenotypes.

We found a *KCNQ2* and *SCN1A* variants, both novel and likely pathogenic, to co-segregate in a subject with an atypical picture of early onset focal drug resistant epilepsy.

CONCLUSIONS: The peculiar phenotypes of individuals we found to carry pathogenic mutations can contribute to further expanding the genotypic and clinical spectrum of these disorders. Our findings highlight the pleyotropy of early onset epilepsy genes and stress the importance of integrating clinical and molecular data to improve genotype-phenotype correlation.

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HYPOPLASTIC ANAEMIA IN *AP3B4* RELATED EPILEPTIC ENCEPHALOPATHY

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We report on a patent affected with early infantile epileptic encephalopathy, EIEE48 [MIM 617276], who also presented with mutation negative, Diamond-Blackfan anaemia, and suggest that this is a rare feature of recessive *AP3B2* related phenotype.

This patient is one of three affected members from a consanguineous, Turkish family, harbouring a homozygous inactivating variant c.445_448delGCTA p.(Ala149fs) in *AP3B2*. The neurological phenotype was very similar in all three patients, and consisted of early onset seizures, profound developmental delay, microcephaly and slowly progressive, choreoathetoid movement disorder. In addition, our patient presented with severe congenital anaemia with bone marrow appearance of Diamond-Blackfan anaemia, requiring multiple transfusions. Sequencing of 85 genes associated with Diamond-Blackfan anaemia was normal. Haematological evaluation of the other affected patients within this family was normal.

AP3B2 is a subunit of the heterotetrameric, neuronal AP-3 complex involved in vesicular transport in neurons. The structurally similar, ubiquitously expressed AP-3, contains AP3B1 instead. *Pearl* mice, deficient in ubiquitous AP-3, manifest abnormal haematopoiesis, but no neurological signs, while *Mocha* mice deficient in both, ubiquitous and neuronal AP-3 exhibit a haematological and neurological phenotype.

The clinical picture in our patient demonstrates an overlap between the neuronal and ubiquitous AP-3 deficient phenotypes. This suggests that neuronal AP-3 may exert effects beyond the nervous system, or that the mutant AP3B2 perhaps interfere with the ubiquitous AP-3. Future reports will help delineate the phenotype and further functional studies clarify the functional relationship between the ubiquitous and neuronal AP-3.

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P09.050B

A novel CNKSR2 deletion associated to epilepsy-aphasia spectrum

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The Epilepsy-aphasia spectrum includes a group of disorders from severe epileptic encephalopathy with continuous spike-waves during sleep (CSWS) and Landau Kleffner syndrome to the mild condition of childhood epilepsy with centrotemporal spikes. Recently, a genetic etiology has been associated with many of these epileptic disorders. Mutations in the RAS-signaling protein encoded by *CNKSR2* have been found in patients with seizures and neurodevelopmental deficits, especially those with unexplained language problems.

Sanger sequencing of *GRIN2A* was carried out to exclude *GRIN2A* variants before performing Sanger sequencing of *CNKSR2* and a customized array-CGH enriched with probes targeting *CNKSR2*, in order to identify alterations in males patients with CSWS (>50%) and language deficit (n = 15).

We identified a novel 10-kb deletion (chrX: 21609392-21619786) in *CNKSR2* in one patient with CSWS and language deficit. In total, we identified 5 disease-causing variants, of which 1 (9%) is in *CNKSR2* and 4 (26%) in *GRIN2A*.

The molecular diagnosis of *CNKSR2* is a guarantee in patients belonging to these types of epileptic disorders (up to 9% in our cohort).

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P09.051C

The searching for new variants in genes *CNTNAP2* and *NRXN1* for patients with epileptic encephalopathies

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Background: epilepsies are highly heterogeneous multifactorial disease. The only way to identify the causal mutations is identification of all genetic variants associated with epilepsy. The aims of study: searching the new genetic variants at *CNTNAP2* and *NRXN1* genes. Methods: 90 samples: 454 GSJunior (NimbleGen SeqCap target enrichment, 34 genes, associated with epileptic encephalopathy), 20 samples: Illumina HiSeq (SureSelect, complete exome sequencing). Informed consents were obtained from legal representatives of patients according local ethical approval. **Results:** Five variants for five samples was found at *NRXN1* gene: two compound heterozygous missense mutations (rs56086732 and rs200074974) were found for

two boys (4 and 6 years old) with preliminary diagnoses symptomatic focal and cryptogenic epilepsies; one heterozygous missense mutation (rs200646155) was found for girl 11 y/o with preliminary diagnoses GEFS+; one new undescribed heterozygous missense mutation (NG_011878.1:g.541548G>C, Asp>His) was found for girl 6 y/o with preliminary diagnoses GEFS+; one new inversions (311b.p. in length) for patient with preliminary diagnosis Aicardi-Goutieres syndrome. An interesting new heterozygous nonsense mutation (2 cases: patient with symptomatic epilepsy (boy, 2 years old) and health control) we have found at the end of *CNTNAP2*`s CDS, NC_000007.14:g.148415611A>Term, resulting in loss of the last amino acid residue (-KKEWLI[Opal]>-KKEWL [Amber]). The authors are grateful to MD Zhylina S., Meshcheryakova T, Ananyeva T., Lukyuanova E., and Ayvazyan S. from Research Center for Children Medical Care for patients selection. The research was supported by the Department of Health of Moscow.

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P09.052D

Improving interpretation of the clinical significance of SCN1A variants in patients with Dravet syndrome using in silico analysis of missense mutations

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Introduction: Interpreting the clinical significance of genetic variants can be challenging, particularly for variants of unknown significance such as missense mutations. To access the rate of success in the classification of missense variants, we assessed algorithms to predict functional effects of *SCN1A* missense mutations in patients with Dravet syndrome (DS) reported in the literature and in our molecular study. **Materials and Methods:** We assessed the use of ten different computer algorithms individually to predict putative deleterious effects of amino acid changes resulting from missense mutations in *SCN1A* in patients with DS. In addition, we developed a classifier to obtain more reliable prediction combining the scores of five prediction algorithms. Furthermore, we evaluated whether amino acid changes are predominant in specific protein regions by performing a permutation test. **Results:** The majority (57.5%) of the 353 *SCN1A* missense variants in patients with DS are predicted as deleterious by all ten algorithms individually. Almost all amino acid changes (92.6%) are considered deleterious by more than half of the algorithms tested. Our classifier combining multiple prediction scores presented high accuracy (0.8873), sensitivity (0.8379) and specificity (0.9191). Moreover, we found a predominance of amino acid changes in the voltage sensor segment (S4), the pore forming region (S5-S6) and adjacent subunit S6 ($p<0.05$). **Conclusion:** We were able to correctly ascertain putative pathogenic effect in the vast majority of missense mutations in *SCN1A* found in patients with DS, thus minimizing the inconvenience of inconclusive reports in the molecular diagnosis of patients with this severe form of epilepsy.

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Targeted gene testing on a cohort of 122 epileptic cases referred to a single institution reveals a high diagnostic rate on Early Infantile Epileptic Encephalopathy

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BACKGROUND. Epilepsy is a common clinical and genetic heterogeneous neurological disorder, with a large number of cases caused by genetic factors. To understand the molecular basis of epilepsy, 145 genes associated to syndromic and non-syndromic epilepsy were analysed on 122 cases. MATERIAL AND METHODS Genomic libraries were generated using the Ion AmpliSeq Exome RDY as exome backbone, combined with an AmpliSeq panel design to improve gene coverage. Sequencing reads generated on the Ion Proton and Ion S5 platform were analyzed using Torrent Suite software. Annotated variants using ION Reporter were prioritized with an in-house

analytical pipeline. RESULTS Among the 90 patients referred as Early Infantile Epileptic Encephalopathy (EEIE). 49%were genetically diagnosed. SCN1A, KCNT1 and SPTAN1 genes, were the most frequently mutated in this group. On the 32 remaining patients, pathogenic variants were detected 19% of the cases. ATP1A2 and TPP1 were the most frequently mutated genes. Out of the 169 identified variants, 66 were associated to genes with an autosomal-dominant inheritance pattern. Variants of uncertain significant category were identified mainly in genes recently associated to epilepsy as RYR3, GPR98 and FASN. Recurrent updating of targeted genes, the familial segregation of identified variants, and the analyses of other tissues in cases with mosaicism (as CDKL5) or associated to an inheritance pattern of cellular interference (as PCDH19) was essential. CONCLUSIONS Epilepsy panels based on WES provide a cost effective and comprehensive strategy that accelerates the identification of a definitive clinical diagnosis, improving the prognosis accuracy and facilitating the therapy selection.

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P09.054B

Confirmation of mutations in the *PROSC* gene as a novel cause of vitamin B6 dependent epilepsy

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Vitamin B6 dependent epilepsy with neonatal seizure onset is a heterogeneous clinical entity with defects in two known genes (*ALDH7A1* and *PNPO*) explaining a major fraction of cases. Very recently biallelic mutations in *PROSC* suggested this gene as a novel cause in five families. The reported patients with *PROSC* mutations showed a rather uniform clinical picture with 6/7 patients presenting with neonatal seizure onset within 24h and a burst suppression pattern in 5/7. 4/7 patients had a neonatal head circumference <10%, cranial imaging was abnormal in 4/7, and none of the patients had normal cognitive function. PLP concentrations in plasma and CSF were abnormal in all patients tested. We now identified four novel unrelated patients harbouring a total of six different mutations, including four novel disease mutations. In contrast to the previous observation, all four of our patients were normocephalic and had normal cranial imaging, and three had a favourable intellectual outcome. Vitamin B6 plasma profiling on pyridoxine of our patients with *PROSC* mutations is currently ongoing and will delineate if it is specific for *PROSC* mutations. The clinical and electroencephalographic phenotype in patients with *PROSC* gene mutations was indistinguishable from the previously known genes *ALDH7A1* and *PNPO*. We therefore confirm the *PROSC* gene as novel gene for vitamin B6 dependent epilepsy and will delineate the according plasma vitamin B6 profile.

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P09.055C

Molecular diagnosis of patients with epilepsy and developmental delay using a customized panel of epilepsy genes

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Pediatric epilepsies are a group of disorders with a broad phenotypic spectrum that are associated with great genetic heterogeneity, thus making sequential single-gene testing an impractical basis for diagnostic strategy. The advent of next-generation sequencing has increased the success rate of epilepsy diagnosis, and targeted resequencing using genetic panels is the a most cost-effective choice. We report the results found in a group of 87 patients with epilepsy and developmental delay using targeted exome sequencing (custom-designed Haloplex panel). Using this gene panel, we were able to identify disease-causing variants in 17 out of 87 (19.5%) analyzed patients, all found in known epilepsy-associated genes (*KCNQ2*, *CDKL5*, *STXBP1*, *SCN1A*, *PCDH19*, *POLG*, *SLC2A1*, A*RX, ALG13*, *CHD2*, *SYNGAP1*, and *GRIN1)*. Twelve of 18 variants arose *de novo* and 8 were novel. The highest yield was found in patients with onset in the first years of life, especially in patients classified as having early-onset epileptic encephalopathy. Knowledge of the underlying genetic cause provides essential information on prognosis and can be used to avoid unnecessary studies, resulting in a greater diagnostic cost-effectiveness. Ministerio de Economía y Competitividad (SAF2010-18586 and SAF2013-48960-P).Fundación Conchita Rábago de Jiménez Díaz

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P09.056D

Impact of a targeted next generation sequencing strategy for the genetic diagnosis of epileptic encephalopathies

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Objective: Epileptic encephalopathies (EE) are clinically and genetically highly heterogeneous conditions. We developed a custom targeted next-generation sequencing (TNGS) strategy for the molecular diagnosis of EE.

Patients: A custom NGS panel targeting 151 genes was applied to a cohort of 92 patients (51 boys, 41 girls) affected by EE classified as known syndromes in 61% and unclassified conditions in 39%. Epileptic seizures started at a mean age of 9 months (range: birth-7 years). Acquired pathologies, metabolic diseases and brain malformations were excluded.

Results: A disease-causing genetic variant was identified in 35/92 (38%) of patients and variants of unknown significance (VUS) in 7/92 (8%). The groups of migrating focal seizures of infancy and unclassified EE had the highest rate of positive findings, respectively 70% and 42%. The most frequently involved genes were *KCNQ2*, *KCNT1* and *SCN2A*. Mutations in *KCNA2, PIGA, ALDH7A1* and *SCN8A* were found in "atypical" phenotypes. TNGS allowed the detection of *SCN1A* mutation at a low rate somatic mosaicism (14%) and of *WWOX* exonic deletions in 2 patients.

Conclusions: With a diagnostic yield of 38%, TNGS is an efficient strategy as first-step genetic screening of EE. Our results allowed prenatal diagnosis in 4 families. Moreover, identifying a genetic diagnosis in patients with EE can orient treatment management and clinical outcome. Even if targeted approaches are restricted to a selected group of genes, interpreting variants generated by TNGS is challenging. TNGS offers a rapid and relevant selection of patients available for WES and WGS studies.

1

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The searching for new variants in genes *SLC2A1* and *RNASEH2A/B/C* for patients with epileptic encephalopathies

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Background: epilepsies are highly heterogeneous multifactorial disease. Identification of the causal mutations is rather difficult task. The aims of study: searching the new genetic variants at *SLC2A1* (GLUT1 deficiency syndrome) and *RNASEH2A/B/C* genes (AGS4/AGS2/AGS3 types of Aicardi-Goutieres syndrome (AGS)). Methods: 90 samples: 454 GSJunior (NimbleGen, 34 genes, associated with epileptic encephalopathy), 20 samples: Illumina HiSeq (SureSelect, WES). Informed consents were obtained from legal

representatives of patients according local ethical approval. **Results:** 6 variants were identified at *SLC2A1* gene for 6 samples: 5 synonymous substitutions (rs146879902, rs2229682, rs11537641, rs1385129, rs34025424; ClinVar) and 1 new indel (6 b.p. in length) at position g.36954 (NG_008232.1) (female, 6 y/o, MIM:606777). Among the 100 samples medical geneticist preselected ten patients with preliminary diagnosis AGS. In *RNASEH2A/B/C* genes we found 8 heterozygous missense mutations: *RNASEH2A*: 5 rs7247284 and 1 rs62619782; *RNASEH2B*: 2 rs144408326 and rs78705382. Also we identified 5 heterozygous synonymous variants (2 at *RNASEH2C*, 3 at *RNASEH2A*). Of particular interest were two cases: compound heterozygous (two missense mutations rs7247284, rs62619782 at *RNASEH2A*) was found for boy 5 y/o without spech. For girl 16 y/o with strong intellectual retardation and speech disorder were found heterozygous rs78705382 (*RNASEH2B*) and rs138809301 (*GRIN2A*) variants. The author is grateful to MD Ayvazyan S., Ananyeva T., Lukyuanova E., Meshcheryakova T, and Zhylina S. from Research Center for Children Medical Care (Moscow) for patients selection. The research was supported by the Department of Health of Moscow.

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P09.058B

Genome-wide association study in essential tremor identifies three new loci

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Introduction: Patients with essential tremor (ET) have a postural and kinetic tremor of the upper extremities and sometimes of other body parts. Twin and family history studies indicate a high heritability for ET. However, the molecular genetic determinants of ET are largely unknown. Previous GWAS studies suggested associations between single nucleotide polymorphisms in *LINGO1* and *SLC1A2* and ET. Replication studies yielded equivocal results. Materials and Methods: We conducted a two-stage GWAS of ET. A total of 2,807 ET-patients and 6,441 controls of European descent were analysed. The Affymetrix Axiom Genome-wide CEU 1 Array Plate genotyping chip was used in the discovery stage. The most significantly disease associated genetic markers were genotyped in the replication stage in an independent case/control sample. We performed expression analysis of the three ET associated genes in cerebellar cortex tissue of patients and controls and mined public brain eQTL database Braineac. **Results:** After Bonferroni correction, two genetic markers were confirmed to be associated with ET in the replication stage. The genes flagged by these markers code for the serine/threonine kinase *STK32B* and a transcriptional coactivator *PPARGC1A*. Another locus in *CTNNA3* met the combined analysis significance threshold. Expression analysis revealed a significant increase in *STK32B* expression in ET patients, while no differences were detected for the other genes. **Conclusions:** We identified ET associated single nucleotide polymorphisms in three loci corresponding to the genes *STK32B*, *PPARGC1A* and *CTNNA3*. Expression analysis and public eQTL data provide additional support for a role of *STK32B* in ET pathogenesis.

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Neurological phenotype for DST-related disorders

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The gene *DST* encodes for the large protein BPAG1 that is involved in the formation of hemidesmosomes. Its alternative splicing gives rise to tissue-enriched isoforms for brain, muscle, and skin. Homozygous *DST* loss-of-function mutations cause an autosomal recessive form of epidermolysis bullosa simplex (EBS). We present the case of a 17-years-old girl with pain insensitivity, recurrent skin blistering, behavioral problems without cognitive impairment. Blisters were localized at the sites of friction and evolved into ulcers, pigmentary lesions, and atrophic scars. In addition, she had recurrent headaches, and syncope episodes, iris heterochromia, cataract, hearing impairment, syringomyelia, chronic diarrhea, osteopenia with multiple fractures, osteomyelitis, and growth hormone deficiency. EMG and nerve biopsy showed sensitive and autonomic peripheral neuropathy. A family-trio whole-exome sequencing unraveled two compound heterozygous variants in the *DST* gene with highly-predicted functional impact: c.806A>G (p.H269R) in exon 29, absent in controls, and c.3886C>T (p.R1296X) exon 7, 1 allele present in ExAC. Interestingly, exon 7 is included in the neuronal isoform whereas exon 29 in all isoforms. Our patient expands the phenotype of *DST*-related disorders that are associated with different phenotypes ranging from isolated skin or peripheral nervous system involvement to more complex phenotypes including a combination of skin and neuronal features, likely as a consequence of the location of the mutation relative to the *DST* isoforms. The identification of further patients with *DST* mutations might allow to better refine this genotype-phenotype correlation and to improve clinical management. Supported by Telethon Italia grant GSP15001/C.

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Mitogenomic analysis in FXTAS

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Introduction: Mitochondria are key regulators of cellular energy production and are involved in cellular processes such as cell cycle progression, differentiation and apoptotic cell death. Mutations in mtDNA accumulate during the aging process in cells and tissues, causing respiratory chain dysfunction and theoretically, compromising cellular function and survival. Some evidence support an association for somatic mtDNA mutations in the development or progression of neurodegenerative disease. Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder that appears in at least one-third of adult *FMR1* premutation carriers. In an attempt to provide new data on the role of mtDNA mutations in FXTAS pathogenesis, we have sequenced mtDNA employing next-generation sequencing techniques in FXTAS patients.

Material and Methods: mtDNA from 26 unrelated *FMR1* premutation carriers (13 FXTAS and 13 non FXTAS) were sequenced using the Nextera XT DNA Sample Preparation kit (Illumina) with a 3000X medium coverage in a MiSeq platform.

Results: No differences were observed in the total number of mtDNA on comparing the FXTAs and non FXTAS group. However, the FXTAS cohort showed a significantly higher number of heteroplasmic variants. An association was found between these variants and their localization (within or outside the D-loop) in FXTAs group.

Conclusion: FXTAS patients seem to accumulate a higher number of heteroplasmic variants in compromised

regions of the mtDNA, thereby suggesting the potential role of these variants in the pathogenesis of FXTAS.

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P09.061A

A novel missense variation (Q220R) of *GNB4* encoding a guanine nucleotide-binding protein, beta-4 in a Japanese neuropathy family

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Dominant intermediate Charcot-Marie-Tooth disease F (CMTDIF) is an autosomal dominant hereditary CMT caused by variations in the *GNB4* gene. We examined two Japanese familial cases with CMT. Case 1 was a 49-year-old male. The chief complaint was slowly progressive gait disturbance and limb dysesthesia which appeared at age 47. On neurological examination, he showed hyporeflexia or areflexia, distal limb muscle weakness, and distal sensory impairment with lower dominancy. Nerve conduction studies demonstrated demyelinating sensorimotor neuropathy with reduced action potentials in the lower limbs. Case 2 was an 80-year-old man, case 1's father, had no complaint. On neurological examination, he showed areflexia in the upper and lower limbs. Distal sensory impairment in the lower limbs was also observed. Nerve conduction studies revealed mainly axonal change. By the exome analysis, we identified a novel heterozygous nonsynonymous variant (c.659T>C; Q220R) in exon 8 of the *GNB4* gene encoding a guanine nucleotide-binding protein, beta-4 through the examination of 67 candidate genes known to be responsible for CMT. By Sanger sequencing, we confirmed that both patients are heterozygous for the variation. Q220R in GNB4 was predicted to be damaging by Polyphen-2. The SNV is located in the highly conserved region in GNB4 among vertebrates. ExAC showed the allele frequency was 0.000008247. This variation was not observed by in-house Sanger sequencing of 502 Japanese control subjects. We conclude that the novel variation in *GNB4* is the causative variant for the CMTDIF patient that is the first record of the disease in the Japanese population.

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P09.062B

With improving technologies, can we sit on the fence with Huntington disease alleles?

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The UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics challenged participants of the 2016 Huntington disease (HD) external quality assessment (EQA) scheme by distributing a patient sample with an allele containing 35 repeats (as determined by sourcing laboratory) i.e. on the normal/reduced penetrance border. Fourteen of the HD EQA reports submitted included the size of the triplet repeat allele and a sizing error range. Half of the participants were deducted marks for failing to state the possibility that taking into account their quoted sizing error meant a diagnosis of HD could not be excluded from the patient.

With advances in modern technology, there has been a significant improvement in the accuracy of triplet repeat allele sizing amongst laboratories. Seventeen of the eighteen participants reported identical allele repeat sizes (28 and 35 repeats) and the remaining laboratory was close to the consensus (28 and 36 repeats). However the implementation of ISO 15189 has resulted in uncertainty of measurement being included in clinical reports resulting in the allele size being portrayed as a range rather than a defined size.

Various testing methods were used for analysis and although there was consistency in the size of the alleles reported, the reluctance to commit to a specific size still remains, contributing to the deduction of marks in the HD 2016 EQA. The question laboratories must ask themselves is - with improving technologies, can we sit on the fence with HD alleles?

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P09.063C

Dissecting the mechanism of action of olesoxime, a potential therapeutic in the treatment of Huntington's disease

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Introduction Huntington's disease (HD) is a fatal neurodegenerative disorder which is caused by a CAG repeat expansion in the HTT gene, which encodes the disease protein huntingtin (htt). An important characteristic of HD is the proteolytic cleavage of mutant htt and formation of toxic protein fragments. Different studies showed that calpains, a calcium-activated class of cysteine proteases, are involved in htt proteolysis. Calcium dyshomeostasis and mitochondrial dysfunction - further features of HD - may amplify calpain-mediated mhtt fragmentation as well. This vicious circle could be interrupted by the cholesterol derivate olesoxime (TRO19622), which binds the mitochondrial calcium channel VDAC1 and may affect its conductance.

Material and Methods Brain samples of six month old Hdh-knock-in mice were collected to investigate VDAC1 and IP3 receptor levels in vivo. HEK 293T and PC12 cells overexpressing wild-type and mutant htt were transfected with cDNA plasmids or esiRNA to overexpress or knock down VDAC1 in the HD context. VDAC1 activity was targeted pharmacologically by the administration of olesoxime and itraconazol. All protein samples were analysed via western blotting.

Results We observed an elevated calpain system activation and an reduced VDAC1 expression in the striatum of six-month-old HD mice. Furthermore, we successfully performed VDAC1 knock-down and overexpression, and pharmacological treatments in cell culture to analyse their impact on calpain activity, and htt cleavage and aggregation.

Conclusion Targeting VDAC1 expression and activity helps to understand olesoxime's mode of action and might by itself represent a promising therapeutic approach for HD.

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P09.064D

Metformin rescues early cognitive symptoms in the HdhCAG150 mouse model and is therefore a promising candidate for treatment of HD patients

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an unstable glutamine (CAG) trinucleotide repeat expansion within exon 1 of the Huntingtin gene and leads to cognitive decline and affects motor abilities. In the prodromal phase patients develop mood swings, personality changes and subtle cognitive impairment. Close understanding of clinical signs and molecular mechanisms behind this early stage of HD is important for the development of a causal therapy. We have analysed a knock-in mouse model that carries 150 CAG repeats and the human exon 1 in the 5′ end of the murine huntingtin gene. By using a novel object recognition test we have found a profound deficiency of hippocampus dependent long-term memory in heterozygous transgenics. This phenotype was detected as early as 12 weeks of age. Motor deficits and intranuclear aggregates are described at much later stages. We have shown previously that in HD patients, mediated through mTOR signaling, translation of mRNA carrying expanded CAG repeats is elevated (Krauss et al., 2013). We have also seen that the biguanid metformin antagonizes mTOR signaling in neurons in-vitro and in-vivo (Kickstein et al., 2010). We show here that metformin, by interfering with the mTOR kinase and its opposing phosphatase, PP2A, regulates local protein synthesis in the brain and suppresses the production of disease making protein in early HD. Furthermore metformin leads to a rescue of early cognitive symptoms in the HdhCAG150 animal model. These data suggest that metformin is a promising candidate for early phase treatment of HD.

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P09.065A

Targeted next generation sequencing in patients with infantile bilateral striatal necrosis and movement disorders

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Introduction: Infantile bilateral striatal necrosis (IBSN) is a heterogeneous histo-pathological condition defined by the involvement of the neostriata that can be observed in a very large number of neurological conditions clinically dominated by the presence of movement disorders (MD). MD are a group of neurological syndromes which affect the ability to produce and control movement.

Materials and Methods: In a prospective study over the course of 1 year, we applied targeted next-generation sequencing (NGS) of 118 genes (76 genes for IBSN and 42 genes for MD), using HaloPlex technology from Agilent Technologies. In addition, we studied biomarkers of known causes of IBSN, including blood interferon signature and free-thiamine and pterins in CSF.

Results: We included 39 patients (26 male, age 1–21 years; mean±SD 8.1±4.3 years). Onset of symptoms was acute in almost all patients. Interferon signature was altered in two patients with Aicardi-Goutierès syndrome. Low free-thiamine and elevated pterins in CSF were detected in two and three patients, respectively. We established firm molecular diagnosis in 28% (11/39) of patients, with mutations in the following genes: *RNASEH2B, PDH1A*, *ADAR*, *SLC25A19, NDUFAF6, CACNA1A, FOXG1, SLC39A14, ATP1A3, TUBBA4* and *SGCE*, including seven novel mutations.

Conclusions: Targeted NGS leads to rapid and cost-effective detection of causative mutations in IBSN and MD. We have reached a diagnostic rate similar to other laboratories. Our study reports new cases of recently described genes and it brings us closer to the use of personalized medicine for routine diagnosis.

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P09.066B

PACS1 vs. GNAO1-single or aggregated effect on the phenotype of a boy with intractable epilepsy and neurological decline

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Whole exome sequencing (WES) is effective in identifying the genetic cause of seizures in patients with early onset epilepsy, with potential implications for specific treatment.

Method: A 10 years old male, presents intractable seizures(up to 100/month), since age 2 years,8 months. He has no dysmorphic features and shows severe regression in cognitive and motor skills. Normal brain structure. Array CGH and WES were performed.

Results: Array CGH was normal. WES detected a de novo heterozygous frameshift variant in the PACS1 gene, c.778dup p.(His260Profs*2); Chr11(GRCh37): g.65983707dup; classified as likely pathogenic. Another heterozygous variant in the GNAO1 gene, c.389G>A p.(Arg130Gln); Chr16(GRCh37): g.56362628G>A; was classified as VUS.

Discussion: A single de novo missense variant in PACS1 gene has been described by Schuurs-Hoeijmakers et al., as disease-causing for an autosomal dominant(AD) disorder characterized by mental retardation, distinct craniofacial

features, intellectual disability and seizures. GNAO1 gene pathogenic variants are associated with early infantile epileptic encephalopathy type 17, a severe AD neurologic disorder characterized by onset of intractable seizures in the first months of life; poor psycho-motor development and brain abnormalities.

Conclusion: This is an unreported PACS1 variant with partial overlap of the phenotype described by Schuurs-Hoeijmakers. The disorders associated with GNAO1 gene also show considerable phenotype overlap with the patient and therefore the variant possibly contributes to his overall presentation. Genetic counseling was done accordingly.

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P09.067C

De novo mutations in C terminal domain of ITPR1 are a frequent cause of Gillespie syndrome

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ITPR1 encodes inositol 1,4,5 tri-phosphate receptor type 1, a transmembrane protein localized at the endoplasmic reticulum involved in the regulation of intracellular Ca^{2+} homeostasis and signaling. *ITPR1* is highly expressed in the cerebellum, and inactivating or dominantly acting mutations in this genes have been identified to cause spinocerebellar ataxia and non-progressive congenital ataxia with or without intellectual disability. Recently, mutations of *ITPR1* have been associated with Gillespie syndrome, characterized by congenital ataxia, mild to moderate intellectual disability and iris hypoplasia. Exome sequencing and targeted resequencing in two families with sporadic Gillespie syndrome allowed to identify a novel (p.N2576I) and a previously reported (p.K2596del) *de novo* heterozygous mutations affecting the C-terminal transmembrane domain of the receptor in both patients. Our study expands the mutational spectrum of *ITPR1* associated with Gillespie syndrome and indicates that *ITPR1* screening should be implemented in patients with congenital cerebellar ataxia with or without aniridia.

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P09.068D

Mutations in *KIAA0753* cause Joubert syndrome associated with growth hormone deficiency

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Introduction: Joubert syndrome (JS) is a type of ciliopathy defined based on mid-hindbrain abnormalities that result in characteristic "molar tooth sign" on brain imaging. Although over 30 JS genes have been identified to date, they do not account for all JS patients suggesting further genetic heterogeneity. Materials and Methods: We performed whole exome sequencing (WES) of a family with two affected individuals presented with JS and severe growth hormone deficiency. We used RT-PCR, immunoblotting and fluorescence microscopy to further investigate the pathogenicity of mutations. **Results:** WES identified novel compound heterozygous mutations in *KIAA0753* (OFIP), a missense (p.Arg257Gly) and an intronic (c.2359-1G>C) mutation, in both affected siblings. OFIP, a component of OFIP-OFD1-FOR20 complex, is a centrosome and pericentriolar satellite (PS) protein essential for ciliogenesis and centriole duplication. p.Arg257Gly is pathogenic according to prediction tools and c.2359-1G>C alters normal splicing by activating a cryptic acceptor splice site, leading to skipping of nine nucleotides that in turn deletes three amino acids from the coding frame (p.Lys787_Gln789del). The missense mutation lies in the PS targeting domain of OFIP which likely disturbs the recruitment of OFIP complex to the centrosome while in-frame deletion resides in the highly conserved C-terminal domain that interacts with OFD1. Analysis of cilia from cells of patients showed significantly lower number of

ciliated cells (~20–30%) and decrease in cilia length (~15–20%). **Conclusions:** Our study highlights two patients with JS with defective KIAA0753, that affects normal ciliogenesis. Funding: This work was supported by the Intramural Research Program at the NHGRI, NIH, USA

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P09.069A

Autosomal dominant Kidd-null blood group with mood disorders is associated to a zinc-finger deletion at *ZNF850*

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Background: The Kidd-null blood group, lacking the urea transporter UT-B1/SLC14A1 in the erythrocyte membrane, is associated with transfusion risk and urine concentration defect in humans, and depression-like behavior in mice. While the autosomal recessive form is due to biallelic *SLC14A1* mutations, the cause of the dominantly inherited form remained unknown. We identified six new families originating from the south of Spain with dominantly inherited Kidd-null. *Methods*: Subjects with Kidd-null erythrocytes underwent medical and psychological evaluation, and two probands were tested for urine concentration. We performed genome-wide linkage analysis, exome sequencing, mRNA and protein expression analyses in samples from patients, and functional studies in cell lines. *Results*: Most Kidd-null individuals (80.77%) fulfilled criteria for mood and/or anxiety disorder, with 7.4-fold increased suicidal risk (95%CI:2.3–16.7, $p=7.9\times10^{-4}$). The tested cases presented a reduced ability to concentrate urea in urine. Kidd-null cells had reduced quantity of glycosylated UT-B1 at the erythrocyte membrane although SLC14A1 mRNA levels were normal. Linkage analyses identified a shared haplotype at 19q13 and exome sequencing identified a deletion of a single C2H2 zinc finger-encoding domain of ZNF850 in Spanish kindreds, absent in controls. An overlapping deletion was identified in a Japanese Kidd-null case. Compared to wild-type, mutant ZNF850 had decreased cytoplasmic location in transfected HEK293T cells. *Conclusions*: A predicted zinc-finger deletion at ZNF850, prevalent in Southern Spain due to a founder mutation, leads to UT-B1 dysfunction and underlies the dominantly inherited Kidd-null blood group. The phenotype associates subnormal urine concentrating ability, mood and/or anxiety disorders, and increased suicidal risk.

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P09.070B

NGS approaches for the molecular diagnosis of leukodystrophies : a french experience

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Introduction The confrontation of cerebral MRI patterns with clinical and neurophysiological data allowed the classification of leukodystrophies (LD), a heterogeneous

group of neurodegenerative hereditary diseases affecting the white matter (WM) and its major component, myelin. The genetic heterogeneity requires high-throughput sequencing approaches (NGS) to optimize the identification of the causal genes of the largest number of patients.

Materials and Methods Targeted resequencing panel of 45 genes associated with recurrent LD was developed to study 224 patients adressed for diagnostic purpose. In parallel, WES (whole exome sequencing) was performed in an independent cohort of 100 patients with LD of unknown etiology.

Results Whereas 56% of cases were diagnosed by WES analyses, targeted resequencing panel allowed the identification of causal mutations in 51/224 patients (23%). In 14 cases (6%), the absence of clinical, MRI data or segregation studies did not confirm the deleterious caracter of the found variants. Interstingly, 19% of the mutations identified by WES affected 4 genes involved in Aïcardi-Goutiere, HABC and polymerase III linked hypomyelinating syndrome in addition to PMD. Similar distribution of thoses diseases was obtened by the targeted resequencing approach suggesting that their clinical spectrum may have been incomplete.

Conclusion A targeted resequencing approach, studying the most frequent genes involved in LD, seems to be good strategy to estabilsh an exaustive molecular diagnosis in, approximatively, a quarter of cases. WES should be indicated as second step particularly for patients with familial forms presenting atypical clinical features. Importantly, clinical spectra of genetic WM abnormalities should be redefined.

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P09.071C

LBSL with intellectual disability: Apparent homozygosity due to segmental uniparental disomy at *DARS2* locus and misdiagnosis as syrinx of spinal cord

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Leukoencephalopathy with Brain Stem and Spinal Cord involvement and Lactate elevation (LBSL) was diagnosed in a 16 year old Australian girl with severe autism and intellectual disability but no neurological motor impairment. Diagnosis was made on review of brain imaging following identification of apparently homozygous *DARS2* mutations on Next Generation Sequencing. MRI was initially reported as showing patchy demyelination suggestive of Multiple Sclerosis, with a syrinx of the spinal cord but no brain stem lesions. The syrinx was correctly identified as demyelination of the posterior columns on review. Parental testing identified a heterozygous *DARS2* mutation in the mother but the father was not available for testing. A SNP array was performed looking for possible uniparental disomy to explain the apparent homozygosity as the parents were unrelated. This identified a region of Loss of Heterozygosity of 5.3Mb at chromosome 1q25 encompassing *DARS2*. Intellectual Disability is uncommon in LBSL and identification of motor disability was hindered here due to previous traumatic amputation of one leg and extreme behavioural problems.

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P09.072D

New RNA polymerase III Leukodystrophy

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RNA polymerase III (POLR3)-related leukodystrophy is an autosomal recessive hypomyelinating leukodystrophy characterized by a progressive cerebellar spastic syndrome with variable age of onset and associated hypo/oligodontia and hypogonadotropic hypogonadism. This disorder is caused by mutations in *POLR3A*, *POLR3B* and *POLR1C*. Here we report two cases of patients with mutation in a gene coding for another POLR3 subunit. The affected individuals from consanguineous Algerian parents present with a Pelizaeus Merzbacher like disease with a sitting position acquired with aid (form 1) in one patient and walking with aid (Form 3) for the other before 24 months of age. A progressive degradation was observed after 5 years of age leading to bedridden patients with severe spasticity and dystonia at respectively 6 and 16 years of age. Despite gastrostomy, severe growth impairment was observed in

both cases with hypogonadism in the oldest one and severe anorexia and hypodontia in the youngest one. Brain MRI showed a diffuse hypomyelinating leukodystrophy without cerebellum and subcortical /cortical atrophy at 4 y of age.

Exome sequencing combined to homozygosity mapping revealed a homozygous mutation. This variant was predicted to be deleterious, located in a highly conserved region and not detected in 492 ethnically matched control chromosomes. In silico structural and functional analysis predicted that this mutation create conformational changes in the POLR3 subunit complex. Functional studies in the patients' fibroblasts revealed an abnormal expression in ribosomal RNAs. This study confirm the role of the polymerase III in the nervous system and particularly in the white matter

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P09.073A

Improving diagnosis in malformations of cortical development using gene panel analysis

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Introduction: Malformations of cortical development (MCD) represent a huge burden to patients and their families. MCD are diagnosed by (fetal) magnetic resonance imaging or post-mortem studies. Molecular diagnosis is significantly hampered by the high degree of phenotypic and genotypic heterogeneity. To optimize diagnosis and facilitate genetic counselling we have developed a next generation sequencing gene panel for MCD.

Materials and methods: The panel includes 193 genes known to be associated with microcephaly, megalencephaly, focal cortical dysplasia, periventricular heterotopia, lissencephaly, cobblestone malformation, polymicrogyria, or vascular brain malformations.

Results: Over a period of 19 months, we have analysed 166 probands with MCD. We were able to find a causative mutation in 29 patients, resulting in a diagnostic yield of 17%. The average coverage is 823x and ~96% of the regions are covered at least 30×. Most mutations occurred *de novo*. The tubulin gene family genes were mutated most frequently in the cohort of patients with polymicrogyria or lissencephaly and included mutations in *TUBA1A* (2), *TUBG1* (2), *TUBB3* (2) and *TUBA2A* (1). The results have enabled us to further delineate the phenotypic spectrum associated with mutations in these genes. Mutations in *FLNA* (5) accounted for the most frequent cause of periventricular heterotopia. The remaining mutations occurred in *ARX, ASPM, CASK, COL4A1, DCX, DEPDC5, DYNC1H1, L1CAM, PTCH1, PTEN, RTTN, TCF4, TREX1* and *TSEN54*. The mutation in *L1CAM* was found in a fetal case.

Conclusion: The implementation of the gene panel in the MCD diagnostic workflow has improved the diagnostic yield and genetic counselling.

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P09.074B

Effect of variable numbers of tandem 30-base-pair repeats on promoter activity of the monoamine oxidase A gene

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Introduction: Monoamine oxidase A (MAOA) is an enzyme that degrades neurotransmitters such as serotonin, dopamine and norepinephrine. The upstream of the promoter region contains a number of polymorphic tandem 30-base-pair repeats, 2.5, 3.5, 4.5, and 5.5 repeats (2.5R, 3.5R, 4.5R and 5.5R), associated with neuropsychiatric disorders. Previous controversial reports have suggested that different repeats have been associated with different promoter activities (PA) of the *MAOA* gene. Materials and Methods: To evaluate the PA of known repeats and a novel 3.3R, we constructed five alleles, 2.5R, 3.3R, 3.5R, 4.5R and 5.5R, and inserted them into the basic pGL3 vectors, and transfected them into two neuroblastoma cell lines (LA-N-5 and SK-N-SH). We triplicately determined the PAs in each basic and constructed vector using the luciferase reporter assay. We used the paired t-test for the statistical comparison of mean PAs between pairs of alleles. **Results:** Vectors with 3.3R and 4.5R had approximately 1.5–2.0 higher PAs than those with 3.5R and 5.5R. The PAs were statistically significantly different among pairs of low and high PA groups with P-value < 0.05 (i.e. 3.3R vs 3.5R; 4.5R vs 5.5R). Although the vector with 2.5R almost always showed similar PAs to those with other alleles in both cell lines, a statistically significant difference between vectors with 2.5R and 5.5R was found only in the SK-N-SH cell line ($P = 0.03$). Conclusion: Our study confirms that the

length of tandem 30-base-pair repeats has an effect on the promoter activity of the *MAOA* gene. Grant: Medicine, PSU

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P09.075C

McLeod syndrome: a novel mutation in an Italian patient

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Introduction: McLeod syndrome (MLS) is a rare X-linked recessive neurodegenerative and multisystemic disease caused by mutations in the XK gene, that has been identified at the Xp21 locus. Clinical manifestations, which include acanthocytosis, chronic anaemia, haemolysis, hyperCKemia, myopathy, cardiomyopathy, chorea, epilepsy, usually appear after the third or fourth decade of life. Patient and Methods: We present a 46-year-old male patient of Italian origin with pharmacoresistant focal epilepsy of unknown cause of adult onset, associated with persistent and asymptomatic increase of creatine kinase (CK). The patient progressively developed mild dyskinetic involuntary movements, mild obsessive tendencies and axonal neuropathy; acanthocytosis was detected on blood smear, and immunoserological analyses confirmed the expression of the Kell antigens supporting the McLeod phenotype. Two cousins on the maternal side both affected by focal epilepsy of unknown cause: one showed asymptomatic CK increase and the other acanthocytosis. Molecular genetic analysis of the XK gene was performed by sequencing the three exons and flanking regions. **Results:** In the proband, molecular genetic analysis of the three exons of the XK gene detected a hemizygous 17 base-pair frameshift deletion at exon 1 (c.248_264delGGCCGAGACAACGGCGG). Discussion: To the best of our knowledge, this variant has not been previously reported and is not present in public databases. Epileptic seizures are reported within this syndrome up to 50% of cases, without specific syndromic features and usually are not the prominent symptom. This family showed epilepsy as the main clinical feature, which is not commonly described in this disease.

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P09.076D

Mitochondrial DNA haplogroups are Associated with psychiatric disease: a nation-wide study of 74,763 Danes

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Disturbed mitochondrial function has been implicated in psychiatric disease. All mitochondria contain a small maternally inherited DNA (mtDNA) of 16.6 kb. Through evolution and genetic drift the mtDNA sequence has become fixed into haplogroups (hgs) with a characteristic population and geographical distribution. Different hgs exhibit variable functional capacity and have been demonstrated to be susceptibility factors for various, predominantly degenerative, diseases. Using dried blood spots from 50,567 Danish psychiatric patients and 24,196 controls we examined the association between psychiatric disease and mtDNA haplogroup in the Danish population. Hg M was associated with affective disorder (n = 17260) with an OR of 0.47 (cfi.95%: 0.39 - 0.58) ($p = 1*10^{-14}$), ADHD (n = 13395) with an OR of 0.50 (0.40 - 0.62)($p = 2*10^{-11}$). Among patients belonging to the macro-hg N, patients with schizophrenia (n = 2589), had a high proportion of hg A (n = 45), OR: 4.52 (cfi.95%: 3.0 - 6.7)($p = 1.2*10^{-12}$). In a mitoGWAS, three mitoSNPs were highly associated with affective disorder (p-values ranging from $2*10^{-25}$ - $2*10^{-21}$), ADHD (p-values: $2*10^{-11}$ - $1*10^{-10}$), and anorexia (p-values: $6*10^{-8}$ - $6*10^{-7}$) and another mitoSNP with schizophrenia ($p = 2*10^{-11}$). In conclusion, psychiatric disease is a bigenomic disease. Haplogroup A is a risk factor for schizophrenia and haplogroup M is a protective factor.

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P09.077A

Mitochondrial DNA SNPs Associated with schizophrenia exhibit highly variable inter-allelic haplogroup affiliation and nuclear genogeographic affinity: Major concerns for link to disease

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Mitochondria play a significant role in human diseases. However, disease associations with mitochondrial DNA (mtDNA) SNPs have proven difficult to replicate. A reanalysis of eight previously schizophrenia-associated mtDNA SNPs, in 23,743 normal Danes and 2,538 schizophrenia patients, revealed marked inter-allelic differences in haplogroup affiliation and nuclear genogeophraphic affinity (GGA), suggesting population stratification. Two SNPs, m.15043A and m.15218G, were significantly ($p < 0.05$) and two, m.3197C and m.10398G, borderline significantly ($p < 0.1$) associated with schizophrenia. However, these associations disappeared when corrected for haplogroup affiliation. The extensive variation in SNP mtDNA affiliation and GGA should be major concerns when interpreting historic and designing future mtDNA association studies.

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P09.078B

Mosaicism of *de novo SCN1A* mutations in epilepsy: an explanation for variable phenotypes?

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Introduction: Phenotypes caused by *de novo SCN1A* mutations are very variable, ranging from Dravet syndrome on the severe end of the spectrum, to GEFS+ on the other end. We investigate whether mosaicism is a major modifier of *de novo SCN1A* mutations. Materials and Methods: We tested 39 patients for mosaicism, from a cohort of 131 patients with *de novo*, pathogenic *SCN1A* mutations. Clinical data were collected from medical records and semi-structured telephone interviews. Next Generation Sequencing with high coverage was performed on DNA from blood, after capture of *SCN1A* by single molecule molecular inversion probes (smMIPs). Only unique reads from single molecules were counted, using the single-molecule tag to remove duplicate reads and provide unbiased estimates of gene copies. The percentages of mutated reads of pathogenic mutations were used to determine whether mosaicism was likely, based on an expected binomial distribution and comparison with heterozygous neutral SNPs. Confirmation by droplet digital PCR and sequencing of buccal DNA is pending. **Results:** In 8 out of 39 patients, mosaicism is suspected (p-values ranging from $2{,}89\times10^{-04}$–$5{,}82\times10^{-36}$). Five patients were classified as "mild", one as "moderate" and two as "severe" (25–42%, 25% and 36–40% alternate allele respectively). **Conclusions:** Mosaicism is suspected in 21% of patients in our cohort, and could explain several mild cases. Confirmational tests and results of the remaining part of the cohort are needed to make definite conclusions about the predictive value of mosaicism in blood.

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P09.079C

De novo mutations in CBL causing early onset pediatric moyamoya cerebral angiopathy

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Background and Aims Moyamoya angiopathy (MMA) is characterized by a progressive stenosis of the terminal part of the internal carotid arteries and the development of abnormal collateral deep vessels. Its pathophysiology is unknown. MMA can be the sole manifestation of the disease (moyamoya disease) or be associated with various conditions (moyamoya syndrome) including some Mendelian diseases. We aimed to investigate the genetic basis of moyamoya using a whole exome sequencing (WES) approach conducted in sporadic cases without any overt symptom suggestive of a known Mendelian moyamoya syndrome.

Method WES was performed in four unrelated early-onset moyamoya sporadic cases and their parents (trios). Exome data were analyzed under dominant de novo and recessive hypotheses. A panel of 17 additional early onset moyamoya sporadic cases was available for mutation recurrence analysis.

Results We identified two germline de novo mutations in CBL in two out of the four trio probands, two females presenting with an infancy-onset severe MMA. Both mutations were predicted to alter the ubiquitin ligase activity of the CBL protein that acts as a negative regulator of the RAS pathway. These two germline CBL mutations have previously been described in association with a developmental Noonan-like syndrome and susceptibility to Juvenile Myelomonocytic Leukemia (JMML). Notably, the two mutated girls never developed JMML and presented only subtle signs of RASopathy that did not lead to evoke this diagnosis during follow-up.

Conclusion These data suggest that CBL gene screening should be considered in early-onset moyamoya, even in the absence of obvious signs of RASopathy.

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P09.080D

Rare RNF213 variants located in the RING domain are associated with moyamoya in Caucasians

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Background and aims Moyamoya angiopathy (MMA) is in East Asia strongly associated with the RNF213 R4810K variant. This variant has never been detected in Caucasian patients. We investigated if rare RNF213 variants are significantly associated with Moyamoya in Caucasian patients.

Method 73 European Caucasian MMA patients and 577 controls from the French EXome project were included. Whole exome sequencing was performed in all subjects. A principal component analysis was performed with > 16,000 SNP to ensure that patients and controls were ethnically matched. Association was tested using the fixed threshold collapsing method. Only functional variants with a minor allele frequency < 0.01 were considered. The likelihood ratio (LR) statistics proposed by Ionita-laza et al. was used to test the presence of a region within RNF213 that would be significantly enriched in rare coding variants

Results We showed a significant association between rare missense RNF213 variants and MMA in Caucasian patients (OR)=2.23, 95% confidence interval (CI)=[1.18–4.08], p=0.01). Probands variants had significantly higher pathogenicity scores and preferentially clustered in a c-terminal hotspot encompassing RNF213 RING domain ($p<10^{-3}$).This association was even stronger in childhood-onset and familial cases (OR=3.76, 95% CI=[1.46–9.29], p=0.003).

Conclusion We provided significant evidence for the role of rare, non-R4810K, variants in the development of MMA in Caucasian patients, especially when located in the c-terminal part of the protein. Elucidation of the functional consequences of these rare missense variants upon the binding of RNF213 to its so far unknown substrates would be of major importance for the understanding of MMA mechanisms.

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P09.081A

Genotyping of insertionally polymorphic HERV-K loci in multiple sclerosis patients using targeted next-generation sequencing

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Introduction: Human endogenous retroviral (HERV) sequences account for 8% of human genome and have been associated with multiple sclerosis (MS) in the scientific literature. The most recently acquired family, HERV-K (HML-2), includes human specific proviruses known to be unfixed in the population. The aim of this study was to assess the presence of selected HERVs with relatively intact ORFs at 48 polymorphic loci, within a group of MS patients and controls.

Materials and Methods: DNA from 30 sporadic MS patients, 40 MS patients from families with recurrent disease, and 30 controls was extracted from whole blood. Biotinylated probes were used to target flanking sequences of putative insertion loci. Enriched libraries were sequenced on Illumina platform and aligned to hg19 reference genome. HERV insertions were genotyped by analyzing read-pairs aligned to insertion breakpoints for LTR sequences of HERVs using BLAT.

Results: HERV insertion at 19p12d(chr19:22414379) was predominantly absent in the MS group (Chi-squared test,p.value 0.01) when compared to controls. Twelve of 48 HERVs were genotyped at both flanks. Amongst them, the right flank LTR of HERV-K103(10p12.1) was predominantly absent in MS patients from affected families (p. value=0.07).

Conclusion: We performed a comprehensive characterization of unfixed HERV loci in our sample of MS patients. Since only minimal additional sequencing is needed, the method employed showcases an interesting extension of the routinely performed exome sequencing. Despite none of the results reaching Bonferroni corrected p.values for significance, the two mentioned results provide ground for further exploration.

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P09.082B

The burden of rare genetic variants in genes involved in tumor necrosis factor (TNF) signalling pathway in multiple sclerosis (MS)

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Introduction: MS is a chronic inflammatory disease of central nervous system with widely studied, however still poorly understood genetic contribution. Several lines of evidence imply an important role of TNF pathway in the pathogenesis of MS including inflammatory demyelination of central nervous system observed in TNF receptor-associated periodic syndrome (TRAPS) caused by rare mutations in TNFRSF1A. Therefore, we hypothesized that an increased mutation burden in genes involved in TNF signalling pathway may trigger inflammation in MS.Materials and Methods: Nextera Coding Exome enrichment was used to perform Whole exome sequencing of 35 patients with familial MS, 43 patients with sporadic MS and 91 population matched controls. Genotypes were called using GATK toolkit. The selection of variants among 110 genes involved in TNF signalling pathway (KEGG database) was narrowed down by evaluation of functional impact of in silico predictors: Meta-SVM (when described as damaging) and/or CADD (when N > 20). **Results:** We identified 56 rare potentially pathogenic genetic variants in 37 genes. We detected a statistically significant increased burden of rare genetic variants in sporadic (x2=5.42, p=0.02), but not in the familial cases of MS (x2=0.13, p=0.72), when compared to controls. **Conclusions:** We found evidence for an increased burden of rare genetic variants in genes of the TNF signalling pathway in sporadic MS patients, which further supports its pathogenetic role in MS.

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P09.083C

Genetic bases of neurodegeneration with brain iron accumulation

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) encompass a wide spectrum of rare and inherited neurologic disorders characterized by progressive movement disorders and accumulation of iron in brain. There are 10 genes causing NBIA that resolve 80% of cases.

Methods: We have investigated a clinical series of 104 index cases. To establish the genetic basis, we have analyzed the most common genes (*PANK2* and *PLA2G6*) by Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA). To analyse all known NBIA genes and other candidate genes, we have designed a gene panel with 500 genes involved in movement disorders, based on SureSelect technologies (Agilent Technologies).

Results: We have achieved the molecular diagnosis for 45 NBIA patients: 31 patients carry mutations in *PANK2* and 11 patients in *PLA2G6*. We have investigated 16 patients by gene panel and no mutations have been detected in the remaining known NBIA genes. The preliminary results have revealed that two patients carry homozygote mutations in *EXOSC3* and in one patient, we have detected homozygote mutations in two genes: *DLD* and *FBXO7*.

Conclusions: The analysis of *PANK2* and *PLA2G6* allows the diagnosis of around 50% NBIA patients.

The data from the gene panel is being filtered and functional assays are ongoing in order to clarify if the detected changes are the causative mutations. The designed gene panel is a cost-effective tool for NBIA and related disorders, since allows the study of the NBIA genes and other candidate genes. *[Funds: Fundació La Marató TV3, Fundació per Amor a l'Art.]*

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P09.084D

The phenotypic spectrum of rare variants in NBIA (neurodegeneration brain iron accumulation) associated genes

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Introduction: Neurodegeneration with brain iron accumulation is a disease resulting in movement disorder. Symptoms may vary greatly. The factors that influence disease severity and clinical phenotype are unknown. Methods: 75 patients with brain iron accumulation, or symptoms characteristic to NBIA, were investigated by targeted Sanger sequencing, MLPA and/or by Wholes Exome Sequencing. Sixteen relatives of patients with identified genetic defects have been investigated too. **Results:** In 7 cases *C19Orf12 (MPAN)*, in 6 cases *PANK2*, in 3 cases *PLA2G6*, in 3 cases *CP*, in one-one family *BPAN* and *FA2H* pathogenic mutations were detected. Further 17 rare variants were observed by WES in NBIA genes. In 8 patients the p.W112X mutation was present in the *C19orf12* gene. Among these patients one had homozygous, one heterozygous form with a coexisting mutation of the *CP* gene. Both had very severe phenotypes. Among the 6 cases with heterozygous mutation, in 3 iron accumulation in the brain and mild extrapyramidal symptoms were present. In 2 patient with atypical Parkinson syndrome the p.Leu72* mutation and a rare variant of the *C19Orf12* heterozygous mutation was detected. Family members having the heterozygous p. Gly69Arg fsTer10 mutation of the *MPAN* did not have any clinical symptoms at their 40s. In persons with heterozygous status of the *PANK2, CP, BPAN* mutations no clinical signs have been observed. Conclusion**:** In MPAN patients a wide variety of clinical symptoms can be seen. Some mutations and rare variants of the *C19Orf12* gene may predict mild extrapyramidal signs after the 60s. .

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P09.085A

Neurodegeneration with brain iron accumulation: PLA2G6-associated dystonia-parkinsonism: clinical and animal studies

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Introduction: Mutations in the phospholipase-A2 group-VI gene (PLA2G6) have been shown to cause a Parkinson-dystonia phenotype (PLAN). Previously we reported a 25-year-old male PLAN patient with a mutation (c.238G>A) in the PLA2G6 gene; who had abnormal pallidal iron by MRI, elevated levels of inflammation, oxidative stress and an 84% elevation in soluble transferrin receptor (sTfR). This investigation provides additional information on the PLAN patient, including antioxidant therapy and characterization of a mouse model of PLAN.

Methods: Antioxidant therapy consisted of combination therapy using N-acetylcysteine (1500mg/day) and/or alpha-lipoate (1200mg/day) for one year. A comparison of iron trafficking proteins to a haematological profile was performed to examine the impact of altered iron trafficking proteins. Finally, the symptomatic presentation in a mouse model (B6.C3-*Pla2g6*^{m1J}/CxRwb) was used to determine its eligibility as a model of PLAN.

Results: No adverse events were report during the antioxidant trial. Neither significant improvements or further motor symptoms were observed during therapy. Despite elevations of sTfR, the haematological profile revealed no significant abnormalities. The mice displayed pronounced motor deficits by 90 days of age: including resting tremor, 20% reduced velocity and distance covered in an open field movement test and an impaired ability to hang suspended by a wire hang test.

Conclusions: Similar to PLAN, *Pla2g6*^{m1J}/CxRwb mice present with early-onset degenerative motor symptoms due to a reduced expression of Phospholipase-A2G6 and represent a potential model to investigate the impact of inflammation and oxidative stress in PLAN and the contribution of changes in systemic iron trafficking to iron accumulation.

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P09.086B

A novel NDE1 mutation causing autosomal recessive primary microcephaly without lissencephaly

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Primary microcephaly is a neurodevelopmental disorder characterized by congenitally small but architecturally normal brain due to disruption of genes regulating neural progenitor proliferation. In comparison, lissencephaly is caused by mutations in genes critical for neuronal migration.

Here, we report a 9-year-old boy with microcephaly, severe intellectual disability and seizure. Combining whole-exome sequencing and linkage analysis identified a novel homozygous mutation (c.658C>T**;** p.Arg220*) in *NDE1* gene.

The Nuclear distribution element 1 (*NDE1*) gene encodes a protein that is localized at the centrosome and plays an essential role in mitosis and neuronal migration. *NDE1* loss-of-function mutations have been reported only in a few cases affected with a wide spectrum of brain malformations including lissencephaly and hydrancephaly. Interestingly, in our case report, the patient presented with less severe phenotype and brain MRI showed reduce brain volume without any structural abnormalities (such as lissencephaly). *Ndel1* (-/-) mice displayed profound defects in cerebral corticogenesis but only modest defects in neuronal migration.

Presumably, *NDE1* truncating mutation (p.Arg220*) leads to the loss of c-terminal domain which is critical for its centrosomal localization. To confirm this, we overexpressed the mutant GFP-*NDE1*, which led to diffuse localization in the cytoplasm and failure of centrosomal localization, whereas the immunoreactivity of wild-type protein was found at centrosome. The analyses of *NDE1* in primary fibroblasts derived from individuals mutated with *NDE1* and control are in progress

NDE1 can be considered as one of the overlapping genes that regulate neural progenitors proliferation and neuronal migration.

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Testing experimental nootropic drug Mitochondrin-2 and Adement on manifestation of SWS/NTE depended glial neurodegeneration in *Drosophila melanogaster*

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Introduction: The Swiss Cheese (SWS) protein is an ortholog of NTE in *Drosophila melanogaster* and shares 39% sequence identify with human Neuropathy target esterase (NTE). The *Drosophila sws* mutants are characterized by progressive degeneration of adult nervous system, glial hyperwrapping and neuronal apoptosis.

Materials and Methods: We created a specific functional knockout of *sws* gene in glial cells. Therapeutic influence of experimental preparations Mitohondrin-2 (M-2) and Adement was assessed on life span, paraffin brain sections and on locomotor activity in "open field" (path length, duration of rest, duration of grooming, number of jumps) experiment in old flies. We investigated two groups: in first group preparation were delivered to the larvae, and in second group, preparations were fed to the adult flies. In paraffin brain sections we calculated the total area of vacuoles in brain using a graphics editor Kompas 13 portable mini.

Results: Our results suggest that M-2 had neuroprotective effect only if it was delivered to larvae. After treatment with M2 size of vacuoles reduced by 31% in brain tissue of old flies with *sws* functional knockout in glia. Life span parameters after M-2 treatment became 15% longer. Nevertheless, we didn't found any changes in locomotor activity. In contrast, after Adement treatment in both groups we observed toxic effect on life span: life expectancy was significantly lower in comparison with flies that did not receive the drug.

Conclusions: Summarizing obtained data we can conclude that in case of glial SWS/NTE-depended pathology in *Drosophila* brain Mitochondrin-2 demonstrate a therapeutic effect.

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BDNF and FGF-2 growth factors synergistically enhances the neural stem cell dedifferentiation in vitro

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Introduction: Adult neurogenesis and Neural Stem Cell (NSC) biology has tremendous potential considering the treatment modalities in neural related diseases and neural injuries. In this study, we aimed to determine the effects of Brain-Derived Neurotrophic Factor (BDNF) and Basic Fibroblast Growth Factor (FGF-2) proteins individually or synergistically on the hippocampal originated NSCs.

Materials and Methods: Hippocampal NSCs that were primarily isolated from rats were utilized in the experiments. NSCs grown in the cell culture medium were treated either with FGF-2 (0.4μgr/ml) and/or BDNF (1μgr/ml) during the day1, day3 and day7 of culture. Afterwards, cells were treated with BrdU and analyzed with flow cytometry. Doublecortin (DCX), Glial Fibrillary Acidic Protein (GFAP), Nestin and β-Tubulin-III (Tubb3) genes expression analysis were performed following RNA isolation and RT-qPCR. Relative gene expression levels were normalized to GAPDH.

Results: Astrocyte marker GFAP, was downregulated all times during the treatment with BDNF and/or FGF-2. Nestin expression, however, was downregulated in the day1 and upregulated during day3 and day7. Upregulated expression levels of Tubb3 and DCX were observed in day1, but downregulated in day3 and day7. BrdU analysis revealed the cells being quiescent in G0/G1 phase upon induction and increasing in day3 and day7, respectively. BDNF induction accumulated the apoptotic cell population in each time points whereas FGF-2 induction saved the apoptotic cells.

Conclusions: These observations suggest that BDNF and FGF-2 induction in the neural cell lineage increases immature neural de-differentiation from neural cells. Supported by grants from Istanbul University BAP; Yüksek Lisans 50267 and BAP THZ-2016-21839

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A comprehensive NGS gene-panel for rapid DNA diagnosis of inherited late onset ataxia

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Aim Our aim was to develop a targeted next generation sequencing (NGS) gene panel that enables to analyze the large number of candidate genes in patients with inherited ataxia at relatively low cost and high speed.

Methods An ataxia-panel of 57 genes involved in spinocerebellar ataxia (SCA) and related ataxia disorders was developed using the Agilent Sure Select Target Enrichment®. The samples were sequenced using 151 base pair paired-end reads on an Illumina NextSeq® sequencer and analyzed using Softgenetics' NextGENe® and Cartagenia's Benchlab NGS® software. Subsequently, for diagnostic purposes, the targeted ataxia-panel was used to analyze a total of fifty unrelated patients. A pre-NGS analysis was offered to test patients for CAG repeat expansions of the *ATXN1 to 3*, *CACNA1A* and *ATXN7*genes.

Results and conclusion In eleven out of forty-eight patients, novel (likely) pathogenic gene mutations were identified which were all confirmed by Sanger sequencing. Additionally, mutations were classified using data from HGMD, frequencies of variants in the ExAC database and gene information and clinical data on corresponding gene variants in OMIM. Sixteen patients were shown to carry heterozygous variants of unknown clinical significance (VUS) in most cases missense mutations. Cosegregation analysis of VUS variants was performed in other affected family members upon availability to elucidate its role in ataxia. Gene panel analysis adds to fine tuning of the clinical phenotype and expands the genotype-phenotype correlations for ataxia. The use of this gene panel facilitates a much faster analysis of the large number of currently known ataxia-genes for mutation analysis.

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P09.090B

Three years molecular diagnosis of hereditary ataxias with NGS: benefits and challenges

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Hereditary ataxias are a group of genetic disorders characterized by slowly progressive incoordination of gait, often associated with poor coordination of hands, speech, and eye movements, that can be inherited in an autosomal dominant, autosomal recessive or X-linked pattern. Next-Generation Sequencing (NGS) allows faster molecular diagnosis, analysing multiple genes simultaneously, with an excellent cost-benefit ratio. This is particularly useful for the rarest and highly heterogeneous forms. A multi-gene panel for hereditary ataxias was the first to be implemented at our centre, using an Ion torrent PGM platform (32 genes) and, later on, exome sequencing with an Illumina platform (144 genes). Over the past 3 years, 58 patients (most previously excluded for repeat-expansions in the more common loci) were screened for mutations using these panels. A diagnosis was achieved in 22%, and variants of unknown significance were identified in 10%. Some of these patients had been molecularly studied for several years. We identified disease-causing variants in genes that are rarely the first choice for testing, such as SYNE1, due its size, or ANO10, due to the small number of families identified so far. Several novel variants have been identified at our laboratory, classified based on clinical and family information, as well as our bioinformatics predictions and clinical judgement. NGS is cost-effective, after exclusion of more common repeat-expansions whenever clinically justified, and allows the genetic diagnosis of patients with rarer types of hereditary ataxia. A great challenge is to conclude about pathogenicity of the novel and rare variants elicited with this technique.

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P09.091C

Genetic variants associated with attenuated niacin flush response in schizophrenia

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Background: Attenuated niacin flush response is consistently found in schizophrenia patients and their non-psychotic relatives, but its relation to clinical features and genetic risk factors remain unclear. This study aimed to 1) examine the association between attenuated niacin flush response and clinical features in schizophrenia; and 2) identify the genetic variants related to attenuated niacin flush response. Methods: A total of 1881 schizophrenia patients who were interviewed with the Diagnostic Interview for Genetic Studies (DIGS) underwent a niacin skin patch test rated on a 4-point scale. Part of the sample (n = 858) was genotyped to search for genome-wide genetic variants. A newly developed polygenic test called the Adaptive Combination of Bayes Factors was applied to select SNPs associated with attenuated niacin flush response. **Results:** Patients with an attenuated niacin flush response score that exhibited robust SNP-based heritability showed lower global functional score ($p<0.005$), higher positive symptoms score ($p<0.005$), and more cognitive impairment ($p<0.05$). No single SNP reached genome-wide significance. However, the polygenic test was statistically significant ($p=0.015$), in which the association signal was enriched by combining 60 common SNPs each with a Bayes factor > 85. **Conclusions:** Attenuated niacin flush response in schizophrenia is characterized by certain clinical features and is influenced by multiple genetic variants with small effects. Further investigation of biological functions is warranted. Grant reference: Supported by NIH/NHGRI grant U54HG003067, NIMH grants R01 MH085521, R01 MH085560, the Gerber Foundation, the Sidney R. Baer, Jr. Foundation, the Brain and Behavior Research Foundation, and the Stanley Center for Psychiatric Research.

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Phase 1/2a trial of intrathecal 2-hydroxypropyl-β-cyclodextrin (VTS-270) for the treatment of Niemann-Pick Disease, type C1

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Introduction: Niemann-Pick disease, type C1 (NPC1) is a lysosomal storage disorder characterized by endolysosomal storage of unesterified cholesterol and progressive neurological impairment. Preclinical studies in mouse and cat models demonstrated significant potential of VTS-270 to slow neurological progression, decrease cerebellar Purkinje cell loss and increase survival time.

Methods: Safety and clinical efficacy of monthly intrathecal VTS-270 (a specific formulation of 2-hydroxypropyl-β-cyclodextrin) were evaluated in an open-label, dose escalation study. 24-hydroxycholesterol was used as a pharmacodynamic marker and CSF protein biomarkers were evaluated. NPC Neurological Severity Scores (NSS) were used to characterize disease progression. Data are from 12 NPC1 participants treated for 18 months and 2 treated for 12 months. Comparison of disease progression is relative to a historical cohort of 21 NPC1 participants of similar age range.

Results: Biomarker studies were consistent with improved neuronal cholesterol homeostasis and decreased neuronal pathology. From a safety perspective, mid- to high-frequency hearing loss was observed. This was an expected adverse event based upon preclinical testing. Post hoc analysis of the total NSS demonstrated significant ($p<0.0002$) decrease in the rate of disease progression (1·22 ± 0·34 points/year) in the treated participants compared to the comparison group (2·92 ± 0·27 points/year). Decreased progression was observed in the NSS subdomains of ambulation ($p=0·0622$), cognition ($p=0·0040$) and speech ($p=0·0423$). Secondary responder analysis demonstrated decreased or stabilized disease progression in 7/14 treated subjects compared to 1/21 untreated subjects ($p=0.003$).

Conclusions: These data demonstrate the potential efficacy of intrathecal VTS-270 to slow neurological disease progression in NPC1 patients.

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NPC1 and *NPC2* gene analysis in Serbian patients with Niemann-Pick disease type C

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Introduction: Niemann-Pick disease type C (NP-C) is a lysosomal lipid storage disorder caused by mutations in either the *NPC1* gene (in 95% of cases) or the *NPC2* gene. It is an autosomal recessively inherited disease most commonly characterized by hepatosplenomegaly and a severe progressive neurological dysfunction with the age of onset ranging from early infancy to adulthood. The large majority of mutations in *NPC1* gene are missens mutations (70%), while nonsense mutation (E20X) appears relatively frequent in *NPC2* gene.

Materials and methods: This study evaluated 135 Serbian adult patients with symptoms that meet the NP-C criteria, and their relatives. Our algorithm for genetic testing involved analysis of 4 selected (mutation prone) exons of *NPC1* (exons 8, 19, 20 and 21) and than, in negative cases, sequencing of all five exons of *NPC2* gene. All analyses were performed on ABI 3500 genetic analyzer.

Results: Genetic testing revealed 13 patients to be either compound heterozygotes (n = 12) or homozygote (n = 1) for mutations in *NPC1* gene. We identified 8 missense mutations, one nonsense mutation, one small deletion, one large deletion of exons 6 to 9, one intronic mutation in *NPC1* gene, and only one heterozygous 5' donor splice-site mutation in *NPC2* gene, all previously described. Heterozygote mutations were identified in 22 relatives of patients. Missense mutations constituted the majority of detected mutations (~ 85%).

Conclusion: These results signify the importance of *NPC1* sequencing as standard genetic test in NP-C. Our findings better characterize NP-C in Serbian population and facilitate future studies into genotype-phenotype correlations.

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P09.094B

Phenotypic and Molecular Characterization of Cockayne Syndrome; A Spotlight to Mild Cases

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Cockayne syndrome (CS) is a rare disorder characterized by cachexia, microcephaly, cognitive deficits, cataracts/pigmentary retinopathy, deafness, and dysmorphism with deep sunken eyes. Mutations in ERCC8, ERCC6 and ERCC2 are the cause. Most of the patients (65%) have mutations in the ERCC6 gene. Here we report the follow up of 7 patients with CS. We detected ERCC8 mutation in two and ERCC6 mutation in five. Of these five of them were novel. One of the novel mutations were detected in two unrelated patients. The patients' age range was 3–17 excluding the patient who died from respiratory insufficiency at 2 years. Although ERCC6 mutations were known to be clinically more severe 5 of 3 had mild phenotype. Longest follow up was 12 years. The patient's height was improved only 3cm while weight and OFC remained the same between 5 and 17 year of age. He had cachectic dwarfism, nystagmus, hypodontia, pectus excavatus, multiple nevi and anhidrosis on last examination,. He had aided walking and talk a few words. Polyneuropathy, basal ganglion and cerebellum calcification and deafness was detected. In contrast to severe cases mild ones had a better intradermal fat storage and body weight. The MRI's revealed isolated nonspecific white matter changes. The oldest mild patient was $9^{4/12}$ years old. Early diagnosis of CS is crucial in order to prolong life expectancy but also to avoid extensive tests and substances that aggravate DNA

breaks. Early genetic counselling with parents concerning future pregnancies is also beneficial.

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P09.095C

Early onset epileptic encephalopathies with suppression-burst EEG pattern: genetic characterization of a European cohort of 70 patients

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Early-onset epileptic encephalopathies (EOEE) are rare and severe neurodevelopmental disorders, characterized by motor seizures occurring before three month of life associated with a very abnormal EEG pattern who can be described as being "suppression-burst" (SB), where bursts of paroxysmal activity alternate with periods of electric silence or flattened tracing. There is a strong and heterogeneous genetic component to these diseases. We studied 70 patients with a SB EEG pattern, which is the most characteristic and easily identifiable for clinicians. Genetic explorations were first performed in a hospital setting (for known genetic causes) and secondly, in a research framework. Trio whole exome sequencing analyses were performed for negative cases. We identified 30 genetic abnormalities. The diagnostic rate was about 40%. An overview of the 70 patients highlights the implication of *KCNQ2* and *STXBP1*, which are the most frequently mutated genes (20% and 7%, respectively). Other mutations were identified in several genes, at a lower frequency: *SCN2A*, *KCNT1*, *ARX*, *GABRB3*, *GABRG2*, *ALGT11*. The vast majority of mutations was heterozygote and arose *de novo*. Interestingly, no patient had a mutation in *SCN8A* gene, which is frequently involved in other EOEE. Deciphering molecular basis of EOEE is the first step to better understanding these severely diseases. Progresses in the field of diagnosis, coupled with development of personalized medicine could modify the quality of life of the patients and their families. This work was supported by Inserm, Aix Marseille University, Marseille University Hospital and the JED Foundation.

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P09.096D

Case of assotiation of the Ondine Syndrome, hyperhomocysteinemia and mitochondrial deficiency - successful metabolic correction

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Introduction: Ondine Syndrome is a congenital central hypoventilation syndrome (CCHS) is a rare disease due to a severely impaired central autonomic control of breathing and dysfunction of the autonomous nervous system. The incidence is estimated to be at 1 of 200 000 livebirths. A heterozygous mutation of PHOX-2B gene is found in 90% of the patients. Description: Girl, 3 years, with the coarse delay of the psycho-speech, motor and physical development, seizures, stereotypes, atopic dermatitis. Convulsions occurred immediately after the birth, there was a dependence on the mechanical ventilation. The Ondine Syndrome was diagnosed by discovery of the gene PHOX-2B mutation. The metabolic examination revealed a high level of homocysteine (blood) - 12 mkmol/l ↑. Molecular testing - MTHFR 677 T/T. Gas chromatography/mass-spectrometry (urine) - disorder of cycle Krebs metabolites. Lactat (blood) - 3,2 mmol/l ↑. Amino acids (blood) - methionine ↑. The treatment included hypomethionine diet, P-5-P, TMG, L-carnitine, coenzyme Q10, riboflavin cropped epileptic seizures and dermatitis. On the background of the therapy (1 month) seizures are absent, the girl began to speak. **Conclusions:** The case demonstrates the syntropy effect, combination of hard rare monogenic syndrome and metabolic disorders. Correction of associated hyperhomocysteinemia and mitochondrial dysfunction were given the opportunity to improve the quality of life of the child.

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MRI-targeted screening for mutation in the WDR45 gene reveals NBIA5 case clarification

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Here we report a female patient with peculiar clinical picture of normal birth history, subsequent psychomotor delay in early childhood with intellectual disability, followed in early adulthood by a neurological regression with parkinsonism and cognitive decline from the already abnormal baseline. The performed RI in 2012 and 2015 revealed no change of the established brain atrophy, low-signal in T2 and FLAIR (fluid-attenuated inversion recovery) areas in substantia nigra and globus pallidus bilaterally. T1-W images showed non-homogeneous signal intensity of these structure changes with low intensity of the center and high intensity in the periphery. The picture was diagnosed as the classical "eye-of the-tiger" sign in neurodegeneration with brain iron accumulation (NBIA) and the diagnosis of Pantothenate kinase-associated neurodegeneration, known as Hallervorden-Spatz disease has been suspected. NBIA1 is caused by mutations in the *PANK2* gene, but the genetic testing for mutations in this gene was negative. This prompted us to reevaluate the MRI (end of 2016). As described earlier, it was found that on T1-weighted imaging, the indisputable iron depositions the substantia nigra and cerebral peduncles have a hyperintense "halo", which is very distinctive features for BPAN (Beta-propeller Protein-Associated Neurodegeneration), which is the only X-linked dominant NBIA disorder and helped us to guide the targeted molecular genetic testing of the *WDR45* gene. The genetic analysis revealed a heterozygous pathogenic variant c.1007_1008delAT, (p.Tyr336CysfsTer4), which confirms the clinical diagnosis of NBIA5. The variant is known in the literature. This is an example how neuroimaging can be applied successfully in guiding the genetic tests.

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Massive parallel sequencing in an autosomal dominant Flanders-Belgian family identified six candidate genes for Parkinson Disease

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Introduction: Molecular genetic studies of familial Parkinson Disease (PD) have provided important insights into the underlying molecular pathomechanisms. To date, only 10 % of familial PD can be explained by mutations in the known genes. We aimed at identifying the genetic defect in a Flanders-Belgian family segregating autosomal dominant PD by combining massive parallel sequencing (MPS) technologies.

Materials and Methods: DNA from three affected relatives was subjected to whole-genome sequencing (WGS). Shared heterozygous variants were prioritized based on quality, frequency in public databases and impact. Segregation was evaluated using whole-exome sequencing (WES) data of two unaffected relatives. We used multiplex amplification resequencing to validate the candidate variants and to screen the Belgian PD patient/control cohorts.

Results: Analysis of the WGS data discovered approximately 3.95 million variants per individual. Applying successive prioritization steps resulted in 52 variants with a high probability of association with PD in the family. Annotations of these variants, including sequence conservation, brain expression and association with other neurological disorders, identified six candidate genes: *PSMD7*, active in the ubiquitin-proteasome dependent proteolysis; *MDGA1* and *HTR1D*, involved in synaptic activity; *EGF*, implicated in the proliferation and differentiation of neurons; *ANK3*, associated with multiple neurological disorders; and *MYOM3*, implicated in neuromuscular disorders.

Conclusions: Using MPS in a Flanders-Belgian family identified potential novel PD genes. Mutation screening of these genes in Belgian PD patient/control cohorts is needed to generate further genetic support for an association with disease, and functional studies to provide insight into the underlying molecular pathomechanism.

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P09.099C

Construction and testing of cell culture based systems to monitor alpha-synuclein aggregation

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Introduction: The Alpha-Synuclein Gene (SNCA) and protein (ASYN) is central in the pathogenesis of Parkinson disease (PD). ASYN is a small (140 aa) intrinsically disordered protein with a tendency to aggregate spontaneously.

Neuronal inclusions containing large amounts of ASYN are the pathological hallmark of PD. However, it is likely that oligomeric ASYN intermediates are the neurotoxic form. The aim of this project is to monitor ASYN aggregation in cell culture in order to develop a high-throughput drug screening tool.

Materials and Methods: We modified a previously published system (Outeiro et al. PLoS One (2008)) monitoring ASYN aggregation based on bimolecular fluorescence complementation (BFC). We inserted both cistrons of the system into a single constitutive or tetracycline inducible expression vector. The cistrons are connected either by a constitutively cleaved 2A protease site or an internal ribosomal entry site (IRES). We used these vectors to construct stable cell lines (HEK293) and tested the system employing one aggregation enhancer (MG132) and a putative aggregation inhibitor (Curcumine).

Results: The cell line characterized by inducible expression and a 2A-site showed the best characteristics in terms of fluorescence brightness and signal to noise ratio. Peak differences in signal intensity after substance treatment were observed after 24h to 36h. MG132 lead to increased fluorescence while Curcumine diminished fluorescence. However, both substances were cytotoxic at much lower concentrations than previously published values. **Conclusions:** After miniaturization and fixed coupling to cell viability measurements our system should be suitable to screen for ASYN aggregation inhibitors.

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P09.100D

Gene discovery and prioritization in recessive Parkinson's disease

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Introduction: Parkinson's disease (PD) is a major public health problem, affecting 1–2% of persons over 60. Eleven genes are now confirmed to cause monogenic forms of PD, but in approximately 50% of the familial and more than 80% of the sporadic cases with early-onset PD, mutations in these genes are not detected. Thus, a significant proportion of cases remain unexplained genetically. Materials and methods: The French PDG group and Mediterranean collaborations assembled a unique collection of autosomal recessive PD families, including a large number with known or suspected consanguinity. We combined whole genome genotyping and whole exome sequencing in 77 cases and 22 relatives, to identify pathogenic mutations in the regions of homozygosity. Sanger sequencing was used to validate the variants and segregation in families, when possible. The recessive candidate genes will be prioritized using a semi-automated pipeline studying multiple parameters relevant to PD pathophysiology (Jansen et al., 2017) in a neuroblastoma cell line. **Results:** We identified about 70 genes with potential pathogenic mutations in the regions of homozygosity of the confirmed consanguineous cases. Little or no replication was found in other PD cases, therefore, functional data to support the pathogenicity of these genes in PD is required. Functional validation is in progress. **Conclusions:** About 70 recessive candidate genes for PD were identified. A wide functional screening will be used to prioritize the genes with the highest overall impact. Further studies will be carried out to complete the body of proofs supporting their involvement in PD.

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P09.102B

Systematic meta-analyses of small RNA profiling studies identify differentially expressed microRNAs in Parkinson's disease

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Background: MicroRNA-mediated (dys)regulation of gene expression has been implicated in many complex traits including Parkinson's disease (PD). Numerous publications have reported a differential expression of specific microRNAs in PD patients versus unaffected controls. However, results have been inconclusive. The aim of this study was to identify microRNAs that show consistent differential expression across all published small RNA profiling studies in PD.

Methods: We performed a systematic literature search on microRNA expression studies in PD. Relevant data were extracted followed by double-checking by an independent investigator. Data were stratified for source of tissue, and overlapping datasets were excluded. Subsequently, we performed *p*-value based-meta-analyses across microRNAs assessed in at least three independent datasets.

Results: Our literature search identified 32 studies eligible for meta-analysis. We performed 145 meta-analyses on microRNAs quantified in blood (n = 11), brain (n = 130), or cerebrospinal fluid samples (n = 4). We identified eleven significantly ($\alpha=3.45\times10^{-4}$) differentially expressed microRNAs in blood or brain. The most significant findings were observed with hsa-miR-15b-5p ($p=1.73\times10^{-16}$), hsa-miR-181a-5p ($p=4.31\times10^{-16}$) and hsa-miR-29c-3p ($p=2.24\times10^{-12}$) in blood, and hsa-miR-497-5p ($p=1.13\times10^{-5}$), hsa-miR-129-2-3p ($p=1.25\times10^{-5}$) and hsa-miR-132-3p ($p=6.37\times10^{-5}$) in brain. Analyses of GWAS data did not show significant enrichment for association signals in target genes of these microRNAs.

Conclusions: We identified several microRNAs that showed highly significant and consistent differential expression in PD in blood and brain tissue. Future studies may assess the potential of differentially expressed microRNAs in blood as possible biomarkers for PD. Our study can serve as a model to merge published results of gene expression studies in other complex diseases.

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Validation of a method for detection of low-level single nucleotide variant mosaicism in sporadic Parkinson's disease

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Neurons are susceptible to develop somatic variations in their genomes throughout development and life. Although their pathological role remains undetermined, the mechanisms of neurodegeneration could be explained by somatic mutations occurring in a limited number of cells. We aim to optimise a robust sequencing method for sensitive detection of somatic variation in Parkinson's disease (PD) genes in sporadic PD samples

Sequencing libraries were prepared by using a Haloplex HS panel (Agilent) targeting *SNCA*, *GBA* and coding exons of 12 PD genes. Molecular barcodes allow for the removal of duplicates and generation of consensus sequences to replicate the original DNA fragments. Six artificial mosaics, consisting of dilutions of a brain sample carrying known variants, were sequenced on a HiSeq 2500. Bioinformatic analysis was done by Surecall's de-duplication tool, aligner and low-level variant caller. LoFreq was used to validate these results.

After analysing low level variants in artificial mosaics at 2050x coverage, four false positives were detected at allele frequency (AF) values under 0.33%. By using our optimised methods, Surecall's sensitivity for detection of variants at 1% and 0.5% is 92% and 78%. LoFreq showed a lower sensitivity for 1% and 0.5% variants (88% and 70%).

Deep sequencing of PD samples using a Haloplex HS allows for sensitive detection of variants at a 0.33% AF level or higher in PD brain samples. These methods will be used for the analysis of 50 samples from different brain regions and other tissues.

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P09.104D

Identification of mutations in *PARK2* gene in Serbian patients with Parkinson's disease

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Introduction: Mutations in the *PARK2* (*PRKN*) gene are the most common cause of autosomal-recessive (AR) juvenile parkinsonism and young-onset recessive Parkinson's disease (YOPD). More than 100 different variants have been reported, including point mutations, small indels and single or multiple exon copy number variations. Materials and Methods: Mutation screening of *PARK2* was performed in 225 Serbian PD patients (143 males and 82 females) with disease onset before 50 years and/or positive family history with apparent AR inheritance. All coding regions and their flanking intronic sequences were amplified and directly sequenced by Sanger method. Whole exon multiplications or deletions were detected using Multiple Ligation Probe Amplification (MLPA) method. **Results:** We identified 12 PD patients with *PARK2* mutations (5.3%). Five patients (2.2%) had biallelic mutations and seven patients (3.1%) were single mutation carriers. Patients with compound heterozygous mutations had earlier onset of the disease compared to non-carriers ($p=0.005$) or heterozygotes ($p=0.001$). Other clinical presentations of the disease in mutation carriers were not different compared to non-carriers. Conclusion: Estimation of *PARK2* mutations frequency and penetrance in different populations is important for efficient genetic testing strategy and counseling. In our cohort sequence and dosage variants were equally represented in patients positive for *PARK2* mutations, inducing their first symptoms mainly before the age of 30, implying that patients with early, especially juvenile onset of PD were strong candidates for both dosage and sequence variants screening in *PARK2*.

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Mutations in DNAJC12, a member of the HSP40/DNAJ family, cause early-onset parkinsonism

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Introduction: Mutations in single genes only explain 30% of familial Parkinson's disease (PD). We looked for novel genes/mutations using whole-exome sequencing (WES) in selected families showing early-onset (<45ys) recessive PD. **Results:** Null homozygous mutations in DNAJC12, a member of the HSP40/DNAJ family, were identified in 2 unrelated families: i) a nonsense mutation (c.187A>T, p.K63X) in a Saskatchewan family (famA); ii) a splicing mutation (c.79-2A>G, p.S26fs13X) in an Italian family (famB). Both variants are absent in ethnically-matched controls and public databases. Proband A had mild non-progressive parkinsonism from the age of 13 and has since come to autopsy (at 74). Proband B (onset at 32) has a similar negligible progression of motor symptoms over 30ys, with mild executive and visuo-spatial dysfunction. Dopaminergic dysfunction was subtle and non-progressive in both probands, who had similar sustained symptomatic benefit on small dose of levodopa and substantial worsening of symptoms after levodopa discontinuation. Neuropathology (famA) revealed no alpha-synuclein pathology and only some mild age-related Alzheimer's disease changes. Functional analyses showed that both mutations ablate DNAJC12 protein expression. **Conclusions:** Our results indicate that loss of DNAJC12, a Hsc70 interactor, is a rare cause of parkinsonism associated with a very slow neurodegenerative process. Very recently, biallelic mutations in DNAJC12 were shown to cause hyperphenylalaninemia with dystonia and intellectual disability in children. Analyses are underway in our patients to verify whether they also show defects in hydroxylase enzymes, which might explain symptoms and pathology data. We thank Fondazione Cariplo for financial support (grant#2015-1017).

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P09.106B

Expanding the phenotypic spectrum of *FLVCR1* mutations

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Posterior column ataxia with retinitis pigmentosa (PCARP) is an autosomal recessive, childhood onset neurodegenerative disorder characterized by retinitis pigmentosa and sensory ataxia and ascribed to FLVCR1 mutations. No cognitive deficits have been reported in the original PCARP families, but two affected siblings have been reported with mild mental retardation. We report the clinical characterization of two unrelated patients harboring biallelic severe FLVCR1. Patient 1 was born at term after uneventful pregnancy, with normal birth parameters. Neonatal hypotonia was noted at birth and he developed epilepsy at age of 3 days. The course was severe, with intractable seizures and severely delayed psychomotor development. Retinitis pigmentosa was diagnosed at 1 year of age. At 8 years, he had no ocular contact with axial hypotonia and absence of deep tendon relexes. OFC was 45 cm (-3SD). Brain MRI showed cerebellar hypoplasia. Electroneuromyography showed a sensory neuropathy. Patient 2 was born at term after uneventful pregnancy. During the first 4 months of life, physical and neurological examination was normal. He presented west syndrome at the age of 5 months. He developed severe psychomotor delay. He walked at 4 years and never developed speech. Retinitis pigmentosa and sensory neuropathy were diagnosed at age of 15 years. At age 16 years, weigh was 32 kg (-3SD), Height 141 cm (-4SD), OFC 50 cm (-4 SD). This report expands the phenotypic spectrum of FLVCR1 mutations to include west syndrome, severe psychomotor retardation, intellectual disability and post-natal microcephaly.

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A zebrafish model of pontocerebellar hypoplasia

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Introduction: Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of congenital neurodegenerative diseases. Patients suffer from severe mental and motor impairments, caused by atrophy of the pons and cerebellum. So far, ten subtypes (PCH1–10) are described based on clinical features and/or genetic causes. Most genetic aberrations are located in genes with a function in transfer RNA (tRNA) metabolism pathways, e.g. tRNA Splicing Endonuclease 54 (*TSEN54)* or Cleavage and Polyadenylation Factor I Subunit 1 (*CLP1*). Hitherto, it is unknown if and how faulty tRNA processing leads to pontine and cerebellar degeneration. Materials and Methods: To elucidate the consequences of aberrant tRNA processing, a zebrafish model with a nonsense mutation in *tsen54* was created using ENU mutagenesis. Survival of homozygous offspring was assessed and pathologically examined. **Results:** *Tsen54* knockout zebrafish died between 15 days post fertilization (dpf) and 26dpf. The total body volume increase between 7dpf and 19dpf was dramatically reduced compared to controls. Brain volumes did not showed to be more severely affected than other tissue. **Conclusions:** Here we show that the knockout of *tsen54* in zebrafish results in early dead between 15dpf and 26dpf. Additionally general hypoplasia, with no specific effect on the brain, was seen. This deviates from findings in humans carrying *TSEN54* mutations were primarily the pons and cerebellum are affected and no other tissue outside of the central nervous system. Further research must show if aberrant amount of tRNA species are present in the knockout fish and if these are comparable to amounts found in PCH patient.

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P09.108D

Genetic and phenotypic spectrum of primary familial brain calcification in 92 patients from 52 families from the French PFBC case series and functional assessment of new *XPR1* variants

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Introduction: Primary Familial Brain Calcification (PFBC) is a rare autosomal dominant calcifying disorder affecting primarily the basal ganglia, associated with diverse neuropsychiatric expression. Variants in the genes encoding the inorganic phosphate (Pi) transporters SLC20A2 and XPR1 or the growth factor PDGFB and its main receptor PDGFRB cause microvessel calcification through a loss-of-function mechanism.

Methods: Thanks to nation-wide recruitment and targeted or whole-exome sequencing screening, we report 24 novel families carrying 16 novel and 12 previously reported rare variants, adding up to a total of 92 patients from 52 families carrying 45 distinct variants. Novel *XPR1* variants were assessed *in vitro* for Pi export.

Results: Following ACMG-AMP recommendations, 27 variants were classified as pathogenic, 7 as likely pathogenic, and 11 of unknown significance. Thirty-one families carried a variant in *SLC20A2* (11 novel variants), 11 in *PDGFB* (3 novel), 8 in *XPR1* (2 novel) and 2 in *PDGFRB*. Overall, 75% patients were symptomatic; main symptoms were psychiatric signs (68.1%), movement disorders (65.2%) and cognitive impairment (59.4%). Ages of onset ranged from 6 to 77 years with first and last quartiles being 20 and 63 years respectively. Regarding the newly-identified gene *XPR1*, the 2 novel missense variants showed a defect in transport function as assessed by efflux of radiolabeled Pi. All 10 *XPR1* variant carriers were symptomatic with similar expression compared to the other groups.

Conclusion: This report is the largest systematic description of PFBC genes variant carriers and helps understanding the phenotypic spectrum of this rare disease with inconstant clinical expression.

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P09.109A

Autosomal dominant progressive myoclonus dystonia resulting from a *PANK2* mutation

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Introduction: With the introduction of next generation sequencing in diagnostics we have to anticipate on the identification of novel gene-disease associations including apparently dominant mutations in suspected recessive disease genes or vice versa. Therefore, functional validation of candidate variants is of utmost importance for clinical decision-making. **Material and Methods:** Whole exome sequencing (WES) was performed in two relatives exhibiting autosomal dominant progressive myoclonus dystonia from a multiplex family followed by bioinformatics analysis. Functional analysis in cell models was used to assess the pathogenic effects of the dominant candidate variant. **Results:** WES identified a novel heterozygous truncating variant c.1317delT;p.Arg440Valfs*10 in *PANK2*. Since recessive *PANK2* mutations cause pantothenate kinase-associated neurodegeneration (PKAN), *PANK2* deletions on the other allele were excluded. p.Arg440Valfs*10 segregated with disease and non-penetrance was observed in 2 relatives. MRI revealed evident iron accumulation in the patient's brains, but less compared to classical PKAN. p.Arg440Valfs*10 led to aberrant processing of precursor PANK2, instable intermediate (i)PANK2 protein, and disrupted dimerization of iPANK2 in HEK293T cells like the known recessive p.Gly521Arg *PANK2* mutation. Additionally, increased levels of the iron exporter, *FPN*, mRNA were detected in patient fibroblasts indicative of loss of PANK2 as reported for PKAN. Conclusion: Our genetic and imaging studies point to pathogenic effects of p.Arg440Valfs*10 in *PANK2*. However, additional functional studies are necessary to prove that it causes myoclonus dystonia through a dominant-negative effect. Funding: DSV; Rosalind Franklin Fellowship, MAFT and TjdK; Actelion pharmaceuticals, MAFT; Prinses Beatrix Muscle Funds, Merz, Ipsen, Allergan Farmaceutics, and Medtronic, OCMS; NWO VICI grant (865.10.012).

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P09.110B

Post-mortem cerebellar methylome of progressive supranuclear palsy

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Introduction; Although Progressive Supranuclear Paly (PSP) is relatively well described clinically and pathologically, much is still unknown regarding the underlying molecular disease mechanisms. We compared genome-wide DNA methylation in the cerebellum of 4 PSP cases and 4 age and sex-matched controls to investigate the genes and mechanisms involved in PSP.

Methods; All frozen post-mortem cerebellar tissue was derived from the Netherlands Brain Bank (NHB). DNA was isolated and processed from macroscopically dissected tissue, followed by Illumina's infinium Human Methylation 450K array. After removing poor quality CpGs and DASEN normalization, methylation values for 434.686 CpGs were compared between cases and controls using two-tailed t-tests. Gene set enrichment for the 500 most significant CpGs, based on Illumina's CpG-Gene annotation, was performed using WebGestalt.

Results; No CpG reached Bonferroni-corrected significance (p<1.2E-7). Two suggestive (p<5E-6) CpGs were identified, one in intron 15 of *WDR60*, the second CpG upstream of both *c19orf44* and *CALR3*. Gene set enrichment showed involvement of several specific GO-terms including *anoikis* (a form of apoptosis, 5 genes, adjP=0.0201), *regulation of microtubule polymerization* (5 genes including *MAPT*, adjP=0.0201) and *neuron projection morphogenesis* (31 genes, adjP=0.0201).

Conclusions; These preliminary results show suggestive differences in cerebellar methylation in PSP brains. Although no single CpG reaches genome-wide significance, analysis of the top signals indicates function specific enrichment, including validated microtubule-related processes and genes previously associated with Tauopathies. We are currently adding methylation data of additional cerebellum samples and other affected brain regions and will replicate these results in an independent cohort.

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Stem cell models for studying the role of epigenetic machinery in abnormal neurogenesis

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In addition to genetic mutations, the role of epigenetic abnormalities is widely appreciated in the development of disorders such as schizophrenia and autism. In support of this notion, elevated levels of DNMT1 and DNMT3A have been reported in post-mortem brain samples from patients with schizophrenia. However, the mechanisms by which elevated levels of DNMT1 or DNMT3 result in formation abnormal neurons is not clear. Towards this goal, we developed a transgenic mouse embryonic stem cell line that overexpresses DNMT1 and observed that neurons obtained from the mutant line shows abnormal branching of dendrites and elevated levels of NMDA receptor activity. To gain more insights into the abnormal effects of increased levels of DNMT1, we performed genome-wide methylation comparisons between wild-type and mutant ES cells to identify loci undergoing aberrant methylation in the mutant ES cells. Results of these experiments will be presented.

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A new hereditary ataxia disorder with prominent cognitive impairment and 1C2 positive inclusions

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Introduction Spinocerebellar ataxias (SCA's) are a heterogeneous group of neurodegenerative disorders characterized by autosomal dominant cerebellar ataxia together with a range of other features such as extrapyramidal symptoms and cognitive impairment. Over 30 genes and loci have been reported to date with the group of polyglutamine

expansions as most common genetic cause. In the present study we report a new Dutch pedigree with late onset ataxia and prominent cognitive decline. Methods After informed consent, DNA was obtained from 8 affected and 4 unaffected relatives from one family. Previous genetic testing excluded mutations in MAPT, PSEN1, PRNP, HTT, DRPLA, FXTAS, SCA1, SCA2, SCA3, SCA6, SCA7 and SCA17. We performed whole exome sequencing (WES) on 2 affected and one unaffected individual. During follow-up, neuropathological examination was performed in three affected individuals. Results Mean age at onset was 64 years and the clinical symptoms included gait ataxia, dysarthria, parkinsonism and prominent cognitive impairment. Neuroimaging in all affected persons showed cerebellar and/or generalized atrophy. WES revealed no mutations in previously reported ataxia genes. Neuropathological examination showed almost complete loss of Purkinje cells. Immunohistochemistry with p62 antibody showed few neuronal intranuclear inclusions in several brain areas, while more widespread diffuse nuclear staining and cytoplasmic inclusions were observed with 1C2 antibody (an antibody that detects long stretches of glutamine). Conclusions We report a new Dutch SCA pedigree with 1C2 positive inclusions and no known genetic mutation. We are currently adding haplotype analysis (array-based) and additional WGS data to identify the underlying genetic cause.

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P09.113A

Evaluation of genotype-phenotype relationship of short NOP56 expansions causing SCA36

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Introduction: Spinocerebellar ataxia 36 (SCA36) is an autosomal dominant ataxia with sensorineural hearing loss and motor neuron dysfunction, usually starting in the fifth or sixth decade. The cause is an intronic hexanucleotide expansion in *NOP56*, with 4–15 repeat normal alleles and over 650 repeats, full-penetrant alleles. The pathogenicity of short expansions is not well documented. **Materials and Methods:** 26 patients with full expansions and 3 patients with short expansions underwent neurologic examination and SARA scoring for the severity of ataxia. Genotyping was carried out by standard and repeat-primed PCR on peripheral blood DNA. **Results:** A 64 yo man with disease onset at 55 had an abnormal 35 repeat allele and SARA score of 9. His 86 yo mother, also with a 35 repeat allele, reported disease onset around 50. Her current SARA score was 15. A maternal uncle, with an expanded allele of 49 repeats, had disease onset around 60, and SARA score of 11 at age 70. The age-adjusted SARA score of patients with short expansions was not significantly different from that of full-expansion patients. The family history did not disclose cases of non-penetrance. **Conclusions:** Short NOP56 expansions (at least ≥35 repeats) are pathogenic. There is no significant difference in the clinical impact of short expansion versus fully expanded alleles. However, more cases with short expansions need to be studied to reach firm conclusions on genotype-phenotype relationships, and thus improve genetic counseling. Penetrance, somatic and gonadal stability of short expansions require further investigations. Funding: PI12/00742.

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P09.114B

A massively parallel reported assay for variants associated with schizophrenia and Alzheimer's disease

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Genome wide association studies (GWAS) have reported on numerous loci for complex disorders including schizophrenia (SZ) and Alzheimer's disease (AD), most located outside protein coding regions. We hypothesize that some of these loci act through differential enhancer activity and test this hypothesis by systematically assaying each locus using a Massively Parallel Reporter Assay (MPRA). Specifically, we investigate all variants in high LD with 71 SZ and 8 AD loci, for a total of 1083 variants. For each variant

we designed a 95 bp sequence centered on the variant and linked it to 5 distinct barcodes, including positive and negative controls. We generated a plasmid library using standard protocols, with final oligo representation of 80% (85% of alleles represented by ≥ 3 oligos) and transfected it 3 independent times into K562 cells; this cell line was chosen because we have observed maximum overlap of open chromatin with SZ loci (transfection of SK-SY-5Y human neuroblastoma cells is under way). Following transfection we extracted RNA and DNA and assayed the transfection efficiency and technical reproducibility. Specifically we found that on average, 80% of barcodes were represented in DNA and 72% in RNA. After summing counts over barcodes, we compute the correlation of counts and activity measures (log ratio of RNA over DNA) across replicates and find high mean correlations: 0.999 for DNA, 0.999 for RNA, and 0.749 for log ratios. Significant overlaps with open chromatin marks validate our results. Specific results of our assay for SZ and AD will be presented.

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Genetic association and functional characterization of a variant in the *MCPH1* gene in bipolar disorder and schizophrenia

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Abstract

The microcephalin 1 gene (*MCPH1*) plays an important role in DNA damage and repair and cell cycle arrest, with the BRCT1 domain being important for centrosomal localization throughout the cell cycle. A low frequency *MCPH1* variant rs61749465 A>G (p.Asp61Gly) showed evidence for association with schizophrenia (SCZ). We further explored this variant in 2,300 bipolar disorder (BPD), 1,930 SCZ subjects and 1,820 normal comparison subjects and report evidence for association with BPD (P=0.0009). Notably the variant allele of rs61749465 was absent in the 1,820 comparison subjects tested. rs61749465 is located in the N-terminal BRCT1 domain of *MCPH1* and bioinformatic analysis predicted the Asp61Gly substitution to be damaging to *MCPH1* protein function. A second *MCPH1* BRCT1 domain variant (rs199422124 C>G; p.Thr27Arg), reported to cause autosomal recessive microcephaly, was also genotyped however the variant was not detected in any of the subjects tested. We sought to characterize the functional effects of these variants on *MCPH1* function. Cell viability and cell count assays indicated that the variant allele of rs199422124 had a larger impact on cell survival compared to the variant allele of rs61749465, however neither of the variant alleles significantly altered DNA damage or mRNA stability. Analysis of gene expression data from RNA sequencing experiments suggested that rs61749465 expression altered the expression of a number of genes involved in protein translation and in the Alzheimer's disease pathway. Confirmation of the findings presented here could further our understanding of the role of genetic variants in the risk of developing a psychotic disorder.

M. aleissa: None.

P09.116D

Genome-wide methylomic analysis of neonatal blood from Danish monozygotic twins discordant for schizophrenia

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Introduction: Emerging evidence implicates altered DNA methylation in psychiatric disorders including bipolar disorder, major depressive disorder and schizophrenia. However, it is unclear whether the DNA methylation changes observed to date are causative or reflect disease progression or treatment. The neonatal period is a time of rapid neurodevelopment during which alterations in DNA methylation may contribute to the risk of psychiatric illness. Hence, we explored whether differences in DNA methylation in blood at birth were associated with monozygotic twin discordance for schizophrenia.

Materials and Methods: Neonatal blood samples obtained from Guthrie cards for sixteen pairs of Danish monozygotic twins discordant for schizophrenia were used

for genome-wide profiling of DNA methylation with the use of Infinium HumanMethylation450 BeadChip from Illumina. Differentially methylated positions (DMPs) associated with schizophrenia were identified using linear regression with sex as a covariate.

Results: The top-ranked schizophrenia-associated DMP (cg08408303, p=3.4e-06) was located in the 5′*UTR* of the *C3orf35* gene, a suspected tumor suppressor. Interestingly, several of the top DMPs map to genes involved in cilia biology and SHH signaling including *TBC1D32*, *DNAH2*, *GPR161*, and *COLGALT1*. In addition, we identified a DMP in the TSS200-island of the *BORCS5* gene encoding one of eight subunits of the BORC-1 multisubunit complex, which regulates lysosome positioning and endosomal trafficking and has known association with risk of schizophrenia.

Conclusions: Our data indicate that DNA methylation differences are quantifiable in neonatal blood from monozygotic twins discordant for schizophrenia later in life and suggest that susceptibility to schizophrenia is conferred by dysregulated neurodevelopmental genes.

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P09.117A

Large family-based exome sequencing study provides new insight into schizophrenia

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Schizophrenia (SCZ) is a severe psychiatric disorder with a prevalence of ~ 1% and a heritability of ~ 65%. Analysing multiply affected families using whole exome sequencing (WES) is a very promising approach to identify new SCZ risk factors as in these families genetic variants with particularly strong effect might co-segregate with the disorder. The present study is the worldwide largest SCZ family study using WES.

We exome sequenced 2–5 affected individuals from 54 families, each. Our analyses focused on rare (allele frequency ≤ 0.1% in the Exome Aggregation Consortium dataset) variants that were *in silico* predicted to be pathogenic (Combined Annotation Dependent Depletion Score ≥ 15) and that were shared between all affected individuals in one family.

Mutations in ~ 800 brain expressed genes were identified. Based on these data, we have performed protein-protein-interaction analyses and have built a SCZ network implicating new genes in the pathogenesis of the disorder and also providing additional genetic evidence for previously reported candidate genes; i.e. co-segregating mutations in *DGCR2* (risk locus 22q11.2) and *CSMD1* (genome-wide significant in worldwide largest genome-wide association study). To further evaluate the relevance of the new candidate genes in the pathogenesis of the disorder, we are currently sequencing these in a large cohort of 5,000 individuals. Our work provides new insight into the genetic architecture of schizophrenia.

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Effects of ectoine and herbal compound (*Rosa canina, Tanacetum vulgare and Urtica dioica*) on behavior and expression of Alzheimer's disease-related genes in streptozotocin-rat model of sporadic Alzheimer's disease

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Possible mechanisms of Alzheimer's disease such as inflammation and oxidative stresses in the brain motivate researchers to follow therapies which would be effective, in the early stages of the disease. Ectoine has been reported prohibits amyloid formation *in vitro*. The herbal extract of *R. Canina, T. Vulgare and U. Dioica* plant species, has anti-inflammatory and protective effects against oxidative stress. In this study eight AD-related genes, *Daxx*, *Nfκb*, *Vegf, Psen1*, *Mtap2*, *Syp*, *Mapk3* and *Tnfα* genes were chosen to investigate the neuroprotective effect of ectoine on gene expression in the hippocampus of rat model of sporadic AD, using qPCR in the treated and control groups. The effects on learning and memory levels were evaluated by Morris Water Maze (MWM) test. All procedures were accomplished for neuroprotective effect of herbal extract on *Daxx*, *Nfκb* and *Vegf*. Our results showed significant down-regulation of *Syp, Mapk3* and *Tnfα* in ectoine comparing to the model group. Significant up-regulation of *Vegf* in ectoine and herbal extract comparing to model group was indicated as well. In MWM, there was no significant change in swimming distance and time for finding the hidden platform in ectoine and herbal-treated comparing to model group. Furthermore, No significant change in ectoine and herbal-treated comparing to model and related groups in memory level was seen, but the same change was detected in the treated comparing to the control group in memory level. It seems that ectoine and herbal extract may have significant effect on gene expression as same as on clinical levels.

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P09.120D

DYT6-associated THAP1 missense mutations affect its function in regulating genes related to synaptic function

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Mutations in *THAP1* (THAP domain containing, apoptosis associated protein 1) were found to be responsible for DYT6 dystonia. Although more than seventy different mutations of THAP1 have been identified in patients with primary dystonia, the pathogenesis of THAP1 mutations causing DYT6 dystonia is still largely elusive. THAP1 is a transcription factor, identifying its target genes might reveal the pathogenic mechanism of DYT6 / THAP1 dystonia. In order to identify target genes of THAP1 and to characterize the pathogenesis of DYT6 dystonia, we performed a microarray analysis on a neuronal cell model for DYT6 dystonia, which stably overexpress wild-type or mutant THAP1 (S21T or F81L mutants). Microarray analysis showed that overexpression of wild-type THAP1 can regulate expression of genes related to cell growth and proliferation. However, DYT6-associated THAP1 missense mutations (S21T or F81L) showed dysfunction in regulating genes related to synaptic function. By using Chromatin immunoprecipitation (ChIP)-qPCR and luciferase reporter assay analysis, we found that THAP1 binds to the promoter region of SYT9 and regulates its activity. Our results indicate that wild-type THAP1 has a function in regulating genes related to cell growth and proliferation in neuronal cells, while synaptic functional alteration might be one of the pathogenic mechanisms of DYT6 dystonia caused by THAP1 missense mutations.

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P09.122B

New cases of de novo truncating mutations of TRIM8 in patients with epileptic encephalopathy, dysmorphic features and nephrotic syndrome

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Mutations of the TRIM8 gene coding for a tripartite motif protein have been reported in a patient with epileptic encephalopathy by Sakai and colleagues. We present here two additional patients with TRIM8 mutations: an eight year old girl with pharmacoresistant epileptic encephalopathy associated with stereotypies and glomerular proteinuria, and further clinical details of a patient reported by the Epi4K consortium. Exome sequencing revealed de novo truncating mutations of TRIM8 in our patient as well as the patient from the trio sequenced by the Epi4K consortium. The de novo mutations were confirmed by Sanger sequencing. Our case presented nephrotic syndrome not reported in the patient of Sakai and colleagues and the Epi4K consortium case. The clinical presentation of these patients overlaps with Galloway-Mowat syndrome, but mutations in the WDR73 gene were absent suggesting a Galloway-Mowat-like phenotype in our cases. Moreover, Galloway-Mowat syndrome seems to result in earlier death than in our cases. These observations suggest that phenotypic variability is observed in patients with TRIM8 mutations and genetic testing of TRIM8 should be expanded to patients with EE associated with dysmorphic features or nephrotic syndrome.

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P09.123C

Compound heterozygous mutations in *UBA5* causing early-onset epileptic encephalopathy in two sisters in Iceland

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Introduction: Epileptic encephalopathies are a group of childhood epilepsies characterized by severe seizures or other paroxysmal eletrical activities that contribute to impaired cerebral function. The recent, extensive use of next-generation sequencing has identified a large number of genes in epileptic encephalopathies, including *UBA5* in which biallelic mutations were first described as pathogenic in 2016.

Materials and Methods: Two sisters with early-onset epileptic encephalopathy were whole-genome sequenced along with their unaffected parents (Icelandic and American). The sisters were born in 2004 and 2006 and first became symptomatic at three months of age when they developed infantile spasms, which later progressed to recurrent, treatment-resistant seizures.

Results: We detected a compound heterozygous genotype in *UBA5* in the affected sisters, a genotype not seen elsewhere in an Icelandic reference set of 30,067 individuals. The maternally inherited missense mutation, p. Ala371Thr, produces a hypomorphic *UBA5* allele and has been reported as pathogenic when in compound heterozygosity with a loss-of-function mutation in *UBA5*. The paternally inherited exonic splicing mutation, c.684G>A, occurs at the last nucleotide of exon 7 of *UBA5* and is predicted to disrupt the splice site with a subsequent loss-of-function effect. Two unaffected brothers of the sisters (born in 2002 and 2014) are each heterozygous for only one of the mutations.

Conclusions: We describe compound heterozygous mutations in the *UBA5* gene in two sisters with early-onset epileptic encephalopathy. To our knowledge, this is the first description of mutations in *UBA5* since the initial discovery that pathogenic biallelic variants in *UBA5* cause early-onset epileptic encephalopathy.

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P09.124D

Functional characterisation of a variant in the *SLC19A1* gene associated with Wernicke-Korskoff syndrome

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Wernicke-Korsakoff syndrome (WKS) is an acute neuropsychiatric disorder characterised by a rapidly progressing Wernicke encephalopathy followed by an irreversible disorder called Korsakoff's psychosis. WKS results as a consequence of low levels of thiamine reaching the brain and is commonly associated with chronic alcohol abuse due to malnutrition and liver damage. Whole exome sequencing was conducted in 30 WKS individuals. We detected 34 truncating mutations in genes predicted to have low intolerance to loss of function variants. Amongst these were genes involved in ciliopathies, "failure to thrive" and with low BMI. Next we used the WKS exome data as a discovery set to identify variants in genes involved in the thiamine and folate pathways. Thirty variants had higher minor allele frequencies in the WKS exome data compared the European 1000 genomes population and exome variant server database. Follow up genotyping in a cohort of 112 WKS individuals compared to 439 alcohol dependent subjects with no WKS found nominal association with the rs1051266 variant in the *SLC19A1* gene (p=0.034). *SLC19A1* encodes a folate transporter which also transports the biologically active forms of thiamine. *In vitro* assays in HEK293 cells show a significant increase of thiamine diphosphate (TPP) in the cells with the mutant versus cells transfected with the wild-type transporter (p=0.023). TPP is an important component of the Krebs and glucose metabolism cycles and altered TPP levels may have downstream effects on these cycles and explain why some individuals who are alcohol dependent develop WKS. Funded by the Brain Damage Research Trust

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P10 Neuromuscular disorders

P10.01A

Col6A2 p.G283E: antisense-induced mRNA knockdown as a possible treatment strategy

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Collagen VI myopathies (prevalence of 10/1,000,000) encompass a broad clinical spectrum from the milder form Bethlem Myopathy (BM; OMIM: # 158810) to the more severe Ullrich Congenital Muscular Dystrophy (UCMD, OMIM: # 254090). Most mutations have a dominant negative effect, with defects in collagen VI chain secretion and assembly. It is generally accepted that, reversely, collagen VI haploinsufficiency has no pathological consequences. Thus, RNA-targeting approaches aimed at preferentially inactivating the mutated COL6 messenger may represent a possible therapeutic strategy.

Exome sequencing revealed a de novo mutation in a highly conserved region of codon 283 of COL6A2 (p. G283E) in a patient with an intermediate phenotype. A cell culture assay with mutant fibroblasts from the patient's skin

biopsy was established in order to compare them to wild type fibroblasts and analyse differences in ECM quality and quantity. We have identified a significant decrease in collagen VI matrix and non-consistent microfibrils from which we concluded the mutation's serious impact on ECM. Specifity of antisense oligo nucleotides (asON) and small interfering RNA (siRNA) was defined after initial transfection carried out against wild type allele showing a significant RNA depletion. Now we aim to transfect with the mutation-specific asON and siRNA to examine their benefit on collagen VI in ECM in the fibroblast assay and perform quantitative PCR for knockdown validation. These studies could further establish antisense induced mRNA knockdown as a possible treatment for collagen VI myopathies.

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P10.02B

Homozygote ADCY6 mutation causing demyelinating polyneruopathy

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Introduction: CMT is a common yet complex diagnosis to work up because of the genetic heterogeneity of the disease. We present a case of two siblings, a boy and a girl, from a consanguineous family who presented with demyelinating polyneuropathy phenotypically assessed as CMT. A syndromal CMT form was suspected because of hearing impairment in one sibling and craniosynostosis in the other.

Materials and Methods: Chromosomal microarray and metabolic testing were normal. A panel of CMT genes was without significant findings. We then applied Whole exome sequencing (WES)

Result: WES revealed a homozygote ADCY6 mutation c.2975A>G, p. Tyr992Cys in both patients. No other likely pathogenic variants were detected.

Conclusion. The present report expands the phenotypical spectrum of ADCY6 related disorder. ADCY6 is gene encoding a protein, which belong to the adenylate cyclase family. This protein is responsible for synthesising cAMP, which has a great variety of functions, and is essential for correct differentiation of peripheral myelinated axons. In the current literature, a mutation in ADCY6 is related to AMC, but these siblings with a homozygote ADCY6 mutation presented CMT like symptoms along with other symptoms, not related to CMT. This might be due to the large variety of the phenotype or due to additional autosomal recessive disorder. Extensive diagnostic work-up based on the assumption that the children had CMT did not lead to a diagnosis whereas the exome approach allowed the clinical team to identify a likely cause and reevaluatethe clinical picture.

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P10.03C

Molecular characterization of skeletal muscle of ALS patients during disease progression: identification of new therapeutic targets

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Introduction: Most of the studies on ALS focused primarily on motor neurons to understand the etiology of the disease. However, there is evidence suggesting that muscle dysfunction and neuromuscular junction degeneration occur long before symptom onset, and may contribute to motor-neurons' death. Our aim is to unravel the molecular pathway connecting nerve and muscle in order to identify the mechanisms that exacerbate the disease.

Methods: Selected genes and miRNAs involved in the innervation and regeneration pathways were analyzed in skeletal muscle and in satellite primary cultures derived from ALS patients and controls. Patients were stratified according to disease progression: long (≥4 years without requiring respiratory support) and rapid (<4 years).

Results: Our data suggested a perturbation in the signals required for an efficient and complete myogenesis in muscle biopsies of ALS patients. HDAC4, MYOG, MYOD, PAX7 and PAX3 resulted significantly upregulated in patients compared with controls.

The most interesting results derived from miRNAs expression analysis: 1) miR206 levels significantly decreased during disease progression; 2) the expression of selected miRNAs changed significantly between the "long" and "rapid" ALS patients groups. The levels of these miRNAs also correlated with fiber types and muscle atrophy.

Moreover, our data indicated that ALS satellite cells are still able to form differentiated myotubes, although this process is delayed.

Conclusion: These data may suggest the use of miRNA mimics in ALS muscles, as a potential therapeutic strategy to enhance motor performance and slow disease progression.

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P10.04D

Genetic heterogeneity of amyotrophic lateral sclerosis in the Hungarian population

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Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder associated with degeneration of upper and lower motor neurons. Affected individuals usually die of respiratory failure within 3–5 years. Genetic factors play a key role in all types of ALS, therefore, our aim was to screen the variation of 7 candidate genes in Hungarian patients with ALS. Patients and methods: Using Sanger sequencing, we screened mutations in *SOD1, ANG, TARDBP* and *UBQLN2* genes in 66 Hungarian patients. A two-step protocol was followed for the detection of the repeat expansion in the *C9ORF72* gene in all patients. Genotyping was used to determine whether the individual carrying the repeat expansion carried the "risk" haplotype. High-thoughput sequencing was used to screen mutations in the coding regions of the *SETX, FUS* and *C9ORF72* genes. **Results:** We identified a novel frameshift (p.Lys91ArgfsTer8) and three recurrent missense mutations (p.Val14Met, p.Leu144Phe and p.Asp90Ala) in the *SOD1* gene. The novel mutation led to a frameshift with the insertion of 8 novel amino acids and the formation of premature stop codon. Mutation analysis of the *TARDBP* gene revealed a recurrent missense mutation (p.Met311Val). Two known mutations (p.Met-24Ile; p.Arg33Trp) were detected in the *ANG* gene. *C9orf72* repeat expansion was identified in one patient, who also carried the rs3849942 risk allele. An additional novel missense mutation (p.Asn264Ser) was identified in the *SETX* gene. Conclusion: Our study adds novel data to the genetic and phenotypic diversity of ALS and indicates that complex approaches are needed to understand the genetic heterogeneity of this disease.

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P10.05A

Elucidating the role of genetic variants in known ALS-associated genes by means of targeted NGS analysis

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Introduction. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by variants in different genes in a consistent proportion of both familial and sporadic cases. The number of ALS-associated genes has been steadily increasing in recent years. Consequently, simultaneous screening of multiple genes is likely to be more efficient than gene-by-gene testing in order to identify possible causative variants in ALS patients.

Materials and Methods. We designed a custom panel of 32 genes (including 5' and 3' UTRs) and performed targeted next-generation sequencing in 300 consecutive Italian ALS patients (both familial and sporadic) and 200 controls.

Results. We evaluated frequencies and relative impact of different categories of variants, including known pathogenic variants (found in 10% of cases), novel coding variants (20%), and variants with MAF < 0.1% (17%). We compared frequencies of both variants in coding regions and in 5′ and 3′ UTRs between patients and controls, thus showing that patients were enriched for rare variants in previously neglected genes (i.e.: *DCTN1* and *EPHA4*, among the others).

Conclusions. Our panel has been proving to be a useful tool for evaluating and defining the relative contribution of genes, the effective role of specific coding variants and the possible involvement of variants in 5' and 3' UTRs in ALS. Furthermore, the integration of all genetic data we obtained allowed us to explore the oligogenic hypothesis in ALS etiopathogenesis, considering that we found a higher frequency than expected of patients carrying two or more rare variants in the studied genes.

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P10.06B

Big Data in genetic research: the example of titin gene and titinopathies

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Exome and genome sequencing will soon be adopted as a first-tier test for all the genetically heterogeneous conditions.

Such extensive screenings will result in the identification of thousands of rare variants which are difficult to interpret with the current knowledge. Because of its sheer size, variants in titin gene (*TTN*) are detected frequently in any sequencing approach. *TTN* variants have been described in so many different clinical conditions that a univocal and unambiguous interpretation of molecular findings is almost impossible, even in monogenic disorders, without access to additional investigations such as segregation studies, protein and cDNA assays.

The interpretation of missense variants in *TTN*, which represent the vast majority of variants identified, is the most significant challenge related to NGS investigation. Our data show that current bioinformatic tools are unable to correctly predict the effect of most of these missense variants.

We carried out a systematic analysis of rare *TTN* variants in our large cohort of patients affected by a skeletal muscle disorder (over 2,500) as well as in publicly available databases (ExAC, 1000G and LOVD).

We identified potentially causative missense variants significantly enriched in our cohort. We also identified harmless rare variants.

Our work represents a crucial first step to prioritise specific missense changes for further functional assays.

Considering the increasing role of titin mutations in skeletal muscle and heart diseases, we strongly encourage a closer cross-field collaboration among all the different specialists: neurologists, cardiologists, pediatric neurologists, geneticists, molecular and cell biologists, etc. working on titin.

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P10.07C

A novel RYR 1 gene mutation in a patient with severe central core disease

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Introduction: Central core disease (CCD) is a congenital myopathy ranging from mild to severe, being relevant to the age of onset. Findings of the disease are proximal muscle weakness, motor developmental delay, kyphoscoliosis, joint contractures and congenital dislocation of the hips. Heterozygous or homozygous mutation in the RYR1 gene, encoding the skeletal muscle ryanodine receptor 1 protein, causes CCD. Here, we present a CCD patient with a novel missense mutation in the RYR1 gene.

Clinical report: The patient is a 1-year old baby boy with hypotonia, feeding difficulty, severe kyphoscoliosis, contractures in hands and hip dislocation.

Results: Whole-exome result analysed with in-house method (SELIM) for variant prioritization revealed de novo heterozygous missense mutation, NM_000540:exon102:c.A14758C:p.T4920P, in RYR1 gene.

Discussion: The RyR1 protein is a calcium releasing channel for the sarcoplasmic reticulum. The mutation detected at codon 4920 is located in the transmembrane C-terminal region which is important for the intact RyR1 channel. Musculoskeletal findings are more prominent in

patients with C-terminal mutations as in our patient, while milder in patients with mutation of the other domains. In literature, a girl with poor fetal movement, hypotonia and proximal muscle weakness, but no skeletal findings has been reported who has threonine-asparagine transformation at codon 4920 (c.14759C> A). The substitution in this patient from threonine to asparagine is from polar to polar, while the alteration in our patient from threonine to proline resulting in polar to nonpolar. So, this difference in amino acid change may explain the more severe skeletal findings in our patient.

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P10.08D

3´splice site mutations in *INF2* cause Charcot Marie Tooths disease without focal segmental glomerulosclerosis: Implication of differential function of alternative splice variants

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Background Mutations in *INF2*, encoding inverted formin-2, cause focal segmental glomerulosclerosis (FSGS) and Charcot-Marie-Tooth neuropathy (FSGS-CMT). The reported disease-causing mutations are located in exon 2 and exon 3, thus affecting most known isoforms of *INF2*. Alternative mRNA splicing results in at least 3 isoforms of *INF2*, but little is known about the function of the individual isoforms. Two of the isoforms are long and differ in the positioning of the translation termination codon due to alternative splicing of exon 22. The longest isoform contains a prenylation site and is termed INF2-CAAX. The isoform that excludes exon 22 is termed INF2-non-CAAX. **Results and methods** We describe how splicing mutations in the last intron of *INF2* (position *INF2* [NM_022489]: c. *1+1/c.3751+1) result in CMT without the development of kidney disease. Two families were identified, each carrying different splice site mutations in the last intron of *INF2* (c. *1+1G>C /c.3751+1G>C and 1G>C/c.3751+1G>A). Experiments using minigene constructs confirm that splicing is affected. cDNA analysis from multiple tissues shows that more than 2 isoforms are expressed in most tissues. **Conclusion** Our data suggests that mutations that cause exon 22 skipping and therefore only affect the long isoform spare the glomeruli. This indicates that the short splice form of *INF2* is required in the kidneys. Thus, the long isoform INF2-CAAX that includes exon 22 must be important for Schwann-cells and causes CMT when mutated. This illustrates how mutations that only affect specific alternative splice forms can cause restricted phenotypes and thereby delineate the functional role of protein isoforms.

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P10.09A

Whole exome sequencing reveals a novel missense mutation in the MARS gene related to a rare Charcot-Marie-Tooth neuropathy type 2U

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Background: Charcot-Marie-Tooth (CMT) is a heterogeneous group of progressive disorders, characterized by chronic motor and sensory polyneuropathy. This hereditary disorder is related to numerous genes and varying inheritance patterns. Thus, many patients do not reach a final genetic diagnosis. Patient: We describe a 13-years old girl presenting with progressive bilateral leg weakness and gait instability. Extensive laboratory studies and brain Magnetic Resonance Imaging were normal. Nerve conduction studies revealed severe lower limb peripheral neuropathy with prominent demyelinative component. Following presumptive diagnosis of chronic inflammatory demyelinating polyneuropathy, the patient received treatment with steroids and intravenous immunoglobulins courses, with no apparent improvement. **Results:** Whole exome sequencing revealed a novel heterozygous c.2209C>T (p.Arg737Trp) mutation in the MARS gene (OMIM 156560). In-silico prediction programs classified this mutation as a probable cause for protein malfunction (3/4 damaging). This gene has recently been related to CMT type 2U. Allele frequency data reported this variant in 0.003% of representative Caucasian population. Family segregation analysis study revealed that the patient had inherited the variant from her mother, reported as healthy. Neurologic examination of the mother demonstrated decreased limb reflexes, with demyelinative and axonal sensory-motor polyneuropathy revealed by nerve conduction studies. Conclusion: Our report highlights the importance of next generation sequencing approach to facilitate the proper molecular diagnosis of highly heterogeneous neurologic disorders.

Amongst other numerous benefits, this approach might prevent unnecessary diagnostic testing and potentially harmful medical treatment.

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P10.10B

Circadian rhythm genes in Duchenne muscular dystrophy

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Introduction: Muscular dystrophies are genetic conditions that cause progressive weakness and loss of muscle mass. Mutations in structural proteins, such as dystrophin, cause muscle fibers' instability with muscle damage.

Circadian rhythm coordinates biological processes with the 24h cycle; its role in maintaining muscle functions is known, both in animal models and in humans. Recently, we disclosed a link between CollagenVI myopathy and circadian genes.

Methods: To define the involvement of circadian circuit in muscle damage, we designed a Fluidic-Card-TaqMan based assay, including 30 genes related to circadian rhythms and muscle regeneration. We tested gastrocnemius and tibialis anterior muscles from unexercised and exercised mdx mice. We subsequently selected 7 most deregulated genes and performed expression analysis by Real-time PCR in 10 DMD patients with different mutations.

Results: We demonstrated the profoundly de-regulation of circadian genes in mdx mice, both exercised and unexercised. Such deregulation was confirmed in DMD patients muscle biopsies. Genes mostly deregulated were CSNK1E, SIRT1, MYOG. In order to explore if the transcript deregulation reflects on plasma, we designed an ELISA assay for the profound up-regulated CSNK1E protein and tested 16 DMD and 5 male control plasma samples. The CSNK1E showed a variable expression profile however, the sample's cohort proves to be rather limited and an enlargement of samples number is needed.

Conclusion: Our preliminary data demonstrate that circadian genes are affected in both DMD patients and mdx mice supporting a correlation between circadian circuit and DMD, open the way to new biomarkers and interesting therapeutic options.

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P10.11C

CMTsubtypes in a cohort of Italian patients enrolled at Messina Neuromuscular Center since 1994 to 2016

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INTRODUCTION: The Neuromuscular Diseases of the University of Messina is a tertiary center for neuromuscular disorders in South Italy and a Regional Reference Center for Rare Diseases. We analyzed the percentage of Charcot-Marie-Tooth (CMT) patients assessed at our Center in the last twenty years, with the aim to evaluate how many patients received a genetic diagnosis and the distribution of the genetic subtypes.

PATIENTS AND METHODS: This was a retrospective study. We analyzed the clinical records from 523 patients with anamnestic and/or clinical suspect of CMT seen since 1994 to 2016. The distribution of CMT subtypes and pathogenic genetic mutations was determined.

RESULTS: 310/523 (59.27%) received a genetic diagnosis. The most common subtypes found were CMT1A/*PMP22* duplication (51.6%), HNPP/*PMP22* deletion (16.1%), CMT1B/*MPZ* mutation (10.6%), CMTX/*GJB1* mutation (8.3%), CMT2F/*HSP27* mutation (5.1%). Other mutations included *PMP22* point mutation (n.7 pts), *MFN2* mutation (n.5 pts), *GDAP1* mutation (n.4 pts), *BSCL2* mutation (n.3 pts), *GARS* mutation (n. 2 pts), *TRPV4* mutation (n. 2 pts), *LITAF* mutation (n.1 pt) and *NEFL* mutation (n. 1 pt).

CONCLUSION: Our results confirm the heterogeneity of CMT. The percentage of genetic confirmation is slightly smaller than the ones previously reported. One explanation might be that the time period we considered is twenty years, with a limited number of known genes in the first decade. The subtypes distribution showed a major prevalence of CMT2F/*HSP27* mutation compared to previous reports, with atypical phenotypic features in some individuals.

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P10.12D

Custom micro-fluidic exome array to detect transcript mutations in undiagnosed patients with ColVI myopathies

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Introduction: Collagen VI-related myopathy is a form of muscular dystrophy caused by mutations in the genes encoding three different Collagen VI (ColVI) chains α1, α2 and α3. About 40% of Col VI patients are however negative for ColVI genes mutations. In these cases analysis of ColVI transcript might be valuable in identifying mutations which escape routine DNA testing.

We designed and validated a customized micro-fluidic exome array (FluiColVI) for the analysis of ColVI transcripts to identify mutations missing the DNA diagnosis.

Methods: We designed a custom TaqMan gene expression assay to profile the entire 115 exons of ColVI genes covering all the exon-exon junctions of the three ColVI transcripts α1, α2 and α3. RNAs were extracted from both muscles biopsies and native/myogenic transformed (MyoD) urine stem cells (USCs). RNA from patients with a known mutations was used to validate the FluiColVI method.

Results: FluiColVI identified the three ColVI transcripts with the detection of all junction–junction systems in both muscle and native USCs of controls. The analysis of MyoD-USCs did not detect any ColVI transcripts, as expected to be absent in myogenic cells. In the patients, the FluiColVI confirmed the presence of the mutations in ColVIα1 identified at the DNA level.

Conclusion: This study demonstrated that FluiColVI is a validated diagnostic tool for molecular profiling of ColVI transcripts in skeletal muscles and can be used in undiangnosed patients. Interestingly, native urine stem cells represent a noninvasive and cost effective human cell model to study ColVI transcripts in myopathies.

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P10.13A

Loss of function mutations in *ASCC1* gene cause autosomal recessive Congenital muscular atrophy with bone fractures

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Introduction: The *ASCC1* gene encodes a subunit of the activating signal cointegrator 1 complex. The ASC-1 complex is a transcriptional coactivator with an important role in gene transactivation by multiple transcription factors including NF-kappa-B, SRF and AP1. A missense mutation in *ASCC1* (p.N290S) was associated with Barrett esophagus and/or esophageal adenocarcinoma in heterozygotes whereas homozygous loss of function truncating mutation of the gene was recently reported in two patients with Congenital muscular atrophy with bone fractures.

Materials and methods: Herein we describe a family of Roma origin with an affected child who deceased 12 days after birth with clinical symptoms of the disease. Osteogenesis imperfecta was initially suspected based on the presence of long bone fractures but absent movements (spontaneous and after stimulation), absent reflexes, respiratory distress syndrome, hepatosplenomegaly, bilateral cryptorchidism and heart insufficiency raised revision of initial diagnosis. NGS using the TruSight One gene panel (Illumina) was applied to search for pathogenic mutations in the proband.

Results: A homozygous substitution was found in donor splice-site of exon 7 of *ASCC1* gene - NM_001198799.2: c.710+1G>A. Results were validated by Sanger sequencing and segregation analysis in the affected family was performed. Both parents were found to be heterozygous carriers of the mutation. The variant is predicted to disrupt splicing of all gene transcripts and result in loss of function.

Conclusions: Our results support the hypothesis that loss of function mutations of *ASCC1* gene are causative for Congenital muscular atrophy with bone fractures - a rare severe form of congenital SMA.

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P10.14B

Modeling of a unique desmin mutation in zebrafish by using genome editing brings new insights into desmin function

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Limb-girdle muscular dystrophies (LGMD) are a diverse group of rare neuromuscular diseases manifesting with proximal muscle weakness. We previously described an ultra-rare LGMD phenotype (LGMD2R, MIM 615325) caused by an unusual desmin mutation predicted to disrupt a poorly described laminB-binding site and affect mechanotransduction in skeletal muscle (1). This phenotype is unique as no desmin accumulation and cardiomyopathy is observed unlike other desminopathies. Our aim is to create a patient-specific disease model in order to understand underlying mechanisms of the disease. First we demonstrated by co-immunoprecipitation and in-situ proximity ligation assay that desmin interacts directly with laminB around myonuclei in wild-type zebrafish. Next, we used genome editing tools in order to create targeted mutations in zebrafish. By CRISPR/Cas9, we knocked-out desmin orthologues (desma and desmb) in zebrafish and described their differential expression profiles. Data showed that desma expression is mainly somitic while desmb is expressed in gut. However in the absence of desmb, desma expression is observed both in somites and gut. By TALEN, we disrupted the laminB-binding domain on desma by creating an early stop codon. Desmin-laminB interaction and calcium influx analyses in mutants are ongoing. In conclusion, modeling of an ultra-rare phenotype in zebrafish by using genome editing tools provided new insights into desmin expression and function in zebrafish and will contribute to the understanding of the role of desmin and the pathophysiology in human.

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P10.15C

Chromatin configuration, RNA and protein studies identified novel DNA elements that influence the dystrophin transcription dynamics

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Introduction: Despite of the several reports describing dystrophin (DMD) gene expression abnormalities, the DMD transcriptional process remains poorly understood. We therefore investigated the transcriptional capacity of the dystrophin locus.

Methods: We designed a DMD locus ChIP-chip array to identify open chromatin regions, and a Chromosome Conformation Capture assay was used for interaction studies. We selected muscle biopsies from 9 BMD patients in order to evaluate the different amount of dystrophin and its link to the novel regulatory regions identified; dystrophin transcript and protein were quantified by FluiDMD card and Western blot.

Results: We identified two novel DMD regions which bind the RNA Pol II, one in intron 52 (DMEi52) and another one in exon 62 (DMEe62). The DMEi52 contains a genuine RNA pol II pausing site used during DMD locus transcription. We also showed that two DMD regions, one in intron 34 (DMEi34) and one in exon 45 (DMEe45), share histone marks of open chromatin, but are unrelated to RNA Pol II activity. The DMEi34 bi-directionally stimulates the Dp427m promoter transcription. Supporting the DMEi34 transcription enhancing function we found lower dystrophin RNA and higher protein levels in BMD patients carrying genomic deletions encompassing the intron 34 compared to other differently deleted BMDs.

Conclusion: We identified the first DMD pausing site which is located in exon 62 and a novel dystrophin muscle enhancer in intron 34. These findings have important repercussions on the understanding of the transcriptional regulation of the DMD locus, as well as for the designing of splicing therapies.

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P10.16D

Becker muscular dystrophy: a natural gene editing

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Introduction Duchenne muscular dystrophy (DMD) is caused by frame-disrupting mutations in DMD gene (DMD; 300377), characterized by progressive muscle weakness and degeneration. In-frame (IF) dystrophin mutations that do not disrupt the open reading frame typically cause a clinically milder disorder, Becker muscular dystrophy (BMD). **Methods** We have characterized a three generations family with Hypertrophic cardiomyopathy (HCM). Index case was diagnosed at five years old with psychomotor retardation and HCM (asymptomatic) developing Long QT at twelve. His father was asymptomatic and his mother had hypertrabeculation and Dilated Cardiomyopathy (DCM). The index case was studied by Next Generation Sequencing and their relatives by Sanger and MLPA (multiplex ligation-dependent probe amplification). **Results** Three mutations were identified in the index case: p.Arg891Alafs*160, a HCM causal mutation; p.Thr263Met in KCNJ5 gene which could be associated to Long QT; and p.Ser2437_-Ile2554delinsPhe in DMD. His father and three uncles (one of them affected of HCM) were carried of p.Arg891Alafs*160. His mother and grandfather (non affected) were carried of the other two mutations (p.Thr263Met and p. Ser2437_Ile2554delinsPhe). The rest of relatives were non-carriers. **Discussion** Our results suggest that although two male patients are carried of mutation in DMD and they had shorter but partially functional protein than those with complete gene, they can be diagnosed as BMD or even be undiagnosed. **Conclusion** The study of patients with these kinds of deletions that naturally mimic those that would be achieved by exon skipping is a way to predict the potential of AOs in exon skipping therapy for DMD.

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P10.17A

32 novel pathogenic sequence variants in 253 *DMD/BMD* patients from Turkey

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Introduction: *DMD* is the only known gene in dystrophinopathies. Mutations in DMD causes X-linked recessive distrophinopathies. More than 3300 different mutations were identified at present, which is consistent with the allelic heterogeneity. Materials and Methods: Two hundred fifty three patients with DMD/BMD clinic were investigated in this study. Deletion/duplication mutations were screened by MLPA (P034, P035), mutation negative patients were further sequenced by next generation sequencing (NGS) on Ion Torrent-PGM, covering all 79 coding exons and other 23 associated intronic and flanking regions adding up to 148 amplicons covering 28.6 kb region. **Results:** Deletions were identified in 125 (49.4%) and duplications in 23 patients (9.1%). Sequence analyses in 103 patients revealed different mutations; 8 splice site, 4 missense, 33 nonsense (including one mosaic), 13 small deletions, 2 small insertions) in 60 patients (23.7%). 32 of these mutations were novel (53%). *De novo* occurrence has been observed in 48.4 % of deletions/duplications and 20.4% of pathogenic sequence variants. **Conclusions:** Causative mutations were identified in 82.2% of DMD/BMD patients with this algorithm. Two single exon deletion in MLPA, presented point mutations at the probe binding region revealed by sequencing. Patients with no identified mutations will be the source for further screening of intronic regions or whole genome sequencing. Mutation detection in DMD/BMD is not only important for genetic counseling and prenatal planning, but also render the mutation specific therapeutic drugs in future.

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P10.18B

A novel point mutation affecting Asn76 of dystrophin protein leads to dystrophinopathy

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Introduction. Mutations in the DMD gene lead to Duchenne and Becker muscular dystrophies (DMD/BMD) characterized by progressive muscle wasting. Missense mutations are rare cause of DMD/BMD. Here we report the detection of a novel missense mutation located in the N-terminal actin binding domain of dystrophin leading to dystrophinopathy.

Materials and Methods. A six-month old male patient showed mild generalized muscle weakness, hypotonia and delayed motor development. Electromyography showed myopathic features. He had elevated serum creatine kinase. MLPA analysis was performed. Pyrosequencing was used for the sequencing the coding region. Dystrophin immunohistochemistry were performed. In silico methods were used to predict the effect of the mutation on protein structure and stability.

Results. MLPA assay showed exon 4 deletion. Amplification of exon 4 did not confirm the deletion. By sequencing exon 4 a novel de novo point mutation (c.227A>T) was detected (p.Asn76Ile). The false positive MLPA result was explained by the fact that the detected nucleotide lies directly next to the ligation site. Sequencing of the whole coding region of the DMD gene proved c.227A>T to be the sole variant being potentially pathogenic. Immunohistochemistry results were suggestive of BMD. The mutation was predicted to be highly destabilizing on the dystrophin structure.

Conclusions. Our results highlight the importance of the confirmatory testing of single-exon deletions detected by MLPA and we describe a novel, destabilizing missense mutation in the DMD gene leading to dystrophinopathy.

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P10.19C

De novo mutation cause compound heterozygosity in DMD gene, resulting mild phenotype in Duchenne muscular dystrophy: a case report

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Introduction: Different muscular dystrophies and myopathies can be inherited in an X-linked, autosomal dominant, or autosomal recessive manner. More than 50 genes have been associated to different forms of muscular dystrophy alone, making accurate diagnosis difficult. The Duchenne and Becker types of muscular dystrophy are two related conditions that primarily affect skeletal muscles and cardiac muscle. These forms of muscular dystrophy occur almost exclusively in males. We are presenting here a case of a 11 years old girl, with healthy parents and with mild symptomps.

Materials and Methods: Total genomic DNA was extracted from the biological sample using a spin column method. We have targeted all of the coding excons with exon-intron boundaries of 51 genes using PCR-based library preparation method. Sequencing reads were mapped to the reference genome (hg19) and after variant calling the variants were classified based on ExAc, ClinVar and 1000G information.

Results: Gene panel test revealed a coumpound heterozygous state of the DMD gene (c.8421G>A, p.(Trp2807*) and c.3954T>A, p.(Asp1318Glu). This two mutations has never been described before, but alterations at this genomic positions are known disease-causing mutations. The family analysis revealed that, the father of the patient doesn't carry any of this mutations and the mother is a carrier of the c.3954T>A alteration. The c.8421G>A variation is a de novo mutation in the patient.

Conclusions: We counclud the mother of our patient is a carrier of a DMD gene mutation, while the patient is a „manifesting carrier" at least, with a de novo compound heterozygous mutation.

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P10.20D

International-DMD (IDMD): a PTC Therapeutics-supported diagnostic project to widely identify Dystrophin mutations by NGS technologies

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Introduction: Extensive molecular diagnosis in genetic diseases is vital to confirm clinical diagnosis and to enable genetic counseling and personalized management. Duchenne muscular dystrophy (DMD) is a rare genetic neuromuscular disease affecting 1/5000 males, due to a variety of dystrophin gene mutations. The first signs and symptoms of DMD include delayed milestones such as walking and talking, and enlarged calves. PTC Therapeutics International Ltd. and the University of Ferrara, Italy, have established a collaboration focused on identifying patients affected by rare genetic disorders through increased genetic testing activities, with an initial focus on DMD. Genetic testing is available to patients throughout European countries, potentially expanding to other regions.

Methods: Diagnostic settings include MLPA (MRC Holland) and NGS dystrophin gene sequencing (Multiplicom).

Results: Currently DNAs from 57 DMD boys were collected. Patients were from Poland (34), Hungary (10), Lituania (5), Romania (3), Russia (1) and Bosnia (4). Among the 30 samples analyzed, 7 deletions, 4 duplications, 11 small mutations (8 nonsense) were identified.

Conclusion: The early identification of the underlying genetic mutation is critical to potentially affecting the course of a disease such as DMD as well as the choice of treatment and aids in the setup of appropriate and effective care and follow up as well as eligibility for clinical trials. Genetic counselling can also be offered to patients and families with important repercussions on reproductive choices and lifestyle planning (full details and contacts at www.ospfe.it/medicalgenetics).

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P10.21A

Genetic diagnosis of severe fetal akinesia syndrome and associated abnormalities by means of whole exome sequencing

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Introduction: The term fetal akinesia represents disorders within a broad spectrum of diseases leading to reduced or absent fetal movements. This clinical entity is often recognized as a sequence of related deformational changes and includes features like intrauterine growth restriction, craniofacial anomalies, limb contractures, together with pregnancy complications such as polyhydramnios. In the last years, gene discovery was revolutionized by implementation of NGS technologies, whereas research mainly focused on postnatally well-defined phenotypes and less on fetal lethal disorders.

Materials and Methods: We included 15 affected fetuses in 11 families. Fetal akinesia syndrome was diagnosed prenatally by ultrasound and/or MRI. Mainly the clinical diagnosis was leading to decision of termination of pregnancy or later to perinatal death. In all cases chromosomal aberrations were excluded. Exome sequencing was performed in the index case of each family.

Results: In 4/11 families, including two consanguineous families, more than one fetus was affected and an autosomal recessive disorder was likely. We correlated exome data with known "33" disease-related genes and found pathogenic variants of *CNTN1*-gene and *RYR1*-gene, respectively in two cases. In one case exome sequencing was negative, but a *DMPK*-gene repeat expansion was identified. In the remaining eight cases no previously reported variant was found.

Conclusions: Fetal akinesia syndrome is a genetically heterogeneous disorder, following different types of inheritance. In a minority, a variant in a known disease-related gene was found. So far, in the majority the underlying genetic cause remained unknown, but several new candidate genes where identified based on our exome data.

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P10.22B

GBE1-related disorders: biallelic pathogenic variation at one codon resulting in both infantile and adult phenotypes

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Recessively inherited *GBE1* pathogenic variants (OMIM#607839) have been linked to: (1) Glycogen Storage Disease IV (GSD4; OMIM#232500), whose clinical picture includes a variable association of hepatic, cardiac, neurological and musculoskeletal involvement; (2) an Adult form of Polyglucosan Body Disease (APBD; OMIM#263570), presenting as adult-onset progressive leukoencephalopathy, spastic paraplegia, cognitive impairment and peripheral neuropathy.

Some genotype-phenotype correlations have been suggested: severe forms result from loss-of-function variants and milder phenotypes from missense mutations. Nevertheless, there is an overlap, since some pathogenic variants have been described as causing different subtypes of GSD4 or APBD. However, to date, no patient has been reported presenting with both phenotypes, infantile and adult.

Here, we report a case of a 45 year-old female with hypotonia and a congenital myopathy, who developed progressive leukoencephalopathy at age 40 years. Cerebral MRI showed hyperintense bilateral frontal lobe, occipital lobe, and periventricular white matter abnormalities on T2 and fluid attenuated inversion recovery (FLAIR) sequences. There were no cardiac or hepatic manifestations.

Exome sequencing identified compound heterozygous missense variants c.785G>A:p.(Arg262His) and c.784C>T: p.(Arg262Cys) in *GBE1*; these variants were respectively classified as likely pathogenic and pathogenic according to the latest ACMG guidelines and each inherited from a different parent. The variant c.784G>A, combined with another missense variant, has been previously published in a young girl with congenital myopathy without cardiac or hepatic involvement.

This case underlines the phenotypic spectrum associated with *GBE1* pathogenic variants.

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P10.23C

Novel mutations of the *GJB1* gene associated with Charcot-Marie-Tooth type 1X in Lithuanian cohort

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BACKGROUND: X-linked Charcot-Marie Tooth disease (CMT1X) is caused by mutations in the *GJB1* gene that encodes a polypeptide that is arranged in hexameric array and forms gap junctions.

OBJECTIVE: To estimate the frequency of the *GJB1* gene point mutations, insertions and deletions in the Lithuanian Charcot-Marie-Tooth (CMT) cohort. To examine the phenotypes of patients with the novel *GJB1* mutations.

METHODS: A cohort of 336 CMT Lithuanian subjects has been screened for mutations in the *GJB1* gene by direct sequencing. Overall, 10 different *GJB1* mutations have been identified, three of which - novel.

RESULTS: In 14 unrelated probands, three novel *GJB1* gene mutations were detected: c.195T>A, c.290A>G and c.596G>A. Proband P5 had a nonsense mutation c.195T>A (p.Y65*) which causes a premature stop codon. Probands P7, P8 and P9 had a missense mutation c.290A>G (p. H97R) which causes a change of the highly conserved histidine residue, while proband P13 had a missense mutation c.596G>A (p.G199D) which causes a change of the highly conserved glycine residue. These mutations have been qualified as a disease causing based on *in silico* analysis (PolyPhen-2, SIFT, PROVEAN, SNPs&GO) and were not detected in the Lithuanian population control group (98 exomes) or in the Exome Aggregation Consortium and 1000 Genomes Project.

CONCLUSION: The c.195T>A, c.290A>G and c.596G>A mutations are novel mutations of the *GJB1* gene that have not been previously reported. The frequency of all *GJB1* mutations is 4.16% in Lithuanian CMT patients cohort.

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P10.24D

The use of zebrafish in functional genetic study of Hirschsprung disease

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Introduction: Hirschsprung disease (HSCR) is a congenital neurocristopathy of the enteric nervous system (ENS) where enteric ganglia were absent in the distal colon. The inheritance of HSCR range from monogenic inheritance to oligogenic effect of the major gene *RET* and other modifiers. Besides *RET* some 20 other genes have been identified however, to date, less than 50% HSCR cases have their disease-causing gene identified. To discover the unknown genetic factors of HSCR, researchers focus on studying patients genome, most lately by whole-exome sequencing. Candidate genes identified from these studies are further assessed by functional genetics to determine their functional correlation to HSCR etiology. In this abstract we describe zebrafish as a model for HSCR functional genetics.

Materials and Methods: Morpholino, CRISPR/Cas9 and mRNA were used to induced gene loss/gain-of-function on reporter zebrafish where fluorescence proteins were expressed in differentiated enteric neurons and/or undifferentiated enteric neural crest cells for direct phenotyping. Transit assay was carried out to evaluate intestinal movement. Heterozygous *ret* mutant zebrafish was characterized to determine its potential in studying *ret*-modifier interaction.

Results: Loss-of-function of zebrafish *ret* phenocopied HSCR in human. Disrupting some of the newly identified candidate genes caused similar phenotype, providing functional correlation to the disease. Overexpression of selected genes on human chromosome 21 revealed possible genetic link in HSCR-associated Down syndrome. The heterozygous *ret* mutant displayed mild intestinal hypoganglionosis that will be useful in *ret*-modifier interaction study.

Conclusions: Zebrafish is a valuable model for HSCR functional genetic that further our understanding of the human genetic data.

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P10.25A

Mutational spectrum of SPG4 (*SPAST*), SPG3A (*ATL1*) genes in Russian patients with hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is clinically and genetically heterogeneous group of neurodegenerative diseases, which characterized by progressive spasticity and weakness of the lower limbs and mild sensory dysfunction, due to axonal degeneration in the pyramidal tract.

It is generally accepted, that SPG4 and SPG3A mutations account for approximately 40% and 10% of all autosomal dominant HSP, respectively.

We screened DNA from 73 unrelated probands (46 familial, 27 sporadic) with clinical symptoms of SPG. DNA of 56 (76,7%) probands were sequenced of *SPAST* gene, and 20 (27,4%) – of *ATL1* gene (some of them have both incoming diagnosis). Patients without mutations were examined by MLPA-analysis.

We found 19 *SPAST* mutations (7 novel), and 8 *ATL1* mutations (1 novel) (Table1). There were found 27 (37%) mutations (74% point mutations; 26% gross deletions/duplications) in a total 73 patients in both genes, 29,6% mutations were not described so far. The origin de novo present in 26%.

Table 1. SPG3A and SPG4 mutations.

Gene	Family	Exon	Mutation
SPAST	2o, 21o	10	c.1291C>T (p.Arg431Term)
	43o**	15	c.1663G>T (p.Asp555Asn)
	44o	8	c.1107A>G (p.Thr369Thr)*
	5**	11	.1391A>G (p.Glu464Gly)*
	19	17	c.1750_1751delGAinsT
	32	8	c.1139T>C (p.Leu380Pro)
	35	10	c.1271delG*
	44	1	c.286delG
	54	9	c.1245+1G>A (IVS9+1G>A)
	73**	9	c.1216A>G (p.Ile406Val)
	75	15	c.1684C>T (p.Arg562Term)

Table (continued)

Gene	Family	Exon	Mutation
	53o	3	c.551A>C (p.Asn184Thr)
	26o	6–16	del.ex6-16*
	33o	1	del.ex1
	59o**	10–12	dup.ex10-12
	20	10–13	del.ex10-13*
	84**	15–16	del.ex15-16*
	88	1	dup.ex1*
ATL1	46, 37o	12	c.1243C>T (p.Arg415Trp)
	13**, 6o	7	c.715C>T (p.Arg239Cys)
	22	8	c.757G>A (p.Val253Ile)
	51	12	c.1483C>T (p.Arg495Trp)
	70**	8	c.773A>G (p.His258Arg)
	42	1–14	del.ex1-14*

*- novel mutation

**- sporadic cases

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P10.26B

Is the *HSPB3* p.Arg7Ser variant really causal? - evidence from three families with different phenotypes

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Introduction: Mutations in the small heat shock proteins HSPB1 and HSPB8 cause distal hereditary motor neuropathy (dHMN)[1]. Kolb et al.[2] searched for variants in other *HSBP* genes (*HSPB2-HSPB10*) and found the p.Arg7Ser variant in the *HSPB3* gene in two affected siblings with autosomal dominant dHMN. They proposed it is pathogenic and causal for dHMN. We sought to determine the relationship of *HSPB3* variants and dHMN.

Patients and Methods: Two-hundred and fifty patients were tested by massively parallel sequencing with a gene panel. Ninety-seven genes (including *HSPB3*) known to be associated with inherited peripheral neuropathies were included.

Results: Variant p.Arg7Ser (NM_006308.2:c.21G>T) was found in heterozygous state in three families from our cohort (table 1).

In our population the variant has frequency 0,06%.

Discussion: Each of our families with the p.Arg7Ser variant has a very different phenotype. Moreover, the variant has a high population frequency (0,05%, 61 alleles in ExAC), which has been observed in our cohort, as well. No additional confirmative reports - except for the original paper by Kolb et al[2] - have been published, so far.

However, additional family members need to be tested for the presence of the variant.

Conclusion: In our three families the variant p.Arg7Ser is very likely not causal for very different diseases and it is probably a rare benign variant.

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Table 1

	Family 1	Family 2	Family 3
Variant	p.Arg7Ser	p.Arg7Ser	p.Arg7Ser
Phenotype	dHMN - distal hereditary motor neuropathy	HSN - hereditary sensory neuropathy	HSP - hereditary spastic paraplegia
Onset and course of the disease	Onset in teens; Axonal neuropathy	Onset at the age of 25 years, foot ulceration	Onset of the disease from the age of 10, rapid worsening
Deep tendon reflexes	L2-S2 no response (0)	L2-S2 no response (0)	L2–4 brisk response (3+), L5-S2 clonus (4+)
Segregation	Not available	Unaffected son not a carrier	Affected daughter also a carrier

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P10.27C

New allelic variant of autosomal recessive hereditary motor-sensory neuropathy type 2S resulted from mutation in *IGHMBP2* gene

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Introduction: Hereditary motor and sensory neuropathy (HMSN, Charcot-Marie-Tooth disease) is a group of genetically heterogeneous diseases caused by mutations in more than 80 genes, including the gene IGHMBP2 responsible for the development of HMSN type 2S (OMIM 616155). Until 2014, mutations in IGHMBP2 were associated exclusively with distal spinal amyotrophy with neonatal respiratory failure (SMARD1, OMIM 604320). Materials and methods**:** exome sequencing on Illumina NextSeq 500 using TruSightOne V1.1 DNA enrichment. **Results:** Exome sequencing revealed two mutations in IGHMBP2 gene in compound heterozygous state: a missense mutation c.1616S> T (p.Ser539Leu) in exon 11, described earlier, and deletion / frameshift mutation c.2601_2602delGA in exon 13, discovered for the first time. These changes were detected in 7-year-old boy who was suffering, starting in infancy, from pronounced muscular hypotonia combined with muscle atrophy in distal extremities, deformity of the hands and feet, pronounced fixed kypho-scoliosis, areflexia and sensory disturbances of polyneurotic type without signs of respiratory disorders and iris dysfunction. The level of CK activity in the serum was 155 U/L. Electroneuromyography revealed signs of distal axonal neuropathy. Discussion: the case, similarly to most patients reported in the literature with HMSN type 2S, manifested in the first year of life with hypotonia in distal extremities and delayed motor development. Features like electroneuromyographic data and increased CK, at some point, can lead diagnostic search in the direction of primary muscle disease with distal distribution of muscle symptoms. Features of HMSN 2S clinical manifestation smake its early diagnosis complex and require differential diagnosis with SMARD1.

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P10.28D

Molecular diagnosis of inherited peripheral neuropathies: gene panel vs. exome sequencing

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Introduction: Inherited peripheral neuropathies (IPNs) encompass a group of disorders highly heterogeneous, clinically and genetically. Charcot-Marie-Tooth (CMT) disease is closely related to distal hereditary motor neuropathy (dHMN) or distal spinal muscular atrophy (DSMA), and some patients show additional signs associated with amyotrophic lateral sclerosis (ALS). Targeted gene panel and exome sequencing are considered to be powerful and cost-effective tools for diagnosis of these disorders. Materials and Methods: We have investigated a clinical series of 178 patients diagnosed of motor or sensory-motor peripheral neuropathy by exome sequencing or using different updated versions of a gene panel: Neuro-104, Neuro-111, and Neuro-119. Each version comprises 104, 111 or 119 IPN genes, respectively, and it shows a high coverage performance. Both genetic approaches are based on SureSelect capture technologies (Agilent Technologies).

Results: Exome sequencing has allowed us to identify causative mutations in 58% of cases. We have identified novel genes and novel mutations in known genes, broadening the phenotypical spectrum associated with IPNs. Gene panel testing has been mostly performed in sporadic cases, and it has allowed us to identify either disease-causing or candidate mutations in 40% of cases. In sum, both strategies have helped us to achieve a more accurate clinical and genetic reclassification of these disorders, an impossible challenge using conventional sequencing methods.

Conclusions: Our study expands the clinical phenotype previously associated to known IPN causing-gene, and emphasizes that gene panels should be considered as a first diagnosis method for unclarified IPN patients.

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P10.30B

Four-hit three event mechanism; investigation of an LZTR1 variant and the possible implications for a woman with schwannomatosis

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A woman presented with chronic pain in her lower back and right leg at 62 years. Magnetic resonance imaging showed four spinal lesions, of which two were excised and confirmed as schwannomas, giving a diagnosis of schwannomatosis but without any known family history. Mutation analysis of blood revealed a heterozygous missense variant c.1394C>T p.(Ala465Val) in exon 13 of the leucine-zipper-like transcription regulator 1 gene (LZTR1) and no variant in the SMARCB1 gene.

Schwannomatosis is usually sporadic, but 15%-25% are familial, inherited in an autosomal dominant manner. A model of schwannomatosis related tumourigenesis describes a four-hit three event mechanism. We speculate that in this case the LZTR1 variant is the predisposing germline mutation, as it lies within a highly conserved amino acid residue and is considered likely to disrupt the N-terminal BTB domain of the encoded protein. Investigation of more than one schwannoma was important to clarify the disease mechanism and provide evidence of pathogenicity of the LZTR1 variant if loss of heterozygosity was demonstrated in multiple specimens. Further analysis of tumour DNA is underway, testing for somatic NF2 mutations in cis and somatic loss of the other 22q allele in fresh frozen tissue from the two excised schwannomas.

Challenges of counselling the family are described in the context of LZTR1, a gene only recently implicated in schwannomatosis with little data available on penetrance and a variant currently classified as of uncertain significance. The process of investigation and the possible consequences are discussed as an illustration of a potential protocol.

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P10.31C

A recurrent mutation in the MUSK gene associated with a more severe phenotype of congenital myastenic syndrome?

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In 2004 mutations in the MUSK gene were reported as a novel cause of a congenital myastenic syndrome. The clinical course mostly comprised limb girdle (most frequently axial and proximal) and facial weakness, stridor or vocal cord palsy, respiratory problems and bulbar symptoms. In 2015, we reported a severe case from Germany with prenatal onset and postnatal «dropped head» sign due to compound heterozygosity for two novel MUSK mutations. We now report on two Turkish siblings with similar severe clinical course sharing one mutation with the case from Germany. Both siblings presented amongst others with congenital diaphragmatic paralysis and tracheomalacia with consecutive long term ventilation, microcephaly, swallowing difficulties and proximal and facial accentuated muscular weakness. The boy additionally showed a rigid spine, limited abduction of both eyes and had been treated for a left-sided club foot, right-sided cryptorchism, chronic pericardial effusion and a central nephrocalcinosis.

Exome sequencing revealed the mutations c.496C>T and c.1779-13A-G in the MUSK gene in both affected children. The mutations were shown to be compound heterozygous, as the parents each carried only one mutation.

As the c.496C>T mutation was also present in the severe case from Germany (Giarrana et al. 2015) with prenatal onset (e.g. polyhydramnion and contractures at birth) we hereby suggest it as a recurrent mutation in the MUSK gene associated with a more severe phenotyp. Additionally, we report the c.1779-13A-G as a novel mutation in the MUSK gene.

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P10.32D

Splicing mutations causing unusual early-onset dominant *MYH7*-related myopathy

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Introduction. Dominant mutations in the *MYH7* gene cause allelic series of diseases including various cardiomyopathies and myopathies usually manifesting in adulthood. **Clinical case**. We had under our observation the boy 2 y.o. manifested with predominant axial muscles weaknesses, "dropped head syndrome", and mild dilatation of the heart chambers. Parents and older brother (10y.o.) are healthy. **Methods**. Clinical investigation was performed by ECG, EchoCG, neurological examination, and muscular biopsy. The DNA-diagnostics had included whole exome sequencing with following Sanger resequencing, bioinformatic analysis, and cascade familial screening. Functional analysis of the variants of interest had included RT-PCR and expression analysis in HEK293N cell line transfected with 4 plasmids containing fragment of the MYH7 gene from exon 37 to exon 39 (*wt*, c.5655+5G>C, c.5655G>A, and c.5655 +1G>A). **Results**. We revealed new *de novo* mutation c.5655+5G>C in the *MYH7* gene in the 2 y.o proband. In-frame skipping of the exon 38 in the proband's mRNA was confirmed. This variant and two previously published mutations (c.5655G>A and c.5655+1G>A) also leading to the 38 exon skipping were studied using expression system. Surprisingly we had found that *wt* plasmid expressed two RNA isoforms: full-length and shortened (skipping of the 38 exon). All mutant plasmids had expressed only shortened isoform. Quantitative difference in expression was shown for mutant plasmids. **Conclusion**. We consider the variant c.5655+5G>C in the *MYH7* gene as a pathogenic mutation causing early-onset myopathy and dilated cardiomyopathy. All patients with those mutations seem to have similar phenotype. **Acknowledgements**.This work was supported by Russian Science Foundation, grant №16-15-10421.

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P10.33A

Next Generation Sequencing (NGS): a useful approach for the genetic diagnosis of dystrophies and myopathies

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Myopathies and muscular dystrophies constitute a a complex and heterogeneous group of more than 50 diseases characterized by muscle weakness. Diagnosis is mainly based on medical examination, biochemical and neurophysiological assessment and muscle biopsy. However, it can be challenging due to the presence of overlapping features among diseases. In this context, genetic testing through NGS could be a key approach to achieve an accurate diagnosis of muscle disorders.

We applied a NGS targeted resequencing 74 gene-panel to a cohort of 87 patients with clinical suspicion of Limb-girdle dystrophy (26), Duchenne/Becker dystrophy (21), RYR1-related disorders (10), Ullrich/Bethlem disease (8), Congenital myopathies and dystrophies (16), Distal myophaties (4) and Emery Dreifuss muscular dystrophy (2). Samples were analyzed using SureSelect kit (Agilent), HiSeq (Illumina) platform and an in-house bioinformatic pipeline. Variant filtering was carried out according to variant effect, familial pattern of inheritance, allele frequency and information available in databases.

Genetic diagnosis was achieved in 24 out of 87 patients (27.6%). Nonsense (31%), frameshift (23%), splicing (19%) or missense variants (27%) were identified in these patients. In 27 out 87 of the patients (31%) no candidate variants were found. Eventually, 4 individuals were heterozygous carriers of a pathogenic/probably pathogenic variant in genes associated with autosomal recessive disorders. In 36 patients only VUS were detected (36.8%).

According to the high diagnosis rate, NGS constitute an effective strategy to study myopathies and muscular dystrophies.

Functional and cosegregation analysis of VUS or extension of NGS analysis in negative patients would be helpful to increase diagnosis rate.

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P10.34B

A novel mutation in MYBPC1 is associated with myopathy, tremor, and spinal rigidity

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Introduction: *MYBPC1* encodes the slow skeletal Myosin Binding Protein-C protein (sMyBP-C), which is expressed in both slow and fast twitch skeletal muscles. sMyBP-C is a modular protein that plays structural and regulatory roles via its dynamic interaction with both actin and myosin filaments.

Materials and Methods: Exome sequencing of four family member samples was performed. The pathogenicity of variants was evaluated using several *in silico* prognostic tools. The NH_2-terminal region of sMyBP-C was amplified and the mutation was introduced *via* site-directed mutagenesis. *In vitro* binding assays were performed.

Results: We describe a three-generation family with a skeletal myopathy, accompanied by tremor and spinal rigidity. Exome sequencing analysis revealed the presence of a novel missense mutation in the *MYBPC1* gene, c.742G>A p.E248K. The E248K mutation is located in a highly conserved linker region in the NH_2-terminus of the molecule. Using *in vitro* binding assays, we demonstrate that the presence of the E248K mutation results in markedly increased binding of the NH_2-terminus of sMyBP-C to myosin (~3.5-fold) compared to wild-type protein.

Conclusions: Identification of the E248K mutation expands the range of myopathies caused by alterations in the *MYBPC1* gene. Based on the familial segregation, absence from the general population, in-silico assessment, and altered binding properties of the E248K mutation, we can classify it as pathogenic by ACMG guidelines.

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P10.35C

Phenotypic variability of myotonia congenita in Lithuanian three generation family with heterozygous mutation in *CLCN1* gene

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Introduction: Myotonia congenita (MC) is an inherited muscle disease due to mutations in the *CLCN1* and associated with delayed skeletal muscle relaxation after contraction, resulting in muscle stiffness. The phenotypic manifestations can have a broad spectrum even among patients carrying the same *CLCN1* mutation. The two forms of MC have different patterns of inheritance: Thomsen disease is inherited in an autosomal dominant pattern and Becker disease is inherited in an autosomal recessive.

Materials and Methods: Five family members in a three generation family underwent a detailed clinical analysis, electromyography (EMG) and genetic analysis (Sanger sequencing of the *CLCN1* gene was performed).

Results: The proband is 12 years old boy complaining of muscle stiffness and pain at lower limbs, difficulties in initiating walking and writing. EMG revealed burst of myotonia. Mother and sister of proband were asymptomatic. Maternal grandmother complained of hand tremors, difficulties in starting speaking and walking. EMG of mother and maternal grandmother were normal. Heterozygous missense mutation NM_000083.2:c.899G>A was identified in exon 8 of the *CLCN1* gene for proband, one of his sisters, mother and maternal grandmother. The *in silico* analysis of the identified variant shows conflicting results: SIFT - 0.02, PolyPhen - 0.853, Mutation Taster - disease causing, Provean - damaging.

Conclusions: The majority of *CLCN1* variants can be associated with reduced penetrance. Family members heterozygous for the same variant may have variable phenotypes. The genotype phenotype correlation analysis in

presented family proves the difficulties in ascertaining the definite diagnosis even based on molecular genetic findings.

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P10.36D

Effect of the expansion of CTG repeats of myotonic dystrophy tipe 1 (DM1) in inducible cellular models of glia and neuron

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Introduction: DM1 is the most common form of inherited muscular dystrophy in adults, with a multisystemic phenotype affecting muscle, central nervous system (CNS) and other tissues. Patients usually present neuropsychological symptoms such as excessive daytime sleepiness, attention deficits, intellectual disability and reduced initiative and apathy. To investigate the role of glia and neuron cells in the CNS alterations we developed two cellular models of DM1.

Materials and Methods: DM1 models were created on MIO-M1 (glia) and SH-SY5Y (neuron) cell lines by using the Tet-On 3G system. A stable cell line with high induction levels of trans-activator protein was first created. Second stable cell lines were made with responsive plasmids constructions, carrying a fragment of the 3′UTR of *DMPK* human gene with 0 and 960 CTG repeats. Models were validated with TP-PCR, RT-PCR and FISH-IF, and alternative splicing and gene expression changes were evaluated by microarrays.

Results: TP-PCR and capillary electrophoresis identified the transgene, expression of the exogenous transcript and modifications in the alternative splicing of the *MBNL2* gene were determined by RT-PCR, FISH-IF experiments revealed nuclear transcript aggregation co-localized with MBNL proteins. Finally, expression microarrays showed changes at alternative splicing and gene expression levels in both cellular models.

Conclusions: These models allow transgene expression in an inducible manner under identical genetic background with specific molecular and cellular markers of DM1. With these models we will be able to study the specific contribution of glial and neuronal cells in the CNS manifestations of the disease.

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P10.37A

The role of chloride voltage-gated channel 1 (CLCN1) screening in molecular diagnostics of patients with suspected/confirmed myotonic dystrophy

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Introduction: Myotonic dystrophies (DM1, CTG expansion in the *DMPK* gene; DM2, CCTG expansion in the *CNBP* gene) are multisystem disorders with prominent neuromuscular symptomatology. Their proper differential diagnostics is, however, complicated because of clinical heterogeneity and symptomatology overlapping other neuromuscular disorders. One of those is *myotonia congenita*, a nondystrophic myotonia caused by mutations in *CLCN1* (chloride voltage-gated channel 1) gene. The aim of our study was to compare the frequency of pathogenic/likely pathogenic *CLCN1* variants in different groups of patients initially suspected to have DM1/DM2.

Materials and methods: We performed complex mutation screening of *CLCN1* exons – MLPA and Sanger or massively parallel sequencing – in four patient groups: 1) 40 patients referred for DM testing but without identified DM1/DM2 expansion; 2) 52 patients with identified DM1 expansion; 3) 55 patients with identified DM2 expansion; 4) 30 individuals representing the general Slovak population.

Results: Pathogenic/likely pathogenic variants were identified in homozygous state in ~8% of patients without DM1/DM2 expansion, in heterozygous state in > 7% of DM2 patients, while there were yet not identified among the DM1 patients and in the group representing the general population.

Conclusions: According to our results *myotonia congenita* can be considered for an alternative diagnosis in a relatively large portion of patients originally suspected to

have DM but without identified DM1/DM2 expansion. In addition *CLCN1* variants seem to be common modifiers of DM2 symptomatology that is, however, not typical for DM1. Consequences of such findings for genetic counselling will be presented in our poster. Financial support: VEGA_2/0115/15.

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P10.38B

Founder Haplotype Associated with DM1 in Yemenite Jews in Israel

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Myotonic dystrophy type 1 (DM1; OMIM160900) is the most common form of inherited adult muscular dystrophy. It is caused by unstable expansion of the CTG repeat in the DMPK gene. The incidence of myotonic dystrophy among Jews from Yemen is 47.3:100,000 as compared to 15.7:100,000 in the general population. Between the years 2011–2015, 29 unrelated families with myotonic dystrophy were referred to the genetics clinic of the Rabin Medical Center. Twelve of the families were of Yemenite Jewish origin. The total percentage of Yemenite Jews in the Israeli population is roughly 2.5%, whereas they represent about 25% of myotonic dystrophy patients referred to our clinic. We performed haplotype analysis in these families to look for evidence of a founder mutation. Analysis of the patients' haplotypes around the DMPK gene revealed that eight of the twelve probands shared the same haplotype based on nine markers . The shared haplotype is located in an 864Kb region, 100Kb 5' of the DMPK gene and 700Kb 3' of the gene. Phasing was performed according to the family members' haplotypes. The aging of the MD mutation in the Yemenite population shows it occurred around 160 years ago.

Our findings suggest that a high prevalence of myotonic dystrophy among Yemenite Jews is consistent with a founder mutation in this population. We recommend a high degree of suspicion for this disease in Yemenite Jews with neuromuscular symptoms. This will enable earlier diagnosis, better follow-up and on-time preconception counseling as well as prenatal or preimplantation genetic testing.

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P10.39C

Swallowing evaluation in Mexican presymptomatic patients with Myotonic Dystrophy Type 1 (DM1)

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DM1 is the most common type of muscular-dystrophy in adults and is characterized by progressive myopathy, myotonia, and multiorgan involvement, that is caused by an unstable CTG repeat expansion in the 3´UTR of *DMPK* gene. Oropharyngeal-dysphagia is highly prevalent in DM1 patients and leads to high mortality rate due to pneumonia and respiratory failure, however there are no reports of this sign in early stages of disease. Here we present a swallowing evaluation in presymptomatic individuals with confirmed diagnosis of DM1.

Eleven asymptomatic subjects between 18–74 years old (mean = 54, SD = 15.62) with confirmed diagnosis of DM1 by TP-PCR and SP-PCR, were subjected to detailed clinical history. Neuromuscular involvement was scored using Muscular Impairment Rating Scale (MIRS). Swallowing assessment was performed by Fiberoptic Endoscopic Evaluation of Swallowing (FEES) protocol using different bolus consistency.

All patients ranging from 50 to 295 CTG repeats perceived themselves as asymptomatic individuals, this condition do not affect their daily activities and quality of life. MIRS reveals no muscular impairment (MIRS 1). Despite absence of distal muscles comprise, FEES showed six patient with decreased sensitivity (54.54%), two with mild impairment to liquids (18.18%), three mild impairment to liquids and solid (27.27%), three mild impairment to all consistencies (27.27%) and two with severe impairment with alteration of security (18.18%). Significant differences

were found between DM1-patients and a healthy control group.

For our knowledge, this is the first report worldwide in presymptomatic DM1-subjects. It is important to detect early clinical manifestations in order to prevent and treat complications.

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P10.40D

Development of a streamlined molecular assay that determines both allele and expanded repeat size in *DMPK* for myotonic dystrophy 1

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Background: Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease characterized by > 50 CTG repeats in the 3′ UTR of *DMPK*. DM1 testing currently requires a combination of PCR and Southern blot analysis because PCR cannot reliably amplify >100 repeats. Here, we describe results using a novel PCR technology that permits assessment of normal and expanded alleles in a single tube.

Materials and Methods: 20 gDNA samples isolated from presumed healthy whole blood donors (Asuragen) or DM1-positive cell-line samples with up to 2000 CTG repeats (Coriell) were acquired. Sample gDNA was amplified using prototype PCR reagents (Asuragen). FAM-labeled amplicons were resolved by capillary electrophoresis on a 3500 xL (Thermo Fisher).

Results: CTG-specific and gene-specific priming were optimized with other PCR components in a single reaction to produce complementary data signatures from repeat-primed and full-length amplicons. These signatures confirmed the genotypes of normal and expanded alleles without the need for separate PCR and Southern blot (SB), resulting in a faster and simpler assay and analysis workflow. Prototype PCR reagents could resolve >200 CTG repeats from 40 ng gDNA and generated repeatable results.

Conclusions: Current methods fail to amplify moderate-to-large repeat expansions in DM1, and allele drop-outs may be indistinguishable from frequently-encountered homozygous samples. We report the first single-tube, long-read PCR technology that can resolve zygosity, identify expansion to >200 repeats, and flag larger expansions. This approach significantly reduces the need for DM1 SB analysis, and may also be multiplexed with PCR for DM2-associated tetranucleotide repeats.

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P10.41A

A novel copy number variation detection array for the diagnostics of neuromuscular disorders

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Introduction: Nemaline myopathy (NM) is caused by mutations in at least ten different genes, but most commonly by recessive mutations in the nebulin gene (*NEB*), a 183 exon gene essential for correct sarcomere structure and function. *NEB* harbors a 32kb triplicate region (TRI), in which eight exons are usually repeated three times (ex 82–89, 90–97, 98–105).

Materials and Methods: We are currently validating a new custom-made 4×180k array-CGH design. It includes the same tiled high-density coverage of the ten known NM genes as our 8×60k NM-CGH-array, but also covers an 170 additional genes, such as titin (*TTN*), that have been related to neuromuscular diseases.

Results: As to date, we have analyzed samples from 259 NM families, and identified 15 different disease-causing aberrations in *NEB* in 31 of these families. The majority of these pathogenic variations were detected only in one to three families each. Copy number variants (CNVs) affecting the TRI region were detected in 16% of the NM families, and in 5 % of the families the CNV was interpreted to be pathogenic. Pathogenic CNVs were found in 12 % of all families analyzed.

Conclusions: The array allows the reliable detection of CNVs also in regions of segmental duplication, such as the TRI regions of *NEB* and *TTN*. Our novel 4×180k array-CGH design allows CNV detection of a broader spectrum of neuromuscular disorders. It can be used for molecular

genetic diagnostics, and is available for mutation analysis in our laboratory.

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P10.42B

Four new pathogenic mutations identified in *SH3TC2* gene responsible for Charcot-Marie-Tooth disease associated with deafness and/or scoliosis

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Introduction: Charcot-Marie-Tooth disease is one of the most frequent inherited peripheral neuropathies (1/2500). So far, mutations in more than 80 genes have been identified causing either the demyelinating form (type 1) or the axonal form (type 2). Duplication of *PMP22* gene is the most frequent cause of autosomal dominant demyelinating form. Autosomal recessive demyelinating form is often due to *SH3TC2* gene mutations. Patients suffer then from early severe neuropathy starting in the first decade. Scoliosis and deafness are often observed.

Material and method: 200 patients suffering from peripheral neuropathy were screened by multiplex-ligation-dependant-probe-amplification, followed by targeted next-generation-sequencing using a 92-gene custom panel designed for the diagnosis of Charcot-Marie-Tooth and associated neuropathies. Mutations of interest were verified by Sanger sequencing.

Results: Diagnosis was positive for 114 patients. As expected, the most frequent mutation was the *PMP22* duplication detected in 30 patients. Deletion of *PMP22* was observed in 18 patients and pathogenic point mutations were detected in 66 patients. *SH3TC2* gene appeared to be the most frequently mutated with nine patients diagnosed. Associated with known mutations, four new mutations have been identified: two nonsense mutations and two missense mutations. All these patients presented deafness and/or scoliosis.

Conclusion: *SH3TC2* appears to be an important gene involved in Charcot-Marie-Tooth disease, often associated with deafness and /or scoliosis. It is important to pay attention to these associated symptoms in Charcot-Marie-Tooth patients in order to guide their diagnosis and to improve their medical care.

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P10.43C

Functional an biochemical characterization of induced Pluripotent Stem cells (iPSc) from patients with Neutral Lipid Storage Disease with Myopathy

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Neutral Lipid Storage Disease with Myopathy (NLSDM) is a very rare disorder characterized by a defect in the degradation of cytoplasmic triglycerides and accumulation as lipid droplets (LDs). This lipid dysmetabolism may determine progressive myopathy, cardiomyopathy, diabetes, hepatomegaly, hepatic steatosis, chronic pancreatitis and short stature. No specific therapy is currently available. All patients carry damaging mutations in the *PNPLA2* gene, which codifies for the adipose triglyceride lipase (ATGL), an enzyme that hydrolyses fatty acids from triacylglycerol. We have previously reprogrammed dermal fibroblasts from two patients and healthy subjects into induced pluripotent stem cells (iPSCs). The iPSCs were tested for pluripotency evaluating the expression of TRA-1-81, SSEA4 and OCT4 markers, and their differentiation ability into three-germ

layers by immunofluorescence analysis (β-III tubulin, ectoderm; SMA, mesoderm; FOXA2, endoderm). Now we demonstrate that NLSDM-iPSCs present an abnormal accumulation of triglycerides into LDs, resembling the hallmark of NLSDM. Indeed, immunofluorescence analysis shows that NLSDM-iPSCs has 20 times more LDs and almost 5 larger LDs then control iPSCs. Moreover, the TG content of NLSDM-iPSCs is significant higher than that of control iPSCs. Oleic acid pulse chase experiments further confirm that lipase activity is impaired in NLSDM-iPSCs compared to control cells. These results demonstrate that iPSCs recapitulate NLSDM specific-lipid metabolism defect and can be considered a valid biochemical disease model. Finally, NLSDM-iPSCs could be differentiated into cardiac myocytes or myoblasts to screen potential therapeutic compounds

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P10.44D

Next generation sequencing technologies in the genetic diagnosis of Congenital Myasthenic Syndrome

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Introduction: Congenital myasthenic syndrome (CMS) is a clinical and genetic heterogeneous condition caused by defect in the neuromuscular junction and characterised by fatigable muscle weakness, bulbar and ocular symptoms. Over 20 disease causative genes have been reported, however 30% of CMS patients remain genetically undiagnosed, suggesting that many novel CMS are yet to be identified.

Materials and Methods: After a hotspot pre-screening step, we have applied next generation sequencing techniques, including target panel, whole exome (WES) and whole genome sequencing (WGS) to investigate a cohort of clinically diagnosed CMS patients (n = 54; 43 pedigrees).

Results: We have identified 17 disease causing variants in known CMS genes in 11 pedigrees. Twelve of these variants are novel. Thanks to the deep coverage of WGS and NGS panel data, we were able to detect a homozygous deletion in *COLQ*, as well as a large heterozygous intragenic deletion in *DOK7*. Surprisingly, we have also found variants in genes that had not been anticipated (*POLG, CPL1, CRLF1, SLC2A1* and *PTPN11*). In addition, two new CMS genes were identified, one of them in two independent families (*SCL25A1* and *MYO9A*, respectively). From the remaining unsolved cases, three strong candidate and modifier genes have emerged and functional investigation is ongoing.

Conclusions: NGS technologies have allowed to genetically diagnose 19 CMS pedigrees (45%) as well highlighted new candidate genes and pathways. This is of extreme value as CMS has effective treatment that depends on the causative defect, and therefore genetic diagnosis has a direct impact on patients' quality of life.

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P10.45A

Two different NGS approaches to address molecular diagnosis of Congenital Neuromuscular Diseases

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Introduction Congenital neuromuscular diseases are early onset muscle disorders encompassing great clinical and genetic heterogeneity so that reaching and accurate genetic diagnosis is still a challenge.

Objectives We aim to evaluate the diagnostic advantage of different NGS approaches in a cohort of congenital neuromuscular disorders and to validate an efficient and cost-effective diagnostic strategy to be incorporated to the National Health System.

Material and methods Sixty-two gDNA samples from patients with clinical suspicion of congenital neuromuscular diseases were analysed by NGS. A custom panel including 118 genes (Nextera Rapid Capture, Illumina) and TruSight One Panel (Clinical Exome, Illumina) were employed. Sequencing data were analysed by Variant Studio and DNA Nexus.

All candidate variants were confirmed by Sanger sequencing and pathogenicity was analysed by using Alamut Software (Interactive Biosoftwares) and LOVD database.

Results The genetic cause of the disease was elucidated in 11/25 samples (44%) by the Clinical Exome and in 28/48 samples (58%) by the custom panel. Eleven samples from the second group (custom panel) had previously been analysed by the Clinical Exome without positive results.

Mutations in *RYR1* were the most common cause of disease in our cohort.

Variants in genes related to Congenital Myasthenic syndromes were identified in 8 patients. This was crucial to choose the appropriate treatment, which depends on the particular molecular defect.

Conclusions The custom panel design has proved to be a more useful tool than the clinical exome to provide a molecular diagnosis of congenital neuromuscular diseases.

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P10.46B

Utility of the X-chromosome exome sequencing for clinical genetic diagnostics of Duchenne and Becker muscular dystrophies

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Introduction: Duchenne and Becker muscle dystrophies are allelic X-linked recessive disorders affecting 1/3500 and 1/18500 males respectively. Both dystrophinopathies are caused by mutations in the *DMD* gene and their clinical diagnostics relays primarily on MLPA/aCGH and Sanger sequencing. Sanger sequencing is laborious and expensive due to large size of *DMD*. Next generation sequencing technologies may provide a cost-effective option. The aim of this study was investigate the utility of a commercial amplicon based X-chromosome exome panel for sequencing the *DMD* gene in the clinical diagnostics. Patients and methods: 50 patients referred for diagnostic Duchenne/Becker testing with normal MLPA results were selected. Target enrichment was performed using the ClearSeq kit (combination of amplicon and hybridization methods) and libraries were sequenced with Illumina MiSeq. Data alignment and variant calling were performed using in-house pipeline. Detected pathogenic variants were confirmed with Sanger sequencing. **Results:** Of the 50 analyzed samples a pathogenic mutation was detected in 19 samples. All detected pathogenic variants were truncating or splice site mutations. **Conclusions:** As our results indicate amplicon-based methods may offer advisable following study MLPA or aCGH being primary study detecting exonic copy number changes. With this approach it is possible to replace Sanger sequencing and hence reduce sequencing costs. However hybridization based methods allow detection of both SNVs and exact break points of exonic deletions/duplication. In the future in addition of detecting SNVs mutation specific treatments may require precise information of break points. Therefore hybridization based methods may provide a better option.

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P10.47C

A novel *GNAO1* mutation in a patient with severe movement disorder

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Introduction: Mutations in the *GNAO1* gene have been recently related to the distinct phenotypes comprising: 1/ early infantile epileptic encephalopaties, and 2/ developmental delay with progressive movement disorders and no

severe seizures. The latter phenotype reported in 14 cases to date, seems to predominantly result from two recurrent substitutions, c.626G>A and c.736G>A. The *GNAO1* gene codes for a specific subclass of guanine-binding proteins, Gαo, that acts as important signaling molecules in the central and peripheral nervous system.

Materials and Methods: Whole-exome sequencing has been performed in a 7-year old boy born to non-consanguineous parents of Polish origin. From the third month of age he presented with progressive axial and limb hypotonia, dystonic spasms, involuntary movement of facial muscles and digits, and absent speech. He was able to sit with support at the age of 4 years. He never stood and walked independently. Brain MRI, EEG, and EMG examinations showed no abnormalities. Muscle biopsy revealed selected atrophy of type II fibers.

Results: The novel c.709G>A p.(Glu237Lys) heterozygous *GNAO1* variant was identified in a proband. The analysis of his parents indicated the substitution occurred de novo. In silico analyses with 10 prediction algorithms indicated its pathogenic character.

Conclusions: The novel pathogenic variant has broadened the mutational spectrum related to *GNAO1* dysfunction beyond two recurrent mutations.

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P10.48D

Functional characterization of *PARK2* mutations: implications for Parkinson disease

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Mutations in *PARK2* are the most common cause of autosomal recessive Parkinson disease (PD). *PARK2* encodes parkin, an E3 ubiquitin ligase that is able to mediate different types of ubiquitination in several substrates. Numerous variants located throughout parkin domains have been shown to be pathogenic for PD. Some of the variants identified were shown to impair parkin E3 ubiquitin ligase activity.

Our group has previously conducted a mutation screening of *PARK2*, in order to evaluate 244 unrelated Portuguese patients with symptoms of PD. This lead to the identification of 18 disease-causing variants, from which c.155delA (p.N52MfsX29) was the most common. Moreover we identified two novel causative variants, including one indel (c.1072_1073delCTinsA; p.L358RfsX77).

Thereby we aim to functionally characterize parkin mutants. For that we generated parkin mutant clones in fusion with EGFP-tag that were transfected in HEK293T cultured cells. The subcellular localization was accessed by immunofluorescence and subcellular fractioning. Parkin E3 activity was evaluated by measuring parkin self-ubiquitination and ubiquitination of DJ1 (a parkin substrate) using co-immunoprecipitation assays in cells co-transfected with parkin clones and HA-ubiquitin.

Our results showed that parkin mutants have different subcellular distribution and are aggregation-prone, conversely to parkin-WT. Moreover, parkin mutants retain their ability to self-ubiquitinate, but seem to have altered activity against DJ1.

In conclusion, we showed that parkin mutants have different biochemical properties and are abnormally localized, but not all result in a complete loss of enzymatic activity. Further studies are needed to fully understand the consequences of PD-associated mutations on parkin cellular functions.

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P10.49A

Mutations in *POLG* and *MPV17* cause a broad spectrum of mitochondrial disorders

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Introduction: Mitochondrial diseases are genetically and phenotypically heterogeneous caused by mutations in both mitochondrial DNA (mtDNA) and nuclear genes. Mutations in nuclear gene *POLG* encoding the mtDNA polymerase gamma required to replicate the mitochondrial genome are reported to affect mtDNA stability such as accumulation of point mutations, multiple deletions and depletion of

mtDNA, and cause progressive mitochondrial disorder. Materials and Methods: We explored the utility of targeted next-generation sequencing with a total of 75 nuclear genes panel and all exons and intron-exon boundaries of the *POLG1* gene was amplified and sequenced. **Results:** We have identified a homozygous p.R627W mutation in three patients from two unrelated families as a cause of an uncommon phenotype with status epilepticus and stroke as dominant phenotype. In addition patient with compound heterozygous p.R627W and p.A781T mutations present with multisystemic clinical presentations. The p.R627W homozygous mutation not previously documented and we report here, for the first time, the p.A781T mutation in *POLG1* as a cause for recessive mitochondrial disease. The parents in all family were unaffected carriers with p.R627W or p.A781T mutations. These mutations were absent in 600 control chromosomes. We also identified homozygous p.R41W mutation in the *MPV17* gene in four patients. The majority of *POLG* and *MPV17* mutations show an autosomal recessive inheritance. Intriguingly, the p.R627W mutation causes severe clinical phenotypes and early death in homozygous condition in contrast to compound heterozygous condition. Conclusion: We conclude that screening for *POLG* mutation is very important for diagnosis, proper medical management and appropriate genetic counseling.

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P10.50B

A missense variant in CACNA1A gene causing familial motor and sensory polyneuropathy with pyramidal signs

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Background: Voltage-dependent Ca(2+) channels mediate the entry of Ca(2+) ions into excitable cells and are also involved in a variety of Ca(2+)-dependent processes, including muscle contraction, hormone or neurotransmitter release and gene expression. Calcium ion channel Alpha -1a subunit gene (CACNA1A) codes for the Alpha1A subunit of P/Q type calcium channel Cav2.1 protein. This protein is expressed mainly in the brain, particularly in the cerebellum. Most CNS synapses rely on Cav2.1 and Cav2.2 calcium channels for fast synaptic transmission. Three allelic autosomal dominant disorders: Spinocerebellar Ataxia type 6, Episodic Ataxia type 2 and Familial Hemiplegic Migraine type 1 have been described with CACNA1A gene mutations. Methods: Whole Exome Sequencing (WES) **Results:** We report a pedigree carrying a rare (0.057% in EXAC database) CACNA1A gene pathogenic variant (c.5897G>A, p.Arg1966Gln). The family presented variable combination of pyramidal signs, peripheral motor and sensory polyneuropathy and cerebellar atrophy. We speculate that voltage-gated calcium channels damage in the CNS and peripheral nerves is the etiology for the familial phenotype. Conclusion: To the best of our knowledge, variants in CACNA1A gene have not been previously associated with this phenotype. This report widens the spectrum of CACNA1A related disorders. We suggest CACNA1A gene mutations should be included in the differential diagnosis of patients presenting the combination of pyramidal signs and peripheral polyneuropathy. Future population researches can determine the variability and penetrance of CACNA1A gene variants regarding "new" neurological conditions.

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P10.51C

POPDC1 gene mutations screening in laminopathies: possible role as a modifier

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Introduction: POPDC1 is the best-studied member of the Popeye domain-containing gene family. The members of this family encode transmembrane proteins, which are abundantly expressed in cardiac and skeletal muscle. The evolutionary conserved Popeye domain functions as a high-affinity 3′-5′-cyclic adenosine monophosphate (cAMP) binding domain. Recently POPDC1 has been identified as causative gene in a family affected by Limb-girdle

Muscular Dystrophy and atrioventricular (AV) block (OMIM LGMD2X). Methods: In order to evaluate if POPDC1 may contribute to phenotype variability in other LGMDs with or without cardiac involvement we screened by Sanger sequencing 240 patients with different phenotypes either orphan of known gene mutations or with known gene mutations.**Results:** We identified 9 different heterozygous POPDC1 mutations in 11 patients, 6 of them do also carry LMNA mutations. Phenotypes vary from classical LGMD phenotype to pure cardiomyopathy with rhythm disturbances. Conclusion: We have demonstrated that POPDC1 mutations do cause rare recessive phenotypes, and we suggest that POPDC1 gene may also act as disease modifiers. Considering that laminopathies are characterized by high clinical heterogeneity and by intra- and interfamilial phenotypic variability, we speculate that POPDC1 may participate to LMNA phenotype modulation.

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P10.52D

A cryptic amyloidogenic element in the 3'UTR of *REEP1* has pathological relevance

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Certain frameshift variants in *NEFH* result in translation of part of the gene's 3′UTR. They have recently been shown to cause autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2) by making the NEFH protein aggregation-prone. The responsible sequences were termed 'cryptic amyloidogenic elements' (CAEs). Whether CAEs in 3′ UTRs of other genes are of pathological relevance remained unclear. Applying a gene panel for neuromuscular diseases, we identified a *REEP1* stop-loss variant in a dominant CMT2 family. This variant had been identified upon exome sequencing of a CMT2 patient in a previous study, but the authors had favored a heterozygous *SETX* non-sense variant as causative. However, truncating *SETX* variants are pathogenic only when present homozygously. It therefore seems more likely that, as in our case, the CMT2 phenotype results from the *REEP1* stop-loss variant. Loss of the *REEP1* stop codon results in translation of the proximal 3′ UTR. *In silico* analyses suggested that the 55 extra residues are highly amyloidogenic. Expression of corresponding constructs revealed cytoplasmic aggregation of the mutant but not the wild-type REEP1 protein. Likewise, fusion of the 55 residues to the C-terminus of several reporters triggered aggregation, and co-overexpression experiments confirmed that the 3'UTR-encoded peptide is both sufficient and necessary for self-aggregation. Our data confirm the previous hypothesis of toxic gain-of-function mutations in *REEP1* to primarily affect lower motoneurons while loss-of-function mutations in this gene result in upper motoneuron axonopathy (causing hereditary spastic paraplegia). More generally, our findings expand the pathologic relevance of CEAs for peripheral axon degeneration.

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P10.53A

Rigid spine myopathy with cardiovascular involvement

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Introduction: Rigid spine myopathy or musclar dystrophy associated with Selenoprotein N1(SEPN1) mutation with is a rare autosomal recessive disease on gene 1p36. It causes contracture of the spinal externsors with severe limitation in the movement of the thoracic cage and whole spine, scoliosis, and restrictive respiratory failure. Cardiovascular involvement is rarely mentioned, mainly cor pulmonare.

Method: A 12 year old girl was admitted in our hospital with hypercapnic coma (PaCO2 =160 mm Hg) due to chronic restrictive respiratory failure, narrow thorax, cachexia (weight = 12 kg), muscular atrophy, hyperlordosis.

As an infant she presented with axial and proximal muscle weakness, delayed motor acquisition, by normal mental development. The feeding difficulties started at 3 month of age and remained the main complain despite of normal gastroenterologic findings. Progressive limitation of the spine mobility resulted in hyperlordosis, scoliosis, thorax deformity, fatigability, respiratory failure.

Echocardiography revealed a severe compression of the left atrium due to the spine, spearing the mitral valve, as well as a moderate pulmonary hypertension PSAP = 52 mm Hg.

Results: Next-generation-sequencing detected 2 heterozygote mutations in exon 10 and 11 of SEPN1 gene- an "null-mutation" exon 10 deletion (c.1308delC) and a "missense" mutation exon 11(c.1397G>A).

After noninvasive BIPAP ventilation was started, a notable improvement in general status occurred.

Conclusions: Although not frequent, cardiac manifestations should be screened in rigid spine myopathy.

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P10.54B

Non coding RNA regulation analyses in the molecular pathways involved in inflammation, senescence, apoptosis and autophagy during the sarcopenic process functional and non-functional elderly population

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Sarcopenia is one of the most deleterious effects of aging. The involuntary loss of muscle mass, strength, and muscular function have a major impact on quality of life in the elderly population. The etiology of the sarcopenia is not clearly established, although, a multifactorial process that develops from the fourth decade of life is proposed. The muscular contractility is a *per se* and independent factor in the sarcopenia development not related with the muscle volume. In order to understand this phenomenon. Our group found that the genetic profiles in muscular tissue showed an increase in genes involved in apoptosis and inflammation in the group of fragile elderly. Surprisingly, gene expression of functional elderly was very similar to young sedentary, while athletes showed expression of genes related to autophagy. Immunohistochemical analysis showed a change in the distribution of actin and myosin in fragile elderly. Our data suggest that in the fragile elderly, the inflammatory and apoptotic process in muscle fiber have a direct impact on the proteins involved in contractility and muscular functionality, which is clinically reflects as lower volume, muscle strength and performance in this population. Here in we present the results of 768 non conding RNAs (miRNAs) regulation analysis over these molecular pathway using a QRTPCR array (RT2Profiler, Qiagen) in muscular tissue. Preliminary results showed a specific group of miRNAS who has differential expression patterns and plays a critical role in these molecular pathways depending the age, the physical activity and the sarcopenic state.

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P10.55C

Diagnosis rate of DNA fragment analysis for patients with SMA symptoms

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Spinal muscular atrophies (SMAs) are autosomal recessive neuromuscular disorders characterized by muscle weakness and atrophy resulting from progressive degeneration of the motor neurons. SMAs are caused by deletions or intragenic mutations of SMN1 and increased number of SMN2 copies usually modify the phenotype. The simplest and most definitive diagnostic tests for SMAs are molecular genetics tests for SMN genes.

We performed molecular genetic analyses of SMN1 and SMN2 genes on blood samples of 23 patients suspected to have SMA based on the clinical findings. We examined 7th and 8th exons of SMN1 and SMN2 genes by DNA fragment analyses.

Out of the 23 patients we examined, no deletion detected on two patients (8,7%). Six were homozygous for deletion of SMN1 (26,1%) and seven were heterozygous for deletion of one SMN1 allele (30,4%). For SMN2 gene, two patients were homozygous for deletion (8,7%) and five were heterozygous for deletion of one allele (21,7%). One patient was heterozygous for deletion of 7th exon of SMN1 allele and 8th exon SMN2 allele (4,3%).

Only 26,1% of the patients suspected to have SMAs were able to diagnose with molecular genetic tests we used. Tests for intrinsic mutations or SMN2 copy numbers may be required to diagnose some SMA patients in case they were unnoticed with DNA fragment analysis that we used; or detailed clinical examinations should be performed before requesting molecular analyses.

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P10.56D

SMN1 deletions among children with spinal muscular atrophy in Bosnia and Herzegovina

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Introduction: Hereditary spinal muscular atrophy (SMA) is an autosomal recessive disease with a prevalence of 1 in 6,000–10,000 live births. It is the result of homozygous mutation of the motor neuron 1 (SMN1) gene on Ch5q13, and characterized by muscle weakness and atrophy. The purpose of study was to determine the frequency of SMN1 deletions and severity of SMA among Bosnian children.

Material and Methods: Retrospectively study was performed among patients younger than 18 years from northeast Bosnia and Herzegovina. The data has been collected from clinical records and molecular genetics tests of patients with suspicion on SMA.

Results: Our data showed that among 1600 patients, our 19 patients (11 female, 8 male) had clinical records (1,19 %) on SMA. Seven of them had diagnosis SMA type I, four SMA type II, and eight SMA type III. Genetics tests confirmed that two patients had muscular dystrophy, and four had no mutations on SMN1 or DMD gene. Among 13 patients, nine patients had homozygous deletion of SMN1 (69,23 %) and one of SMN2 gene, eight had duplication of SMN2 gene, eight patients had both, deletion and duplication of SMN genes. One patient had deletion, and two patients had duplication of NAIP gene. Three patients with SMA type 1/2 are died before genetic confirmation.

Conclusion: We present the first paper of frequency of SMN1 deletions in SMA Bosnian children. Anticipating future gene therapy for SMA, this paper is a starting point for developing policies and strategies for SMA in Bosnia and Herzegovina.

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P10.57A

Percentage of *FL-SMN* and *Δ7-SMN* transcripts as biomarker of spinal muscular atrophy treatment efficacy

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Introduction: Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by mutations within the *SMN1* gene. All SMA patients have at least one copy of *SMN2* gene - a nearly identical copy of *SMN1* - that produces low amount of full-length *SMN* (*FL-SMN*) transcripts. All the rest transcripts lack exon 7 (*Δ7-SMN*) and encode non-functional protein. Correction of *SMN2* gene splicing is one of the principal SMA therapeutic goals. To access the efficiency of fast-developing SMA therapy approaches there is a need for reliable biomarker whose score differs between patients, carriers and healthy individuals. **Material and Methods:** Detection of *FL-SMN* and *Δ7-SMN* transcripts was carried out by means of RT-PCR. Blood cell RNA samples from 28 SMA patients, 33 SMA carriers and 15 control individuals were analyzed. SMA patient-derived fibroblasts were transfected with antisense oligonucleotides (ASO) aimed at correction of *SMN2* exon 7 splicing. The percentage of exon 7 inclusion was determined using ImageJ software measuring the intensity of each transcript band in electropherogram. All data were verified by means of QF-RT-PCR. **Results:** Significant differences in percentage of *FL-SMN* and *Δ7-SMN* transcripts were found among three examined groups. Mean percentages of *FL-SMN* transcripts in blood cells were following: 16% for SMA patients, 28% for SMA carriers and 56% for control individuals. Two-fold increase in exon 7 inclusion was detected in SMA fibroblasts treated with ASO. Conclusion: *FL-SMN* and *Δ7-SMN* transcripts percentage is potentially useful biomarker for evaluation of SMA therapy efficacy by correction of *SMN2* splicing

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P10.58B

Progressive SCAR14 with Unclear Speech, Developmental Delay, Tremor, and Behavioral Problems caused by a Homozygous Deletion of the SPTBN2 Pleckstrin Homology Domain

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Introduction: SCAR14 is autosomal recessive spinocerebellar ataxia caused by mutations in *SPTBN2*. To date, only two other *SPTBN2* mutations with recessive effect

causing SCAR14 that manifest with developmental ataxia and cognitive impairment, or cerebellar ataxia, mental retardation and pyramidal signs have been reported. We report on nine members of a consanguineous Pakistani kindred with primary presentation of intellectual disability, developmental delay, limb and gait ataxia, behavioural and speech problems, and tremor.

Materials and Methods: The disease locus was identified by linkage mapping using SNP genotype data of 10 family members. Whole exome sequencing for one affected subject was evaluated for rare/novel variants.

Results: We identified novel homozygous splicing variant c.6375-1G>C in *SPTBN2* that encodes beta-III spectrin, which forms tetramers with alpha-II spectrin, predicted to lead to the truncation of the encoded protein.

Conclusion: The mutation we identified would lead to the deletion of just the pleckstrin homology domain; thus, the earlier onset and more progressive nature of the disease in the presented family, as compared to earlier reports, were unexpected. No other mutation that could possibly explain the features that were unusual for SCAR14—arched palate, climacophobia, and behavioural problems—was identified. Our findings expand the recessive *SPTBN2* mutation phenotype. We also provide a review of *SPTBN2* mutation phenotypes.

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P10.60D

Wieacker-Wolff syndrome in a tenth family

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Wieacker-Wolff syndrome (WWS) is a rare X-linked neurodevelopmental disorder, first described in 1985 in a family segregating developmental delay and congenital contractures in boys. In 2013, Hirata identified causative *ZC4H2* mutations in five families. Our patient was detected prenatally by second trimester ultrasound screening: bilateral club feet and a questionable tethered cord in a male fetus. The pregnant woman had an unremarkable family history, herself undergoing surgery in infancy for a right clubfoot. CS delivery occurred at term with normal growth parameters, except length at -3SD. However, a severe hypotonia with limbs hypertonia and respiratory distress requiring assisted ventilation, bilateral clubfeet and distal arthrogryposis were observed. Brain MRI was remarkable for fronto-temporal cortical atrophy. He died at 21 weeks. The autopsy confirmed the clinical findings in addition to auricular septal defect. An exome targeted to 70 genes known to be associated with arthrogryposis or fetal akinesia was performed and identified a *ZC4H2* missense mutation (c.625A>G; p.Lys209Glu) inherited from his mother. This mutation is predicted to be pathogenic with a high score (PolyPhen-2 score of 0.94), was not annotated in the 1000Genomes or ExAC Browser databases and was confirmed by Sanger sequencing. A diagnosis of WWS was made and the females in childbearing age were counselled accordingly. This is the tenth family described with WWS mainly characterized by *arthrogryposis multiplex congenita* and developmental delay with variable expression in affected boy. Carrier female can be either asymptomatic or symptomatic with generally less severe manifestations. Our family illustrates nicely this "new" X-linked condition.

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P11 Multiple Malformation/anomalies syndromes

P11.001A

Somatic activating *PIK3CA* mutations cause CLAPO syndrome

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Introduction: CLAPO syndrome, described so far in 7 patients, is a combined vascular disorder of unknown cause, characterized by capillary malformation on the lower lip, lymphatic malformation predominant on the cervicofacial area, asymmetric face and partial/generalized overgrowth. Due to the distribution of the affected tissue as well as to its phenotypic overlap with the *PIK3CA*-related overgrowth spectrum (PROS), we studied 13 CLAPO patients under the hypothesis that it could also be caused by low somatic mosaic *PIK3CA* activating mutations.

Materials and Methods: A custom NGS panel and an in-house bioinformatic pipeline for low mosaics detection were used to test paired blood/affected tissue samples from 9 CLAPO patients. Candidate variants were validated according to their degree of mosaicism by Sanger Sequencing (>15%), pyrosequencing (>5%), and/or Droplet Digital PCR for mosaics as low as >0.1%. Gain of function of *PIK3CA* variants not previously described was verified by directed mutagenesis, transfection into in HEK293T cells, and western blot for Akt phosphorylation, its natural target.

Results: Five different low somatic mosaic mutations (5–16%) in *PIK3CA* were identified in affected tissues from 6/9 CLAPO patients. Three of the mutations were previously described in PROS. The other two showed gain of function compared to wildtype.

Conclusions: We describe *PIK3CA* mosaic activating mutations as the causing mechanism in CLAPO and suggest including this syndrome into the PROS spectrum. These findings will allow a better diagnosis of the syndrome, which will also benefit from any possible future treatment targeting the AKT-PI3K-mTOR pathway.

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P11.002B

Biallelic mutations in *TBCD*, encoding the tubulin folding cofactor D, perturb microtubule dynamics and cause early-onset neurodegenerative encephalopathy

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Microtubules are dynamic cytoskeletal elements coordinating a variety of neuronal processes, including cell division, migration, and signal transduction. Mutations in genes encoding tubulins and microtubule-associated proteins have been reported to cause neurodevelopmental and neurodegenerative disorders. Growing evidence suggests that altered microtubule dynamics may also underlie or contribute to neurodegeneration. We report that biallelic mutations in *TBCD*, encoding one of the five co-chaperones required for assembly and disassembly of the αβ-tubulin heterodimer, the structural unit of microtubules, cause a neurodegenerative disease characterized by early-onset cortical atrophy with microcephaly, developmental delay and intellectual disability, seizures, and spastic quadriplegia. Molecular dynamics simulations predicted substantial local structural perturbations associated the disease-causing mutations. Biochemical analyses documented variably reduced levels of TBCD, indicating relative instability or insolubility of mutant proteins, and defective β-tubulin binding in a subset of the tested mutants. Reduced/defective TBCD function resulted in decreased

soluble α/β-tubulin levels and accelerated microtubule polymerization in fibroblasts from affected subjects, demonstrating an overall shift towards a more rapidly growing and stable microtubule population. These cells also displayed an aberrant mitotic spindle with disorganized, tangle-shaped microtubules and markedly reduced aster formation, which however did not alter appreciably the rate of cell proliferation. Our findings establish that defective TBCD function underlies a recognizable neurodegenerative disorder and causes accelerated microtubule polymerization and enhanced microtubule stability, underscoring a tight link between aberrant microtubule dynamics and neurodegeneration. M. Niceta and E. Flex equally contributed to this work.

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P11.003C

Recurrent Cushing syndrome, severe growth retardation with dysmorphic features and skeletal abnormalities as a result of *de novo* microdeletion 17q24.2-q24.3 - a case report

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Microaberrations of chromosome 17 are frequently reported as a cause of birth defects, but microdeletions involving q24.2-q24.3 are sporadic. Up-to-date only a few cases have been published, mostly as an isolated Carney complex. In some of them dysmorphic features were described. We present an 8-year-old girl who was born from the first pregnancy with oligohydramnios after 42 weeks of gestation with IUGR and dysmorphic features of Pierre-Robin sequence. No inborn defect was diagnosed except from patent foramen ovale. During follow-up severe growth retardation was observed. On physical evaluation round face, small mouth, high palate, micro-rethrognathia, chest asymmetry, diastasis recti, limited pronation and suspicion, drum-stick fingers were present. Radio-ulnar synostosis was radiologically confirmed and additionally there were features of bone dysplasia. Array-CGH, confirmed by FISH, revealed *de novo* 3,4 Mb microdeletion of chromosome 17q24.2-q24.3 containing *PRKAR1A, MAP2K6, KCNJ2* genes. Independently, due to polycythemia and growth delay, endocrinological assessment was performed, with the suspicion of secondary causes of above complaints. After careful anamnesis it appeared that recurrent episodes of growth arrest were accompanied by hyperphagia and weight gain. Laboratory assays performed in symptomatic periods revealed disturbed daily rhythm and lack of suppression of cortisol secretion in the high-dose dexamethasone test. MRI of adrenal glands repeated in symptomatic and non-symptomatic periods did not show abnormalities. Head MRI revealed cortical dysplasia and additionally there was suspicion of pituitary microadenoma. However, based on clinical picture and laboratory findings, the child was qualified for the unilateral adrenalectomy. Phenotype-genotype correlation will be discussed.

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P11.004D

22q11.2 deletion influences expression levels of hemizygous and normal copy number genes: possible disruption of cis and trans regulation

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22q11.2 deletion syndrome (22q11.2DS) results from hemizygous deletions of chromosome 22, usually flanked by low copy repeats (LCR), which represent a substrate for aberrant recombination. The LCR sequences that flank the 22q11.2 deletion region define the common breakpoints, being the 3 Mb deletion the most frequent one. Even though most 22q11.2DS patients presents similar size deletions, the

phenotype is highly variable among individuals. Presuming that the phenotype observed is not only caused by the hemizygous genes, but also by variants outside the deleted region, we investigated whole-genome gene expression in peripheral blood from patients with 3 Mb deletion and from controls, to investigate the expression levels of 22q11.2 genes and to investigate whether 22q11.2 deletion can influence the expression of genes in other genomic regions. We found downregulation in hemizygous genes and in normal copy number genes flanking the deleted region. Considering the whole genome expression data, genes from different chromosomes were found to be both down and upregulated. These genes differentially expressed were investigated taking into account the pathways relevant to the syndrome. Genes located on chromosomes 22, 7 and 11 enriched the pathways that are associated to immune response, dopamine receptor mediated signaling and adrenaline and noradrenaline biosynthesis. Based on these data, we propose that the 22q11.2 deletion is responsible for the disruption of important interactions between chromatin in the nucleus, causing an imbalance of gene expression in a genome wide scale. Financial support: FAPESP, Brazil.

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P11.005A

Investigation of genetic variants in patients with 22q11.2 Deletion Syndrome

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The 22q11.2 deletion syndrome is the most common microdeletion syndrome in humans with an incidence of 1 in 2000 live births. Major clinical characteristics are intellectual disability, congenital heart anomalies, velopharyngeal abnormalities, characteristic facial appearance, and psychiatric disorders. Most affected individuals have a hemizygous 3Mb deletion on chromosome 22q11.2, which includes at least 50 known genes. Highly variable phenotypic severity is characteristic for the syndrome. Clinical features are varied and exhibit variable expressivity even in the patients carrying identical deletions. Potential genetics mechanisms underlying this variability include: additional copy number change elsewhere in the genome, allelic variation of genes within the 22q11.2 region of the non-deleted chromosome, or modifier genes outside of the deleted region.

To address copy number change in the genome, besides deletion of 22q11.2, aCGH analysis was performed on over 50 patients. Next, whole exome sequencing (WES) is being applied to identify both recessive gene mutation on the second chromosome and candidate genes that lie outside of the 22q11.2. Preliminary results from the first 29 exomes identify 290,318 variant positions, of which 74,221 are protein altering. 53% of these are rare, as defined by < 5% in 1000 genomes data. Within the 22q11.2 deletion region, we identified 33 rare nonsynonymous variant positions and 6 rare frameshift insertion positions spread across 14 different genes. Detailed phenotyping of all tested patients was performed and correlation between WES results and specific phenotypes is ongoing.

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P11.006B

Subtelomeric 2q deletion - evocative features, new signs, genotype - phenotype correlation

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2q deletion syndrome is a common subtelomeric rearrangement (some cases still unrecognized), defined by typical dysmorphic face, hand/foot anomalies and intellectual disability (ID). We present 7 cases to discuss the clinical features suggestive for this diagnosis, to present new features and the genotype-phenotype relationship.

Case 1: male (24 years), short stature, truncal obesity, dysmorphic face, short neck, dorsal kyphosis, lowset nipples, small/puffy extremities, hypermobile hands, proximal 4th finger/toe, moderate ID; normal karyotype; MLPA, arrayCGH: 2qdel;

Cases 2, 3 (siblings): male (17) and female (13), mild short stature, truncal obesity, dysmorphic face, short neck, dorsal kyphosis, lowset nipples, skin with visible small vessels and hirsutism, normal hands/feet, mild ID, autistic features, seizures; normal karyotype; MLPA: 2qdel, 9qdup;

Case 4: female (5 years), tall stature, obesity, dysmorphic face, short neck, dorsal kyphosis, lowset nipples, proximal 4th toe, hypotonia, moderate ID; normal karyotype; MLPA: 2qdel, 15qdup;

Case 5: female (14 years), normal height/weight, microcephaly, dysmorphic face, proximal 4-5th toe, mild ID; normal karyotype; MLPA: 2qdel;

Case 6: female (5 years), normal height/weight, dysmorphic face, wideset nipples, 5th finger clinodactyly, joint hypermobility, umbilical/inguinal hernia, VSD, mild ID; karyotype: 46,XX/46,XX,t(2,9); MLPA: 2qdel;

Case 7: male (4 years), normal height/weight, dysmorphic face, short neck, deviated hands with flexed fingers, varus equin foot, umbilical hernia, hypospadias, undescended testes, moderate ID; karyotype: 46,XY,add2; MLPA: 2qdel.

Features are illustrated and clinical data are correlated with the size of the deletion.

In conclusion, we present 7 cases of 2q-del to underline evocative features, illustrate particular features and discuss genotype-phenotype correlation.

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Familial Williams-Beuren syndrome: case report and overview

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Williams-Beuren syndrome (WBS, MIM194050) caused by 7q11.23 deletion manifested by recognizable pattern of multiply abnormalities with heart malformations high frequency. Inherited WBS cases are extremely rare. We presented WBS maternal transmission (3 relatives in 3 generations, FISH confirmation) and published cases overview focused for cardiac abnormalities spectrum. Proposita (G1,P1 at 38 weeks, BW = 2390g), 2-years-old boy (W = 10,5kg, L = 82cm, OFC = 46cm, <3p.c.) presented borderline growth delay, microcephaly, muscular hypotonia, typical facial dysmorphisms, nasolacrimalis canals obstruction, scoliosis, pyeloectasia, hypospadia, inguinal hernia. Heart: supravalvular aortic stenosis (SVAoS), pulmonary stenosis (PS), right pulmonary branch hypoplasia, foramen oval (FO), bicuspid aortic valve (BAoV), mitral valve prolapse, regurgitation (MVPR). Prenatally detected moderate extremities shortness was uncommon feature. Normal motor and speech development. Mother (BW = 1800g at 36 weeks), firstly was counseled at age 27 years manifested a similar facial features, gallbladder polyp, borderline mentality. Heart: BAoV, MVPR, atrial septal prolapse. Grandmother (52-years-old) showed intellectual disability, mild dysmorphisms. Heart: mitral, aortic, tricuspid valves regurgitation. Reproduction: G1-miscarriege, G2-WBS, G3-healthy girl. Both woman presented microcephaly (OFC=51cm), muscle weakness, nephroptosis, hypertensia, normal growth. We analyzed 15 published families' data (33 WBS patients): 15 parents, 18 children. Maternal transmission detected in 11 reports, including one prenatal case, two families with 2 and 3 affected siblings. Cardiac abnormalities detected in 12 families (cases without echocardiography were excluded). Children (11/13 cases) presented SVAoS/AoS (4/11), SVAoS + PS (3/11), PS (2/11), FO (1), MVPR (1). Parents (3/13) showed PS (1), MVPR (1), mitral valve insufficiency (1). Intrafamilial variability for heart defects, facial dysmorphisms, growth/mental delay and malformations was found.

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Differential cytogenomic diagnosis in five patients with 8p23.1 deletion suggesting 22q11.2 deletion syndrome

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Cytogenomics has become a fast-moving field, with potential to improve the characterization of clinical distinguishable phenotypes associated with a common genomic

region, however, for some cases the establishment of their correct diagnostic is challenging. We studied five patients with initial clinical suspicion of the 22q11.2 deletion syndrome using MLPA (multiplex ligation-dependent probe amplification) technique with P064 kit for multiple microdeletion syndromes and P250 kit specific for the DiGeorge syndrome and others control regions. Unexpected the molecular analysis revealed the presence of the 8p23.1 deletion involving the genes *PPP1R3B*, *MSRA* and *GATA4* in all patients. The 22q11.2 and 8p23.1 deletions show similar clinical signs as cardiac malformations, facial dysmorphisms and behavioral changes. However the patients with 8p23.1 deletion may present congenital diaphragmatic hernia, convulsions, ophthalmic problems, aggressive behavior with abrupt changes that usually appear in childhood, and eczemas. The *GATA4* gene is associated to cardiac abnormalities and the deletion or duplication in this gene can generate several different cardiac defects with variable penetrance and expressivity. The MLPA screening was efficient to accomplish the differential diagnosis, since it discarded the initial suspicion and also detected the genomic alteration associated with phenotype, improving the clinical conduct and allowing the adequate genetic counseling.

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P11.009A

Unraveling the genetic diagnosis in a large cohort of Adams-Oliver syndrome patients

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Adams-Oliver syndrome (AOS) is a rare developmental disorder, with an estimated prevalence of one in 225,000 individuals. AOS is typically characterized by aplasia cutis congenita (ACC) and transverse terminal limb defects. Additional abnormalities affect the heart, vasculature, eye and brain. Several genes causing AOS were described over the past few years. The autosomal dominant form of AOS is caused by mutations in *ARHGAP31*, *RBPJ*, *NOTCH1*, or *DLL4*, while the autosomal recessive form is related or linked to mutations in *DOCK6* or *EOGT*. Because of the rarity of this disorder, no reports are available on the distribution of the frequency of mutations in the currently known AOS genes in large cohorts. Here, we report on the molecular diagnostic screening of a cohort of 178 AOS/ACC patients and families.

The majority of the samples were screened using targeted next generation resequencing of the six AOS genes. A small proportion was screened by either whole exome sequencing, whole genome sequencing or Sanger sequencing. In total, we identified 61 mutations, including 20 novel mutations, providing a molecular cause in 55 of the 178 patients (31% diagnostic yield). Besides pathogenic mutations, we also identified 6 variants of unknown significance. *NOTCH1* was the major contributor to the phenotype, with mutations in 11% of the patients, followed by *DOCK6* (6%), *DLL4* (6%), *ARHGAP31* (3%), *EOGT* (3%) and *RBPJ* (1%). No major genotype/phenotype correlations emerged.

In conclusion, the majority of AOS probands remain unresolved, indicating that additional AOS genes await their identification.

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P11.010B

Fetal akinesia deformation sequence caused by recessive null mutations of the AGRN gene

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Fetal akinesia deformation sequence (FADS) is a clinically and genetically heterogeneous disorder involving reduced fetal movements, intrauterine growth retardation, and a range of developmental anomalies including arthryogryposis and skeletal abnormalities. It is usually fatal in utero. We report a case of a cytogenetically normal fetus affected with severely reduced fetal movements, pleural and pericardial effusions, and flexion deformities of the wrists, hips, knees and elbows apparent on 20-week scan.

Following termination of pregnancy, reduced muscle bulk and pterygia were noted on post-mortem examination. Clinical exome sequencing identified a homozygous frameshift mutation (LRG_198t1:c.4792_4799del) in the AGRN gene. AGRN encodes agrin, a synaptic regulator known to stimulate phosphorylation of MuSK receptors, thereby facilitating the formation and stabilisation of the acetylcholine receptor complex at the postsynapse of neuromuscular junctions (NMJ). Hypomorphic (largely missense) mutations in agrin and other NMJ proteins have been shown to cause a related, milder phenotype, congenital myasthenic syndrome, however, this is the first report of null mutation in this gene causing FADS. Mutations in genes encoding other proteins of this complex (DOK7, MUSK, RAPSN) have been associated with this disorder, and a knockout mouse model demonstrates an analogous lethal phenotype showing severe abnormalities of synaptic differentiation and fetal akinesia.

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Gene discovery by Array- Comparative Genomic Hybridization

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INTRODUCTION: Array-ComparativeGenomicHybridization(aCGH) is designed to detect well-characterized microdeletion/microduplication syndromes, unbalanced chromosomal-rearrangements. It serves as a powerful diagnostic tool in chromosomal diseases. Recently the technique has also been proven to be a method of gene discovery.Here we report 5 cases of independent MonogenicDisorders detected by a-CGH analysis. **PATIENTS: Y.B.E**.:A male patient with signs of **CysticFibrosis** with additional dysmorphism/mental retardation. CFTR gene mutation screening by reverse-hybridization and Sanger-Sequencing failed due to unamplified PCR products. aCGH revealed 100kb homozygous deletion on chromosome 7q31.2 encompassing theCFTR gene locus.**E.T.:**A female patient with clinical signs of **CysticFibrosisDisease** and mild-retardation. CFTR gene mutation screening by reverse-hybridization and SangerSequencing failed due to unamplified-PCR products. aCGH analysis revealed a 78 kb homozygousDeletion on chromosome7q31.2 encompassing the CFTR-gene locus.**O.D.:**A male patient with CongenitalAdrenalHypoplasia, DMD and GlycerolKinaseDeficiency. ArrayCGH analysis revealed a 4.8MB deletion on chromosome Xp21.1-21.2 region encompassing the **Xp21.1DeletionSyndrome** gene locus.**I. P.:**A 6-month old-male-patient with severe hypotonia. Follow up impressed findings of **InfantileParkinsonism**. aCGH showed 7 kb homozygous deletion encompassing the SLC6A3 gene on 5p15.33. DNA-sequencing of SLC6A3 gene was normal except exon-4 which was not amplified. **Z. V**.:A female patient with genital ambiguity and clinical signs of **CongenitalAdrenalHyperplasia**(CAH), 21-hydoxylaseDeficiency with additional dysmorphism, mental-retardation. CYP21A2 gene mutation-screening by reverse hybridization method failed to amplify PCR products. aCGH revealed a 2.7Mb deletion on chromosome6p21.32 encompassing the CYP21A2gene locus. **DISCUSSION:**Array-CGH is a powerful disease gene identification strategy. This strategy is most likely to be successful in patients with a monogenic condition in combination with mental-retardation or in patients with two or more unrelated genetic conditions.

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Hydrocephalus due to multifocal atresia-forking of the ependyma is caused by mutations in the *MPDZ* gene

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Introduction: Congenital hydrocephalus is either syndromic or non-syndromic, and in the latter, no cause is found in more than half of the patients. In patients with isolated hydrocephalus, *L1CAM* mutations represent the most common aetiology. In the last years, mutations have been reported in the *CCDC88C* gene, which cause hydrocephalus with mental retardation. A founder mutation has also been reported in the *MPDZ* gene in foetuses presenting massive hydrocephalus, but the neuropathology remained unknown.

Materials and Methods: We sequenced the *MPDZ* gene by targeted NGS sequencing and detailed the neuropathological data in mutated cases.

Results: We describe here three novel homozygous null mutations in the *MPDZ* gene in foetuses whose postmortem examination has revealed a homogeneous phenotype characterized by multifocal atresia-forking foci along the aqueduct of Sylvius, the third and fourth ventricles as well as the central canal of the medulla, with immature cell accumulation in the vicinity of ependymal lining, probably detached from the ventricular zone.

Conclusions: MPDZ also named MUPP1 is an essential component of tight junctions which are expressed from early brain development in the choroid plexuses and ependyma. Alterations in the formation of tight junctions within the ependyma very likely account for the lesions observed and highlight for the first time that primary atresia-forking of the ventricular system is genetically determined in humans. Therefore, *MPDZ* sequencing should be performed when neuropathological examination reveals atresia-forking of the aqueduct of Sylvius, of the third and fourth ventricles and of the central canal of the medulla.

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P11.013A

Aymé-Gripp syndrome. Case report and new mutation in MAF

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Introduction Mutations impairing the GSK3-mediated MAF phosphorylation cause a syndrome characterized by mental retardation (MR), cataracts, sensorineural deafness, brachycephaly, flat facial appearance and short stature, also known as Aymé-Gripp syndrome and delineated by Niceta et al (2015). We report a patient with phenotype suggestive of a MAF-related disorder, whose molecular studies showed a novel mutation in MAF.

Materials and Methods: A 10 years-old boy was evaluated for sensorineural hearing loss and dysmorphic features. He was the only child of healthy non-consanguineous parents. Antecedents of bilateral cryptorchidism and inguinal hernia that were surgically repaired. Sensorineural deafness was detected at 3 years because of speech delay. A cochlear implantation was performed at 4 years of age. At examination his weight and height were below 3rd percentile and his occipito-frontal circumference was over 50th percentile. He had a flat midface, hypertelorism, short nasal tip, small mouth, fine lips, brachycephaly, low-set ears and nail dystrophy. An autistic spectrum disorder and mild MR was diagnosed.

Radiological study showed nasal bones hypoplasia. CGH-Array and sequencing of COL2A1, COL11A1, COL11A2 (Stickler Syndrome) were negative.

Results: In exon 1 of the MAF gene a novel c.170C > T transition that predicts a p.Ser57Phe was detected. Both parents were negative for the mutation. All *in silico* predictors indicated this change as probably pathogenic.

Conclusion: We describe a patient with Aymé-Gripp syndrome (OMIM:601088) due to a novel mutation in *MAF*. Our patient showed a clinical phenotype that overlaps with a patient originally described by Fine and Lubinsky in 1983.

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P11.014B

Baraitser-Winter syndrome: two novel mutations of the *ACTB* gene

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The *ACTB* gene encodes one of six different actin proteins, beta (β)-actin. Heterozygous mutations in *ACTB* have been identified in patients with Baraitser-Winter syndrome (BWS), a recognizable entity, characterized by intellectual disability, distinctive facial features, bilateral ptosis, ocular coloboma, short stature, and pachygyria. We have performed exome sequencing analysis on two unrelated patients, a seven-year-old boy and a four-year-old girl, both with unclear genetic condition and found two different novel *de-novo* missense *ACTB* mutations, c.208C>G; p.Pro70Ala and c.511C>T; p.Leu171Phe. Both mutations lay in a highly conserved site of the *ACTB* gene and are possibly involved with subunit interactions near the polymerization interface. While the boy presented with a typical facial appearance, the girl showed facial features difficult to recognize as a BWS gestalt. Both patients showed mild intellectual disability and normal growth parameters. The boy presented shortly after birth with a ventricular septal defect which closed spontaneously. Brain MRI revealed a low-grade Chiari 1 malformation. The dysmorphic features include hypertelorism, epicanthus, prominent nasal bridge, mild ptosis, anteverted nares, smooth philtrum, thin upper lip, small ears, broad hands, short fingers, single palmar crease, multiple café-au-lait spots, low-set nipples and an overriding scrotum. The girl presented with prenatal extrasystolia, diagnosed after birth as ventricular arrhythmia as well as with thrombocytopenia. Additionally, she showed deeply-set-eyes, mid-face hypoplasia, broad nose, micrograthia, isolated cleft palate, dysplastic ears, long slender fingers, astigmatism, hyperopia, mild grey-matter heteropia and PVL.The phenotypical spectrum of *ACTB* mutations is very broad and may not necessarily be recognized as BWS.

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P11.015C

BBS10 frameshift mutation in a turkish girl with bardet biedl syndrome

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Introduction: Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by retinitis pigmentosa, rod-cone dystrophy, turuncal obesity, postaxial polydactyly, cognitive impairment, renal dysfunction and hypogonadism. BBS is a defined ciliopathy notable for extensive allelic and genetic heterogeneity and oligogenic inheritance. The most common mutations are in genes BBS1, BBS2 and BBS10. **Case:** Our patient is a 2 year-old girl born at term with normal physical parameters to consanguineous parents. Postnatally she was hypotonic. In her physical examination, length was 78 cm (<3P), weight 14 kg (90–97P), and head circumference 47 cm (25P). Her dysmorphic features were a broad face, narrow forehead, hypertelorism, narrow palpebral fissures, mild synophrys, dry rough hair, long eyelashes, broad and depressed nasal root, broad nasal ridge, long and smooth philtrum, small mouth, thin upper vermillion, small ears, microdontia, short neck, atypical palmar crease, turuncal obesity, postaxial polydactyly of hands and feet and pes valgus. Her eye examination showed retinal pigment epithelial degeneration. Her pelvic and renal ultrasonography, echocardiography and cranial MRI were normal. Her high resolution banded karyotype was normal. **Method:** BBS1, BBS2 and BBS10 genes were sequenced via Sanger sequencing. **Results:** We found homozygous c.270_271insT (p.Cys91Leufs*5) mutation in BBS10 in exon 2 resulting in a highly truncated protein. Her parents are heterozygous for the same mutation. **Conclusions:** Our patient has typical clinical features of BBS. The mutation found is the most common mutation in the BBS10 gene. We reported our patient to contribute to genotype-phenotype correlations in BBS.

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P11.016D

BCOR mutation a boy with multiple anomalies and large eyeglobes

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A one-month-old boy, second child of non-consanguineous parents, was admitted because of respiratory insufficiency. He was born term with normal birth weight, length and OFC. On cardiac ultrasound, a VSD, ASD, PDA, persistent left vena cava and non-compaction left ventricle were diagnosed. He suffers from bilateral grade 4–5 vesicoureteral reflux. The eyespecialist noted high myopia (-15) with large globes, embryotoxon posterior and nystagmus. His motor milestones are severely delayed and he does not speak. From the age of 1 years he has seizures. SNP array, FISH-analysis (Pallister Kilian syndrome) and analysis of CHD7, ASXL1 and SETBP1 was normal. WES analysis revealed a hemizygous variant in the BCOR (BCL-6 corepressor) -gene: NM_001123385.1:c.4741+1G>A p.(?). This variant is located within the donor splice site of intron 12, most likely resulting in aberrant splicing. His healthy mother is a carrier and showed 100% skewed X-inactivation upon analysis. The variant was not present in both maternal grandparents. We consider this variant in BCOR likely causative. To our knowledge, this is the first report on a BCOR variant in BCOR in a child without microphtalmia, but with high myopia and large eyeglobes. The organs involved in the phenotype in this boy correspond with oculo-facio-cardio-dental syndrome and Lenz microphtalmia . However, his phenotype is different to the phenotype of those two disorders and most described patients with oculo-facio-cardio-dental syndrome are females with skewed X-inactivation. This case illustrates a broader phenotypic spectrum of oculo-facio-cardio-dental syndrome/ Lenz microphtalmia and that female carriers may be asymptomatic due to skewed X-inactivation

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Beckwith-Wiedemann Syndrome Caused by Non-Mosaic Uniparental Disomy of the Complete Chromosome 11

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Beckwith-Wiedemann Syndrome (BWS) is an overgrowth disorder involving imprinted genes on chromosome 11p15.5. Clinical features are varied and can include macroglossia, somatic overgrowth, abdominal wall defects and neonatal hypoglycemia. A concern for infants and children with BWS is the predisposition to develop embryonal tumors which is estimated at about 7.5%. There are numerous mechanisms that can result in BWS, including mosaic paternal uniparental disomy (UPD) of chromosome 11p15.5 which accounts for about 20% of cases. Paternal UPD involving more than just the short arm of chromosome 11 is very uncommon and non-mosaicism for paternal UPD 11, to our knowledge, has not been reported and is hypothesized to be lethal. Here we present on the prenatal diagnosis of a female fetus with non-mosaic UPD of the entirety of chromosome 11. Prenatally there was macroglossia and an abdominal circumference and kidneys greater than the 95th percentile. SNP-based microarray on DNA from amniocytes and DNA from peripheral blood after birth identified non-mosaic paternal UPD spanning the entire length of chromosome 11. SNP-based array on DNA from placenta identified paternal UPD for the entire length of chromosome 11 in a mosaic state. She was born at 31 weeks 6 days gestation with nephromegaly and hepatomegaly, but no detectable Wilm's tumor or hepatoblastoma. Her clinical course was complicated by respiratory difficulties, a bowel perforation, a patent ductus arteriosis, persistent hypoglycemia, and sepsis. At 4.5 months of age, hepatoblastoma was detected and she died at 7 months due to respiratory difficulties caused by the severe hepatomegaly.

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P11.018B

Cantu syndrome caused by a novel heterozygous ABCC9 mutation: Description of a Malaysian boy with predominant cardiorespiratory features

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Introduction: Cantu syndrome, a rare autosomal dominant disorder characterised by congenital hypertrichosis, distinctive facies, osteochondrodysplasia, cardiomegaly and cardiovascular abnormalities, is largely caused by heterozygous mutations in the ABCC9 gene, and rarer in KCNJ8.

Case Report: We present a 3 year-old boy, first child of a consanguineous couple. Born premature at 32weeks, he had a large PDA, inguinal hernia and undescended testes at birth. His infantile period was stormy, with frequent severe respiratory infections and hyperactive airways. Clinically,

Summary of clinical features of patients with 6q22.1-q22.31 deletions

	Patient 1	Patient 2	Patient 3	Patient 4
Age, sex	5 years., male	4 years., male	1 month, female	3 months, female
Developmental delay	+	+	+	−
Seizures	Only epileptiform activity on EEG	+	−	−
Craniosynostosis	−	−	+	+
Brain malformation	−	+	−	+
Dysmorphic facial features	+	+	+	+
Size, Mb	9,8	7,9	13,9	11,9
Molecular karyotype	arr[hg19] 6q22.1q22.31 (114650304_124411267)x1	arr[hg19] 6q22.1q22.31 (117939083_125843984)x1	arr[hg19] 6q21q22.31 (109428325_123335803)x1	arr[hg19] 6q21q22.31 (113684116_125569047)x1

he had relative macrocephaly, hypertrichosis, thick scalp hair, coarse facies, long eyelashes, flat nasal bridge, full lips, and also tachypnoea, pectus carinatum, mild hypotonia and motor delay.

Chest radiographs showed cardiomegaly and broad ribs. Echocardiogram demonstrated a small PDA, hypertrophy of both ventricles and biventricular diastolic dysfunction. CT thorax revealed features of pulmonary hypertension and bronchiolitis obliterans. His immune screen was normal. Molecular testing of ABCC9 gene identified a novel de novo heterozygous missense Q481H mutation in exon 9.

His clinical course was challenging with very labile respiratory reserve and prolonged oxygen dependency. Frequent infections associated with immune dysfunction have been reported in Cantu syndrome but none so severe as to result in bronchiolitis obliterans. Another unique feature is his biventricular diastolic dysfunction as cardiac function in prior reports has always been normal, thus potentially expanding the phenotypic spectrum.

Conclusion: We wish to highlight the unique cardiorespiratory complications and the challenges managing them. Molecular confirmation directs accurate genetic counselling and vigilance for other potential complications like conduction defects, vascular abnormalities, scoliosis and lymphoedema.

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P11.019C

Cat-Eye Syndrome: Phenotype and Cytogenetic analyses of a cohort of 44 patients from an international collaborative study

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Cat Eye Syndrome (CES) is a rare genetic disease. It has been observed that most patients diagnosed with CES carry a small supernumerary bisatellited marker chromosome (sSMC), which results in partial tetrasomy of 22p-22q11.21.

Individuals with CES are characterized by three main clinical features: preauricular pits and/or tags, anal atresia, and iris coloboma. However, clinical features reveal an extreme phenotypic variability, including variable congenital heart and kidney defects, skeletal abnormalities, growth delay, associated or not with variable mental deficiency.

This work studies and compares to the literature data 44 patients who carry a cytogenetically proved CES. This is the largest cohort of CES patients reported to date (excluding meta-analyses).

On phenotypic level, we confirm the high frequency of pre-auricular anomalies (84%). But anorectal anomalies (43%) and iris coloboma (35%) are less frequent than expected. Furthermore, 35% of patients presented with only one of the three major sign, and 10% did not present any of them. Heart defect (46%) and intellectual deficiency (46%)

are the other most frequent features. Other recurrent reported anomalies are urogenital anomalies (30%), hormonal deficiency (31%), and growth delay (19%).

On cytogenetic level we highlighted high prevalence of mosaic cases (37%). We also highlight the unexpected high prevalence of parental transmission of sSMC (35%), whereas only 5 cases have been reported so far. Most often, the transmitting parent is pauci(a)-symptomatic and carries the mosaic marker at a very low rate (<10%) making it difficult to detect without FISH analysis.

These data could significantly modify genetic counseling.

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P11.020D

New clinical features in patients with 6q22.1-q22.31 deletions

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Chromosomal microarray analysis (CMA) of 3299 Russian patients with congenital malformations, developmental delay and distinctive facial features detected novel pathogenic copy number variations (CNVs), unclassified in OMIM database, in 4,9% cases. Here we describe 4 deletions overlapping 6q22.1-q22.31 region. Size of deletions varied from 7,9 Mb to 13,9 Mb. The smallest region of overlap is 5,4 Mb and includes 16 genes. Clinical manifestations included developmental delay (3/4) and brain malformations (2/4). All patients had dysmorphic facial features. Unlike the previously described patients with 6q22.1-q22.31 deletions, 2 patients had craniosynostosis, whereas only 1 patient had seizures and another one had only epileptiform activity on EEG. Deletions of 6q22.1-q22.31 region were previously associated with variable clinical features. We suggest that craniosynostosis is a part of this wide clinical spectrum.

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P11.021A

Deletion, duplication and ring of chromosome 13 due to different rearrangements and implication to phenotype

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Patients with duplication, deletion, and ring chromosome 13 may present overlap of clinical characteristics making difficult to delineate a specific phenotype for each group, mainly due to the variable size of the deleted segments. We describe eight patients with different abnormalities involving chromosome 13 characterized by G-banding karyotype, FISH, and array techniques. Cytogenomic analysis revealed: three patients with ring chromosome 13, three patients with interstitial, and one with terminal 13q deletion, and one with concomitant 13q deletion and duplication. Array revealed the breakpoints and genomic coordinates in all cases. FISH with BAC probes revealed a duplicated and inverted segment in one of the ring chromosomes. All patients showed neuropsychomotor development delay, but only one had deletion of the *ZIC2* gene (13q32.3), important for normal development of the forebrain. Among the patients who had deletion including 13q32.2-q33.2, a region associated to Dandy-Walker malformation, only one presented this feature. The *ARHGEF7* gene, which is mapped in 13q34 and plays a role in the development of human cerebral cortex, is duplicated in one patient and deleted in four patients who present microcephaly and neuropsychomotor development delay. Our data suggest that the region responsible for genital abnormalities in male is between 104.2 Mb (13q31.1) and 111.5 Mb (13q34) and includes the *EFNB2* gene. This work shows the relevance of the study of chromosome rearrangements with similar genome imbalances for a better cytogenetic-phenotype correlation, helping the diagnosis, prognosis, and genetic counseling. Financial Support: FAPESP, Brazil.

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P11.022B

DOCK6: novel gene involved in ciliopathies

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Introduction: Molecular diagnosis of ciliopathies becomes complicated due to the high genetic and phenotypic heterogeneity associated with these syndromes, it is therefore necessary to use the variety of genetic techniques currently available. The objective of this study was to identify the genetic defect responsible for the pathology of a consanguineous family with BBS-*like* phenotype, after discarding mutations in predominant *BBS* genes.

Materials and Methods: We performed whole exome sequencing (WES) followed by data filtering. Given the consanguinity of the family, we also carried out homozygosity mapping. Data analysis of both approaches led us to one candidate gene, which was silenced by siRNA transfection to perform immunofluorescence assays to check possible ciliary defects.

Results: Homozygosity mapping revealed several LOH regions, one of which contained *DOCK6* gene. WES data filtering led us to the homozygous variant c.1289G>A (p.(Arg430His)), located in the same gene and classified as potentially pathogenic by several prediction tools. This variant, confirmed by Sanger, segregates within the family, confirming the autosomal recessive inheritance. Given its possible relation to primary cilium through actin cytoskeleton, gene expression was suppressed. Immunofluoresence assays allowed us to confirm F-actin destabilization and, surprisingly, detect nearly 40–50% of defective primary cilia.

Conclusions: The combination of WES and homozygosity mapping was useful for the molecular diagnosis of this family and the identification of a novel candidate gene which seems to be related to defects in primary cilia structure. Further functional studies will clarify its role in cilia function.

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P11.023C

Extending the phenotype in nephronophtisis-related ciliopathies : severe fetal presentation with homozygous *ANKS6* mutations

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Ciliopathies are a large group of congenital diseases related to ciliary dysfunction. These are highly heterogeneous in features and severity with substantial phenotype and genotype overlap.

We report here the first pregnancy of a young consanguineous couple without peculiar medical history. First trimester prenatal ultrasound found a small chest, short and curved femora, hyperechogenic and enlarged kidneys, oligohydramnios, absent corpus callosum and congenital heart malformation. Pregnancy was terminated at 18+6 WG. At most-mortem examination, the male fetus had normal size and body weight, a *situs inversus totalis* with outflow tract anomaly, large kidneys with interstitial fibrosis and widespread cysts, voluminous and fibrotic pancreas, hepatic fibrosis and ductal proliferation. Fetal X-ray showed split lumbar vertebrae and curved long bones.

Fetal DNA was analysed using a ciliary genes targeteted panel with next-generation-sequencing and allowed to identify a homozygous frameshift variant in *ANKS6* gene.

ANKS6 mutations were first described in nephronophthisis cohorts. Almost all nephronophthisis related genes have already been identified in syndromic ciliopathies, such as Joubert, Sensenbrenner, Jeune or Meckel syndrome. ANKS6 protein is localized at the basal segment of the cilia and interacts with NEK8, NPHP3 and INVS. To date, 18 patients with *ANKS6* mutations have already been described in patients with cystic kidney disease due to nephronophthisis with variable phenotype and severity. Patients with loss of function mutations seem to have more severe phenotype. Here we describe the most severe form of

ANKS6-related diseases, refining the far end of the spectrum by adding brain, skeletal and pancreatic anomalies.

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P11.024D

Comprehensive genetic analysis of patients with (non)-syndromic cleft lip with or without cleft palate

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a common congenital malformation caused by both genetic- and environmental changes. The underlying genetic causes of isolated forms of nsCL/P is restricted to a limited set of genes but primarily by common risk variants, and is only explaining a fraction of patients with nsCL/P. On the other hand, CL/P occurs frequently as part of a syndromic spectrum of congenital abnormalities. Over >200 genes are associated with syndromes for which CL/P is one of the representations, but comprehensive genetic analysis of these patients is challenging since incomplete penetrance and variable expressivity has been described for a substantial subset of these genes. We performed routine diagnostic testing for a set of genes associated with (non-) syndromic forms of CL/P in a cohort of >40 patients with a broad spectrum of phenotypes. We identified (likely) pathogenic mutations in the LRP4, IRF6, MSX1 and SMAD4 genes confirming the clinical diagnosis. In 1 family, a paternally inherited SIX3 mutation resulted in a variable morphological phenotype in two siblings, where the father did not show apparent abnormalities. Mutations in SIX3 are associated with autosomal dominant inherited holoprosencephaly type 2. MRI analysis of the brain did confirm structural abnormalities in the index patient, supporting the assumption that a mutation in the SIX3 gene is underlying to the clinical phenotype. These preliminary results suggest that mutations in genes associated with syndromic forms of CL/P can be clinically represented as nsCL/P, underlining the importance of comprehensive genetic testing.

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P11.025A

Exome sequencing reveals a recessive mechanism involving interacting genes in persistent cloaca

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Aim of the study: Persistent Cloaca (PC) is characterized by the convergence of the rectum, vagina, and urethra into a single common channel as a result of septational anomalies during development. PC is rare (1/20,000 live births), occurs sporadically and exclusively in females. The causes of this developmental failure are not known, yet there is strong evidence for a genetic component. The aim of this study is to investigate the genetic etiology of PC patients. **Methods:** Whole exome sequencing was conducted on 21 PC trios following the standard protocol. **Main Results:** Two novel and damaging *de novo* mutations in caudal type homeobox 2 gene (*CDX2*) were found in two PC patients. *CDX2* is a transcription factor that defines urothelial differentiation. Different rare damaging mutations in interacting genes relevant for caudal region development such as laminin, nidogen and integrin were found in several patients. Similarly, mutations in the gene pair *GPRASP1* and *LRP2* were recurrently identified. *GPRASP1* and *LRP2* directly interact with members of the sonic hedgehog (SHH) and bone morphogenetic protein (BMP) pathways, which are essential in the development of the caudal region. Overall, the mutated genes were over-represented in the extracellular matrix and basement membrane gene sets (Gene enrichment test $p<4.58\times10^{-10}$). **Conclusion:** 1: Our data supports a strong genetic basis for PC. 2: PC is heterogeneous requiring mutations in interacting genes to give rise to the anomaly : different mutated genes in different patients. 3: PC is likely to be a recessively inherited disorder. Grant acknowledgement: RGC **HKU 778213**

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P11.026B

A case of familial Coffin - Lowry syndrome associated with left ventricular noncompaction cardiomyopathy

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Introduction: Coffin-Lowry syndrome (CLS) is a rare X-linked disorder characterised by mental and growth retardation, distinct facial dysmorphism, short and broad hands, skeletal and cardiac anomalies in men Female carriers are normal or only mildly affected. CLS is caused by mutations in the *RPS6KA3* gene located at Xp22.2 which encodes RSK2, a growth-factor-regulated protein kinase.

Materials and Methods: We report a family with CLS with eight patients in two generations. The patients in this family, a mother and her seven children - four males and three females, all except one had severe mental retardation with the distinct clinical features of CLS: short stature, prominent forehead, hypertelorism, down-slanting palpebral fissures, a flat nasal bridge, a wide mouth with full lips, short hands with stubby, tapering fingers, kyphosis and/or scoliosis.Two of the male siblings had a left ventricular noncompaction (LVNC) with a restrictive pattern, as documented by echocardiography. The oldest sister presented with a mild facial dysmorphism and a normal intelligence. Mutation screening was performed on the *RPS6KA3* gene in this family.

Results: All patients tested (the mother and her seven children) had the same pathogenic mutation in the *RPS6KA3* gene - c.1672C>T (R558X).

Conclusions: This report confirms that some CLS females may present not only mild clinical features such as facial and digital dysmorphism and learning difficulties, but can have well noticeable CLS symptoms typical for affected males. This case underlines that patients with CLS should, be screened for LVNC cardiomyopathy which is a potentially life threatening condition.

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P11.027C

Different and atypical presentation of Cohen syndrome in two sisters

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Introduction: Cohen syndrome (CS) is a hereditary condition, consisting of obesity, hypotonia, mental retardation, characteristic craniofacial dysmorphism and abnormalities of hands and feet. It is transmitted in an autosomal recessive pattern, with considerable variability of expression. No genotype-phenotype correlation has been established. CS is caused by different mutations in the *COH1* gene, located at 8q22-q23. This gene encodes a specific transmembrane protein which is involved in vesicle-mediated sorting and transport within the cells.

Clinical case: We report two sisters who were diagnosed with CS. The elder sister had mental retardation, obesity, truncal hypotonia, and some of the distinct features of Cohen syndrome: hypertelorism, prominent central incisors, coarse hair and thick eyebrows, long eyelashes, wave-shaped palpebral fissures, broad nasal tip, smooth or short philtrum, small and narrow hands and feet, with slim fingers. The younger sister had conical teeth, hypertelorism, and congenital heart defect. Both sisters had short stature, dolichocephally without microcephally, kyphosis, and scoliosis.

Results: Both patients are found to be compound heterozygotes carrying one point mutation (c.5086C→T), and deletion of 5 exons (DelEX46–50).

Conclusions: This report confirms the great variability of phenotypic expression of Cohen syndrome, and the need for thorough physical examination of these patients. Considering the possibility of atypical clinical manifestation, the Cohen syndrome deserves utmost attention and care.

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P11.028D

Copy number variation spectrum in children with combined congenital heart defects and renal malformations

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Introduction: Combined congenital abnormalities of cardio-vascular system and renal-urinary tract belong to severe and relatively rare clinical conditions, the genetic etiology of which is still poorly characterized. In the present study we evaluated the impact of copy number variations in 30 children with combined heart and kidney structural defects without additional severe organ manifestations.

Materials and methods: Molecular-cytogenetic analysis was performed using array-based comparative genomic hybridization (array-CGH) with an Agilent 60K platform.

Results: In 2 out of 30 patients, pathogenic microdeletions of the same size (~2.47 Mb) were identified in 22q11.2 region known as DiGeorge/Velocardiofacial locus. Besides, a newborn with hypoplastic left heart syndrome, mitral atresia, ventricle sept defect and congenital megaloureter harbored a microduplication at 15q26.2 region encompassing *MCTP2* gene. Given that *MCTP2* was recently identified as a dosage-sensitive gene essential for proper left ventricular outflow tract development, the revealed duplication seems to be responsible for at least a cardiac component of the patient's phenotype. We also took notice of genes *NNT*, *DHFR*, *ZNF649* and *NPHP1* within copy number gains at 5p12, 5q14.1, 19p13.33 and 2q13 regions. The genes are shown to widely express in heart and kidney and play important roles during embryogenesis. To assess the clinical significance of these variants unambiguously, further comprehensive functional studies are required.

Conclusions: Our findings support the utility of array-CGH screening of patients with combined structural cardiorenal malformations for accurate individual diagnosis, genetic counseling, and new candidate genes identification.

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P11.030B

A new recognizable recessive syndrome with distinct dysmorphism, lymphedema and sensorineural hearing loss caused by Carboxypeptidase D mutations

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Lymphedema is the consequence of inefficient uptake and/or reduced lymph flow, which leads to the accumulation of high-protein fluid beneath the skin and chronic swelling of extremities. Approximately 20 genes of VEGFR3 pathway have been identified as responsible for related phenotypes. We report three individuals from two families with a new recessive syndrome presenting with a recognizable facial dysmorphism, sensorineural hearing loss and lymphedema of the lower limbs. The index patient and her affected second cousin once-removed showed growth retardation of prenatal onset, prelingual sensorineural hearing loss, short feet, narrow hands, and distinct facies; dispersed eyebrows, upslanting palpebral fissures, prominent nasal bridge and broad ridge, smooth/long philtrum, thin/inverted vermilion of the upper lip and short neck. Both had mild intellectual deficit. After 10 years of age, they developed persisting lower-limb lymphedema.14-year-old female patient from an unrelated family, presented with similar distinct dysmorphic features along with short stature, prelingual sensorineural hearing loss but had no sign of lymphedema. Whole-exome sequencing in the first family revealed three different pathogenic mutations segregating with the phenotype, including a protein-null allele, in *CPD* gene which encodes for Carboxypeptidase D. Sequencing *CPD* gene in the affected of second family, showed a novel homozygous *CPD* mutation. CPD is a circulating protease and to date, no endogenous substrates for CPD have been identified. Patient-derived primary cells and zebrafish knockout animals will enlighten the pathogenesis of this new syndrome for the identification of potential therapeutic options for lymphedema.

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P11.031C

A novel variant of *GNAI3* gene in a Japanese girl patient with malformed ears, microstomia, abnormal temporomandibular joint, and mandibular condyle hypoplasia

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Auriculo-condyularsyndrome (ARCND, OMIM #602483) is a rare craniofacial disorder involving the first and second pharyngeal arches and is characterized by malformed ears (question mark ears), prominent cheeks, microstomia, abnormal temporomandibular joint, and mandibular condyle hypoplasia. The *GNAI3, EDN1* or *PLCB4* gene has been reported to be responsible for ARCND. We report a Japanese girl who was clinically diagnosed as Pierre Robin syndrome. She underwent tracheostomy due to upper airway obstruction. In order to make genetic diagnosis, whole exome sequencing (WES) analysis was performed in the patient and her parents using Human All Exon V6 kit (Agilent) and HiSeq 2500 (illumina).

Then, we found a novel *de novo* variant of the *GNAI3* gene (NM_006496) in the patient, which was also

confirmed by Sanger sequencing. The variant causes an amino acid change at the GDP/GTP binding site in the catalytic domain of GNAI3. Another amino acid change at the same position has been reported in a Caucasian patient of ARCND. All three prediction program of functional affects of variations, SIFT, PolyPhen-2 and Mutation taster, estimated the amino acid change as damaging. The novel variant found in the patient might be pathogenic for ARCND.

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P11.032D

A novel *CTNNB1* mutation in a patient with teratoma and multiple malformations - expansion of the phenotypic spectrum and possible new gene for Currarino phenotype

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Introduction: We present three-year-old girl with a complex phenotype including severe developmental and growth delay, microcephaly, anal atresia and the massive teratoma of pelvis, which infiltrates sacrum and bladder. The patient has undergone several surgical interventions and chemotherapy due to relapse of the teratoma. She also has facial dysmorphism including gestalt of primary microcephaly and bilateral mild ptosis. Based on this clinical presentation we suspected Currarino syndrome possibly caused by (micro)deletion of 7q containing *MNX1* gene.

Materials and Methods: We performed chromosomal microarray (CGH, Agilent Technologies) and the clinical exome sequencing (Illumina TruSight One) which targeted genes related to observed clinical presentation.

Results: Unexpectedly, chromosomal microarray showed normal result. Subsequently, clinical exome sequencing showed presence of heterozygous pathogenic nonsense variant in *CTNNB1* gene (c.1876G>T, p. Glu626X). Pathogenic variants in *CTNNB1* gene represent a known cause of *CTNNB1*-related mental retardation, autosomal dominant, 19 (OMIM: 615075). Furthermore, disruption of the beta-catenin (CTNNB1) signaling pathway has been implicated in the pathogenesis of anorectal anomalies, possibly explaining the anal atresia in the patient. Beta-catenin also functions as the gatekeeper in differentiation and tumorogenesis in embryonic stem cells and could possibly play a role in development of teratomas.

Conclusion: *CTNNB1*-associated teratoma has not been described in humans so far. If associated with anal atresia and sacrum abnormalities, Currarino syndrome could be easily suspected. Thus, we propose germline *CTNNB1* gene mutations as a novel cause of human teratomas and Currarino syndrome phenotypic spectrum, which could be of interest for clinical geneticists, as well as further research.

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P11.033A

De novo pure 1q42.11qter duplication syndrome: a case report

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Introduction: 1q42.11qter duplication syndrome is a rare chromosomal disorder associated with mild mental retardation, neurodevelopmental delay, macrocephaly, cardiac and urogenital malformations. According to the literature, most of the cases are related to unbalanced inheritance of balanced chromosomal rearrangements, particularly translocations derived from parental origins. Here we report a case of de novo pure 1q42.11qter duplication without parental chromosomal abnormalities. Our report may be helpful to better define the partial 1q duplication syndrome. Materials and Methods: Metaphases obtained from peripheral lymphocyte culture were analyzed after GTG banding. 1p and 1q subtelomeric probes were used for FISH analysis, DNA microarray analysis was examined using Affymetrix Cytoscan Optima. **Results:** Karyotype analysis was reported as 46,XX,der(1)add(1)(p3?6). FISH analysis revealed 1p and 1q subtelomeric signals consecutively on the 1p terminal region which was shown as .ishder(1)add(1)(p3?6) (CEB108/T7+,D1S3738+). Karyotype and FISH analysis

did not approve any parental origin. On the other hand, DNA microarray analysis was reported as arr[hg19] 1q42.11q44(224,433,219–249,224,684)x3. As a result, a pathogenic duplication of 1q42.11q44 region was determined. Conclusion: Phenotypic features of our patient are generally consistent with previously reported cases. Besides, our patient has lateral ventricles enlargement seen in some cases and arachnoid cyst which is not described before. Duplication region contains 94 OMIM genes. According to the literature, some of these genes are responsible for pathogenesis such as *AKT3* is associated with macrocephaly, *TBCE, DISC1, TRAX* genes are related with neurodevelopment, whereas *WNT3A,WNT9A* are associated with craniofacial morphogenesis. But, functional studies are required to verify the relation between these genes and pathogenesis.

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P11.034B

Duplication of HTR 7 gene in a patient: Is it a possible cause of autism and congenital cataract ?

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Introduction: HTR7 gene, encoding one of the serotonin receptors, is shown as a candidate region for autistic disorder and other some neuropsychiatric diseases. Here, we present a patient with a HTR7 duplication, in terms of bringing a new perspective to copy number variations of this gene.

Clinical report: The patient was an 11-yeard-old boy with autistic behaviors, bilateral congenital cataract and surgery-resistant glaucoma developed after cataract operation. Maternal family history included severe myopia and adult onset cataract.

Results: Array-CGH (Agilent, 8×60K ISCA) revealed a 453 kb duplication including the HTR7 gene. This CNV was confirmed in the patient and shown to be present in the mother using qPCR and qRT-PCR.

Discussion: HTR7 gene is a candidate locus in autism based on functional studies. Similar duplication of HTR7 gene have been reported in DECIPHER patients with autistic behavior or other neurological findings. Recently, compound heterozygote mutation in HTR7 gene have been reported in dizygotic twins with autism. So, we consider that duplication of HTR7 gene may be one of the genetic causes of autism. Other remarkable findings in our patient were bilateral congenital cataract and surgery-resistant glaucoma. HTR7 gene is expressed in retina, lens, iris and ciliary body, and 5-HT7 agonists have been shown to increase intraocular pressure. We suggest that HTR7 duplication may be related to congenital cataract and predisposition to secondary glaucoma. But further knowledge is needed to clarify these relationships.

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P11.035C

Dyskeratosis congenita (DC) caused by mutations in PARN

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We report on a 10 year-old boy with developmental delay, microcephaly, cerebellar atrophy and hypoplasia of the corpus callosum. Furthermore he showed short stature, deep-set eyes, thin eyebrows, sparse, fine hair, dry, translucent and abnormally pigmented skin, small, dystrophic nails and carious teeth. Because of bone marrow failure a transplantation was performed.The telomere length in bone marrow cells was shortened, but panel diagnostic for 10 dyskeratosis congenita (DC) genes could not identify a pathogenic mutation. Finally, exome sequencing uncovered the mutation c.1262+2_1262+3dupTA in the gene PARN. No unaffected allele was detectable. Mucosal cells were taken and RNA-analysis revealed a partial skipping of exon 18 of PARN. The mother showed a heterozygous state for the mutation. A sample of the father was not available, so that we cannot distinguish true homozygosity from a deletion of the second allele by segregation analysis. Further investigations (qPCR, RNA of the mother) are in progress. The protein encoded by PARN (poly(A)-specific ribonuclease), a deadenylase enzyme is involved in the regulation of mRNA stability. Moreover, it has recently been shown that PARN is also involved in the regulation of the expression of different telomere-biology related genes. In 2015, Tummala et al. were the first to describe biallelic PARN-mutations, two of them splice-site mutations as well, as the cause of severe DC in three families. The findings in our patient support the assumption that PARN represents a new disease-causing gene in patients with a severe DC.

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P11.036D

EAST syndrome - expanding the clinical phenotype of this recently delineated, rare autosomal recessive multisystemic disorder caused by mutations in the potassium channel gene *KCNJ10*

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Biallelic mutations in the *KCNJ10* gene were recently reported to cause a multisystemic disorder characterised by epilepsy, ataxia, sensorineural deafness, and salt-wasting renal tubulopathy - EAST syndrome. To date, only a few clinical reports have been published detailing the phenotype. We report 2 unrelated cases of Pakistani origin expanding on the clinical features of this disorder, including the first reported case of EAST syndrome with structural renal abnormalities. The first case is the first child born to first cousin parents after an uneventful pregnancy. He presented with global developmental delay, sensorineural hearing loss, marked cerebellar dysfunction, and tonic-clonic seizures. MRI brain at 1 year showed signs of mild volume loss. The second case is the 3rd child born to non-consanguineous parents at 35 weeks by emergency section following a pregnancy complicated by gestational diabetes, pre-eclampsia and severe intrauterine growth restriction. He presented with infantile focal seizures, sensorineural deafness, failure to thrive, and global developmental delay. He initially had mild axial hypotonia and then developed marked ataxia with spastic paraplegia by age 3 years. Brain MRI at age 8 years showed mild degree of generalised volume loss with normal cerebellum. Muscle biopsy showed selective type B fibre atrophy. Renal tubular dysfunction with persistent hypokalaemia was diagnosed. At 14 years he developed heavy haematuria and a renal ultrasound scan showed very echogenic kidneys containing several small cysts bilaterally with loss of normal corticomedullary differentiation. Both cases were found to be homozygous for a previously reported pathogenic missense variant c.194G>C (p.Arg65Pro) in *KCNJ10*.

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P11.037A

The impact of ERCC6 or ERCC8 genes in two turkish patients with clinical findings of cockayne syndrome

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Introduction: Cockayne syndrome (CS) is a rare recessive disorder characterized by developmental delay, severe growth failure, photosensitivity, pigmentary retinal degeneration and/or cataracts.CS is progressive. There are two major subtypes: (1) with classical findings and (2) a severe form.Type 2 is also referred as cerebrooculofacial-skeletal [COFS] syndrome because of similar clinical findings with CS.Premature aging is the major feature of both types. CS is linked to mutations in CSB/ERCC6 and CSA/ERCC8 genes. Here we report two cases of CS syndrome and discuss results of molecular testing. Case 1 and 2:Both patients were referred because of microcephaly and congenital cataract.They both presented beaked nose, enophthalmia and contractures at joints.Both were not able to sit without support, to walk or talk. Clinical findings were more severe for case 2. She had early detected, severe contractural joints. Cranial MRI showed cortical atrophy and dilatated lateral ventricles.Sequencing with 17 genes, revealed a novel homozygous nucleotide variation in the ERCC8 (CSA) gene c.300C>G (exon 4)(p.Tyr100*), in case 1 and a homozygous nucleotide variation in the ERCC6 (CSB) gene c.25551T>A (exon 13) (p.Trp851Arg), in case 2. **Conclusions:**In people with CS, DNA damage is not repaired normally. Due to DNA errors, cells malfunction and eventually die. It is been suspected by researchers that DNA errors contribute to the several features of CS.There is yet no genotype-phenptype correlation in CS.It is unclear how *ERCC6* or *ERCC8* gene mutations cause all features of this condition.More cases with molecular testing are needed to clarify the issue.

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P11.038B

Application of exome sequencing to reverse dysmorphology: case report of one patient with pathogenic variant in *HDAC8*

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Introduction: The application of New Generation Sequencing is enabling the delineation of new syndromes that have less distinctive clinical features by a process that can be termed "reverse dysmorphology." Here, the classical approach of identifying the phenotype first and analyzing the genotype second is reversed.

The Cornelia de Lange syndrome (CDLS) is a multisystem malformation syndrome recognized primarily on the basis of characteristic facial dysmorphism, in association with mental retardation and, in many cases, upper limb anomalies. However, there is wide clinical variability in this disorder, with milder phenotypes that may be difficult to ascertain on the basis of physical features. Up to now there are five different types of CDLS and 19 genes related to them. Here we describe a patient with multiple dysmorphisms and a previous clinical diagnosis of Ohdo Syndrome that underwent exome sequencing.

Methods: A 6 years old female patient with IUGR, neonatal seizures, cardiac malformation, horse-shoe kidney, developmental delay, hypothyroidism and bilateral deafness had her exome sequenced in the Illumina™ platform and analysis performed with acess through the PhenoDb website.

Results: A rare missense variant was found in exon 4 of *HDAC8* (c.C356T; p.T119M) with a SIFT score of 0 and a GERP score of 4.89.

Conclusions: This new approach allows the recognition of common clinical features that were initially too subtle or too variable to enable a new syndrome to be identified solely on clinical grounds. This approach may be particularly valuable for the investigation of disorders with high locus heterogeneity, like CDLS.

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P11.039C

Familial gastroschisis in Greenland

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Background. Gastroschisis is an abdominal wall defect located laterally to a normally closed umbilical ring with herniation of uncovered organs. The cause of gastroshisis is unknown. Greenland is the world's largest island, but 80% is covered by an ice cap, and it has a small populations of around 56.000 peoples (2016). We have studied gastroschisis in Greenland during the 27 years (1989–2015). We found 28 cases. The prevalence was 10.7 per 10.000 liveborn-and stillborn infants, the highest described so far. **Material and Methods:** We have used two registries to create extended pedigrees of the 28 infants.1. CPR registry (Civil Registration System). 2. The Parish registers. **Result**. Two families had family occurrence. **Family 1**. The ancestor was a man born in Europe in 1779. In Greenland and had four children with a local woman. One, a boy born in 1819, had two descendent with gastroschisis, born in 1996 and 2015. **Family 2**. The ancestor was a man born in Europe in 1769. He came to Greenland and had seven children with a local woman. One of their son's was married twice. His descendent with the first wife had a girl with gastroschisis born in 1990, and with the second wife three gastroschisis infants born in 2006, 2007 and 2013 respectively. **Conclusion**. Six infants out of 28 (21%) born with gastroschisis in Greenland in the period 1989–2015 belongs to two families. Familial factors may represent exposure to a similar environmental factor, a genetic factor, or a combination of the two.

M. Bugge: None.

P11.040D

Mutation spectrum of Fanconi anemia associated genes in eleven patients from Turkey

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Introduction: Fanconi anemia (FA) is phenotypically heterogenous, rare multigenic chromosomal instability syndrome that predisposes children and adults to life-threatening bone marrow failure, congenital malformations and cancer predisposition. Presently, 18 autosomal and one X linked genes are held responsible from >85% of the disease. *FANCA* mutations accommodate the highest share with 60–70% in genetically heterogeneous pie. Materials

and Methods: 11 patients, with FA clinic are included into this investigation. Targeted panel-gene test is designed to cover all the coding exons and exon-intron boundaries of 17 FA associated genes. Genetic analysis is performed on Ion Torrent PGM platform by next generation sequencing (NGS), and detected mutations and/or alterations considered to be pathogenic are verified by Sanger. Mutation un-identified patients and patients carrying heterozygous pathogenic variants are further tested by MLPA for deletion/duplication in *FANCA*, *FANCD2*, *FANCN* (*PALB2*) and *RAD51C* genes. **Results:** four known mutations in five alleles (c.[2638C>T];[2638C>T], c.[3492C>T];[3163C>T], c.3754G>T), and three *novel* variants in five alleles (c.[2938G>C];[2938G>C], c.[3026G>A];[3026G>A], c.776C>G) in *FANCA*, and a *novel* variant in two alleles (c.[2890G>A];[2890G>A]) in *FANCM* are identified. **Conclusions:** Two patients with single heterozygous alteration and four patients with un-identified pathogenic variant by NGS are presently subject to MLPA testing. Comprehensive results will be acknowledged at the poster presentation upon completion of the algorithmic testing procedures. We hope to put forward mutation frequencies of FA genes in our population.

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P11.041A

Fanconi anaemia in South African patients with Afrikaner ancestry

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Introduction: Fanconi anemia (FA) is a rare genetic disorder of impaired DNA repair resulting in physical and hematological abnormalities in affected individuals. In South Africa, individuals with Afrikaner ancestry are at an increased risk of inheriting disease causing FA mutations, owing to the three common *FANCA* founder mutations present in this population subgroup. **Methods:** We evaluated the physical phenotype of seven patients with compound heterozygous and homozygous *FANCA* founder mutations and one patient with confirmed *FANCA* complementation analysis using a hospital-file based review and a comprehensive clinical examination. We compared the South African Afrikaner phenotype to other FA cohorts, including a previously clinically characterized South African Black FA cohort. **Results:** The results indicate a significantly earlier age of diagnosis of FA in Afrikaner patients compared to Black patients (3 years vs. 7 years 8 months), a high frequency of somatic anomalies (with high average IFAR and PHENOS scores) and higher than expected incidence of the VACTERL/H phenotype (25%). **Conclusion:** Based on our findings, recommendations for the care of FA patients with Afrikaner ancestry in South Africa are made including renal ultrasonography and hearing tests.

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P11.042B

Screenning Fanconi anemia assosiated genes with next generation sequencing technology in FA patients

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Introduction: FA is recessively inherited chromosomal instability syndrome characterized by bone marrow failure, congenital malformations and cancer predisposition. Pathogenic variants of *FANCA* are attributed to 60–70%, while *BRIP1* is associated in approximately 2% of the FA patients.

Material and method: Targeted gene panel is design to cover all the coding exons of 17 FA genes plus flanking exon-intron regions up to10 bp. Genetic analysis is performed on Ion Torrent PGM platform, and detected mutations and/or alterations considered to be pathogenic are verified by Sanger and screened in family for segregation with inheritance pattern.

Results: 6 out of 14 alleles found to carry mutations. Two different homozygous mutations (c.894-2A>G and c.4261-2A>C) in *FANCA* and one compound heterozygous [(c.205+5G>T)+(c.761_764delAGCA)]mutations in *BRIP1* gene are identified, accounting to three DEB positive patients.

Conclusion & Discussion: 72 different mutations of *BRIP1* gene are known and out of 14 are involved in germ line bi-allelic FA.c.761_764delAGCA identified in our study is novel and attained to be the second deletion that is

reported in the context of FA, striking the DEAD-2 domain of the encoding protein that is different than the former (c.2255_2256delAA), damaging the HELIC domain.

The results of the limited number of individuals tested in this study, reveals that the DEB positivity assures the definitive diagnosis and if the DEB test is ignored, mutation detection rate is calculated to be 42%, when presently known FA genes are analyzed, in which that the 28% holds for *FANCA* and the 14% holds for *BRIP1* associated mutations.

G. Bagirova: None.

P11.043C

First Turkish Patient with Feingold Syndrome Type 2 with severe motor mental retardation, epilepsy

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Feingold syndrome type 2 which was first described by Pontual et al. in 2011 is caused by germline heterozygous deletions of *MIR17H (*13q31.3). To our knowledge, until today 11 affected patients has been described. Feingold syndrome-1 is an autosomal dominant syndrome characterised by short stature, microcephaly, brachymesophalangy, brachysyndactyly of the toes, oesophageal and duodenal atresias and intellectual disability. It is caused by mutations in or deletions of *MYCN* (2p24.1). Manifestations common to both Feingold syndrome-1 and Feingold syndrome-2 include microcephaly, short stature, and brachymesophalangy; but those with Feingold syndrome-2 lack gastrointestinal atresias. Here we describe the first Turkish patient with micocephaly, short stature, upslanting palpebral fissures, epicanthal folds, broad nasal bridge anteverted nostrils, absence of eyebrows-eyelashes, brachydactyly, 5th finger clinodactyly, hypoplastic nails, single palmar crease, proximally placed thumps, deafness, patent foramen ovale, motormental retardation and epilepsy. He was operated for Hirschsprung's disease in infancy. Sequence analysis of *MYCN* was normal. Comparative genomic hybridisation array testing confirmed a 22.9 Mb deletion in chromosome region 13q21.33-q.31.33 corresponding to the *MIR17HG* gene. Herein we present a new patient with large deletion and additionally. Different from the other patients Our patient has severe mental retardation and epilepsy.

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P11.044D

Use of CGH-array in paraffin-tissue samples of fetus with congenital defects

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Introduction: In fetuses with one or more CD (congenital defect) and with normal karyotype, the application of CGH-array, can diagnose chromosomal alterations in an additional 10–15% of cases. This technique requires little quantity of DNA and it can be obtained from non-viable cells, this is important in the study of abortions because the rate of karyotype failure is very high. Post-mortem study of fetus with CD is needed to provide relevant information for diagnosis and reproductive counseling to the family. Materials and methods: Forty-four fetus were selected for CGH analysis and classified according to type of CD. The best preserved blocks of paraffin-tissue were chosen and processed for genomic DNA ($n = 40$). The array that has been selected for the study is the Cytochip Focus Constitutional (Cambridge BlueGnome^{TM}). In 4 cases, the DNA could not be used because it was degraded. **Results:** The result was normal in 90% of cases (36/40), and pathological in the remaining 10% (4/40). In 3 cases the chromosomal alteration is considered causative, with a clear phenotype-genotype correlation. In one case, the chromosomal alteration is associated with two locus of genetic susceptibility for neurodevelopmental alteration. In the post-mortem study of fetuses with CD, the use of new diagnostic techniques, such as comparative genomic hybridization, allows the detection of 10% of chromosomal imbalances not diagnosed with conventional techniques. On the other hand, the exhaustive study of the phenotype of these patients, it is necessary for the correct interpretation of the array data.

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P11.045A

Identification of new genes responsible for syndromic developmental abnormalities using whole exome sequencing

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Introduction : Multiple Congenital Anomalies (MCA) are defined by the association of at least 2 malformations. In fetuses, the diagnostic rate in MCA is about 30%. From 2014 to 2015, over 12 new Mendelian disease genes were published per month mainly due to the use of WES, however about 1600 phenotypes described in OMIM still have an unknown molecular basis. Since WES has not been fully evaluated in fetopathology, we aimed to assess the contribution of WES to diagnose and identify new genes responsible for fetal MCA.

Material and methods : We recruited 100 polymalformed fetuses from 10 prenatal diagnosis centers in France and performed WES with the solo strategy. We first performed a targeted analysis of known OMIM disease-causing genes and then extended the analysis to other genes in the negative cases.

Results : To date 46 fetal DNA have been sequenced. A diagnosis has been done in 13 known genes in 15 fetuses (33%), these diagnosis were aspecific, atypical or extreme. 3 candidate genes are suspected in 4 fetuses (9%), 2 of them are from recessive inheritance (one homozygous null variation in a consaguineous family, one compound heterozygous variation in a case of intrafamilial recurrence and one X-linked variation), 2 variations of unknown significance have been identified in 2 fetuses in recently described genes.

Conclusion : WES is a powerful tool to diagnose fetal MCA, to extend our knowledge of the phenotype spectrum of known disease-causing gene, and to discover new disease-causing genes with international collaborations through data-sharing.

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P11.046B

A dual molecular diagnosis: two syndromes in one case?

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Background Frank-Ter Haar syndrome (FTHS; OMIM 249420) and opsismodysplasia (OPSMD; OMIM 258480) are two distinct skeletal dysplasias with some overlapping dysmorphic features. FTHS is a rare, autosomal recessive condition characterized by multiple skeletal abnormalities, dismorphic facial features, congenital heart defects and macrocornea caused by mutations in *SH3PXD2B*. Opsismodysplasia is an uncommon skeletal dysplasia presented by short stature, characteristic craniofacial abnormalities, skeletal anomalies and susceptibility to respiratory infections caused by mutations in the *INPPL1* gene. Here, we describe a case of multiple congenital anomalies with likely pathogenic homozygous mutations in both *SH3PXD2B* and *INPPL1* identified by whole exome sequencing (WES). **Clinical Report** 12-day-old baby who was the child of consanguineous parents was referred to our genetics clinic because of her facial dysmorphic features including prominent forehead, flat occiput, micrognathia, exophthalmos, hypertelorism, downslanting palpebral fissures, flat nasal bridge, nuchal edema and multiple congenital malformations (pes equinovarus and atrioventricular septal defect). Chromosome analysis showed normal karyotype. Since her phenotypic features were consistent with Robinow syndrome, *ROR2* and *WNT5A* genes were screened and no mutation was found. WES was carried out for further genetic analysis and a homozygous frameshift mutation (c.969delG; p.G323fs) in *SH3PXD2B* and a novel homozygous missense mutation (c.G1636A; p.V546I) in *INPPL1* were determined. Unfortunately, the patient was deceased because of recurrent respiratory infections before her radiological examinations were completed. **Conclusion** The identified homozygous variants in this case suggest that perturbed *SH3PXD2B* is responsible for dismorphic features and, heart defect while *INPPL1* variants may cause nuchal edema and recurrent respiratory infections.

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P11.047C

Clinical delineation of a subtype of frontonasal dysplasia with creased nasal dorsum and limb anomalies: report of six novel cases and review of the literature

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Introduction: Frontonasal dysplasias (FND) are rare congenital malformations of the frontonasal process-derived structures, characterized by median cleft, nasal anomalies, hypertelorism, and cranium bifidum occultum. Several entities of syndromic FND have been described, among which, to date, only the craniofrontonasal syndrome (CFNS, MIM 304110), ALX-related FND (MIM 136760, 613451, 613456), and acromelic frontonasal dysostosis (AFND, MIM 603671) have identified molecular bases. **Materials and methods**: We clinically ascertained a cohort of 124 individuals referred for isolated or syndromic FND. **Results**: We identified six individuals (five males and one female) with a very similar and easily recognizable phenotype. Facial features were remarkable by nasal deformity with creased dorsum and absent tip, hypertelorism, narrow palpebral fissures and downturned corners of the mouth. All had normal psychomotor development. In addition, unilateral transverse upper limb agenesis, brachydactyly and widely spaced nipples were observed in one individual each. Interestingly, one patient had an unaffected monozygotic twin. We identified five reports in the literature of cases presenting with the same facial phenotype, associated with transverse limb agenesis, Poland anomaly and clinodactyly. **Conclusion**: We report six novel sporadic cases of a specific FND entity associating recognizable facial features, limb defects, and normal intelligence. The identification of discordant monozygotic twins, as well as the negativity of the whole exome sequencing approaches in various FND, supports the hypothesis of a mosaic disorder. Although previous cases had been reported, this is the first series of patients, allowing delineation of this syndrome and paving the way toward the identification of its molecular etiology.

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P11.048D

A novel *ITPR1* deletion in a consanguineous Egyptian family with Gillespie syndrome

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Introduction: Gillespie Syndrome (GS, OMIM entry: #206700) is a rare congenital autosomal disorder characterized by partial or complete aniridia, hypotonia, cerebellar hypoplasia, ataxia and mild to moderate developmental delay. GS has recently been associated with mutations of *ITPR1* gene, encoding an intracellular inositol 1,4,5-triphosphate-responsive calcium channel involved in mediating calcium release from the endoplasmic reticulum.

Materials and methods: WES was performed on the five members of an Egyptian family recruited at the Pediatric Highly Intensive Care Unit of Policlinico Hospital in Milan, composed of consanguineous healthy parents, two GS-affected siblings and an unaffected son.

Results: we identified a novel *ITPR1* variant, the c.278_279+2delACGT, present in both the alleles of the two GS-affected siblings. Their parents were carriers, whilst it was absent in the unaffected son. The c.278_279+2del affects the last two 3′-nucleotides of the exon 5 (AC) and the two first 5′-bases of the respective intron (GT), abolishing the exonic splice donor site. Using functional *in-silico* prediction we proposed the likely disease-causing mechanism of the c.278_279+2del. The analysis revealed that the presence of this variant may lead to the retention of the intron 5 in the mature ITPR1 mRNA, with the generation of a premature STOP codon and the production of a non-functional protein.

Conclusions: these results corroborate the role of the homozygous recessive variant c.278_279+2delACGT as the underlying genetic defect for Gillespie syndrome in this family, further extending the spectrum of the *ITPR1* pathogenetic variants.

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P11.049A

Bi-allelic loss-of-function variants in *HACE1* lead to a rare autosomal recessive syndromic developmental disorder with emerging metabolic features

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HACE1, a previously reported tumour suppressor gene, encodes a HECT domain– and ankyrin repeat-containing E3 ubiquitin protein ligase that is expressed in the brain and involved in neuronal and photoreceptor differentiation and cerebellar development. Two recent publications suggest that bi-allelic loss-of-function mutations in *HACE1* is a cause of a new autosomal recessive neurodevelopmental disorder without cancer disposition.

We report a non-consanguineous family originating from Pakistan with two boys affected with severe global developmental delay, intellectual disability, hypotonia, bilateral cryptorchidism, inner retinal dystrophy, strabismus and morbid obesity with hyperphagia and abnormal thyroid function. Whole exome sequencing identified homozygosity for *HACE1* nonsense variants c.805C>T, p.Arg269* in the two siblings and confirmed their unaffected parents as being heterozygous.

This variant has previously been reported in one of four families with *HACE1* bi-allelic variants from the UK DDD study cohort. This report and one other published report of two other families show that bi-allelic loss-of-function variants in *HACE1* are associated with a severe syndromic developmental disorder characterised by global developmental delay, intellectual disability, ataxia, hypotonia, dystonia, spasticity, brain atrophy, hypoplasia of corpus callosum, epilepsy, hypoplastic genitalia, ocular abnormalities, sensorineural deafness, obesity and skeletal features. All reported variants are in either the ankryn repeat domain or in the catalytic HECT domain, predicted to result in a truncated protein lacking the catalytic HECT domain. Our seventh family provides further evidence that *HACE1* is an important neurodevelopmental gene with multi-systemic effects, including metabolic features of morbid obesity and abnormal thyroid function that merit further future investigation.

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P11.050B

Novel *TP73L* variants in two patients with Ankyloblepharon-Ectodermal Defects-Cleft Lip/Palate (AEC) Syndrome

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Introduction: Ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) syndrome, also known as Hay-Wells syndrome, is a very rare ectodermal dysplasia autosomal dominant genetic disorder characterized by Ankyloblepharon Filiforme Adnatum (AFA), abnormalities of ectodermal tissues (skin, hair, nails, teeth and sweat glands) and cleft lip and/or palate. Mutations in *TP73L* gene, member of the p53 family of transcription factors are responsible for the syndrome.

Materials and Methods: We report two new cases with AES syndrome. Case 1: A 9-months-old male presented with previously repaired bilateral ankyloblepharon, lack of lacrimal ducts, cleft soft palate and ectodermal dysplasia findings including onychodystrophy, hypodontia and sparse, fair hair. Morphological features such as mid-face hypoplasia, micrognathia and low set ears were obvious. Psychomotor development was normal. Case 2: A 14-years-old boy presented with features of severe ectodermal dysplasia such as total alopecia, absent eyebrows and eyelashes, dry – eczematoid skin, hypohidrosis, hypodontia and dysplastic nails. The patient also had lacrimal ducts atresia and urogenital abnormalities with dysplastic right kidney. Ankyloblepharon and cleft lip/cleft palate were surgically repaired during neonatal period and childhood respectively.

Results: In case 1, a novel, de novo, heterozygous TP73L:C.951C>A missense pathogenic variant in exon 7 was identified by sequencing. In case 2, a novel, de novo, heterozygous TP73L:c.1751T>G pathogenic variant was detected in exon 14 by sequencing.

Conclusion: The identification of novel mutations in rare genetic disorders such as AEC syndrome is crucial and must be reported because it allows more precise genotype-phenotype correlation of these conditions. A multi-disciplinary follow-up of these patients is important.

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P11.051C

Extensive skin manifestations in a girl with a severe ´HDAC8-phenotype´

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HDAC8-gene had been implicated in a cohesin-complex disorder Cornelia de Lange syndrome (CdLS) as the fifth known gene (CdLS5). It is an X-linked gene and the skewed inactivation contributes to the great variability of clinical symptomatology in females. The authors refer on a 4,5 y-old girl with notable alopecia areata and extensive pigmentary mosaicism, which were, apart from the cardinal features like severe developmental delay (no speech, walking since 26 mo.), short stature and microcephaly (-4SD, -4,3SD), heart defect (ASD I, II, AV-canal, MI) and bilateral hearing loss (40–60%), the most striking clinical sings. The girl did not really have a typical "CdLS-face" and the limbs showed no major anomalies. A heterozygous de novo stop mutation in *HDAC8*-Gene had been identified by WES and confirmed by Sanger sequencing, the X-inactivation ratio was 30:70. The personality of the child was very friendly, cooperating. Other anamnestic details consistent with CdLS(5) involved delayed closing of fontanelles, lacrimal duct obstruction and small birth size. The areas of alopecia involved a large portion of scalp, the hair was brittle and the remarkable whorl-streaky lines of skin-hyperpigmentations covered the trunk, neck and limbs. The pigmentary mosaicism had been by CdLS5 mentioned just one time as a minor feature (Kaiser et al, 2014), the areas of scalp-alopecia not yet and these features are also not in the Omim clinical-synopsis. Thus it is important to think of a *HDAC8*-mutation by girls with mosaic pigment and integument changes together with variable growth and developmental delay.

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P11.052D

A novel homozygous mutation in MMP21 is associated with heterotaxia & cardiac defects

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Introduction: Heterotaxia is a rare congenital autosomal recessive defect in which the major visceral organs are distributed abnormally within the chest and abdomen. The mixed organ orientation found in situs ambiguus often leads to severe complications mainly of the heart and/or lungs. We have characterized a consanguineous Bedouin family from Israel with 2 affected siblings with heterotaxia and complicated heart defects and detected a novel MMP21 mutation.

Materials and Methods: Genotyping of both patients and their parents was performed using Affymetrix microarrays and kinSNP analysis. Regions of homozygosity were identified. Exome sequencing was performed on 1 affected DNA.

Results: Exome sequencing revealed 50 potential variations within the homozygous regions. Following bioinformatics predictions, segregation in the family and analysis of prevalence in the Bedouin population, one candidate remained - a homozygous variation in the MMP21 gene that is involved in the breakdown of extracellular matrix for normal physiological processes, such as embryonic development, reproduction, and tissue remodeling. The variation causes an amino acid change that is predicted to be highly damaging.

Conclusions: The novel MMP21 mutation presented here, reinforce the role of this gene that only recently has been described with association to heterotaxia and is in line with an ENU-induced mutant mice with LR asymmetry and major cardiac defects. Further studies are required to verify whether this gene is a candidate for complicated heart defects without LR asymmetry.

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P11.053A

HIRA, a candidate gene for the neurodevelopmental phenotype of 22q11 deletion syndrome

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The 22q11.2 deletion syndrome (22q11DS) is a neurocristopathy associated with a wide phenotypic spectrum. Candidate genes, located in the 3 Mb typically deleted region (TDR), such as TBX1, have been proposed to explain part of the features. However, to date none of them explains the entire phenotype and the cause of the neurodevelopmental problems remains speculative. Here we report a 28kb intragenic deletion in the HIRA (Tuple1) gene, which is located in the TDR, in a 5-year-old female patient with features highly suggestive of 22q11DS. To our knowledge this is the first description of an intragenic deletion of the HIRA gene. This gene is highly expressed in neural crests and encodes a histone chaperone involved in histone H3.3 deposition in nucleosomes and thereby, plays roles in the regulation of gene expression. The similarities between the phenotype of the present case and the classical 22q11.2 deletion syndrome strongly suggest that this gene could be a candidate for the neurodevelopmental phenotype and the dysmorphic traits of the 22q11.2 deletion syndrome. To explore the implication of HIRA in the neurodevelopmental phenotype of 22q11DS we are performing gene silencing using shRNA on murine primary neuronal cultures to assess the impact on neuronal morphology and synaptogenesis. We are also studying the expression of HIRA in murine primary neuronal cultures at different times of embryonic and postnatal development using RT-qPCR and Western-Blot in order to evaluate the pattern of brain expression. We will present our final results at the ESHG meeting.

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P11.054B

Homozygous deletion in RECQL4 caused by the rare identical haplotype

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Introduction: RECQL4 is a responsible gene for three diseases; Baller-Gerold syndrome, RAPADILINO syndrome, and Rothmund-Thomson syndrome. Those are autosomal recessive diseases.

Materials and Methods: Patient was born as the second child from healthy and nonconsanguineous parents. She has short limb, absent radius, absent thumb, clubfoot, scoliosis, high-arched palate and corpus callosum hypoplasia.

Results: Homozygous deletion in RECQL4 was detected by whole exome sequence. Deletion size was about 1.5kb. Homo SNPs were detected in about 1.3Mb region around the deletion. Therefore, we build the following hypothesis, homozygous deletion caused segmental isodisomy or identical haplotype. To test this hypothesis, PCR analysis using primer pair for region including the deletion performed in parents. Two bands correspond to a normal allele and a deletion allele were detected in both her father and mother. This result showed that identical haplotype derived from parents caused homozygous deletion in RECQL4. SNP array analysis did not indicate that there was a close blood relationship between her father and mother. Same haplotype were not detected in our in-house data consist of 257 samples.

Conclusions: Homozygous deletion in RECQL4 caused by the rare identical haplotype. Her parents born in a relatively small area. It is possible that the haplotype only presents in the area and her father is distantly related to mother.

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P11.055C

Effects of PAX9 and MSX1 gene variants to hypodontia, tooth size and the type of congenitally missing teeth

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Introducton: Tooth agenesis, affecting up to 20% of human population, is one of the most common congenital disorder. The most frequent form of tooth agenesis is known as hypodontia, which is characterized by the absence of one to five permanent teeth excluding third molars. It was considered that hypodontia is especially related with gene mutations which play role in tooth formation. Additionally mutations in *PAX9* and/or *MSX1* have been identified as the defects responsible for missing permanent molars and second premolars. In some studies it was also found that *PAX9* and *MSX1* gene mutations may change tooth size. Therefore in this study all of these factors were investigated.

Materials and Methods: Thirty one patients and 30 controls were enrolled to the study. Information about tooth sizes and type of congenitally missing teeth were collected. *MSX1* and *PAX9* gene mutations were investigated by direct sequencing. Results were evaluated statistically.

Results: Twenty two variations were detected in *PAX9* in which 18 of them are novel. In addition, 7 variations were found in *MSX1* in which 5 of them are novel and one of them lead to amino acid change. Statistically significant relations were found between detected variations and tooth sizes. Any relation between mutations and type of congenitally missing teeth were not detected.

Conclusion: Especially new mutations which may cause hypodontia, effect tooth size and type of congenitally missing teeth, should be investigated with other researchers for clarifying the mechanism.

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P11.057A

Inverted duplication deletion of 8p : about 3 French cases

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Array-CGH is a technique used for genetic testing of individuals with unexplained intellectual disability and multiple congenital anomalies; it offers a diagnostic yield between 15%-20%. Inverted duplication deletion of the short arm of

chromosome 8 (inv dup del (8p)) is a rare cytogenetic disorder (less than 1/10.000) characterized by a wide range of clinical features and cognitive impairment that depend on the size of the deleted and duplicated regions. The underlying mechanism is complex and explains the variability in the size of the identified chromosomal abnormalities.

We report here 3 cases with 8p inverted duplication deletion and various manifestations. The first case is a fetus presenting with antenatal hygroma colli for which array CGH revealed a 6.8Mb deletion and 31.8Mb duplication of chromosome 8p. The foetopathologic exam revealed olfactory bulb agenesis and right ventricular hypertrophy. The second case was newborn diagnosed with agenesis of the corpus callosum, microcephaly and feeding difficulties. The array-CGH revealed a deletion of 7.6Mb in 8p23 associated with a 25.8Mb duplication. The third case is a 15 years old male with moderate intellectual disability, lower limb acrocyanosis, livedo and normal MRI. Array-CGH identified a 6.8Mb terminal deletion and a 5.7Mb interstitial duplication. For each case, we explain cytogenetic mechanism underlying the origin of these chromosomal abnormalities.

Our three cases illustrate the large clinical variability associated with inv dup del (8p) and highlight the correlation between the size of the duplicated region and severity of the phenotype.

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P11.058B

Clinical spectrum of Kabuki-like syndrome caused by HNRNPK haploinsufficiency. Case report and literature review

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Kabuki syndrome is a genetically heterogeneous disorder characterized by postnatal growth retardation, skeletal abnormalities, intellectual disability, facial dysmorphisms and a variable range of organ malformations. In approximately 30% of affected individuals, the underlying genetic defect remains unknown. A small number of inactivating heterozygous HNRNPK mutations has recently been reported to be associated with a condition partially overlapping or suggestive of Kabuki syndrome. Here, we report on an 11 year-old girl with a complex phenotype in whom the diagnosis of KS was suggested but molecular testing for the known causative disease genes was negative. Whole exome sequencing identified a previously undescribed de novo truncating mutation in HNRNPK as the molecular defect underlying the trait. Analysis of available records of patients with HNRNPK haploinsufficiency was performed to delineate the associated clinical phenotype and outline their distinguishing features in comparison with the KS clinical spectrum. The clinical profile associated with inactivating HNRNPK mutations supports the idea that the associated disorder should be considered as a distinct nosologic entity clinically related to KS, and that the condition should be considered in differential diagnosis with KS, in particular in subjects exhibiting brain malformation (nodular heterotopia), craniosynostosis, and polydactyly.

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P11.059C

A systematic analysis of missense variants in *KMT2D* in Kabuki syndrome and cancer

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Introduction: KMT2D, a H3K4 methyltransferase, is a large protein of 5,537 amino acids. Germline loss-of-function *KMT2D* mutations cause Kabuki syndrome (KS). Similar to a number of other developmental genes, somatic *KMT2D* mutations are detected in many cancers. Missense variants (MV) can help understand protein structure and function, but determining their clinical significance can be challenging. We present a systematic comparison between *KMT2D* MV in KS and cancers.

Methods: From various sources, we compiled *KMT2D* MV with information regarding their frequencies, evolutionary conservation, thermodynamic properties and protein-interaction disruption when available.

Results: We identified 1,535 germline MV in unaffected controls (Control-MV), 201 in individuals with KS (KS-

MV) and 584 somatic MVs detected in cancers (Cancers-MV).

When compared with Control-MV, KS-MV and Cancers-MV were significantly enriched in the PHD3, PHD4, RING4, FYRC, and SET domains, and for only KS-MV in the FYRN domain.

Enrichment of only KS-MV was observed in residues between 4,995–5,090 and between 5,328–5,396; and of only Cancers-MV between 3,043–3,248. These regions are not part of any known functional domains.

No significant enrichment of either KS-MV or Cancers-MV was detected in RING1, PHD1, PHD2, PHD5, SPPPEPEA region, HMG Box, Coiled coils, LXLL motifs and Post-SET domains.

KS-MV and Cancers-MV affect more conserved residues.

KS-MV, but not Cancers-MV, increase the folding/interaction energy of KMT2D.

Conclusions: This analysis will assist in the interpretation of *KMT2D* MV in cancer and patients with KS. Identification of regions of the protein with enrichment of disease MV provides novel insights into functionally important areas of the protein.

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P11.060D

KAT6A Syndrome: The results of an international collaborative study of phenotype and genotype

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Through next generation sequencing studies, pathological germline KAT6A mutations are emerging as a cause of approximately 1% undiagnosed syndromic intellectual disability.

We have collected data from 43 cases (25 unpublished) through international collaboration, the DDD study and the patient support group established by a parent of an affected child, more than doubling the number of reported patients and providing a unique opportunity to review the phenotype and natural history together with mutation details.

Key features of KAT6A syndrome include a variable degree of intellectual disability, hypotonia, expressive speech delay, early feeding difficulties, reflux, constipation and craniofacial dysmorphism including a thin tented upper lip and a widened nasal tip. Newly identified features include behavioural difficulties and sensory issues that overlap traits seen in autistic spectrum disorder. We also report on the high frequency of recurrent infections and several patients have proven immune or haematological dysfunction. We expand our knowledge of previously reported features including the mechanisms of speech delay and gastrointestinal dysfunction, the prevalence and type of associated congenital cardiac defects and the high prevalence of visual problems including strabismus.

KAT6A encodes an enzyme involved in post translational protein modification via acetylation. It thus regulates the transcription of many other genes and cellular processes. Our data suggests that the severity of the phenotype varies according to the site and type of mutation. The KAT6A phenotype show striking overlap with the 22q11 Di George

phenotype and also the phenotypes associated with KAT6B mutations and other disorders of histone modification.

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P11.062B

Novel truncating recessive mutations in KLHL7 gene causing Bohring-Opitz like syndrome

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Biallelic mutations in *KLHL7* gene were recently reported in four families associated with Crisponi/cold-induced sweating syndrome type 1-like syndrome (CS/CISS1). CS/CISS1 characterized by extremities abnormalities, feeding difficulties, inconstant palate defects and respiratory difficulties. Here, we report on six cases from four families, carrying autosomal recessive truncating variations in *KLHL7*. None of them had received a clinical diagnosis of CS/CISS1. They presented with microcephaly (6/6), facial dysmorphism including prominent eyes (X/6), flammeus naevus (5/6) and brain abnormalities (6/6). These individuals presented with fixed contractures and distinctive posture of elbows and wrist called "BOS posture", suggestive of a Bohring-Opitz syndrome (BOS). Evolution was marked by severe feeding problems, seizures and pulmonary infections. BOS is caused by heterozygous mutation in the *ASXL1* gene encoding for chromatin-binding protein. *KLHL7* encodes a BTB-kelch protein implicated in cellular cycle and protein degradation by ubiquitin-proteasome pathway. Additionally, KLHL7 may be implicated in the regulation of gene expression by its interaction with the Polycomb Repressive Complex 1 (PRC1). PRC1 is a multisubunit complex interacting with ASXL1, that is able to recognize several epigenetic marks and enables the ubiquitylation of the lysine 119 of the histone H2A, in order to block the repressive state of the chromatin and inactivate gene expression. In conclusion, we expend the clinical spectrum of *KLHL7* autosomal recessive variations, responsible for CS/CISS1 and a new syndrome with features overlapping the BOS.

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P11.063C

Knobloch syndrome - a rare but distinct phenotype - two patients with novel mutations in *COL18A1* and review of the literature

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Knobloch syndrome is an autosomal recessive condition with mainly ocular manifestations and usually normal intelligence. Additionally to the severe ocular phenotype an occipital encephalocele and occipital scalp defects have been described as a specific feature. Since the first clinical report in 1971 by Knobloch et al. more than 60 patients with Knobloch syndrome have been reported. In 1996 the *COL18A1* gene was identified to cause Knobloch syndrome. Yet the clinical diagnosis remains challenging, especially in patients lacking encephalocele. Due to this, in the last years mutations in *COL18A1* gene were found using modern sequencing techniques, leading to expanded knowledge on the phenotype of Knobloch syndrome with additional neurological features, such as malformations of the brain cortex and intellectual impairment.

We report on two siblings in a non-consanguineous German family with typical ocular findings (myopia magna, nystagmus, macular hypoplasia, defects of the retinal pigment epithelium, retinal detachment, posterior subscapular lens opacity) and occipital meningocele in one patient as well as occipital area of alopecia in the other patient. The findings lead to the clinical diagnosis of Knobloch syndrome, which was confirmed by Sanger sequencing of the *COL18A1* gene, showing compound heterozygosity for two novel mutations (a frameshift mutation c.1727_1740dupGGAGGGACGGCACC and a splice site mutation c.3618+1 G>T) in the index patient, confirming the clinical diagnosis of Knobloch syndrome. The parents were shown to be heterozygous for each *COL18A1* mutation.

In addition we review the literature, presenting the clinical and molecular findings of all 64 patients with Knobloch syndrome described up to date.

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P11.064D

HOXC4 gene is possibly responsible for Lin-Gettig syndrome

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Lin-Gettig syndrome, described by Lin and Gettig in 1990, is a very rare autosomal recessive disease. The syndrome is characterized by craniosynostosis, severe mental retardation, absence of corpus callosum, dysmorphic facial features, camptodactyly and hypogonadism. The molecular etiology of the syndrome has not yet been identified yet. In this report, we present a patient diagnosed as Lin-Gettig syndrome via clinical findings. Molecular genetic studies have revealed that HOXC4 may be the responsible gene responsible for this syndrome. Due to motor-mental retardation and abnormal facial features a 15-month-old boy was referred to our department for genetic conselling and differantial diagnosis. On physical examination, his weight, height and head circumference were measured to be 9.4kg (10^{th}-25^{th} centile), 74cm (3th-10^{th} centile), 43cm (<3th centile), respectively. He had microcephaly and trigonocepohaly, proptosis, downslanting palpebral fissures, midface hypoplasia, depressed nasal bridge, short columella, micrognatia and low-set dysplastic ears. His genital examination showed micropenis, bifid scrotum and cryptorchidism. Craniosynostosis was diagnosed using 3D computerized tomography. Brain magnetic resonance imaging (MRI) revealed a Chiari I malformation. Exome sequencing of the proband showed a homozygous c. 410C>G (p. P137R) mutation in HOXC4 gene. The parents carried this mutation heterozygously. It has been considered a mutations in HOXC4 gene is the most probable candidate responsible for the underlying molecular etiology in the syndrome. This is the first study in the literature defining a gene considered to be responsible for Lin-Gettig syndrome.

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P11.065A

Lissencephaly, osteopenia, and recurrent bone fractures caused by a novel *de novo* CUT2 domain mutation of the *SATB2* gene

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Introduction: The proband is a 19-month-old Chinese boy who presented with global developmental delay, epilepsy,

teeth anomalies and severe osteopenia with recurrent fractures. MRI brain showed pachygyria, lissencephaly, white matter hypoplasia and partial agenesis of corpus callosum.

Materials and Methods: This case was enrolled in our Undiagnosed Diseases Program for ending diagnostic odyssey. We initiated genetic analysis for neurodevelopmental impairment using whole-exome sequencing (WES).

Results: WES revealed a novel heterozygous missense variant in *SATB2* gene, NM_001172509.1:c.1555G>A (p.Glu519Lys) which is predicted to be probably damaging (PolyPhen-2) and deleterious (SIFT). The mutation was not detected in the parents. No pathogenic variants were detected in other neurodevelopmental genes. The history of recurrent fractures also raises the suspicion of osteogenesis imperfecta (OI). Through WES, no pathogenic variant in all known OI-causing genes was found.

Conclusions: This is the first report describing *SATB2*-associated lissencephaly. The cardinal features of *SATB2*-associated syndrome (SAS) include developmental delay/intellectual disability (ID), craniofacial abnormalities, dysmorphic features, behaviour problems and palatal/teeth anomalies. Recently, *SATB2*-associated bone abnormalities had been reported and is potentially treatable by osteoclast inhibitors. SATB2 is a Special AT-rich Binding protein 2 which regulates transcription and remodels chromatin by binding to specific genomic matrix. This gene is vital for craniofacial development and bone regeneration. *SATB2* haploinsufficiency is now regarded as a common cause for syndromic ID. *SATB2* is highly expressed in cortical neurons which regulates migration of neuronal axons in the corpus callosum. The p.Glu519Lys, locating in the CUT2 domain, probably interfere the SATB2 mobility and result to lissencephaly-pachygyria spectrum.

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P11.066B

Acute treatment decision based on whole exome sequencing result

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Case report A term boy presented with icterus due to a prenatal intracerebral haemorrhage, cardiac malformation (VSD, ASD and overriding aorta) and congenital cutaneous vascular malformations. Besides telecanthus, no major dysmorphic features were seen. Lymphangioendotheliomatosis (skin biopsy) and closed lip schizencephaly (MRI-brain) were identified. Whole exome sequencing (WES) was performed. Just before WES results were known, the patient presented with proptosis due to an intraorbital venous malformation and several cerebral venous malformations. Treatment with corticosteroids or rapamycin was considered. Methods WES of the patient, the father and egg donor was performed. Horizontal coverage was 90.9% at 30x read depth. Variants identified in the index fitting a de novo, autosomal recessive or X-linked inheritance were analysed. Results A de novo probably pathogenic missense variant in the TEK-gene was identified (c.3361G>A; p.(Glu1121Lys)). Discussion The tyrosine kinase receptor (TEK) is located in the vascular endothelium and plays an important role in blood vessel formation and cardiac development. Patients with activating missense mutations in this gene have been reported to have cutaneous and mucous membrane venous malformations. Also ventricular septum defects have been described in some patients with a germline TEK mutation. Although our patient has different cutaneous vascular malformations, the TEK mutation is very probable the cause of his disease. Supported by good results in mice and patients with somatic TEK mutations, treatment was started with rapamycin. Six months after initiation of therapy the cutaneous malformations were unchanged, but the intracerebral malformations were significantly decreased in size.

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P11.067C

Next generation sequencing identifies missense mutations in *EPHB4* causing autosomal dominant generalised lymphatic dysplasia

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Generalised lymphatic dysplasia (GLD) is a rare form of primary lymphoedema characterised by widespread oedema including systemic or visceral involvement. The lymphatic dysfunction often presents prenatally as non-immune hydrops fetalis. Three autosomal recessive forms of GLD have been reported previously which suggest high genetic heterogeneity. The aim of this study was to investigate what appear to be an autosomal dominant form of GLD.

We studied two multigenerational families with a heterogeneous and variable phenotype. Both presented a large number of *in utero* and neonatal deaths associated with non-

immune hydrops fetalis. Whole-exome sequencing (WES) and Sanger sequencing analysis identified novel missense variants in the gene encoding Ephrin receptor B4 (*EPHB4*), known to play an important role in lymphangiogenesis. The two mutations occur at highly conserved residues located in the tyrosine kinase domain of the EPHB4 protein and are predicted to compromise protein function. Site-directed mutagenesis was performed to generate expression constructs for the mutant proteins and the phosphorylation activity was analysed by western blot after transient transfection in HEK293 cells. Mutated proteins showed lack of phosphorylation compared to wild-type protein indicating that these novel mutations in the *EPHB4* gene inactivate the tyrosine kinase receptor.

Therefore, mutations in EPHB4 can cause a new form of GLD with a high mortality. This also suggests not only is GLD genetically heterogeneous, but it should also be considered in an autosomal dominant form. This work was funded by British Heart Foundation (SP/13/5/30288) and Newlife Foundation for Disabled Children (12-13/01).

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P11.068D

Marshall-Smith syndrome in a newborn boy presenting with respiratory distress and micrognathia

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Introduction: Marshall-Smith syndrome is a very rare congenital malformation syndrome with less than 100 cases reported to date. The patients usually show characteristic facial features, a dysharmonic bone maturation with advanced bone age, respiratory difficulties due to upper airway obstruction and a moderate to severe developmental delay. Heterozygous mutations and deletions in the nuclear factor 1/X gene (NFIX) are the only known cause for Marshall-Smith syndrome. Case report: We report a newborn boy with the initial diagnosis of Pierre-Robin sequence. He was referred to the neonatal intensive care unit with acute airway obstruction for a treatment with an orthodontic palate plate (Tuebingen soft palate plate). In addition to his respiratory problems and micrognathia he presented with a long face, proptosis and a short nose with anteverted nares. Hands and feet were large with very long fingers and toes. A radiograph of a hand showed a very advanced bone age with a mean of 2 years. The suspected diagnosis of Marshall-Smith syndrome was supported by a *de novo* heterozygous missense variation in the *NFIX* gene (c.955G>C). The orthodontic palate plate proved to be a very satisfactory treatment for the child's airway obstruction. Conclusion: Very young patients with milder forms of Marshall-Smith syndrome might be misdiagnosed as isolated Pierre-Robin sequence because the prevalent clinical features, airway obstruction and micrognathia, are similar in both entities.

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P11.069A

Question mark ear as a clue for loss of *MEF2C* function in patients with intellectual deficiency

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Auriculocondylar syndrome (ACS, OMIM # 602483 and # 614669) is a rare disorder of the first and second pharyngeal arches (PAs), combining mandibular hypoplasia, condylar abnormalities and a distinctive anomaly of the pinnae described as a question mark ear (QME). Isolated QME (OMIM # 612798) has also been reported. The disease-causing genes act within the endothelin 1 (EDN1)-endothelin receptor type A (EDNRA) signaling pathway, and include phospholipase C, beta 4 (*PLCB4*), guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 (*GNAI3*) and endothelin 1 (*EDN1*). Here we report 2 patients with a unilateral or bilateral QME, developmental delay and intellectual deficiency. This combination is highly suggestive of *MEF2C* loss-of-

function (deletion encompassing *MEF2C* in one case and a frameshift mutation in the other, NM_002397.4, c.146dup, p.Asn49Lysfs*29). Indeed, on the one hand, heterozygous deletions encompassing *MEF2C* or loss-of-function mutations have been identified in patients presenting congenital encephalopathy with intellectual disability, severe hypotonia, poor communication and stereotypic hand movements with or without epilepsy and non-specific brain anomalies. On the other hand *Mef2c* is essential for development of the craniofacial skeleton in mice and zebrafish and is a transcriptional target of endothelin signaling in neural crest cells. The penetrance of QME in patients with *MEF2C* haploinsufficiency can be estimated at 4% (2/54). It is thus a rare but highly distinctive feature among patients with severe encephalopathy. Thus, mutations in *EDN1*, *PLCB4*, *GNAI3* and *MEF2C* disrupt the endothelin pathway and lead to ACS or QME in humans.

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P11.070B

Expanding genotype and phenotype of MEIS2-related neurocristopathy

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Loss of function variants in *MEIS* genes may be linked to neurocristopathies as MEIS proteins are involved in neural crest cell development. *MEIS2* intragenic gene variants have been described in individuals with congenital heart defects, cleft palate, dysmorphic features, autism, developmental delay, and gastroesophageal reflux disorder (GERD). A similar presentation is seen in 15q14 deletion syndrome, a multi-gene deletion that includes *MEIS2*. Fourteen patients with *MEIS2* aberrations from twelve families have been reported. Aberrations include multi-gene deletions, an intragenic single exon deletion, an intragenic duplication, an in-frame deletion and a nonsense variant. We present a patient with a de novo germline frameshift *MEIS2* variant and previously undescribed clinical features.

Our patient has developmental delays and dysmorphic features including bifid uvula and possible submucosal cleft palate. Echocardiogram is normal. He had cryptorchidism, inguinal hernia and meatal stenosis. He also has precocious adrenarche with a slightly advanced bone age, mild vitamin D deficiency, hyperlipidemia and episodes of GERD. He developed iris nevus/melanocytosis at 7 years of age.

Trio whole exome sequencing was performed through an institutional review board approved research study at the Institute for Genomic Medicine of Columbia University.

A de novo frameshift variant (c.868dupA, p.Ile290AsnfsTer40) in *MEIS2* was identified in our patient. To date, frameshift variants in MEIS2 have not been reported.

The phenotype of individuals with *MEIS2* variants has not been completely delineated. Additional features will be identified as more patients are reported. This case expands both phenotype and range of molecular aberrations for this rare, recently described neurocristopathy.

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P11.071C

ARCN1 haploinsuficiency causes a microcephalic dwarfism due to COPI-mediated transport defects

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We report on 4 patients in 3 independent families with a newly described autosomal dominant form of microcephalic dwarfism, associated with micrognatia, shortening of forearms and legs and mild intellectual delay. Exome

sequencing revealed heterozygous nonsense and frameshift mutations in *ARCN1*, leading to gene haploinsufficiency.

ARCN1 encodes the subunit delta of the heptameric coat protein I (COPI), a complex required for retrograde transport of proteins and lipids from the Golgi apparatus to the endoplasmic reticulum (ER). Down-regulation of the gene in cell lines or in patient's primary fibroblasts resulted in overexpression of stress response genes such as *ATF4*, *DDIT3* and *HSPA5*, cytosolic accumulation of BiP/GRP78 and reduced secretion of type I collagen, suggesting the involvement of ER stress response and transport defects in the pathogenesis of this disorder.

Our findings demonstrate the importance of COPI-mediated transport in human development, including skeletogenesis and brain growth.

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P11.072D

MIRAGE syndrome - a new form of syndromic adrenal hypoplasia caused by a novel heterozygous activating mutation p.Leu641Pro in the SAMD9 Gene in mosaic form

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We report a 14 months old Austrian boy with a features of syndromic adrenal hypoplasia. He was born at $32+0^{th}$ pregnancy week per caesarean section because of prenatal growth retardation and Oligohydramnios. The infant presented with birth weight of 996 g (-2.25 s.d.) and birth length 39 cm (-1.44 s.d.), OFC 26 cm (-2.55 s.d.), penoscrotal hypospadia and APGAR 6/7/8. He developed respiratory and catecholamine-dependent circulatory insufficiency. After administration of cortisol it took a turn to immense clinical improvement. and by ultrasound an adrenal hypoplasia was diagnosed. Causative genes for syndromic adrenal hypoplasia as DAX1 and CDKN1C were without finding of any germline mutation. At the age of 1 month he presented disturbed myelopoesis with thrombocytopenia and anemia. Since birth he was treated for recurrent infections (more than 16 times) and for the chronic watery diarrhea with severe wound healing perianal and chronic ulcers. At the age of 10 month we performed Exome sequencing and during the analysis we investigated the first information about a novel multisystem disorder named MIRAGE (Myeolodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes, Enteropathy) published in May 2016 by *Narumi at al*. Considering this we supposed a germline mutation in the SAMD9 gene. The Exom-analysis revealed a heterozygous Mutation p.Leu641Pro in mosaic form (22% pathologic cell line). It has probably evolved postzygotic and arised *de novo* as all previously published 11 Corian and Japanese cases by *Narumi* et al. We here, to our best knowledge, present the first report about European infant with MIRAGE Syndrome.

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P11.073A

The case of double diagnosis - *MKRN3*-related central precocious puberty diagnosed after *CHD8*-related autism spectrum disorder in a father and his daughter

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Central precocious puberty syndrome (CPP) is a rare condition characterized by premature activation of the hypothalamic-pituitary-gonadal axis resulting in an early development of secondary sexual characteristics. Loss-of-function mutations in the imprinted MKRN3 gene are found to be the most frequent cause of familial CPP affecting both girls and boys. Because the maternal allele of MKRN3 gene is normally silenced, the disease manifests only in the case of paternal transmission. Here, we present a case of familial CPP in combination with autosomal dominant CHD8 gene-related syndrome.

The proband is a girl first referred to genetic counselling at 2.5 years of age due to mild intellectual disability, autistic

behaviour, epilepsy, macrocephaly, dysmorphic features and atrial septal defect. Her father and younger brother have similar developmental problems and macrocephaly. NGS analysis (TruSight One panel) revealed a heterozygous mutation c.2423_2424del (p.Arg808Lysfs*12) in the CHD8 gene. Mutation appeared de novo in father and was detected in the brother.

At the age of 4.5 years, the breast growth started in the proband. Menarche occurred at 6.5 years. As the precocious puberty is not typical for CHD8 gene mutations, reanalysis of NGS data was performed and a heterozygous mutation c.326G>A (p.Cys109Tyr) in MKRN3 was found. Familial segregation analysis showed that both proband and her father inherited the MKRN3 gene mutation from their fathers. In addition pituitary microadenoma was visualised in MRI scan.

In conclusion, in the case of atypical symptoms of genetic disorder, additional genetic testing or data reanalysis should be performed to test for double diagnosis.

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P11.074B

Mosaic tetrasomy 8p associated with mild phenotype

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Introduction: Tetrasomy 8p is a rare chromosomal disorder generally detected in mosaic form, with 15 cases reported so far. The phenotype is characterized by agenesis of corpus callosum (ACC), enlarged cerebral ventricles, congenital heart defects (CHD), mild facial dysmorphisms, rib and vertebral abnormalities, and mild to moderate developmental delay. Here we report on a 4.5-year old girl with mosaic tetrasomy 8p that came to our attention due to mild facial dysmorphia and failure to thrive.

Results: She had moderate global developmental delay since birth. She started to walk at 21 months of age and used few words with meaning at 3.5 years. Failure to thrive due to feeding difficulties was first noticed at one year. Clinical examination showed mild facial dysmorphia (high forehead, hypoplastic malar region, narrow palpebral fissures, wide nasal root, high palate, micrognathia), scoliosis and hypotonia. Extensive evaluation including neuroimaging, ultrasound examination of heart and kidneys and skeletal X-rays didn't show additional anomalies. Cytogenetic analysis revealed mosaic karyotype: 47,XX,i(8)(p10)[18]/46,XX [82]. The isochromosome identity was confirmed and further characterized by chromosomal microarray.

Conclusion: Most cases of tetrasomy 8p described so far had at least one congenital anomaly, such as CHD, ACC, rib and/or vertebral anomalies. Still, 3 patients, including our patient, showed only minor facial anomalies, hypotonia, and developmental delay, affecting mainly gross motor skills, speech and language development. Our patient supports the observation that mosaic tetrasomy 8p can present with mild clinical phenotype and highlights the potential of missing the diagnosis that might be more common than previously assumed.

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P11.075C

Identification of a denovo novel missense mutation in ZEB2 gene in a Turkish boy with Mowat Wilson Syndrome

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Mowat-Wilson syndrome (MWS) is a multiple congenital anomaly syndrome characterized by moderate or severe intellectual disability, a characteristic facial appearance, microcephaly, epilepsy, agenesis or hypoplasia of the corpus callosum, congenital heart defects, Hirschsprung disease, and urogenital/renal anomalies. It is caused by de novo heterozygous loss of function mutations including nonsense mutations, frameshift mutations, and deletions in ZEB2 at 2q22. A 4 months old boy was referred to genetic counselling because of congenital anomalies and dysmorphic features. He was borned to healthy consanguineous parents. The child had had typical facial findings; square shaped face with a prominent but narrow triangular chin, hypertelorism, deep set but large eyes, broad nasal bridge, saddle nose, prominent, rounded nasal tip, open mouth, full or everted lower lip, posteriorly rotated ears, and large uplifted ear lobes with a central depression. Further, there was no evidence of dysmorphic findings in any relative. A mutation analysis of *ZEB2* gene was requested. The results revealed a previously unpublished sequence variant a missense c.1660_1664delATAAA in a heterozygous state. As a consequence of this sequence variation, a reading code shift occurs that generates stop codon 13 amino acids downstream of the deletion, predicting the translation of a truncated protein. Sequencing of *ZEB2* gene of both parents was performed, but the variant was not found, confirming a denovo event.Thus, clinical data of this family were

presented by moderate or mild form of the disease. Fuctional studies and more reports are required to understand for the affect of the mutation and genotype-phenotype correlation.

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P11.076D

Mowat-Wilson syndrome in Polish patients

R. Smigiel[1], M. Badura-Stronka[2], K. Szczałuba[3], A. Jakubiak[4], M. Wi□niewska[2], E. Obersztyn[5], A. Kutkowska-Ka□mierczak[5], T. Chilarska[6], J. Pilch[7], M. Kugaudo[8], K. Wicher[9], A. Jakubiuk-Tomaszuk[10], J. Wierzba[11], G. Bugaj[2], A. Latos-Biele□ska[2], M. Sasiadek[4], M. Krajewska-Walasek[12]

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800×600 Normal 0 21 false false false PL X-NONE X-NONE MicrosoftInternetExplorer4 /* Style Definitions */ table.MsoNormalTable {mso-style-name:Standardowy; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:10.0pt; font-family:"Times New Roman",serif;} Mowat-Wilson syndrome (MWS) is a rare multisystem congenital disorder. Incidence of MWS is currently unknown, estimated birth incidence is 50000–100000. MWS is caused by mutations in gene *ZEB2* or deletions in region 2q22-23. Facial dysmorphy in MWS is very characteristic and it changes with age. Among congenital anomalies most often appears: Hirschsprung disease, heart defects, brain anomalies, genitourinary system anomalies and eye abnormalities. Developmental delay is present, with subsequent intellectual disability. Language develop tardily, and usually is restricted to few words. Behavioral phenotype is similar to Angelman syndrome. We report on clinical and genetic findings of 18 Polish patients with diagnosis of MWS. All presented patients have classical facial phenotype (uplifted ear lobe with central depression, pointed chin and prognathism in elders), severe developmental defects, intellectual disability and at least one congenital anomaly. Language development was impaired, older patients do not develop expressive language. Hirschsprung disease was present in 3 patients (it suggests that MWS is underdiagnosed in Polish patients with Hirschsprung disease), 85% patients - heart defect, 55% - brain abnormalities. 45% patients - urogenital or renal anomalies. All male patients had cryptorchidism and only two of them had hypospadias. We observe genotype-phenotype correction between point mutation and exons deletion of *ZEB2* gene and severity of phenotype.

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P11.077A

Smith-Kingsmore Syndrome: four new cases, clinical and molecular review, and testing recommendations

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Introduction Smith-Kinsgmore (SKS) is a rare macrocephaly/intellectual disability/epilepsy syndrome caused by *mTOR* germline mutations. We add four new patients to the 24 previously reported and review the syndrome.

Material and methods Two unrelated patients and two siblings were assessed and studied by deep coverage NGS designed to study somatic mosaic syndromes, since they were originally referred with clinical diagnosis of Macrocephaly-Capillary Malformation —caused by somatic mosaic *PIK3CA* mutations— or Macrocephaly.

Results Major diagnostic criteria for SKS are intellectual disability (24/26), macrocephaly/megalencephaly or hemimegalencephaly (25/28) and seizures (18/23). Nevertheless,

some less frequent features reported for SKS are also present in other PI3K-AKT-mTOR syndromes. In our study, one patient showed the common mTOR p.Glu1799Lys variant —present in 13/28 patients—. The variant p. Phe2202Cys was detected in two siblings, and was not present in their parents, thus gonadal mosaicism is suspected —described before in other 3 families—. Last patient showed the p.Cys1483Tyr variant in mosaic (2% blood, 11% saliva, 32% skin), so it cannot be strictly considered a case of SKS caused by germline mutations. No clear genotype-phenotype correlation was found.

Conclusions SKS must be suspected in patients with intellectual disability, macrocephaly/megalencephaly or hemimegalencephaly, and seizures. *mTOR* germline mutations can be detected by Sanger sequencing; however, due to the great variety of syndromes with highly overlapping clinical features which comprise the differential diagnosis in SKS, as well as to the presence of both germ and low somatic mosaic mutations, we recommend performing deep coverage NGS for the detection of possible mosaic variants.

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P11.078B

New intragenic rearrangements in non-Finnish MULIBREY nanism

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Prenatal growth is a complex dynamic process controlled by a variety of genetic and environmental factors. Among genetic syndromes characterized by growth restriction, MULIBREY nanism represents a rare autosomal recessive condition presenting with severe pre-and post-natal growth failure, characteristic dysmorphic features but normal neurological development. The phenotype of MULIBRREY nanism is variable and overlaps to such as the Silver Russell syndrome. We report here three patients in two distinct non-finnish families who were first clinically suspected to have Silver-Russell syndrome. Molecular analyses did not confirm a Silver-Russell syndrome. Clinical features in the three patients led us to also consider the diagnosis of MULIBREY nanism. Sequencing of the TRIM37 gene showed the same novel nonsense mutation (c.181 C>T p. Arg61*) in the three patients in a heterozygous state. We used a quantitative fluorescent multiplex PCR approach to explore potential gene rearrangement in TRIM37 which led to identification of a new deletion of exons 15 and 16 in one isolated patient and of another deletion of exon 9 in two siblings. Breakpoints of both the deletions were characterized and revealed implication of Alu sequences. Given the high number of Alu sequences, which predisposes to gene rearrangements, in introns of TRIM37, one should always consider such genetic mutations in the molecular diagnosis of MULIBREY nanism. The clinical study confirms the usefulness of a precocious and careful cardiologic follow up of the patients, even if they are no sign of heart disease.

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P11.079C

Familiar case of Myhre syndrome

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INTRODUCTION. Myhre syndrome (MS) (*MIM*#139210) is an autosomal dominant disorder mainly characterized by dysmorphic facial features, short stature, skeletal anomalies, muscle pseudohypertrophy, restricted joint mobility, thick skin and variable intelectual disability. It is often sporadic, due to gain-of-function mutations in *SMAD4*. We report on a new patient with suspected maternal origin.

CLINICAL CASE. 22-year-old boy, born to non consanguineous parents. His mother and sister presented with short stature, brachydactyly, mild cognitive impairment, and similar facial phenotype. Uneventful pregnancy, born at term small to gestational age. His childhood was marked by

growth and developmental delay. IQ: 48. Associated congenital anomalies were right renal agenesis and epigastric hernia. Clinical phenotype with macrocephaly (2.18 SD), short stature (-4.6 SD), facial asymmetry, hypertelorism, prognathism, small ears, broad and short neck. Hoarse voice. Brachydactyly with slightly progressive finger and elbow contractures. Brain MRI, echocardiogram, metabolic test, arrayCGH, and Fragile-X molecular analysis were normal. Targeted exome sequencing revealed a c.1498A>G (p.Ile500Val) mutation at SMAD4, consistent with the diagnosis of MS. Familiar study is ongoing.

CONCLUSION. We describe the first familiar case of MS. The index patient presents with a classical phenotype of MS with progressive contractures of fingers as a key feature for its diagnosis. This uncommon disease should be bear in mind in the differential diagnosis of patients with brachydactyly, short stature and macrocephaly. Clinical suspicion allows earlier diagnosis and proper medical follow-up to anticipate possible life-threatening complications such as high blood pressure, pericardial effusion and recurrent infections.

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P11.080D

Myhre syndrome with novel findings: bilateral congenital cortical cataract, bilateral papilledema, accessory nipple and adenoid hypertrophy

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Introduction: Myhre syndrome is a rare autosomal dominant disorder, includes intellectual disability, short stature, muscular appearance, skeletal abnormalities, decreased joint mobility, thickened skin, early-onset deafness of mixed conductive and sensory type and characteristic facial features. This syndrome is caused by *SMAD4* mutations. Here we report a 14-year-old boy with multiple features consistent with Myhre syndrome and novel findings.

Case Report: The 14-year-old boy was referred to our clinic with mild intellectual disability, short stature, cataract, hearing loss. He has short strature, characteristic facial features, adenoid hypertrophy, dry skin, generalized rash, accessory nipple, joint contractures, brachydactyly and fifth finger clinodactyly on both hands. He has also bilateral congenital cortical cataract, bilateral papilledema and hypermetropic astigmatism in eye exam. He operated from right cryptorchidism. He has obsessive-compulsive disorder and attention deficit hyperactivity disorder. His brain MRI shows hyperintense plaque on the right cerebral hemisphere. We identified heterozygosity for an A-to-G transition at position c.1498, which leads to I500V mutation on *SMAD4* gene. This mutation was described before and was associated with Myhre syndrome (HGMD: CM110610).

Discussion: Novel clinical findings in our patient are accessory nipple, bilateral congenital cortical cataract, bilateral papilledema and hypernasal speech because of adenoid hypertrophy. Bilateral cataract was described once as subcapsular type in Myhre syndrome, and it was not indicated as congenital or acquired. We suggest that accessory nipple, bilateral congenital cortical cataract, bilateral papilledema and hypernasal speech because of adenoid hypertrophy may evaluate as minor findings of Myhre syndrome, in addition to main findings.

A. Tatar: None. **H.B. Erdem:** None. **I. Sahin:** None.

P11.081A

PTC124 (Ataluren) treatment of nonsense mutations in *BMP4*

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Pharmacological treatments that impact the morphology of birth defects have not been readily available. Nonsense suppression therapy (NST) utilizes compounds such as PTC124 (Ataluren) to induce translational read-through of stop mutations by promoting the insertion of near cognate aminoacyl tRNAs that yield functional proteins. We used PTC124 to determine if we could rescue the phenotypic defects resulting from stop mutations in *BMP4*. We generated p.Arg198* and p.Glu213* mutations in human *BMP4* cDNA and obtained $bmp4^{st72/+}$ zebrafish. After transfection of 293T/17 cells with wildtype or mutant *BMP4* cDNA and treatment with 0–20μM PTC124, we found a small, non-significant increase in BMP4 expression for both mutations at 20μM measured with an in-cell ELISA assay. We then examined the effects of PTC124 treatment on the $bmp4^{st72}$ nonsense allele in zebrafish. Phenotyping an in-cross of $bmp4^{st72/+}$ *Danio rerio* confirmed the mild to severe tail fin deformation and cloacal defects previously reported. Doses of 0–2 μM PTC124 did not affect Mendelian inheritance. Ventroposterior defects were significantly more frequent in homozygotes compared to other genotypes, consistent with recessive inheritance of the $bmp4^{st72}$ allele. For 1μM PTC124 treatment, there was an increase in homozygous larvae without phenotypes (non-penetrance), but the difference was not statistically significant. Although the

increase in non-penetrance suggests rescue by PTC124 could have occurred, non-specific toxicity was observed, possibly due to the early timepoint at which treatment was commenced. We conclude that the utility of PTC124 is likely to be mutation dependent and that toxicity can occur at relatively low concentrations in *Danio rerio*.

A. Slavotinek: F. Consultant/Advisory Board; Modest; OptumRx. **S. Htun:** None. **M. Krall:** None.

P11.082B

New mutation in NRAS in familial Noonan syndrome

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Introduction: Noonan syndrome (NS) is a heterogeneous developmental disorder associated with variable clinical expression including short stature, congenital heart defect, unusual pectus deformity and typical facial features, is caused by activating mutations in genes involved in the RAS-MAPK signaling pathway. We present a familial case of a NS patient, her affected brother and father with NRAS mutation and NS. Materials and Methods: Comprehensive mutation analysis of NF1, PTPN11, SOS1, CBL, BRAF, RAF1, SHOC2, MAP2K2, MAP2K1, SPRED1, NRAS, HRAS and KRAS was performed using targeted next-generation sequencing. **Results:** Analysis revealed a recurrent mutation in NRAS, c.182A>T (p.Gln61Leu) in the index patient, both father and brother were carriers too. She was assessed by a clinical geneticist at the age of 3 years due to a peculiar facies in a child with a heart murmur. Her height and OFC were at the 20th centile and she presented with a distinctive phenotype suggesting NS: high and broad forehead, hypertelorism, sparse eyebrows, posteriorly rotated ears, curly scalp hair, broad chest, and hyperkeratotic palmar skin. No congenital cardiac anomalies were found on echocardiography. Her affected father and brother exhibited anthropometric characteristics similar to the proband, however the boy was discordant for facial features and had straight hair. Neither of the affected individuals in this family presented with juvenile myelomonocytic leukemia (JMML). The clinical and genetic heterogeneity observed in RASopathies is a challenge for genetic testing. To date, only eight unrelated individuals with NS and three NS families have been identified with mutations in NRAS.

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P11.083C

Rapid mutational analysis of RASopathy related genes in prenatal diagnosis - our first experience

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Introduction: RASopathies represent a group of syndromes (e.g. Noonan, Costello, Cardio-Facio-Cutaneous, Legius and LEOPARD syndrome, neurofibromatosis 1 and 2 etc.) caused by mutations in genes encoding components of the signaling RAS/MAPK pathway. While postnatal symptoms of these conditions are well described, the prenatal features are more difficult to clearly determine. Reported are prenatal cases with indication of suspected RASopathy.

Materials and Methods: Targeted resequencing of 20 selected genes involved in pathogenesis of RASopathies using HaloPlex HS enrichment system (Agilent) followed by massive parallel sequencing was performed on 17 prenatal cases.

Results: We implemented an approach for molecular diagnostic of RASopathies with features required in prenatal setting, i.e. fast turnaround time, limited input amount of DNA, few samples per run and high diagnostic yield (many genes analysed in parallel). In the first group of cases with increased linea nuchea and/or hygroma colli only (7/17; 41,2%) we did not detect any pathological variant. On contrary, in the group with severe ultrasound findings, i.e. increased linea nuchea, hygroma colli and one or more additional features (hydrothorax, polyhydramnion, renal anomalies, cardiac defects, pes equinovarus or polydactyly), a *de novo* mutation was detected in 4 cases (4/17; 23,5%). Diagnostic yield in this group is 40 % (4/10).

Conclusion: In general our findings correspond to previously published studies. With our approach the molecular genetic diagnosis of RASopathies can be performed rapidly and in cost-effective manner with high diagnostic yield. This brings a great benefit into genetic counseling and management of prenatal as well as neonatal period.

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P11.084D

Noonan Syndrome and portosystemic shunt: a rare presentation

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Background: Noonan syndrome is a common autosomal dominant disorder, mainly characterized by short stature, webbed neck, chest deformity, craniofacial dysmorphism and cardiac abnormalities. However, less typical phenotypic findings might entangle the clinical diagnosis. Case report: We describe a female baby, sixth child to non-consanguineous parents. Prenatal sonographic examination demonstrated cystic hygroma, polyhydramnios, right ventricular hypertrophy and suspected portosystemic shunt. The parents refused invasive prenatal testing. Following uneventful delivery, imaging testing confirmed the prenatal findings. At the age of seven weeks postfeeding hyperammonemia was diagnosed, apparently related to circulatory liver bypassing. Postnatal chromosomal microarray analysis yielded normal results. Several dysmorphic signs were noted during examination in our genetics clinic at the age of two months, including hypertelorism, downslanting palpebral fissures, depressed nasal bridge with upturned nares, low posteriorly rotated auricles, low posterior hairline and bilateral transverse line. **Results:** Sequencing of selected RAF1 gene exons was performed, due to its significant correlation with congenital hypertrophic cardiomyopathy in patients with Noonan syndrome. This test revealed a heterozygous c.770C>T heterozygous p.(Ser257Leu) mutation, previously repeatedly reported as variant causative for this disorder. **Conclusions:** Our case provides valuable information to the scarce literature evidence describing the association between RASopathies and portosystemic shunts. This finding suggests that RAF1 mutations should be considered in the differential diagnosis of fetal vascular anomalies (such as portosystemic shunt), particularly when accompanied by characteristic features of Noonan syndrome.

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P11.085A

Okamoto syndrome has considerable similarities with Au-Kline syndrome and caused by *HNRNPK* mutation

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Au-Kline syndrome (OMIM#616580) is a new syndrome due to loss-of-function variants in the heterogeneous nuclear ribonucleoprotein K gene (*HNRNPK*). It is characterized by intellectual disability, distinctive facial dysmorphism and skeletal/connective tissue abnormalities. We report a new patient with a novel *HNRNPK* mutation. The 4-year-old male was the first child of healthy and non-consanguineous Japanese parents. His birth weight was 3,300 g, length was 48 cm, and head circumference was 33.6 cm, at 35 weeks of gestation. Surgical closure for omphalocele was carried out after birth. Cleft palate and bilateral hydronephrosis was noted. He was hypotonic and his development was delayed. He could not creep or stand alone. He spoke no meaningful words, with no cognitive or language perception. He showed severe intellectual disability. Physical examination revealed dysmorphic features including hypertelorism, epicanthal folds, low set ears, flat nasal bridge, high arched palate, macroglossia, and open mouth. He showed joint hyperextensibility and talipes equinovarus. Initially, this patient was considered to have Okamoto syndrome (OMIM604916). Okamoto syndrome is characterized by severe ID, generalized hypotonia, stenosis of the ureteropelvic junction with hydronephrosis, cardiac anomalies and characteristic face. So far, five patients have been reported. Okamoto syndrome has considerable similarities with Au-Kline syndrome. A novel splicing variant (c.1361+1G>A) was found in the patient *HNRNPK* by Sanger sequencing. We suggest that Okamoto syndrome and Au-Kline syndrome is the same syndrome due to *HNRNPK* mutation. The present research protocol was approved by the local institutional board review. Written informed consent was obtained from the parents.

N. Okamoto: None.

P11.086B

Unveiling the heterogeneous molecular basis of Opitz C syndrome

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Introduction: Opitz C syndrome (OTCS, MIM #211750) is an extremely rare genetic disorder characterized by multiple malformations (e.g. trigonocephaly, congenital heart defects), contractures, variable intellectual and psychomotor delay and a high mortality rate. Different patterns of inheritance and genetic heterogeneity have been suggested.

Material and Methods: We studied a cohort of 13 patients (10 unrelated pedigrees) with clear or tentative diagnosis of OTCS. Patients and parents were analysed by means of whole exome sequencing (WES).

Results: We identified the disease-causing mutation in 8 of the 10 families in 8 different genes sharing demonstrated roles in development and cancer. Three of them are still in the final validation stage. All these genes were associated with other diseases with phenotypic similarities to OTCS. For example, *MAGEL2* truncating mutations were shared by one of our OTCS patients and all patients with Schaaf-Yang syndrome reported so far.

Conclusions: WES is a very powerful approach to identify OTCS related mutations, since it was successful in 80% of our cases. Genematcher has been an essential tool to connect with other researchers who identified mutations in the same gene in other patients, a necessary way to solve the cause of extremely rare diseases in the context of a high genetic heterogeneity. Our results point to OTCS as a causally heterogeneous phenotype instead of a specific entity.

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P11.087C

Identification of a denovo novel missense mutation inSPECC1L gene in a Turkish boy with Opitz GBBB Syndrome Type II

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Opitz G/BBB syndrome, is a rare genetic disorder that will affect physical structures along the midline of the body. There are two different forms of Optiz G/BBB syndrome: X-linked (recessive) syndrome (Type I) and dominant autosomal syndrome (Type II). However, both result in common physical deformities. Features of the Opitz GBBB syndrome type II include hypertelorism or telecanthus, laryngotracheoesophageal cleft; clefts of lip, palate and uvula; swallowing difficulty and hoarse cry; genitourinary defects, especially hypospadias in males, mental retardation; developmental delay; and congenital heart defects. It is caused by a heterozygous mutation in the *SPECC1L* gene on chromosome 22q11.23. A 4 months old boy was referred to genetic counselling because of congenital anomalies and dysmorphic features. He was borned to healthy non-consanguineous parents. The child had had typical laryngotracheoesophageal manifestations, swalloving difficulties, hypertelorism, and a broad prominent nasal root and bridge. Further, there was no evidence of dysplasia, respiratory abnormality, or hoarse voice in any relative. A mutation analysis of *SPECC1L* gene was requested. The results revealed a previously unpublished sequence variant a missense c.1382G>A (p.R461Q) in a heterozygous state. In silico analysis (Mutation Taster, SIFT and PolyPhen) predicts this mutation as potentially deleterious. Sequencing of *SPECC1L* gene of both parents was performed, but the variant was not found, confirming a denovo event. Thus, clinical data of this family were presented by moderate or mild form of the disease. Fuctional studies and more reports are required to understand for the affect of the mutation and genotype-phenotype correlation.

K. Karaer: None. **D. Kan Karaer:** None. **T. Bahsi:** None.

P11.088D

New insights in human overgrowth and intellectual disability

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Introduction Human overgrowth (OGS) is mainly characterized by an increase of any combination of the following parameters: height, weight and head circumference above +2sd or 97th-centile of the mean for age, sex and ethnic group. This overgrowth may be accompanied by other clinical features, from which intellectual disability (ID) is one of the most common. Thus, in patients with non-syndromic OGS+ID we decided to perform CNV analysis through SNP-microarrays with the aim of identifies new dosage-altered regions, large rearrangements and LOH.

Material and Methods We have selected a cohort of patients from the Spanish Overgrowth Syndromes Registry. The two initial clinical features from which we selected the

patients were overgrowth and intellectual disability as presumptive diagnostic.

CytoSNP-850K was applied in 151 patients with OGS +ID and the results were analyzed with GenomeStudio-v2011.1.

Results Preliminary results showed a mean of 19-LOH per patient and 19 possible pathogenic CNV: one translocation, two uniparental disomy (one with low mosaicism encompassing the whole short arm of chr11), ten deletions and six duplications that could potentially be related to the phenotype after the in silico analysis. The CNV size ranged from 4,326bp to 44Mb.

Conclusions SNP-microarrays is a powerful tool to study patients with overgrowth and intellectual disability, due to the high positive rate obtained (approximately 12,5%). Twelve CNVs were not previously reported and included a cluster of growth-related and ID genes probably related to the phenotype. Furthermore, SNP-microarrays are also recommended to study UPD, microdeletions and low-level mosaicism undetectable with other similar techniques.

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P11.089A

Phenotype expansion for Kosaki Overgrowth Syndrome

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Skeletal overgrowth is a characteristic of several genetic disorders that are linked to specific molecular signaling cascades. Recently, we established a novel overgrowth syndrome (Kosaki overgrowth syndrome, OMIM #616592) arising from a *de novo* mutation in *PDGFRB*, i.e., p. Pro584Arg. Subsequently, other investigators provided *in vitro* molecular evidence that this specific mutation in the juxtamembrane domain of *PDGFRB* causes an overgrowth phenotype and is the first gain-of-function point mutation of *PDGFRB* to be reported in humans. Here, we report the identification of a *de novo* mutation in *PDGFRB*, p. Trp566Arg, in two unrelated patients with skeletal overgrowth, further confirming the existence of PDGFRB-related overgrowth syndrome arising from mutations in the juxtamembrane domain of *PDGFRB*. A review of all four of these patients with an overgrowth phenotype and *PDGFRB* mutations revealed postnatal skeletal overgrowth, premature aging, cognitive impairment, neurodegeneration, and a prominent connective tissue component to this complex phenotype. From a functional standpoint, hypermorphic mutations in *PDGFRB* lead to Kosaki overgrowth syndrome, infantile myofibromatosis (OMIM #228550), and Penttinen type premature aging syndrome (OMIM #601812), whereas hypomorphic mutations lead to idiopathic basal ganglia calcification (OMIM #615007). In conclusion, a specific class of mutations in *PDGFRB* causes a clinically recognizable syndromic form of skeletal overgrowth.

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P11.090B

Phenotypic overlap of partial trisomy 4q due to a complex maternal chromosome rearrangement with Cornelia de Lange Syndrome

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We report on a 5 year old boy, second child to a healthy non-consanguineous couple. He was born near term. Length, weight and head circumference at birth were in the lower normal range. There was postaxial hexadactyly of the left hand. He showed dysmorphic features with microcephaly (-3,67z), highly arched eyebrows, depressed nasal bridge, long philtrum and a thin upper lip. No structural brain abnormalities in MRI. Psychomotor development was significantly retarded especially concerning language. The clinical picture resembled Cornelia de Lange syndrome.

G-banding showed an unbalanced karyotype with a derivative chromosome 12 and a marker chromosome in all metaphases. FISH-analysis clarified that the marker chromosome was formed of chromosome 12 material originating from an interstitial deletion of the long arm of chromosome 12. At the site of the deletion, the derivative chromosome 12 contained an insertion of chromosome 4 material. As shown by array-CGH, there was trisomy of the chromosomal region 4q24-q28.1 but no visible imbalance of chromosome 12. Karyotyping of the parents was performed and the healthy mother also exhibited the marker and the derivative chromosome 12. Additionally, she

possesses a derivative chromosome 4 with an interstitial deletion 4q resulting in a balanced situation.

Interestingly, the supernumerary marker was mitotically and meiotically stable indicating that there should be a neocentromere. Clinically, our case illustrates, that still a chromosome analysis should be performed first in all syndromic children, even if the phenotype fits with a specific genetic diagnosis.

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P11.091C

A complex homozygous mutation in ABHD12 responsible for PHARC syndrome (Polyneuropathy, Hearing loss, cerebellar Ataxia, Retinis pigmentosa and early-onset Cataract) discovered by NGS

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Introduction: PHARC syndrome (MIM612674) is an autosomal recessive neurodegenerative pathology leading to demyelinating Polyneuropathy, Hearing loss, cerebellar Ataxia, Retinis pigmentosa and early-onset Cataract. These various symptoms can occur at different ages, so that PHARC syndrome can be a differential diagnosis of Charcot-Marie-Tooth disease (CMT) associated with deafness. Only 13 *ABHD12* mutations have been reported in 33 patients. We described the 14th mutation and compared our results to the literature data. Materials and Methods: Genomic DNA was extracted by standard methods. Next Generation Sequencing (NGS) strategy was performed by using a targeted CMT and associated neuropathies 92-gene panel. **Results:** A 36-year old male has suffered from demyelinating sensory and motor polyneuropathy and ataxia since the age of 15. Bilateral sensorineural deafness was diagnosed at the age of five. Bilateral congenital cataracts were operated on at the age of 28. A new large complex homozygous mutation, with one deletion of seven base pairs and one insertion of 38 base pairs, was detected by NGS. In PHARC syndrome, sensorineural deafness always occurs as the first feature in late teens. The ophthalmological symptoms are cataracts that occur at a mean age of 25 yo and then retinis pigmentosa at a mean age of 29. Demyelinating sensory-motor polyneuropathy is the most variable characteristics, which occurs in the thirties. Conclusion: We report the first large complex homozygous mutation in PHARC syndrome, which is certainly underdiagnosed. Therefore, it seems interesting to include *ABHD12* in the panels of the five symptoms, especially deafness ones.

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P11.092D

Ophthalmological features in a patient with Pierpont syndrome

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Pierpont syndrome (OMIM 602342) is a rare disorder characterized by developmental delay, feeding problems, facial dysmorphisms, and palmar and plantar fat pads with deep grooves. In 2016, the mutation c.1337A>G, p.(Tyr446Cys) in *TBL1XR1* (transducin β-like 1 ×-linked receptor 1) was identified in six unrelated patients with Pierpont syndrome.

We report on a 3-year-old boy with clinical signs of Pierpont syndrome. The patient had hypotrophy at birth and prolonged jaundice. Subsequently, he developed feeding problems that required percutaneous endoscopic gastrostomy. He was unable to speak or to stand at the age of 3 years. He also had the typical facial characteristics, microcephaly, short stature, and palmar and plantar fat pads. Ophthalmological examination revealed microphthalmia, pendular nystagmus, and hypermetropia. Using clinical exome sequencing, we identified the *de novo* mutation c.1337A>G, p.(Tyr446Cys) in *TBL1XR1*.

This patient corroborates the assumption that Pierpont syndrome is exclusively caused by this specific *TBL1XR1* missense mutation, and the ophthalmological features broaden the phenotypic spectrum of this rare disorder.

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P11.093A

PIK3CA related overgrowth spectrum (PROS): Molecular diagnosis and phenotype delineation

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Introduction: *PIK3CA* related overgrowth spectrum (PROS) —MCAP, CLOVES, macrodactyly…— is characterized by segmental overgrowth and vascular anomalies. It is caused by somatic mutations in *PIK3CA*. We present recommendations for molecular testing and review the phenotype in 63 patients.

Material and methods: A custom deep coverage NGS panel and a bioinformatic pipeline were designed to test 20 genes related to somatic overgrowth. DNA samples from blood, saliva and/or affected tissue were studied. Variants were validated by Sanger (mosaics >20%), pyrosequencing (between 5–20%) and Droplet Digital PCR (ddPCR, <5%).

Results: We detected 17 different *PIK3CA* mutations in 13/36 MCAP (1 germline), 10/25 CLOVES and 1/2 macrodactyly. Mutations in "pure" CLOVES/macrodactyly are only present in affected tissue, as expected. In MCAP, mosaicism percentage is higher in affected tissue, but low-mosaic mutations can also be found in saliva and blood. In addition to *PIK3CA*, we detected mutations (mosaic and germline) in two other genes included in the PI3K-AKT-mTOR pathway in 4 patients referred as MCAP or PROS. Clinical characteristics are not always consistent and there is quite phenotypic overlap, making the clinical diagnosis tricky in some cases.

Conclusions: Combination of NGS and ddPCR allows detecting mosaics as low as 1%. Testing saliva as first choice avoids unnecessary biopsies in some MCAP cases. Syndromes within PROS are part of a continuous clinical spectrum whose manifestations depend on the time/tissue of the embryonic development in which the mutation arose. This also predicts the future inclusion of more syndromes —not yet associated but belonging— to this group.

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P11.094B

Pitt-Hopkins syndrome identified by trio whole-exome sequencing in a boy with severe intellectual disability, postnatal microcephaly, myopia, scoliosis and congenital abnormalities of the elbow joint

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Pitt-Hopkins syndrome (PTHS, OMIM #610954) is a neurodevelopmental disorder characterized by severe developmental delay, distinctive facial features (including deep-set eyes, a broad nasal base, widely spaced teeth, a wide mouth with a tented, upper lip and fleshy ears), postnatal microcephaly, seizures, constipation and breathing anomalies. It is caused by mutations in the *TCF4* gene on 18q21. Here we report on a six-year-old boy with severe intellectual disability, postnatal microcephaly, distinctive craniofacial features, myopia, astigmatism, scoliosis, obstipation and congenital bilateral radial head subluxation. Genetic testing including conventional cytogenetic analysis, Microarray-CGH and Sanger sequencing and MLPA (multiplex ligation-dependent amplification) of *COH1* was unremarkable. Subsequent trio whole-exome sequencing identified a de novo heterozygous mutation in *TCF4* (c.1459C>T / p.Arg487*) leading to a premature stop codon that has already been described in two other patients with PTHS. Although the patient presented in this report shows clinical features typical for PTHS we missed to make a specific diagnosis. This might be explained by the absence of two characteristic symptoms such as seizures and abnormal breathing patterns. Additionally, and so far we know not yet described as a characteristic feature for PTHS, the patient showed congenital bilateral radial head subluxation. A retrospective image analysis with face2gene suggested PTHS as the most likely diagnose. Therefore, this case supports that computer-assisted facial recognition should be used in the routine diagnostic workflow.

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P11.095C

Two pathogenic heterozygous mutations in *PNPLA6* but which is the syndrome?

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Introduction: Mutations in *PNPLA6* gene results in a wide spectrum of clinical presentations, mostly involving ataxia, neuropathy, chorioretinal dystrophy, hypogonadotropic hypogonadism, hair anomalies, and impaired cognitive functioning. Sometime features can cluster in specific syndromes like those of Boucher-Neuhäuser, Gordon-Holmes, Oliver-McFarlane, Laurence-Moon, and spastic paraplegia type 39.

Materials and methods: We present a female patient with pigmentary retinitis and polyneuropathy of sensory-axonal type, developing between 4th and 6th years of life. At age of 6 y she presented with chronic kidney disease, related to kidney hypoplasia. MRT at age of 10 y showed mild atrophy of the cranial part of cerebellar vermis. She presents no learning disability, no polydactyly, and no obesity till age of 10 y. In her long medical history many diseases have been considered in differential diagnosis, and were exclude by specific methods. Finally because of vermis anomaly, retinitis pigmentosa, ataxia, and neuropathy, NGS-analysis was performed.

Results: NGS of genomic DNA of the patient revealed two pathogenic heterozygous point mutations within intron 5 (c.199-2A>T) and exon 29 (c.3152G>A) of the *PNPLA6* gene, coding neuropathy coding esterase. Both mutations were subsequently confirmed by conventional DNA capillary sequencing.

Conclusions: This clinical case shows the overlapping between many different syndromes, caused by mutations in *PNPLA6* gene, as well as the gradual appearance of symptoms which can mislead to other "closer diagnosis" at certain moment of patient's life.

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P11.096D

Compound 16p12.2 microdeletion and hemizygous missense mutation in *POLR3E* associated with syndromic neurodevelopmental impairment and near-fatal cardiac arrhythmias

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Introduction: Microdeletion at 16p12.2 is associated with developmental delay, sometimes in presence of additional variants. *POLR3E*, located at 16p12.2 has been suggested to be important for phenotypic manifestation but no disease-causing mutation has yet been reported.

Methods and results: We evaluated a newborn boy with natal teeth, bilateral club feet, dysphagia, bilateral cerebral palsy, axial hypotonia and Pierre Robin sequence with obstructive sleep apnea. During the first week of life, cardiac evaluation for respiratory distress showed a Brugada syndrome electrocardiogram without structural abnormalities and with recurrent ventricular arrhythmias requiring extra-corporeal membrane oxygenation. During follow-up, severe myopia, growth retardation and global developmental delay were noted, with ongoing improvement in motor functions and communication abilities. Brain magnetic resonance imaging at 4 months showed a thin corpus callosum, cerebral atrophy and slightly delayed myelination. Array genotyping identified a 16p12.2 microdeletion inherited from a healthy mother. Trio whole-exome sequencing revealed a novel predicted deleterious hemizygous mutation in *POLR3E* (ENST00000299853; c.985A>G; p.Ser329Gly), inherited from a healthy father. Exome aggregation consortium constraint metrics suggest that *POLR3E* is intolerant to double loss-of-function. *POLR3E* codes for a subunit of the RNA polymerase III (Pol-III). Dysregulation of other Pol-III genes is involved in leukodystrophies, sharing some clinical features with the reported case.

Conclusion: We identified a hemizygous *POLR3E* missense mutation in a patient with the 16p12.2 microdeletion which may account for a severe multisystem phenotype. Missense mutations in *POLR3E* may provide a second hit in the 16p12.2 microdeletion syndrome. Additional cases are needed to unequivocally implicate *POLR3E* to disease.

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P11.097A

Unravelling proximal 6q deletions with the help of Facebook

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Introduction: Social media are widely used by parents of children with rare disorders to seek and exchange information and are therefore a powerful source for case-finding. In the Chromosome 6 Project a successful collaboration with a Facebook group was realised. Most chromosome 6 aberrations are extremely rare, thus a world-wide collection of patients is needed to perform genotype-phenotype studies. Here, we describe the results on proximal 6q deletions.

Materials and methods: Parents of children with a deletion within the region 6q11-q16 were approached via the private Chromosome 6 Facebook group and asked to complete an online questionnaire, available in multiple languages, on the phenotype of their child. Descriptive data analysis was performed and for specific features the smallest region of deletion overlap (SRO) was defined.

Results: Data of 16 children from Facebook and 24 literature cases were used to describe the observed phenotypes. Most frequently reported features were hypermobility, hypotonia and developmental delay. In the more proximal deletions (6q11-q14) an SRO for the combination of club feet, hyperlaxity, delayed motor development and umbilical hernia revealed COL12A1 as a candidate gene for the connective tissue disorder-like phenotype. In the more distal deletions (6q14-q16) an SRO for congenital heart defects revealed the candidate gene TBX18, which has been shown to be involved in cardiac development in mice. Further studies are being performed to confirm the role of TBX18 haploinsufficiency in heart defects.

Conclusions: Our study proved that social media is a powerful source for case-finding, resulting in better information to counsel families.

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P11.098B

Family case of pure 6p25.2–24.2 duplication

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Introduction: Distal 6p trisomy is very rare. Most patients have variable clinical findings with duplications that usually range from 6pter to 6p21–6p25. We report on a family case with distal interstitial 6p duplication and distinctive phenotypic features.

Results: Our proband was born small for gestational age with dysmorphic features, hypoplastic left kidney, hypospadia and uretral stenosis. At present he is 16.5-years old, tall and obese. His intellectual functioning is normal. His 37-year old mother has similar phenotype, obesity, and is diagnostically investigated for osteopetrosis. Chromosomal microarray analysis identified pure interstitial duplication 6p25.2-p24.2 in both subjects, confirmed by GTG-banding of chromosomes. Size of the duplicated segment is 8,2 Mb and it contains 31 OMIM genes. Duplications of similar size that partially overlap this region have been described in 3 patients so far. One patient has only mild skeletal anomalies and other two presented with dysmorphia, microcephaly, ocular, cardiac and renal defects, growth impairment and developmental delay.

Conclusion: Presented family with interstitial 6p25.2-p24.2 duplication provides evidence for phenotypic variability in this region. It is of interest to note that the case described in the literature as having only mild skeletal anomalies has inherited the duplication from his mother. This points to the possibility that familial cases of distal duplication 6p tend to have mild phenotype. As intellectual functioning in so far described familial case is normal, there is possibility that the diagnosis is sometimes missed.

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P11.099C

RLIM is a new candidate gene for congenital diaphragmatic hernia

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We describe a three generation Belgian family with three affected males displaying congenital diaphragmatic hernia, polycystic kidneys and sexual ambiguity. Whole exome sequencing revealed a new rare and predicted pathogenic RLIM variant (RLIM:NM_183353:c.G1792A:p.D598N) in 2 second cousins. The clinical phenotype and the variant co-segregate, with a LOD score of 1.8 for linkage to a shared region of 24.4 Mb in the X chromosome. No other shared rare variants are located in this common region. All the female carriers showed a 100% skewed X inactivation. RLIM encodes ring zinc finger protein 12 (RNF12) ubiquitin ligase, which is essential for embryonic development through its involvement in imprinted X-inactivation in mice, and its function as a co-regulator of LIM homeodomain transcription factors. In addition, RNF12 has been shown to regulate TGFbeta and BMP pathways in embryonic stem cells and in zebrafish. These pathways are important for diaphragm and kidney development. We confirmed RLIM expression in diaphragm at the transcriptional and proteic levels. This suggests a possible involvement of this gene in diaphragm genesis. Breeding statistics of RLIM C57bl/6 knockout female with a wild type male indicate a perinatal lethality in KO males. The investigation of the underlying phenotype and the molecular mechanism of this perinatal death is still ongoing.

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P11.100D

Unraveling clinical and genetic heterogeneity in Robinow syndrome

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Robinow syndrome (RS) is a clinical and genetically heterogeneous disorder characterized by mesomelic limb shortening, genital hypoplasia and distinctive facial features. Recessive RS is characterized by severe skeletal defects and is explained by *ROR2* biallelic variants. Autosomal dominant RS has been associated with mutations in *WNT5A, DVL1* and *DVL3* indicating that noncanonical Wnt pathway is perturbed in RS. Though some genotype-phenotype correlations have been proposed the paucity of patients described so far does not allow any firm conclusions. Other genes remain to be identified since several patients do not have mutations in any of these genes. Since Robinow-associated genes have defined roles in non-canonical Wnt pathway, we hypothesized that variants in other genes within the pathway may underlie the 'unsolved' cases. To address this, we recruited 31 unrelated subjects diagnosed with Robinow or Robinow-like phenotypes. Preliminary analysis confirmed that *DVL1*/*DVL3* indels contribute to ~20% of cases. One individual had a *WNT5A* variant. Additionally, variants in *FZD2* gene which has been associated with omodysplasia in one case, contributes to 13% of the DRS cases. Importantly, FZD2 is a relevant protein partner in the WNT5A interactome, supporting its role in skeletal development. In summary, our study illuminates the molecular causes of this rare Mendelian disorder, provides insights relevant to clinical diagnosis and management, and reveals the contribution of genes from the same pathway elucidating locus heterogeneity in Robinow Syndrome. Grant: National Human Genome Research Institute-National Heart, Lung, and Blood Institute grant U54 HG006542 to Baylor Hopkins Center for Mendelian Genomics.

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P11.101A

Clinical utility of next-generation sequencing in the recognition of well-known syndromes with atypical phenotypes

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Introduction: Diagnosis of patients with non-specific intellectual disability (ID) presents challenges in daily clinical practice. In the absence of specific diagnostic/clinical markers, whole-exome sequencing (WES) is the method of choice for the detection of gene mutations responsible for ID.

Material and methods: The study was conducted on a cohort of 21 families (63 individuals) with a child presenting with sporadic ID (with or without additional minor physical and neurological/metabolic abnormalities), and comprised integrated clinical data, WES and the use of bioinformatics tools like PhenIX2 that utilize Human Phenotype Ontology.

Results: Among 21 examined patients, an etiological diagnosis was made in 8; in 3 of them unexpectedly some "old" syndromes were detected, such as Rubinstein-Taybi 2, Wiedemann-Steiner, and Cornelia de Lange 2 syndromes. These patients presented with ID associated with atypical features not suggestive of a known monogenic syndrome. Re-evaluation of these patients confirmed that the diagnosis remained difficult on clinical grounds. In five others, Glass, Spastic Paraplegia 50, Bosch-Boonstra-Schaaf Optic Atrophy, Cerebral Creatine Deficiency 3 and Mental Retardation 31 syndromes were proved.

Conclusions: The introduction of WES led not only to the description of new syndromes, but also to the recognition of well-known syndromes with atypical phenotypes. Our study emphasized that old syndromes can have a phenotype that is heterogeneous and more variable than originally thought. The use of WES shows that such patients may be more readily diagnosed on the basis of genotype rather than phenotype.

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P11.102B

Expansion of the phenotypic spectrum in Rubinstein-Taybi syndrome associated with novel variants in *EP300* and *CREBBP*

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Introduction: Rubinstein-Taybi syndrome (RSTS) is a rare congenital neurodevelopmental disorder. Clinical diagnosis is complicated by widely varying signs and symptoms. Mutations in two genes, *CREBBP* and *EP300*, have been identified in 55%-78% of RSTS individuals.

Methods and Results: We report the clinical and genetic data of 11 Polish RSTS patients. Causative mutations in 7 patients with a distinct and moderate RSTS phenotype were identified. In addition, in four patients with an obvious RSTS phenotype, no molecular defect was found. Molecular analysis (MLPA, WES) revealed five different *CREBBP* alterations, such as frame-shift (in two cases), nonsense, missense, and a large deletion, as well as nonsense and frame-shift mutations in *EP300*. They were mostly unreported variants (6/7). Four patients with a *CREBBP* mutation presented the classical RSTS phenotype, including specific facial dysmorphy, broad thumbs and big toes, while two patients with an *EP300* mutation were significantly less typical (unspecific facial phenotype, somewhat broader great toes but normal thumbs and mild intellectual disability). As a result of such an atypical phenotype, in one of them Floating-Harbor syndrome was initially considered in the differential diagnosis.

Conclusions: Our 63.6% mutation detection rate in RSTS patients with an either classical or atypical phenotype, supports the concept that RSTS is a genetically heterogeneous disorder and indicates that RSTS may be caused by gene/s other than *CREBBP* or *EP300* in up to 36% of cases. These data confirm the need for expanding the studies on molecular mechanisms leading to unspecific RSTS manifestations.

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P11.103C

Segregation pedigree analysis of t(2,13)(p25.1,q33.1) carriers and description of the resulting partial trisomy 2p and monosomy 13q phenotype in progeny

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Introduction: We present the proposal of genetic counselling for carriers of an unique reciprocal chromosome translocation t(2,13)(p25.1,q33.1). In addition, the phenotype spectrum associated with the chromosomal imbalance in progeny at birth with resulting unbalanced translocation is provided.

Material and Methods: A combination of several cytogenetic techniques, including routine karyotype, FISH, and array CGH [CytoSure ISCA UPD (4×180k), Oxford Gene Technology] were applied. Probability rate estimation for different categories of pregnancy outcomes was studied by direct method of pedigree segregation according to Stene and Stene-Rutkowski. Phenotype analyzes of three affected children were performed according to Munchen Dysmorphology Database (MDDB) methodology.

Results: The common morphological features observed in three affected relatives with monosomy 13q33.1→qter (loss of 13.1Mb) and trisomy 2p25.1→ pter (gain of 7.1Mb) were: microcephaly, long forehead, ptosis, long nasal bridge, short philtrum, retrogenia, skeletal malformations and hypogenitalism. In addition, the affected children presented muscle hypotonia, bleeding disorders, intellectual disability, vision problems, speech delay and hyperactivity. Segregation pedigree analysis of t(2,13)(p25.1,q33.1) carriers allowed to estimate the probability rate of having a child with unbalanced karyotype 13.0±7%, the risk for miscarriage as 39.1±10.2% and for a stillbirth/neonatal death as 4.3±4.2%.

Conclusions: The chromosomal imbalance in the form of monosomy 13q33.1→ qter and trisomy 2p25.1→ pter does not limit survival of the offspring to term. Chromosomal translocation t(2,13)(p25.1,q33.1) carriership is high risk factor for miscarriages and unbalanced progeny at birth.
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P11.104D

A novel familial *SIX1* mutation with incomplete penetrance in Branchio-otic Syndrome

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(* contributed-equally) Introduction: The Branchio-oto-renal spectrum disorders include branchio-oto-renal (BOR) and branchio-otic syndromes (BOS). Main clinical features include otologic, second branchial arch and renal anomalies. Pathogenic variants in three genes [*EYA1*(40%), *SIX5* (2.5%),*SIX1*(2%)] have been reported.

Patient report and Methods: A three-year-old girl was observed to have unilateral cleft lip and palate, anomalies of the second branchial arch, and a right pre-auricular pit. Cardiac, renal, ophthalmology and otorhinolaryngology assessments are normal. She has two paternal cousins with renal problems from childhood.

Sanger sequencing of *SIX1* (NM_005982) identified a frameshift mutation. Site directed mutagenesis was used to generate the identified mutation and other related constructs. MEF3 luciferase reporter assays were performed in MCF7 cells after co-transfecting WT or mutant Six1 and its co-activator Eya2.

Results: A novel heterozygote frameshift variant [c.561_564delGGAG(p.Arg188fsTer63)] was identified in exon 2 of *SIX1* gene. Parental studies showed it was inherited from her healthy father. Although the expression level of wildtype and mutant Six1 were similar, the ability of p.Arg188fsTer63 Six1 to activate transcription in a reporter assay was significantly impaired.

Conclusions: The lack of transcriptional activity of the identified Six1 mutant fits well with the observed phenotype in the patient. However, the lack of phenotype in the father is intriguing, because most families with BOR spectrum appear to have 100% penetrance. We speculate that the father may compensate by overexpressing another Six family member. Our data also argue that the C-term of Six1 is required for its transcriptional activity, although its specific role is still under investigation.

T. Carminho-Rodrigues*: None. **M. Guney*:** None. **B. Carvalho:** None. **L. Zhang:** None. **R. Zhao:** None. **H. Ford*:** None. **R. Oliveira*:** None.

P11.105A

The mutation spectrum of DHCR7 gene and two novel mutations

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Introduction: Smith Lemli Opitz syndrome (SLOS) is a rare autosomal recessive syndrome. It is one of the 46, XY disorder of sexual development. Molecular defects in *DHCR7* gene are responsible for this syndrome. In this study, mutation spectrum of the *DHCR7* gene in SLOS patients has been evaluated. **Material and Methods:** In this study 13 patients carrying mutations in *DHCR7* gene were evaluated. Molecular analysis was performed using Sanger sequencing or next generation sequencing. Clinical and laboratory findings of all patients were obtained from their hospital records. Clinical severity scores of patients were calculated according to Kelley and Hennekam for phenotype-genotype correlation. **Results:** In 13 patients 7 different *DHCR7* gene mutations (4 missense: p.T93M, p. R352W, p.Y432C, p.E448K; 2 nonsense: p.W151X, p. Q259X; and one splice site: IVS7+1) were detected. p. T93M was the most frequent (57% of all allels) mutation. Two of the seven mutations (p.Q259X, IVS7+1) defined for the first time in this study. Conclusion: This study defines the mutation spectrum and genotype phenotype correlation of *DHCR7* gene in the Turkish SLOS patients. As seen in other Mediterranean populations, p.T93M mutation is the most frequent mutation observed in our patients.

E. Isik: None. **H. Onay:** None. **B. Akgun:** None. **T. Atik:** None. **A. Aykut:** None. **A. Durmaz:** None. **M. Dundar:** None. **Y.B. Kurtbay:** None. **E. Mihci:** None. **B. Nur:** None. **M. Elmas:** None. **A.E. Solmaz:** None. **I. Erol:** None. **T. Sahin:** None. **Z. Gokmen:** None. **O. Cogulu:** None. **F. Ozkinay:** None.

P11.106B

Absence of major eye malformations further expands the phenotype of SOX2 deletions

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Introduction: Syndromic microphthalmia-3 (MCOPS3; OMIM 206900) is a rare dominant disorder characterized by anophthalmia or microphthalmia. Iris coloboma and various extraocular abnormalities such as growth retardation, developmental delay, central nervous system malformations, sensorineural deafness, esophageal atresia and genital abnormalities are also involved in the spectrum. Heterozygous point mutations in *SOX2* are responsible from approximately 20% of MCOPS3 phenotype. Widespread use of microarray analysis has increased the detection rate of *SOX2* mutations in up to 40% of cases. In this study, we report on 2 patients with diverse ocular and extraocular findings.

Material and Methods: Patients were screened for copy number variation by Agilent SurePrint G3-CGH 8×60K and 4×180K Human Microarray Kit.

Result: Array CGH analysis revealed *de novo* heterozygous deletions ranging from 12 Mb (3:180,660,194–192,709,474) to 33 kb (3:181,418,149–181,451,562) on chromosome 3q26.33 encompassing the entire *SOX2*. First patient with 12 Mb deletion, the largest deletion reported so far in this region, was a 6 month-old-female. She had iris coloboma, cleft lip/palate, tracheoesophageal fistula,

esophageal and anal atresia, hydrocolpos and pons hypoplasia, of which the latter two had not been reported previously in deletions of this region. Interestingly, anophthalmia or microphthalmia was not present in this patient. The second patient with 33 kb deletion demonstrated anophthalmia, micropenis and cryptorchidism.

Conclusion: This study expands the phenotypic spectrum of *SOX2* abnormalities and provides evidence that complete *SOX2* deletion might not result in major eye abnormalities. Thus, *SOX2* genetic analysis should be considered in patients presenting with ocular and/or extraocular findings consistent with the phenotype.

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P11.107C

Phenotypic spectrum of SPECC1L mutations encompasses Teebi hypertelorism syndrome but not Opitz BBBG syndrome: report of new families and critical review of the literature

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Anomalies of midline craniofacial development are observed in several syndromes that were originally delineated on clinical basis. Opitz BBBG syndrome was associated with mutations in MID1, Baraitser-Winter craniofrontofacial syndrome with mutations in ACTB and ACTG1, and recently, Teebi syndrome with mutations in SPECC1L. SPECC1L plays a role in in gap junctions involved in cell adhesion, in actin cytoskeleton organization, microtubule stabilization and spindle organization, and in cytokinesis. It modulates PI3K-AKT signaling and controls cranial neural crest cells delamination and facial morphogenesis. SPECC1L mutations were first identified in patients with oblique facial clefts (OBLFC1; OMIM 600251). Recently, mutations in SPECC1L were reported in a pedigree reported in 1988 as Opitz syndrome. We report three further pedigrees with SPECC1L syndrome, including a 3-generation family and one fetal case. Our patients confirm that SPECC1L syndrome is clinically similar to Teebi syndrome. Critical phenotypic analysis of previous patients published with SPECC1L and MID1 mutations lead us to consider that SPECC1L syndrome should not be described as "dominant Opitz syndrome" : hypospadias and laryngeal anomalies that characterizes Opitz syndrome are never observed with SPECC1L mutations, whereas branchial anomalies, parietal hernias, uterine anomalies or vascular anomalies characterize SPECC1L mutants.

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P11.108D

Split hand/foot malformation associated with 20p12.1 deletion: a case report

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Introduction: Split hand/foot malformation (SHFM) or ectrodactyly is a rare congenital disorder affecting limb development. It is characterized by clinical and genetic heterogeneity. It is usually inherited as an autosomal dominant trait with incomplete penetrance. Isolated and syndromic forms are described. The extent of the malformations associated with SHFM is highly variable and multiple syndromes with clinical and genetic overlap have been described.

Material and methods: We report here a 28 year-old caucasian man born to unrelated parents who presented with bilateral deep median hand cleft, oligodactyly, right syndactyly and bilateral forearm hypoplasia. Additionally, sparse hair and extended freckles were noted. Physical and systemic examination was normal without facial dysmorphism or intellectual disability.

Results: qPCR analysis of *BHLHA9*, *TP63* Sanger sequencing and next generation sequencing of more than

400 genes implicated in bone disorders did not reveal any significant sequence alteration. By contrast, Array-CGH identified a 450 kb *de novo* 20p12.1 microdeletion encompassing three exons (exon 6 to 8) of *MACROD2*.

Conclusion: *MACROD2* deficiency has not been reported to be associated with limb malformation until now. However, it is located next to *KIF16B*, which is involved in fibroblast growth factor receptor (FGFR) signaling. Additionally, the deletion encompassed a histone modification H3K27ac mark, which provides a quantitative readout of promoter and enhancer activity during human limb development. Altogether, these relationships suggest that the CNV is causative in the present case through a positional effect.

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P11.109A

A case of syndromic split hand/foot malformation type 1 with *de novo* 7q21.3 deletion

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Background: Split hand/foot malformation (SHFM) is a nonsyndromic or syndromic congenital malformation of limb development. A locus in chromosomal region 7q21.3 associated with SHFM is referred to as SHFM1. Case: A 4-year-old patient was monitored since birth because of multiple dysmorphic features and complaints about development delay. A boy is born from the 2nd pregnancy and delivery (polyhydramnios) via C-section, Apgar score 10/10. Phenotype of the newborn: craniofacial dysmorphism, left hand ectrodactyly, syndactyly of I-II, III-IV toes in right foot, bilateral cryptorchidism. Later, mild bilateral conductive hearing loss, severe astigmatism, hypermetropia, unclear hyponasal speech, recurrent respiratory tract infections were observed. He was diagnosed with language and psychomotor developmental delay. 7q21.3 deletion, 985 kb in size (arr[GRCh37] 7q21.3 (96222790_97207764)x1) was identified by SNP-array analysis. *De novo* origin of the deletion was confirmed for the patient by RT-PCR as both parents are not the carriers of the deletion. On recent assessment at 4 years of age, his psychomotor development shows no significant improvement, he has social interaction difficulties and food related obsessions. **Conclusions:** Phenotype and chromosomal rearrangement of presented patient confirm the role of known candidate genes that underlie the pathogenesis of this disorder. The impact of *SHFM1*, *DLX5* and *DLX6* genes is known to have a function in SHFM1. This has been previously described in cases with 7q21.3 deletion. Our results support the idea that imbalances in some genomic regions are associated with variable phenotypes. Further investigation of genetic and environmental modifiers may explain variable expressivity.

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P11.110B

A family with 17p13.3 duplication encompassing *BHLHA9* gene in four generations and with reduced penetrance in females

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Introduction: Duplications in the 17p13.3 chromosome region encompassing *BHLHA9* gene have been associated with split hand/foot malformation with or without tibia hemimelia. It is shown by Klopocki et al (2012) that this duplication serves as a susceptibility factor for a highly variable phenotype with reduced penetrance, particularly in females.

Materials and methods: We are presenting a family with a duplication of *BHLHA9* gene in four generations. There are four affected males with split hand/foot malformation, tibia hemimelia and/or syndactyly. Index patient is a 10-month-old boy with bilateral ectrodactyly. All carrier females were healthy and four of them were investigated. At first, all individuals were investigated with chromosomal microarray analysis (CMA). For some, additional MLPA analysis of *BHLHA9* gene was performed to confirm the finding.

Results: CMA of the index patient showed two small sequential duplications in the region 17p13.3. The first one, ~70-kb in size, included *BHLHA9* gene, but the meaning of the second one, ~95.5-kb in size, remained unclear. Unfortunately, the first pathogenic duplication of *BHLHA9*

gene was not visible on CMA in other investigated affected male and unaffected carrier females. Therefore, additional MLPA analysis was done to three individuals, revealing a heterozygous duplication encompassing the entire gene.

Conclusions: We present an additional family with a duplication of *BHLHA9* gene and non-Mendelian inheritance of split hand/foot malformation characterized by a high degree of non-penetrance with sex bias. The identification of small copy number variations can sometimes be challenging on CMA due to low coverage in some regions.

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P11.111C

STIL compound heterozygous mutations cause microcephaly via centriolar lengthening

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STIL (SCL/TAL1 interrupting locus) is a core component of the centriole duplication process. *STIL* mutations have been associated to both Autosomal Recessive Primary Microcephaly (MCPH) and holoprosencephaly (HPE). In this report, we describe a family with multiple miscarriages and two terminations of pregnancy due to marked fetal microcephaly, delayed cortical gyrification and dysgenesis of corpus callosum. Whole exome sequencing allowed to identify novel compound heterozygous mutations in *STIL*. M-phase synchronized amniocytes from both affected fetuses did not display an aberrant number of centrioles, as shown previously for either STIL-depleted or overexpressing cells. However, we observed a significant elongation of at least one centriole for each duplicated centrosome. Since the mutations found in the affected fetuses lie respectively in the CPAP/CENPJ and the PLK4 interacting domains of STIL, we suggest that centriole elongation represents a novel mechanism causing MCPH and embryonic lethality in humans.

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P11.113A

A study of minor splicing defects associated with *RNU4ATAC* pathogenic variants

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- **Introduction:** We and others have shown that biallelic variants of *RNU4ATAC*, a gene transcribed into the U4atac small nuclear RNA component of the minor (U12) spliceosome, cause Taybi-Linder syndrome (TALS, OMIM #210710). TALS is a rare autosomal recessive disorder characterised by dwarfism, severe microcephaly, brain malformations and other developmental defects. Minor splicing uses a specialised machinery composed of U4atac and 4 other snRNAs, along with multiple proteins, and concerns ~800 U12-type introns harbouring specific splicing recognition sequences. Minor splicing may play a regulatory role on gene expression and is evolutionarily conserved. Recently, another rare disorder, Roifman Syndrome (RFMN, OMIM #616651), characterised by growth retardation, intellectual disability and antibody deficiency, was also associated with compound heterozygous RNU4ATAC variants, with one variant located in the TALS region and the other located elsewhere in *RNU4ATAC*. Our goal is to assess splicing efficiencies of *RNU4ATAC* variants in a cellular model and to characterize minor intron splicing and gene expression defects at the transcriptomic level.

- Materials and Methods: We engineered a cellular model aiming at assessing the pathogenicity, i.e. the splicing efficiency, of *RNU4ATAC* variants. We also analysed and compared transcriptomes of cells derived from patients with biallelic *RNU4ATAC* pathogenic variants.

- **Results:** U12-type intron splicing efficiency depends on the tested *RNU4ATAC* alleles and correlates with TALS disease severity. Preliminary transcriptomic studies show generalised U12-intron splicing defects in patients' cells.

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P11.114B

A novel mutation of *TBL1XR1* in individual with Pierpont syndrome

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Background: Exome sequencing is an effective diagnostic strategy to identify genetic causes of multiple congenital anomalies and intellectual disabilities (ID). We report on a Japanese girl with ID, facial dysmorphism, and multiple congenital anomalies associated with a novel mutation in *TBL1XR1*. **Case Report:** The proposita was a 9-year-old female. She presented with progressive postnatal growth failure, developmental delay. On closer examination, nothing was to be found and G-Banded karyotype showed a normal female karyotype 46,XX. At the age of 5 years, she was referred to our hospital to determine the existence of the underlying disease. At the age of 7 years, she complained of precocious puberty. In the course of the examination, brain magnetic resonance imaging revealed Arnold Chiari malformation. At the age of 8 years, she underwent surgery for a Chiari malformation. **Methods:** Written informed consent was obtained from the parents. A clinical whole exome sequencing was performed by using MiSeq (Illumina Inc. USA) with TruSight One sequencing panel (Illumina Inc. USA). The detected variants were confirmed by sanger sequences. **Results:** Array comparative genomic hybridization analysis had a normal result. De novo heterozygous nucleotide substitution for the missense mutation in exon14: c.A1339C:p.S447R in *TBL1XR1* was identified. **Conclusion:** Up to now, several different phenotypes caused by the pathogenic variants of *TBL1XR1* have been reported. While the recurrent and unique variants of *TBL1XR1*represent each unique phenotype, it has not been known well about the mutation specific mechanism. To reveal the phenotypes associated with *TBL1XR1* mutations required further functional analysis.

M. Minatogawa: None.

P11.115C

2 new cases of Temple syndrome caused by isolated hypomethylation of the 14q32 imprinted DLK1/MEG3 region

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Introduction Temple syndrome is a genetic condition caused by altered imprinted genes expression at chromosome 14q32 (maternal UPD 14, paternal deletion within the locus or loss of methylation (LOM) at DLK1/MEG3). Although the clinical manifestations are variable, pre-postnatal growth retardation, poor feeding, hypotonia, variable motor delay, premature puberty and obesity characterize it. To date only 10 patients have been described caused by isolated LOM.

Material and Methods We describe 2 cases diagnosed in the last 7 months. They were born to healthy and non-consanguineous parents and one was conceived by assisted reproductive techniques. Prenatal data: one intrauterin growth retardation; the other increased nuchal translucency and hypoplastic nasal bone with normal karyotype 46,XY. Both presented feeding problems (1 gastrostomy), hypotonia, neurodevelopmental delay, big low-set posteriorly rotated ears, almond eyes, small hands, fifth clinodactyly and triangular face. Other features (1): hypoplastic nasal alae, short stature, relative macrocephaly, facial asymmetry, truncal obesity and high-pitched voice. Molecular studies: arrayCGH, MS-PCR 11p15, UPD 7, Prader-Willi. S: normal. MS-PCR 14q32: LOM at DKL1/MEG3, biparental chromosome 14 origin.

Conclusions These 2 new patients diagnosed since MS-PCR 14q32 is available in our centre suggest that this disorder may be under-diagnosed.

The fact that one child was conceived by ART (third case described) supports the ART- imprinting disorders association.

Both patients presented with hypotonia, feeding problems, fifth cliniodactily and triangular face, and other manifestation as relative macrocephaly, facial asymetry, obesity; features also present in Silver-Russell and Prader-Willi patients, so it should be included in their differential diagnosis.

M. Sánchez Soler: None. **V. López-González:** None. **M. Ballesta-Martínez:** None. **A. Serran-Antón:** None. **L. Rodríguez-Peña:** None. **G. Glóver-López:** None. **M. Martínez-Romero:** None. **I. López-Expósito:** None. **E. Guilllén-Navarro:** None.

P11.116D

Analysis of the sequence of *Cereblon* gene in people with Thalidomide Embryopathy shows its coding region being highly conserved

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Introduction: Cereblon protein was proposed as a primary target of thalidomide teratogenicity. Here, we sequenced *CRBN* whole gene in Brazilians with thalidomide embryopathy (TE) to identify variants of susceptibility that may help to elucidate Cereblon's role in thalidomide teratogenesis. Materials and Methods: Thirty-six Brazilians with TE participated of this exploratory study. *Cereblon* whole coding, untranslated regions, and 50bp of adjacent introns were sequenced through next-generation sequencing. Functional prediction of the variants was performed *in silico*, evaluating microRNA, transcription factors (TFs), and splicing sites. **Results:** Forty-one variants were identified; thirty-nine previously described. Only two alterations were exonic (exons 4 and 6), both synonymous. Twenty-three variants occurred in 3′UTR, sixteen in the region shared with *TRNT1* gene. rs551497808 (3′UTR), rs199995326 (Intron 9) and rs6793531 (Intron 8) were present in two individuals, despite their low frequency in genomic databases (<0.001). Four polymorphisms identified alter TFs Ikaros and Aiolos binding sites (important in thalidomide therapy); three other variants modify alignment sequence of three mature miRNAs identified in human embryonic stem cells. Results regarding splicing sites alterations were controversial and will be further evaluated. **Conclusions:** TE individuals do not present coding variants in *CRBN* that could drastically alter the protein structure; hence, we did not evidence a high impact alteration in *CRBN* that could be determinant for TE development. Our approach identified variants in regulatory regions that could help to a better understanding of genetic susceptibility to TE. These results must be further evaluated and may help to elucidate Cereblon's role in TE. Grant: INAGEMP

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P11.117A

Search for the mutation causing the ThoracoAbdominal Syndrome (TAS) an x-linked dominant disorder

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Introduction: The Thoracoabdominal Syndrome (TAS) is a very rare congenital X-linked dominant disorder presented only in one Jewish North African family. The features of the syndrome are ventral herniae (midline abdominal wall defect), antero-lateral diaphragmatic herniae (manifested almost exclusively in affected males), hypoplastic lung, and associated cardiac anomalies.

The aim of this study is to identify and characterize the mutation causing TAS.

Materials and Methods: The initial search for the mutation causing the syndrome was done by linkage analysis on the X chromosome using 27 individuals of the TAS family. Genome sequencing of two of the family members was performed on the identified TAS interval on chromosome X.

Results: The TAS interval was localized to Chr:Xq27.1 in an interval of 1.06 Mb. Three genes and two miRNA are encoded in the interval. No mutation causing variants were identified in these sequences. Large deletions, insertions and translocations were negated by the sequence. Therefore, further search for indel variants in the non-coding DNA was pursued. Variations presenting in more than 1 person in the population databases and in Short Tandem Repeats (STR) regions were excluded, leaving 4 variations that are further characterized for the possibility of causing the TAS phenotype.

Conclusion: Further analysis using the Chromosome Conformation Capture-on-Chip (4C-seq) technology will be held on the mutation causing variant for determining long-range chromatin interactions and the gene that may be affected.

P. Majdalani: None. **R. Parvari:** None.

P11.118B

Solving the diagnostic enigma of Toriello-Carey syndrome

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Introduction: Toriello-Carey syndrome (TCS) is characterised by a combination of corpus callosal agenesis, Pierre-Robin sequence (PRS), facial dysmorphism, intellectual disability and other anomalies. Since the first case was described in 1988, over 50 patients have been reported yet a single molecular mechanism underlying these diagnoses has remained elusive.

Materials and Methods: Exome sequencing was performed on ten families with a diagnosis of Toriello-Carey syndrome following clinical geneticist consultation. Photographs and clinical features of patients were compared to determine whether there was uniformity of dysmorphic features and congenital anomalies.

Results: Of the ten kindreds tested, five had *de novo* variants identified in genes linked to other Mendelian disorders. These included variants in BAF-complex disorder genes, SMARCA4 and SMARCE1; as well as variants in KAT6B, THOC2 and TUBA1A. In the remaining five "negative" families, no novel candidate genes were identified. Comparison of patient data and photographs revealed considerable heterogeneity in facial dysmorphism, although almost all exhibited the features of PRS, corpus callosal agenesis, intellectual disability and cardiac septal defect.

Conclusion: This study suggests that many characteristics of TCS, rather than being a distinctive phenotype with a unifying molecular cause, may in fact fall in the spectrum of other recognised syndromes. This shift in perspective is likely to impact future diagnostic approaches and recurrence risk counselling for patients with this constellation of features. The possibility remains that there is a true TCS phenotype and a future direction may be to better delineate the facial dysmorphology to separate this from the mix.

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P11.119C

Clinical and molecular findings of seven Turkish non-photosensitive trichothiodystrophy patients with two novel mutations in *MPLKIP*

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Introduction: Trichothiodystrophy (TTD) is a rare, autosomal recessive disease, recognizable by sulphur-deficient brittle hair with typical tiger-tail pattern on polarizing microscopy. TTD patients also display variable neuroectodermal features including cogntitive deficit, ichthyosis, photosensitivity, ocular findings, infections, and decreased fertility. Approximately half of TTD patients exhibit photosensitivity. Non-photosensitive TTD (TTDN) is caused by mutations in *MPLKIP* and *GTF2E2*. An X-linked form was described in a family with two male affecteds with *RNF113A* mutations. We here present the clinical and molecular findings of seven patients from five unrelated families with non-photosensitive TTD, including two novel mutations in *MPLKIP*. Materials and Methods: Sanger sequencing of *MPLKIP* was performed in seven clinically diagnosed TTDN patients from five unrelated families, consulted in the Medical Genetics Department of Istanbul Medical Faculty between 2008–2016. Their clinical and molecular findings were reviewed. **Results:** All our patients had characteristics of TTDN including the tiger-tail hair pattern, dysmorphic findings, cognitive deficit or developmental delay, short stature, ichthyosis and hypogonadism. Notably, four patients displayed hipo/anhydrosis and temperature intolerance, indicative for ectodermal involvement, one had hypoplasia of corpus callosum, and one had a single central incisor. Previously reported c.505dupA (p.T169Nfs*75) was detected in one patient, novel c.85G>T (p.G29X) in three families, and novel frameshift c.61delT (p.W21Gfs*132) in one family. Conclusion: This study adds to the clinical and mutational spectrum of TTDN, with two novel mutations, the previously unreported finding of single central incisor, and confirmation of callosal abnormalities as a finding of TTDN.

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P11.120D

A 3-year-old girl with 46,XX,upd(14)mat/47,XX,+14 mosaicism

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Introduction: Chromosome 14 contains a cluster of imprinted genes at 14q32.2. Consistent with this, Temple syndrome caused by maternal uniparental disomy of chromosome 14 (upd(14)mat) presents discernable clinical features such as pre- and postnatal growth failure, and hypotonia. Although clinical phenotype of mosaic trisomy 14 is varied, pre- and postnatal growth failure has been frequently reported. Furthermore, a combination of complete trisomy 14 mosaicism and upd(14)mat is rarely reported.

Subject: A 3-year-old girl, born small for gestational age, was referred to our hospital due to severe growth failure (height: -5.1 SD, weight: -3.3 SD), failure to thrive, hypotonia and developmental delay together with deafness.

Methods and Results: Plasma hormone levels and growth hormone provocation test showed a normal range. To clarify the genetic cause of this patient, we first performed comparative genomic hybridization and SNP array analysis, and detected a copy number change of chromosome 14. Fluorescence in situ hybridization analysis in blood showed a mosaic trisomy 14 in 46.8% of cells. To detect the origin of extra chromosome 14, we performed methylation analysis and identified the mild hypomethylated *MEG3*-DMR at the 14q32.2 imprinted region. Then, we performed microsatellites analysis for chromosome 14, and compared the peak area under the curve between maternal inherited allele and paternal inherited allele. Finally, we confirmed that this patient had 46,XX,upd(14)mat/47,XX,+14 mosaicism.

Conclusions: We report the third case of complete trisomy 14 mosaicism with upd(14)mat. Severe short stature in this case may be caused by synergic effect of trisomy 14 mosaicism and upd(14)mat.

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P11.121A

Partial trisomy 15q21.2-q26.3 and partial deletion Xp22.33-p11.4 3p in a female patient with amenorrhea and normal intelligence

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Duplications that affect the distal part of chromosome 15 (dup15q24-q26) have variable phenotypes and are characterized by developmental delay, palpebral ptosis and short stature. However, duplications in the more telomeric part of chromosome 15 (15q26.3) have been associated with an overgrowth syndrome characterized by large size and renal abnormalities in 45% of cases (polycystic or renal agenesis, hydronephrosis, vesicourethral reflux). Cases with overlapping duplications reported range from generalized developmental delay to cardiac involvement. Partial deletions of the short arm of the X chromosome in female patients present clinical heterogeneity; typical physical abnormalities are often mild or absent. In all cases there is a short stature. In the present study we describe a female patient with amenorrhea and normal intelligence and a gain of 49,93 Mb in 15q21.2-q26.3 and loss of 40,05 Mb in Xq22.33-p11.4. Patient attends to physician due to amenorrhea, mammary gland tanner 1, hypergonadotropic hypogonadism, absence of right gonad and hypoplasia of left gonad. No other clinical manifestations were found. The patient's phenotype may be a consequence of the interaction between the effects of both alterations, this may vary with respect to the phenotype described for each of the rearrangements when presented in an isolated way. There is a case described in the DECIPHER database which presents very similar rearrangements and whose reported phenotype is global developmental delay, conversely to our case that presented normal intelligence. This data enriches the spectrum of clinical manifestations in Partial trisomy 15q21.2-q26.3 and partial deletion Xp22.33-p11.4 3p.

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P11.122B

Trisomy 4q22.3-q35.2 in a patient with additional material in 16q24 due to a de novo chromosomal rearrangement

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4q duplication syndrome is characterized by developmental delay, medium to severe intellectual disability, growth retardation, microcephaly, facial dimorphism (wide and prominent nasal bridge, low implantation of ears, palpebral fissures sloping downward, epicantic fold and hypertelorism) and digital anomalies (thumb hypoplastic and clinodactyly of the 5th finger). Less frequently, cardiac malformations, renal, unilateral or bilateral cryptorchidism, umbilical hernia, and epilepsy may occur. Occasionally, choanal atresia, preaxial polydactyly and neonatal coleostasis are present. Because duplication of the proximal first third of chromosome 4q is infrequent and the clinical effect is mild, the phenotype described for 4q duplication syndrome seems to be associated with more distal region, specifically to the 4q33-q34 region. It has been postulated that the genes contained in this region may affect as follows: whereas *GLRA3* and *GMP6A* could be related to developmental delay motor and language and with intellectual disability, *HAND2* would be associated with craniofacial and digital anomalies in these patients. Interestingly, we describe a patient with a duplication of 94.1MB in chromosome 4q22.3–4q35.2 product of a de novo chromosomal rearrangement of chromosome 16q.

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P11.123C

Identification of *TTC21A* as a novel cilia gene for Joubert syndrome by whole exome sequencing

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Introduction: The proband is a Chinese girl who presented with hypotonia and central apnoea at birth. She had no dysmorphic feature. Serial MRI and CT brain studies showed features of Dandy Walker variant, severe hydrocephalus, agenesis of corpus callosum, pontocerebellar hypoplasia and absent cerebellar vermis. Her brother was similarly affected and succumbed at 1 year.

Materials and Methods: This case was enrolled in our undiagnosed diseases program for ending diagnostic odyssey. Given the clinical features, we initiated genetic analysis for Joubert syndrome (JS). To-date, there are >30 disease-causing genes for JS. Instead of a "gene-after-gene" approach, whole-exome sequencing (WES) was applied to streamline the genetic analysis.

Results: WES revealed a novel heterozygous splice site variant in *TTC21A* gene, NM_145755.2:c.27+2T>G and a missense pathogenic variant, c.3676C>T (p.Arg1226Cys). *In silico* prediction of the splice site variants suggested a damaging effect on the donor splice site of exon 1. The variant c.3676C>T is a rare variant with allele frequency of 0.0001373 (total population) and 0.0002746 (East Asian) in the ExAC database, and is predicted to be probably damaging (PolyPhen-2) and deleterious (SIFT). No pathogenic variants were detected in other known JS-causing genes.

Conclusions: The diagnosis of JS in this case is challenging because the MRI hallmark, "Molar-tooth sign" was absent secondary to the severe Dandy Walker malformation. *TTC21A* encodes for tetratricopeptide repeat domain-containing protein 21A. It is a novel cilia gene for JS and is widely expressed in nervous system, which probably interacted with *TTC21B*, a known disease-gene for JS in fetal development.

C. Lam: None. **C. Law:** None.

P11.124D

Genotype-phenotype correlation in Czech patients with tuberous sclerosis complex

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Introduction: Tuberous sclerosis complex is an autosomal dominant disorder manifested by benign tumors in many tissues and organs (skin, brain, kidney, heart etc.). Pathogenic mutations are in TSC1 and TSC2 gene. This study is focused on analysis of TSC genes in selected group of patients and subsequent genotype/phenotype correlation.

Materials and Methods: Using MLPA, Sanger or NGS on Ion Torrent platform we analyzed TSC genes in 118 patients (99 probands and 19 cases of disabled family members of some probands).

Results: In group of 99 probands were found 75 mutations in TSC2 and 24 mutations in TSC1 gene. TSC2 mutations were associated with more severe phenotypes than TSC1 mutations. No statistically significant correlation was observed between different types of mutations and specific phenotypes in a group of TSC2 patients. In group of

TSC1 patients there were almost exclusively only protein-truncating mutations. We also revealed significant correlations between hypomelanotic spots findings and incidence of cortical tuber, the facial skin angiofibromas involvement and finding of renal angiomyolipomas, between hamartoma of the retina and heart rhabdomyoma or between epilepsy and mental retardation in the group of patients with TSC2 mutations. Conversely, in individuals with TSC1 mutations have been rarely observed angiomyolipomas and multiple renal cysts, head fibrous plaques and retinal hamartomas.

Conclusions: In our cohort the variability of clinical expression among patients was substantial, even within families or between unrelated individuals with the same mutation. In total 27 revealed mutations has not been described in LOVD TSC1/TSC2.

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P11.125A

Phenotypic and molecular characterization of partial 2q trisomy and partial 9p monosomy resulting from a maternal balanced translocation. A case report

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We report a female infant presenting with several congenital malformations at birth. The baby was born to a 33-year-old G2P0A1 mother at full-term (41+1) by eutocic delivery. All the follow-up ultrasound scans were normal. Her birth weight was 3340 g (PC 49) with a length of 50 cm (PC 52) and a head circumference of 33.5 cm (PC 37). Her Apgar score was 3/5.

Our patient presented with development delay. Facial dysmorphisms included round face, progressive microcephaly, hypertelorism, alternating exotropia, low-set ears, long flat philtrum, thin lips and thick neck with redundant skin. Echocardiogram showed an interventricular communication. Vesicoureteral reflux, trombopenia and nomal external female genitalia were present.

The CytoScan array 750K revealed 2q-duplication and 9p-deletion, 2q36.3q37.3(229,586,529–242,782,258)x3, 9p24.3p24.1(208,454–6,211,500)x1. Duplicated region on chromosome 2 was 13196kbp including 91 OMIM genes whereas deleted region on chromosome 9 was 6003kbp including 27 OMIM genes. Maternal karyotype showed a balanced translocation between chromosomes 2 and 9,[46, XX,t(2;9)(q36.3;p24.1)].

Ocular problems were described in 9p-deletion similar cases. Psychomotor, cardiac and renal issues were attributable to both changes. Trygonocephaly, nystagmus, sex reversal, respiratory, swallowing and otitis problems, for 9p-deletion and limb-finger alterations for 2q-duplication were not observed.

The clinic of duplication-deletion syndromes due to inherited translocations is complicated to integrate in a unique syndrome. The phenotype is resulted from two overlapped variants and it is difficult to find each change described separately. It depends on the chromosomes involved, the size and genes implicated. It is helpful to characterize and share with the scientific society each case.

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P11.126B

Rhombencephalosynapsis in a Fetus with WAGR Syndrome: Extending the Spectrum of Associated Central Nervous System Anomalies

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WAGR syndrome (Wilms tumour - Aniridia - Genitourinary anomalies - Intellectual disability mental retardation) is a rare genetic disorder caused by cytogenetically visible deletions of 11p13 or cryptic deletions of the *PAX6* and *WT1* genes. In up to one third of patients additional neurological anomalies (either functional or malformative) can be identified, including hypertonia or hypotonia, epilepsy, enlarged ventricles, corpus callosum agenesis, and microcephaly. Here we report the case of a fetus at 21+5 GW with a 11p13 deletion and rhombencephalosynapsis, a rare malformation not previously reported in the WAGR syndrome. Prenatal invasive analysis by array-CGH on DNA from amniocentesis, performed after the ultrasonographic detection of hypoplasia of the corpus callosum, the cerebellar vermis and a query Dandy Walker Variant, revealed the following deletion: arr[GRCh37] 11p14.1p13 (30,000,728×2, 30,034,712–35,323,086×1, 35,380,819×2) dn. Parents opted for termination of pregnancy at 21 + 5 GW. Autopsy revealed hypoplasia of the iris, a small right sided postero-lateral diaphragmatic hernia, testicular

dysgenesis and a complex malformation of the central nervous system, characterized by partial agenesis of the corpus callosum and the cerebellar vermis, temporal polymicrogyria and a partial rhombencephalosynapsis (RS). RS is a rare malformation of the cerebellum, only around 50 cases have been reported to date, characterised by the association of total or partial agenesis of the vermis and fusion of the cerebellar hemispheres. Numerous additional malformations - cerebral and non - have been reported and syndromic associations have been observed in some cases. RS however, has never been reported in cases of WAGR syndrome.

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P11.127C

WDR26 haploinsufficiency causes a recognizable syndrome of intellectual disability, seizures, abnormal gait, and distinctive facial features

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Introduction: Using exome sequencing, we have identified *de novo* heterozygous loss of function *WDR26* gene variants in individuals with intellectual disability. The purpose of this work was to fully characterize the clinical spectrum, specific features and molecular pathology that result from pathogenic *WDR26* variants. Materials and Methods: We utilized a network of collaborators and GeneMatcher to identify individuals with *WDR26* variants, carefully assessed clinical information and correlated findings with cellular and molecular data. **Results:** We identified eleven individuals with *de novo* pathogenic variants in *WDR26*. Eight carry loss-of-function mutations and three harbor missense substitutions. All have intellectual disability with delayed speech, a history of seizures and abnormal gaits. Common facial features include a prominent maxilla, widely spaced teeth, and a broad nasal tip that together comprise a recognizable facial phenotype. These features are consistent with those seen with heterozygous chromosome 1q41q42 microdeletions, suggesting that haploinsufficiency of *WDR26* contributes to the pathology. To support this, *WDR26* loss-of-function single nucleotide variants lead to nonsense-mediated decay with subsequent reduction of RNA expression and protein levels. Finally, structural modeling of missense mutations also supports haploinsufficiency as a mechanism of pathogenicity by disrupting conserved domains of the protein.**Conclusions:** Haploinsufficiency of *WDR26* causes an emerging clinical syndrome of intellectual disability, seizures, and distinctive facial features.

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P11.128D

Intragenic deletion of *WDR63* results in a dominant negative ciliopathy disorder

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Dysfunctional cilia result in a heterogenous group of human disorders termed ciliopathies. Using a customized CGH array and pair-end WGS sequencing we identified a rare intragenic heterozygous in-frame *WDR63* deletion in a fetal case with occipital encephalocele and inconsistent brain lobulation. No other pathogenic CNVs or rare SNVs were identified. The deletion spans exons 14–17, interrupting the 3rd and 4th WD repeat domains. RT-PCR showed expression of this deleted variant, confirmed by Western blot analysis. Functional studies in zebrafish using overexpression of *WDR63* RNA encoding the deleted variant resulted in abnormal development (46%, n = 255, P<0.001) compared to overexpression of wild-type RNA (3%, n = 177). In some embryos, a sac like protrusion of the brain was identified (5%, P<0.01). These findings were further confirmed by CRISPR/Cas9 mediated mosaic somatic deletions in zebrafish. Two sets of sgRNAs targeted at intron regions flanking orthologous exons were designed. As the individual sgRNAs only targeted intron sequences, injection of a set of sgRNAs was required to cause the deletion. Injection of these two different sgRNA pairs resulted in an increase of abnormal embryos with malformations (41%, n = 161, p<0.01 and 62%, n = 224, p<0.01 respectively) compared to injection of single sgRNAs (2–7%, pooled n = 227). Embryos presented with phenotypes similar to RNA overexpression experiments, including sac-like brain protrusions (7% and 9%, p<0.01) not observed in control embryos. We predict the in-frame *WDR63* deletion to result in a dominant negative protein contributing towards encephalocele formation. These are the first findings supporting a role for WDR63 in a ciliopathy-like disorder.

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P11.129A

KMT2A mutation is a major gene inintellectual disability

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Intellectual disability (ID) is a common neurodevelopmental disorder characterized by an extreme genetic heterogeneity with more than 500 genes implicated in mendelian forms. Wiedemann-Steiner syndrome (WSS) (MIM 605130) is a rare syndromic condition associating ID with hypertrichosis cubiti, short stature and a characteristic facial appearance. Since the identification of the causative

gene *KMT2A* in 2012, 25 patients with a mutation and a phenotypic description were reported.

We report herein 32 French individuals with a *KMT2A* mutation identified by targeted gene sequencing or new generation sequencing. We identified 25 new mutations (11 frameshift, 7 missense, 7 nonsense and 1 splice mutations) and 2 already reported ones. We describe the first autosomal dominant transmission (a mother and her son affected) and the first mosaïcism with two affected children and a non-affected father (mutation found with a rate of 1% in the blood).

We observed a wide clinical spectrum in severity of ID (mild to severe), facial appearance (typical or not) and in associated malformations (bone malformations, cerebral, renal, cardiac and ophthalmologic anomalies). We compared our results to the literature: our findings are close to those reported except for hypertrichosis, only found in 69%, and growth retardation, found in about 50%, whereas these symptoms were reported as almost constant. We make the hypothesis that *KMT2A* is a major gene of ID as the clinical spectrum is wide and the gene was identified recently. Recent publications reporting several *KMT2A* mutations in large cohorts of patients with neurodevelopmental troubles strengthen this hypothesis.

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P11.130B

Characterization, by array-CGH and whole-exome sequencing, of 6 patients with Williams-Beuren syndrome and Autism Spectrum Disorder

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Williams-Beuren Syndrome (WBS), caused by 7q11.23 microdeletion, is characterized by multiple congenital abnormalities, intellectual disability (ID) and a typical behavioral profile ("overfriendliness", excessive talkativeness), which is generally considered as the polar opposite phenotype to Autism Spectrum Disorder (ASD). Nevertheless, the prevalence of ASD has been reported to be significantly higher (12%) in WBS than in general population (1%): this study aims at characterizing the basis of the ASD observed in a subset of WBS patients.

We performed array-CGH and whole-exome sequencing (WES) in 6 patients with WBS and ASD (diagnosed by ADOS/ADI-R) to evaluate the possible presence of I) unusually-large 7q11.23 deletions; II) a coexistent pathogenic CNV; III) a point mutation in a gene localized in the non-deleted 7q11.23 allele; IV) a mutation in genes causing ID/ASD localized in other chromosomal regions.

Array-CGH confirmed the presence of classical 7q11.23 microdeletions in all patients and did not show any other pathogenic CNVs. WES found no recurrent SNVs either in genes located in the non-deleted 7q11.23 allele or in a panel of 450 genes causing ID/ASD.

Further studies are currently ongoing to evaluate: I) the possible presence of a rare inherited or de novo variant in the 6 WBS/ASD patients II) the functional dysregulation in disease-relevant pathways using Induced Pluripotent Stem Cells obtained from skin fibroblasts from 3 among the 6 patients.

This study may provide new insights into the WBS phenotypic variability of social communication, possibly ranging from excessive talkativeness and "overfriendliness" to absence of verbal language and social deficit.

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A double cryptic chromosome imbalance as a cause of phenotype variability in Wolf-Hirschhorn syndrome

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We report on a 5-year boy with extreme short stature, underweight, microcephaly, psychomotor development delay, triangular-shaped face with pointed chin, low anterior and posterior hairline, massive nose depressed inside, long philtrum, mild hypospadias and low muscle tone. The child has always been growing steadily and speaks syllables only. Cytogenetic testing revealed normal male karyotype. Performed CGH testing revealed genomic deletion of 4p16.3 (4,2Mb) and genomic duplication of 8p23.1–8p23.3 (7,3Mb): arr4p16.3(85743.3–3770271)x1,8p23.3-p23.1 (194617–6873437)x3. FISH analysis confirmed the findings of Wolf-Hischhorn (WHS) syndrome in our patient. To date, only three recurrent constitutional non-Robertsonian translocations have been described in humans. Translocation (4;8) is the third by prevalence along with t(11;22) and t(8;22). Several literature reports point to a great variability of the WHS phenotype that is dependent on the genomic defect (terminal versus interstitial deletions; pure deletions versus unbalanced translocations; size of the deletion), consisting in the association of severe growth delay, intellectual disability, peculiar facial dysmorphisms and seizures. The deleted and duplicated regions include multiple genes, containing WHSC1, WHSC2, and LETM. The latter reported as an excellent candidate gene also for seizures although our patient doesn't perform epilepsy. features. On the other hand trisomic part of 8p may be responsible for the clinical variability in our patient. While the interpretation of terminal duplication of 8p23 is difficult as no overlapping terminal duplications in literature exist. Duplications of this region has already been reported as a possible benign variant.

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P12 Cancer genetics

P12.001A

Integrative approach and eQTL analysis identify breast cancer risk genetic variants affecting microRNA binding sites

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Genome-wide association studies (GWAS) found ~ 80 risk loci associated to breast cancer (BC). Approximately 90% of single nucleotide polymorphisms (SNP) associated to complex diseases are in non-coding regions of the human genome, including target sequences of microRNAs (miRNAs). MiRNAs act by imperfect base-paring to 3' untranslated regions (3'- UTR) of target messenger RNAs (mRNA), leading to translation repression, mRNA degradation or both. SNPs located at the 3'UTR of miRNA targets called miRSNPs may reduce effectiveness or abolish miRNA-mediated repression.

Here we used a naïve Bayes classifier approach to integrate results from three tools (Polymirts, miRSNP and miRSNPscore), which use different methods to predict for miRSNPs. Further, we integrated miRSNPs predictions with summary statistics from breast cancer GWAS data. In addition, to prioritize miRSNPs, miRNAs and targets genes, we performed a tumour-specific eQTL analysis (~ 2,000 samples from METABRIC), and a Pearson correlation analysis in 847 BC samples (mRNA-seq and small RNA-seq from TCGA).

We found 338,894 candidate miRSNPs (score > 0.7). Of those, 43 were associated to BC ($P < 5 \times 10^{-8}$) and predicted to affect interaction of 160 miRNAs with 20 target genes. By using a tumour-specific eQTL analysis we could prioritize 5 miRSNPs with cis-eQTL effect (FDR < 0.05). Additionally, correlation analysis confirmed predictions of 18 miRNAs with their 17 targets ($P < 0.05$).

Our integrative approach revealed new insights into miRNAs and BC associated target genes. Thus, it helped to prioritize BC noncoding risk SNPs that might be involved in the causal mechanisms.

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P12.003C

Landscape of pathogenic variations in a panel of 34 genes in 5131 HBOC families and cancer risk estimation

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The integration of gene panels in the diagnostic of hereditary breast and ovarian cancer (HBOC) requires a careful evaluation of the risk associated with the genetic variations detected in each gene. We analyzed by NGS 34 genes in a series of 5131 index cases suspected to present HBOC.

Only variations strongly suspected to be pathogenic were considered. Patients were excluded if one variant genomic position did not reach 20x of coverage. We used as control data sets, ExAC and 574 individuals from the French Exome Project (FREX). We controlled stratification bias using correspondence analysis of 1000Genome data and FREX genotypes to exclude non-European patients. The probability than an individual from ExAC carries a pathogenic variation was simulated and compared to the estimated frequency in the HBOC population.

The incidence of pathogenic variations in *BRCA2*, *BRCA1*, *CHEK2*, *ATM*, *PALB2*, *RAD51C*, *TP53*, and *BRIP1* was 3.9%, 3.7%, 1.1%, 1.0%, 0.9%, 0.5%, 0.5%, 0.5%, respectively. Odds-ratio (OR) conferred by variations within *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *ATM*, *BRIP1*, *CHEK2* and *MSH6* were 13.48[10.22–17.58], 8.07 [6.39–10.08], 8.21[4.9–13.02], 4.40[2.46–7.22], 5.23[1.44–13.04], 3.20[2.14–4.55], 2.49[1.41–3.97], 1.67[1.18–2.28] and 2.50[1.12–4.63], respectively. Variations within *RAD51C*, *RAD51D*, and *BRIP1* were predominantly associated with ovarian cancer (OR: 14.17[5.24–28.49], 11.85 [1.09–40.27] and 3.76[0.75–9.36]). *PALB2* variations were associated with bilateral breast cancer (OR 16.17[5.48–34.10]).

The OR evaluated in this large series validates the integration, besides *BRCA1* and *BRCA2*, of *PALB2*, *RAD51C*, *RAD51D* in the molecular diagnostic of HBOC and the lower values observed for the other genes suggests that they are involved in an oligogenic determinism.

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P12.004D

Impact Of Genome Variation On Functional CRISPR Screens And Mapping Variant Data To Disease Biology

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The wealth of data output from next-generation sequencing (NGS) technologies have led to tremendous progress in the study of human genetic variation and its contribution to human disease. Several consortia have been developed to map human genetic variation, producing genome-wide association studies and rare variant analyses in the context of common and rare diseases. However, many of these variant databases lack information on the function of these variants and their contribution to the pathophysiology of human diseases. Without elucidating the functional contribution of these mutations to cellular activity, these studies yield little benefit to human health. Clearly, investigation of the molecular and cellular activity of genetic variation is increasingly important for understanding the mechanistic function of the human genome. At Desktop Genetics, we have developed a CRISPR design platform accounting for genomic variants including single nucleotide polymorphisms, small insertions/deletions, translocations and copy number variants. Using NGS data and our optimized CRISPR design rules, we demonstrate a bespoke methodology for *in vitro* functional assays in cultured human cells of a defined genotype. We show how model-specific genomes in conjunction with customizable guide RNAs can be used to precisely target genomic sites of interest to induce loss-of-function mutations and validate predicted variant function. This may provide a unique approach for downstream variant interpretation and interrogation of their role in the pathogenesis of rare and common genetic diseases.

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High resolution Chromosomal Microarray Analysis (CMA) as a tool for the genetic profiling of children with B-cell Acute Lymphoblastic Leukemia (B-ALL)

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Advances in the genetic profiling of patients with ALL continue to transform diagnosis, prognosis and treatment, by constantly adding to our knowledge on the biology of the disease and improving clinical management with the means for better risk stratification and novel therapeutic approaches. With most leukemia patients harboring acquired clonal chromosomal aberrations, we here aim to investigate the potential benefits of high resolution CMA in B-ALL patients.

The study material consisted of bone marrow samples obtained at diagnosis from 27 children (median age 5 years old) with B-cell ALL, that were analyzed with the high-resolution genome-wide G3 CGH+SNP 2×400K microarray platform (Agilent Technologies), with a median CGH probe spacing of 4.6kb (Refseq genes). Karyotype and FISH analysis for the most common translocations were also performed for all patients.

Overall, clinically relevant CNVs were detected in 22/27 cases (81,4%). The CMA revealed 8 cases with hyperdiploidy, 1 case with hypodiploidy and 13 cases with complex karyotypes. The gene most frequently involved in aberrations was *ETV6*. The most notable small size CNVs were a 87kb deletion encompassing *IKZF1*, a 9 kb duplication involving *NF1* and a 28kb deletion involving *PAX5* gene. Genomic regions that harbored hematopoiesis (*RUNX1*) and cell-cycle regulation (*CDKN2A/2B*) genes, as well as oncogenes and tumor suppressor genes were also affected.

In conclusion, higher resolution CMA is able to detect small CNVs with clinical consequences in a significant number of ALL cases while also proving to be a robust tool for the identification of gross chromosomal imbalances.

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P12.006B

ETV6 and *NOTCH1* germline variants in adult acute lymphoblastic leukemia - a case report

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INTRODUCTION: Inherited mutations of *ETV6* have recently been associated with susceptibility to acute lymphoblastic leukemia (ALL). In particular, germline *ETV6* gene variants were reported in a relation with thrombocytopenia and predisposition to childhood ALL (Moriyama et al., 2015), although little is known about *ETV6* variant status in adult ALL. Also there are no studies about the role of *NOTCH1* germline variants in adult ALL. CASE REPORT: A 19-year old Caucasian male was diagnosed with bifenotypic leukemia (T/myeloid). He was born in a family of non consanguineous healthy parents and has two elder siblings. No family history of cancer or thrombocytopenia was identified. At diagnosis, next generation sequencing (NGS) analysis was performed on patient's bone marrow using TruSight Myeloid sequencing panel (Illumina Inc., CA). Two *ETV6* gene variants c.216G>A (p.W72Ter) and c.304delT (p. R103AfsTer19) and one *NOTCH1* gene variant c.7130C>T (p.P2377L) were detected. qPCR assay was designed to confirm these variants in leukemic and non-leukemic DNA. Data analysis confirmed *ETV6* W72Ter and *NOTCH1* P2377L mutations as germline variants, whereas *ETV6* R103AfsTer19 - as somatic mutation. The proband's both mother and father had *ETV6* W72Ter and *NOTCH1* P2377L germline variants, respectively. Notably, one of the proband's siblings had ETV6 W72Ter and the other - NOTCH1 P2377L germline variant. Our findings suggest that the inheritance of both *ETV6* and *NOTCH1* germline variants and the acquisition of an additional somatic *ETV6* mutation may have contributed to the malignant trasformation to ALL.

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P12.007C

New DNA methylation markers in pediatric acute myeloid leukemia

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Violation of the distribution of methylation markers is the process of carcinogenesis and it can play a significant role in the initiation and progression of neoplastic degeneration of the genome. We aimed to establish diagnostic markers based on DNA methylation for acute myeloid leukemia (AML) in children, to be used for detection of AML subtypes having different sensitivity to therapeutic regimens.

We used 107 samples of the bone marrow from 39 children with AML to determine DNA methylation of 6 genes that we previously identified as abnormally methylated in AML. DNA samples were analyzed by multiplex methylation-sensitive polymerase chain reaction before treatment and at different stages of treatment.

We demonstrate that epigenetic therapy with decitabine before the course of chemotherapy reduces DNA methylation notably.

Gene	Group I (n = 10) before treatment/ after decitabine (% of samples with methylation)	p	Group II (n = 29) before treatment/ after AIE/ after AIE and decitabine (% of samples with methylation)	p
CLDN7	30/30	1	*20.7/80/66.7*	*<0.0001*
SOX8	50/10	0.1409	*51.7/0/0*	*<0.0001*
EGFLAM	20/10	1	10.4/20/13.3	0.4487
THEM176A/176B	60/30	0.3698	62/68/53.3	0.7772
CXCL14	90/80	1	96.6/100/100	1
GSG1L	10/0	1	10.4/8/0	1

There are an increase of *CLDN7* methylation frequency and a decrease of *SOX8* methylation frequency significantly in group II. After chemotherapy treatment there is increase in the methylation frequency of 4 (*CLDN7, EGFLAM, THEM176A/176B, CXCL14*), and a decrease in 2 genes, indicating clonal evolution of tumors (*SOX8, GSG1L*).

Chemotherapy causes destabilization of the methylation profile, frequency of methylation begins to decline, though not pronounced, with the introduction of decitabine after AIE. Our new 6-gene panel is useful to estimate the progression of malignant cells, as blast cells after chemotherapy, considered morphologically normal, exhibit abnormal methylation profile of tumor cells.

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P12.008D

A rare familial APC deletion in a Tunisian family revealed in a 18-month-old boy with paravertabral Gardner fibroma

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UR17ES36 Genomics of signalopathies in the Service of Medicine, Medical University, Sfax, Tunisia, [2]UR12ES15, Medical University, Sfax, Tunisia, [3]Molecular of Genetics, University Hospital, Lille, France

We report the case of an 18-month-old boy who underwent resection of a left thoracic paravertabral deep fibromatous tumor contracting intimate adhesions to the paravertebral muscles. Histological evaluation revealed a benign Gardner fibroma. The family history revealed the presence of a deep-seated dorsal soft tissue tumour in his 7-year-old brother. His father as well as his uncle present bone masse at a same localization which is the left mandible angle. The orthopantamograph revealed a well-defined mass that was consistent with an osteoma (for both the boy's father and uncle). Molecular investigation of all members of this Tunisian non consanguineous family was conducted, using genomic DNA and bidirectional sequencing of the APC gene. Molecular analysis showed heterozygous exon 15 deletion of the APC gene: c.4652_4655delAAGA (p. Lys1551Argfs) in the patient, his brother, his father and his uncle. This pathogenic 4 pb deletion leading to a truncated APC protein, is a rare variant which was already described in association with FAP. However, no details are given about the patients and their families (https://www.ncbi.nlm.nih.gov/clinvar/17533765/). Gardner fibromas and osteomas were the only clinical features of this germline APC variant and this unrecognized FAP. Our family is under surveillance to detect adenomatous polyps and prevent colorectal cancer. Somatic variants of CTNNB1 and APC genes will be investigated to identify biallelic inactivation of Wnt components/regulators, in a possible recurring or newly diagnosed soft-tissue tumours to prevent desmoids and to adopt prophylactic and therapeutic measures.

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'Sinapic acid induces apoptosis in LNCaP human prostate cancer cell line'

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Introduction: The results of experimental studies have shown that natural compounds can have been anticarcinogenic effect. Sinapic acid is one of the natural compounds and found in various vegetables and fruit species. Prostate cancer is quite often seen in industrialized countries and the second causing of cancer related death in men. The aim of the study was to investigate the apoptotic effect of sinapic acid, in the LNCaP human prostate cancer cells. Materials and Methods: Cytotoxic effect of sinapic acid was determined by using XTT assay. Total RNA isolation of control and dose groups (IC_{50} dose of sinapic acid) was conducted using TRIzol Reagent. Relative mRNA expression levels of important genes in apoptosis including *CASP3, CASP7, CASP8, CASP9, BAX, BCL2, FAS* and *CYCS* were quantified in control and dose groups using qPCR. **Results:** IC_{50} dose of sinapic acid was detected as 1 mM for 72h in LNCaP cells. According to qPCR results, significant increases in the expressions of *CASP3, CASP7, BAX* and *CYCS* genes were determined as 3.93, 4.92, 8.54 and 2.71 folds respectively, compared with the control group cells. **Conclusions:** Sinapic acid can affect apoptosis by regulating the expression of *CASP3, CASP7, BAX* and *CYCS* genes in LNCaP cells. Further studies are required to demonstrate the molecular mechanism of apoptotic effect of sinapic acid in prostate cancer. *Financially supported by N. E.U. Scientific Research Projects (BAP #151218023).*

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P12.010B

Using whole exome sequencing to explore germline variation in BRCA1/BRCA2/PALB2 negative contralateral breast cancers from the Women's Environmental Cancer and Radiation Epidemiology (WECARE) study

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Background: BRCA1/2-negative asynchronous contralateral breast cancer (CBC) is associated with familial history, suggesting a heritable component in CBC beyond the BRCA1/2 mutations. However, no datasets have yet been generated to study germline variants associated with BRCA1/2-negative CBC.

Methods: DNA extracted from blood was obtained from 256 CBC and 256 matched unilateral breast cancer (UBC) patients of European ancestry enrolled in the WECARE study. Women with *BRCA1*, *BRCA2* or *PALB2* mutations were excluded. Whole exome sequencing (WES) was performed using Illumina library preparation and sequencing technologies. VCF files were generated following GATK Best Practices workflow. Rare variants were aggregated per gene and statistical associations were assessed using SKAT R package. Trends in crude allelic frequencies (AFs) were evaluated by comparing AFs in CBC, UBC and non-Finish European cases from 1-thousand genomes project.

Results: 235 CBC and 245 UBC were successfully sequenced to ~60x average depth on targets. Focused analysis in established breast cancer genes suggested that *ATM* and *CHEK2* showed crude AFs trends consistent with CBC association. Small sample and effect sizes precluded exome-wide detection of candidate genes that would pass multiple testing-correction in SKAT analysis. However, combination of SKAT analysis, the crude AFs assessments and manual prioritising by biological functions allowed for the selection of candidate genes, which could be used for further genotyping in a larger cohort or for experimental validation.

Conclusion: The study reports a new WES dataset for the identification of candidate genes potentially associated with asynchronous contralateral breast cancer.

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P12.011C

Mutations of ATR in male breast cancer predisposition

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Introduction: Male Breast Cancer (MBC) is a rare disorder representing less than 1% of all breast cancers. BRCA1 and 2 mutations are not found in all high risk families with at least one male breast cancer, and the involvement of other predisposing genes are likely.

Methods: To identify new predisposing genes for breast cancer, we used an "extreme phenotype" strategy, by performing whole exome sequencing in 3 families with several MBCs and negative diagnostic panel screening. A replication cohort of 161 patients, including MBC cases and families with high breast cancer risk, was constituted in order to sequence the full coding sequences of candidate genes.

Results: We identified a truncating mutation in exon 32 of *ATR (*p.Leu1808*) in one proband. This mutation was not described in public databases. *ATR* is implicated in cell cycle regulation and DNA repair. Functional validation revealed a decrease of ATR expression and phosphorylation of CHEK1 in cells exposed to hydroxyurea. No truncated transcript neither loss of heterozygosity were detected in the tumour. Within the replication cohort, we identified a second patient with a mutation in the splice-acceptor site of the last exon of ATR (c.7762-2A>C), and 7 missense and near-splice VUS.

Conclusion: Including these two novel truncating *ATR* mutations, 8 constitutional loss-of-function mutations have been described in the literature in high-risk families. Our results converge towards a model in which more moderate replicative stress due to the haploinsufficiency of ATR allows the survival of the cells but generates sufficient genomic instability to induce cancerous transformation.

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P12.012D

A novel gross deletion involving *PTEN* and *BMPR1A* genes produces a syndromic case of colorectal cancer

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Introduction: We present a 4 year old patient with suspected of having Bannayan-Riley-Ruvalcaba syndrome. She has severe anemia, juvenile polyposis, mild cardiomegaly, lipoma and Hypoalbuminemia.

Materials and Methods: A next-generation sequencing (NGS) panel containing 15 genes related to colorectal cancer (CRC) was carried out. *TruSight One kit (Illumina)* was used with a *HiSeq4000 sequencer*. Alignment of sequences against hg19 genome followed by *GATK* variant calling and *ExomeDepth* as Copy Number Variants (CNVs) predictor was performed. Confirmation of CNVs was done by using Multiplex Ligation-dependent Probe Amplification (MLPA) test (P-225-D2 kit).

Results: No relevant variants were found after variant calling. Nevertheless, *ExomeDepth* predicted a high confidence deletion in heterozygous state (Bonferroni Score: 73,1 for *PTEN* and 60,8 for *BMPR1A*) involving minimal coordinates [chr10:88635766-91007415] and maximum [chr10:88492744-91192810]. This deletion includes *PTEN*, *BMPR1A, KLLN* and *FAS* genes, among others. This alteration was further confirmed by MLPA test. Smaller deletions inside this region have been described in DECIPHER database associated with genotypes including

polyposis, lipomas, macrocephaly, and intellectual and behavioral findings.

Conclusions: In cases of syndromic forms of CRC, with others abnormalities, several genes can be involved, producing higher number of clinical findings, such as in this patient, where a gross deletion is affecting *PTEN* and *BMPR1A* genes. When a negative result is found in a point mutations analysis, a CNVs test should always be performed. Gross deletions can be detected by *in silico* predictors using NGS data, however, any result should be confirmed by other alternative methodology as NGS isn't the gold standard.

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P12.013A

BAP1 germline mutations in Finnish uveal melanoma patients

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Introduction: Germline pathogenic variants of the BRCA-1 associated protein-1 (*BAP1*) gene predispose to uveal melanoma and several other cancers. Testing for germline *BAP1* mutations should be performed if typical BAP1 cancer predisposition syndrome tumors have been diagnosed in the family. We report the frequency of germline pathogenic variants of *BAP1* in consecutive Finnish uveal melanoma patients.

Methods: In Finland, uveal melanomas are treated centrally in the Ocular Oncology Service, Helsinki University Hospital. We collected clinical data and genomic DNA from 284 of 417 consecutive patients diagnosed from January 2010 to June 2016. The exons and exon-intron junctions of *BAP1* were sequenced.

Results: We found two pathogenic germline variants, a T-insertion in exon 14 in three patients and donor splice site mutation in a highly conserved region immediately after exon 2 in 2 patients. The families of these patients also had typical BAP1-related cancers (cutaneous melanoma, mesothelioma, and renal cell carcinoma). The mutations were not found in 61,486 controls from the ExAC (http://exac.broadinstitute.org). The overall frequency of BAP1 pathogenic variants was 1.8% (5/284; 95% confidence interval, 0.6–4.0).

Conclusions: The frequency of BAP1 germline pathogenic variants in consecutive Finnish patients with uveal melanoma who come from a high-risk region for the development of this cancer is comparable with reports from other populations.

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P12.014B

Follow-up of a *CFTR* association with Barrett's esophagus and esophageal adenocarcinoma

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Esophageal adenocarcinoma (EA) represents one of the most rapidly increasing cancer types in high-income countries. Barrett's esophagus (BE) is a premalignant precursor of EA. In a recent GWAS meta-analysis (6.167 BE patients, 4.112 AE patients, 17.159 controls) we identified 14 genome-wide significant risk loci. The strongest associated signal was observed for an intronic variant in the *CFTR* gene. *CFTR* encodes a chloride channel protein and is mutated in patients with cystic fibrosis (CF). Interestingly, CF patients show increased incidence of gastroesophageal reflux (GERD), a major risk factor for BE and AE. In view of the phenotypic overlap for GERD and CF, and for GERD and both BE and AE, a common pathophysiological mechanism triggered by *CFTR* seems plausible. In order to test this hypothesis, we analyzed the association of the most common CF mutation ΔF508 in a European case-control cohort (1.037 BE patients, 1.609 AE patients and 941 controls) using a single-marker genotyping assay. We could not observe a significant association. This might be (i) due to insufficient sample power or (ii) due to the fact, that not ΔF508 but other genetic variants at the locus might explain the underlying pathological mechanism. Fine-mapping of all genetic variation at the *CFTR* locus and functional analysis are needed to elucidate the interference of the *CFTR* locus with BE and AE pathomechanism. A recent functional study indicated *CFTR* as a tumor suppressor gene in murine and human intestinal cancer, providing further evidence for *CFTR* as a true disease gene for BE and AE.

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P12.015C

Genomic analysis of sporadic basal-cell carcinoma by comparative genomic hybridization (CGH): PTCH1 alterations and beyond

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Introduction: Basal-cell carcinoma (BCC) is the most common malignant tumour in Caucasian population. Several tumour suppressor genes and proto-oncogenes are implicated in the pathogenesis of BCC, being *PTCH1* the most important of them with mutations in 48–83% of all sporadic BCCs. New techniques such as comparative genomic hybridization (CGH) facilitate the analysis of the tumour genome, but, to date, only two reports have analysed sporadic BCCs. The main aim of this work was to identify genomic and chromosomal imbalances of sporadic BCC. Our secondary goal was to find punctual mutations in the *PTCH1* gene.

Materials and methods: 13 BCC samples were obtained by surgery and subsequently histologically analysed. Tumor DNA was studied by CGH and *PTCH1* gene by PCR and Sanger sequencing.

Results: Aneuploidies were found in 10 tumours (77%), with simultaneous alterations in two o more chromosomes in 9/10. Most frequent trisomies were +22, +15, +17 y +20 and the most common monosomie was -4. Partial chromosome gains and losses were also characterized, being the most usual -9q22.33 y -9q33. The 9q22.32 region, where *PTCH1* is placed, was lost in 7 cases (53,85%). Gains in the SHH and SMO locus were detected. Punctual mutations in *PTCH1* gene were found in 10 (76,9%) tumours. Six of these mutations are firstly described in this work.

Conclusions: Our data confirm the importance of *PTCH1* as well as chromosomic imbalances in the pathogenesis of sporadic BCCs. However, further analysis of these regions must be carried out.

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P12.016D

Evaluation of BAT40, NR-24 and D17S250 MSI Markers in Tumor and Tumor Marginal Samples of Sporadic Colorectal Cancer

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Background: Microsatellites, also known as Short Tandem Repeats (STRs), are small repeating stretches of DNA scattered throughout the entire genome and account for approximately 3% of the human genome. Due to their repeated structure, microsatellites are prone to high mutation rate. Microsatellite instability in tumor DNA is defined as the presence of alternate sized repetitive DNA sequences that are not present in the corresponding germ-line DNA. Microsatellite instability (MSI) is a molecular phenotype due to a defective DNA mismatch repair system. The presence of MSI is found in sporadic colon, gastric, endometrial and the majority of other cancers. Determination of MSI status in colorectal cancer has prognostic and therapeutic implications. As well, MSI can be used diagnostically for tumor detection and classification.

Material and methods: In this study we investigated tumoral and tumor marginal DNA of 50 sporadic CRC patients who have not received chemotherapy. Two mononucleotide markers (BAT40 and NR-24) and one dinucleotide marker (D17S250) were used as a triplex PCR panel to evaluate microsatellite instability status.

Results: Our primary findings showed that MSI was detected in almost 25% of samples. Instability was observed in the tumoral DNA compared to the normal DNA samples. Moreover, our primary results showed that the instability frequency of NR-24 marker was more than others and also the frequency of instability in BAT40 and D17S250 were almost similar.

Conclusion: Instability of NR-24 can be a promising potential marker for determination of MSI status in patients with sporadic colorectal cancer comparing to other investigated markers.

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P12.017A

Anti-tumor effects of bemiparin in HepG2 and MIA PaCa-2 cells

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Introduction: In this study, we examine the effects of bemiparin on apoptosis and cell cycle related gene expression, viability, colony formation and migration/invasion of the cultured MIA PaCa2 pancreatic and HepG2 hepatocellular cancer cells. Materials and Methods: IC_{50} dose of bemiparin in MIA PaCa2 and HepG2 cells were performed by using XTT assay. Total RNA was isolated by using Trizol Reagent. Effects of the bemiparin on apoptosis and cell cycle related genes were determined via RT-PCR. Cell invasion, colony formation and cell migration status were detected by matrigel-chamber, colony formation assay and wound-healing assay. "RT^2 Profiles PCR Array Data Analysis" through "Student t-test" was used for statistical analysis. **Results:** IC_{50} dose of bemiparin was found to be 200 IU/mL in the MIA PaCa2 and 50 IU/mL in the HepG2 cell lines on 48th hours. In HepG2, *CCND1* expression was reduced and *p53*, *caspase-3*, *p21*, *caspase-8* expressions were increased in the dose group cells when compared with the control group cells. In MIA PaCa2 dose group, *CCND1*, *CDK4* and *CDK6* expressions were reduced and *p53* expression was increased. Cell invasion and migration was significantly inhibited and colony formation was significantly decreased through bemiparin treatment in both cell lines. **Conclusions:** In present study, bemiparin inhibits cell proliferation by inducing cell cycle arrest, apoptosis and also decreases invasion, migration and colony formation in HepG2 and MIA PaCa2 cells. Bemiparin may be a novel agent for treatment of hepatocellular and pancreatic cancers as a single agent or in combination with other agents.

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P12.018B

Birt-Hogg-Dubé syndrome as a cause of childhood pneumothorax

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTA2-AS1	ADAMTS9-AS2	AFAP1-AS1	AIRN	BANCR	BCAR4	BLACAT1	CAHM	CBR3-AS1	CCAT1	CCAT2	CDKN2B-AS1
B	CRNDE	DGCR5	DLEU2	DLX6-AS1	EMX2OS	FTX	GACAT1	GAS5	GAS6-AS1	GNAS-AS1	H19	HAND2-AS1
C	HEIH	HIF1A-AS2	HNF1A-AS1	HOTAIR	HOTAIRM1	HOTTIP	HOXA11-AS	HOXA-AS2	HULC	IPW	UniSp4 CP	JADRR
D	KCNQ1OT1	KRASP1	LINC00152	LINC00261	LINC00312	LINC00538	LINC00887	LINC00963	LINC01233	LINC01234	LISNCT5	LUCAT1
E	MALAT1	MEG3	MIR155HG	MIR17HG	MIR31HG	MIR7-3HG	MRPL23-AS1	NAMA	NBR2	NEAT1	NRON	PANDAR
F	PCA3	PCAT1	PCGEM1	POU5F1P5	PRNCR1	PTCSC1	PTCSC3	PTENP1	PVT1	RMRP	RMST	RPS6KA2-AS1
G	SNHG16	SPRY4-IT1	SUMO1P3	TERC	TRERNA1	TSIX	TUG1	TUSC7	UCA1	WT1-AS	XIST	ZFAS1
H	ACTB	B2M	RPLPO	RN7SK	SNORA73A	HGDC	RTC	RTC	RTC	PPC	PPC	PPC

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Introduction: Birt-Hogg-Dubé syndrome (BHD) is a rare autosomal dominant cancer predisposition syndrome caused by heterozygous variants in the *FLCN* gene. Phenotypically it is characterized by fibrofolliculomas, spontaneous pneumothoraces and renal cell carcinoma. We present a case of BHD in which pediatric pneumothorax led to the diagnosis.

Case presentation: A fourteen-year old otherwise healthy boy was admitted to hospital due to sudden onset of unprovoked chest pain. Chest X-ray was normal, but echocardiography was with remarkably poor visualization and this caused suspicion of free air in mediastinum. A second chest X-ray confirmed pneumomediastinum. Several relatives had suffered from spontaneous pneumothoraces, including the patient's father, paternal uncle, paternal grandmother and a brother of the grandmother. A clinical geneticist was consulted and the diagnosis BHD proposed. Genetic testing showed that the patient is a heterozygous carrier of the pathogenic variant *FLCN*, c.1062+2T>G. Age range of first pneumothorax in the family was 14–73 years. Two relatives had skin lesions consistent with fibrofolliculomas. None had renal cell carcinoma.

Conclusion: Diagnosis of BHD in this case of pediatric pneumothorax has enabled predictive genetic testing in the family and renal cancer surveillance for mutation carriers. In the literature there are only few previous reports of BHD and pediatric pneumothorax. Based on the present case and our experience from an additional 106 BHD cases, we hypothesize that pediatric pneumothorax in BHD may be more frequent than previously assumed. Along with this case results from our cohort study will be presented.

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P12.019C

Regulation of immune checkpoint genes revealed by a bladder cancer data from The Cancer Genome Atlas (TCGA) - bioinformatical analysis followed by an experimental validation

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Introduction: The complex immunological synapse between T lymphocytes and cancer or antigen presenting cells (C/APCs) contains both co-inhibitory and co-stimulatory checkpoint proteins, modulating the signal transmitted to T lymphocytes. Despite the antineoplastic activity of immune checkpoint inhibitors in many malignancies, the majority of bladder cancer patients do not respond. Since of the function of miR-15/16 miRNAs family were shown to target *PD-L1* in ovarian cancer, in bladder cancer it remains unknown. Our aim was to study the associations between these miRNAs and checkpoint mRNAs in bladder cancer. Materials and methods: Bioinformatic analyses of the expression of 15 mRNAs and 8 miRNAs from 403 bladder cancer samples from the TCGA database were performed. Statistical analysis: Spearman rho correlation method. **Results:** Among mRNAs of 15 checkpoint genes known to be expressed on the C/APC, the expression of six was positively correlated to any of the other five mRNAs. These mRNAs encode 3 co-stimulatory proteins (CD86, TNFSF4, TNFSF9) and 3 co-inhibitory proteins (PD-L1, PD-L2 LGALS9). The expression of mir-

15b was negatively correlated with 3 of these mRNAs: CD86, TNFSF4 and PD-L2. **Conclusions:** A joint transcriptional regulation of a several co-stimulatory and co-inhibitory mRNAs on the C/APC side of the synapse in bladder cancer was suggested, in line with the 'tidal wave' hypothesis of immune checkpoint regulation, where co-stimulation and co-inhibition occur simultaneously. mir-15b might negatively regulate expression of checkpoint genes, and this is now experimentally studied in our lab. Our results may point to novel methods to enhance the immunogenicity of bladder cancer.

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P12.020D

Clinical usefulness of non coding RNA markers in bladder cancer

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Introduction & Objectives Bladder cancer (BC) is the fourth commonest male malignancy and one of the most expensive human cancers to manage. Long noncoding RNA (lncRNA) could play a role in urothelial carcinogenesis. We analysed expression of 84 most relevant to tumorigenesis lncRNAs discovered to date in patients with various cancer as well as associated with cancer-related miRNA predicted with bioinformatics.

Material & Methods We examined tissue samples from patients with urothelial cancer histopathologically confirmed T2G3 and normal urothelium (as a control). The cDNA was used on the real-time Human Cancer Pathway finder RT² Profiler PCR Array (QIAGEN) in combination with RT² SYBR® Green qPCR Mastermix . CT values were exported to an Excel file to create a table of CT values which was then uploaded on to the data analysis web portal at http://www.qiagen.com/geneglobe. Fig. 1 RT^2 lncRNA PCR Array (96-well format); Human Cancer Pathway Finder (Qiqgen).

Results We found significantly altered expression of many lncRNAs: **PPC, ACTA2-AS1, LINC00887, CCAT2, GACAT1, ADAMTS9-AS2, PVT1, LSINCT5, RPLP0, RPS6KA2-AS2, MRPL23-AS1, CDKN2B-AS1, AFAP1-AS1, EMX2OS, LINC01234, HNF1A-AS1**. Some of them were already described as characteristic for BC while others have so far been detected only in different types of tumors.

Conclusions We have identified a group of new lncRNAs potentially related to the development or progression of BC.

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P12.021A

Methylation and clinicopathological markers for predicting progression of non-muscle invasive bladder cancer

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Most patients with non-muscle invasive bladder cancer (NMIBC) experience recurrences and up to 25% - progression to muscle-invasive disease. The risk of progression is estimated by several clinicopathological parameters which lack high objectivity and reproducibility. Therefore, the development of novel prognostic biomarkers is of particular importance. The aim of our study was to determine the prognostic value of *TBX4*, *SOX1*, *HOXA9*, and *ISL1* abnormal methylation with regard to progression in a prospective cohort of 258 NMIBC patients. Quantitative methylation analysis was performed using Ms-SNuPE.

We found statistically significant association of higher methylation levels with lamina propria invasion (*TBX4*, *SOX1*, *ISL1* genes), high-grade (*TBX4*, *SOX1* genes), and large tumor size (*SOX1*, *ISL1* genes). However, only the high ratio of *TBX4* methylation (≥ 44.5%) was significantly linked to disease progression, being able to discriminate between progressors and non-progressors with 92.3% sensitivity, 58.3% specificity, and 0.76 AUC (p=0.002). The multivariate Cox regression analysis, after adjusting for clinical parameters, confirmed an independent effect of high *TBX4* methylation on NMIBC progression (HR 13.1; 95% CI 1.7–101.8; p=0.014). Using logistic regression, we built a prediction model, comprising *TBX4* methylation marker, tumor grade, tumor size, and previous recurrence episodes, which resulted in improved specificity of 72.2%, sensitivity of 92.3% and AUC of 0.87 (p<0.001) for tumor progression. The data obtained demonstrate that the combination of *TBX4* methylation and clinicopathological markers is highly informative for the selection of patients with more aggressive disease course who are recommended early cystectomy. The work was financially supported by the BRFFR (Agreement M15-046).

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P12.022B

Gene expression and relapse of superficial cell carcinoma. A microarray analysis

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Treatment of bladder superficial tumours is dependent on the risk of recurrence and it is therefore clinically important to identify bladder cancer with high risk of intravesical recurrence after transurethral bladder tumour resection. For the improvement of recurrence prognosis we applied gene expression microarray analysis to two groups of superficial bladder tumours (with no or late relapse during the period of two years versus early relapse ones). Data from microarray containing 29,019 targets (Applied Biosystems) were subjected to a panel of statistical analyses to identify bladder cancer recurrence-associated gene expression changes. After validation 33 genes manifested significant differences between both groups. The significant expression was observed in the group of patients without recurrence by 30 genes of which the highest differences were detected by NINJ1, GNE, ANXA1, TNFSF15, WDR34, ARHGEF4, PRICKLE1, PSAT41, RNASE1, TM4SF1, TSPAN1, PLOD2 and WDR72. These genes code for proteins involved in signal transduction, vascular remodeling and vascular endothelial growth inhibition, mainly. Specially, PRICKLE1 and TNFSF15 were described to be linked with WNT/beta-catenin signaling and angiogenesis regulation and MTOR pathway. Loci of genes with significant changes of gene expression were located on characterisric chromosomes for bladder cancer: 9q, 17q, 2q and 16p. On the basis of this findings we documented a number of expression changes among which some seem to form clinically useful relapse markers of superficial bladder tumours. Research was supported by MSM21620808 and Diana Lucina.

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P12.023C

Alterations in TIMP-2 and MMP-9 expression levels in urinary bladder cancer

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Introduction: Tumor invasion and metastasis are the most decisive factors in the diagnosis and prognosis of bladder cancer. Genes regulating extracellular matrix play significant roles in invasion and metastasis of bladder tumors. Therefore the aim of this study was to investigate the deregulation of TIMP-2 and MMP-9 genes in association with bladder tumor invasion and metastasis.

Materials and Methods: 50 bladder tumors and 50 paired cancer free tissues were collected for RNA isolation. Following isolation and cDNAsynthesis, RT-PCR with gene specific primers was used to determine mRNA expression levels. Significant alterations in expression levels were determined by Student's t test.

Results: MMP-9 and TIMP-2 were significantly upregulated in tumors compared to cancer free tissue. Significant upregulation was also observed for MMP-9 in invasive and metastatic tumors (Table 1).

Conclusions: According to our results TIMP-2 and MMP-9 expressions are significantly upregulated in association with cellular events during tumorigenesis, invasion and metastasis. Upregulation of MMP-9 gene, which is known to enhance tumor invasion and metastasis, results in the significant yet insufficient upregulation of TIMP-2 to counter the effects of MMP-9. This imbalance between the expression levels of MMP-9 and TIMP-2 may reflect the invasiness and metastatic potential of bladder tumors.

MMP-9 and TIMP-2 expressions in bladder tissue

Gene	Comparison	Fc	95%CI	p
MMP-9	Tumor vs Cancer Free Tissue	7.078	3.08.-11.07	0.026
TIMP-2		2.434	1.21-3.66	0.038
MMP-9	Invasive vs Non-invasiveTumor	3.0951	1.50-4.69	0.037
TIMP-2		2.3729	1.10-3.64	0.071
MMP-9	Metastatic vs Non-Metastatic Tumor	4.0935	3.51-4.68	0.000
TIMP-2		1.9453	1.79–2.11	0.000

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P12.024D

Next-Generation Sequencing-based genomic profiling of brain metastases of primary ovarian cancer

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Introduction: Ovarian cancer represents the most common gynecologic malignancy and has the highest mortality of all female reproductive cancers whereas brain metastases (BM) from ovarian carcinoma are uncommon. In this study, we evaluated the mutational profile of ovarian cancer metastases through Next-Generation Sequencing (NGS) with the aim of identifying potential clinically actionable genetic alterations with options for small molecule targeted therapy.

Methods: Library preparation was conducted using Illumina TruSight Rapid Capture kit in combination with a cancer specific enrichment kit covering 94 genes. BRCA-mutations were confirmed by using TruSeq Custom Amplicon Low Input Kit in combination with a custom-designed *BRCA* gene panel.

Results: In our cohort all 8 sequenced BM samples exhibited a multitude of variant alterations, each with unique molecular profiles. The 37 identified variants were distributed over 22 cancer-related genes. In total, 7 out of 8 samples revealed either a *BRCA1* or a *BRCA2* pathogenic mutation. Furthermore, all 8 BM samples showed mutations in at least one DNA repair gene.

Conclusions: Our NGS study of BM of ovarian carcinoma revealed a significant number of *BRCA*-mutations beside *TP53*, *ATM* and *CHEK2* mutations. These findings strongly suggest the implication of BRCA and DNA repair malfunction in ovarian cancer metastasizing to the brain. Based on these findings, pharmacological PARP inhibition could be one potential targeted therapeutic for brain metastatic ovarian cancer patients.

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P12.025A

MCG-International: Increasing global availability of BRCA testing for cancer patients

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BRCA testing can provide useful clinical management information for patients with ovarian and/or breast cancer and their relatives. NGS has made BRCA testing fast and affordable, but access remains limited in many countries. Through the Mainstreaming Cancer Genetics (MCG) programme we developed and validated a rapid, robust, cost-effective testing pathway in which genetic testing is undertaken by the cancer team with cascade testing to relatives performed by genetics. The MCG pathway offers a means of increasing global availability of BRCA testing, which we are hoping to foster through a new initiative, MCG-International.

As a first step we performed a review of current testing activity using a non-systematic search of open-access data sources: Google, Google Scholar, PubMed, GeneTests, Orphanet, Genetic Testing Registry (NCBI). We used search terms to determine BRCA testing availability, resource availability and research activity. Terms were applied to each country in turn.

Eleven countries are introducing mainstream BRCA testing. Thirty countries reported interest in increasing access to BRCA testing with some supporting infrastructure. Barriers reported in these latter countries were similar to those we had to overcome, including lack of knowledge and confidence amongst healthcare professionals and patients, insufficient laboratory resources and inadequate financing.

These data suggest there is demand for increased BRCA testing access. The practical, efficient MCG pathway may have global utility in this regard. MCG-International aims to foster this through direct support of countries wishing to implement mainstream testing and through sharing experiences and best practice. Please contact mcg@icr.ac.uk if interested in participating. Funder, Wellcome:098518/Z/12/Z

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P12.026B

Delivering fast, affordable, high-throughput BRCA gene testing

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BRCA1 and *BRCA2* (BRCA) gene testing is one the most commonly requested tests in clinical genetics. The results are used to personalise management in cancer patients and to determine cancer risk and cancer surveillance management in unaffected patients. Delivering fast, affordable, high-throughput BRCA gene testing is therefore a key objective in genetic medicine. TGLclinical laboratory, a medical laboratory accredited to ISO15189, delivers germline BRCA testing to cancer and genetic services, using the TruSight Cancer Panel (Illumina). We run 96 samples every week, ~one-third are clinical BRCA test requests. We call both small mutations and exon deletion/duplications from NGS data, using the SALSA software package, verifying pathogenic and rare variants by Sanger and MLPA respectively. We have reported 2,504 BRCA tests. The overall mutation detection rate in 2,369 individuals with cancer was 10%. All ovarian cancer (OC) patients are eligible for testing (12% had mutations). Patients with BC<40 years, BC+ OC, bilateral BC <60 years, triple-negative BC, male BC or for whom testing would alter management are eligible (9.5% had mutations). Unaffected individuals with a strong family history of BC/OC cancer are eligible for testing, but the mutation detection rate was only 2% (3/135). Our VUS rate is 0.1% using an integrated interpretation pipeline that leverages gene and variant-specific information. Median turnaround is 18 days and cost is £355 These data show that, fast, clear, affordable, large-scale, high-throughput BRCA testing is achievable and suggests that services with limited resources should prioritise testing in affected individuals to maximise mutation detection. Funder, NIHR-RM/ICR-BRC

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P12.027C

Spectrum of BRCA1 and BRCA2 mutations in Romanian patients with ovarian cancer

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Ovarian cancer (OC) represents the fifth cause of cancer deaths among malignancies in European women. Mutations in the *BRCA1* and *BRCA2* genes play an important role in OC pathogenesis and confer more sensitivity to platinum agents than wild-type *BRCA*-OC.

The aim of our study was to identify and characterize the spectrum of germline sequence mutations in the *BRCA1/2* genes in order to provide knowledge of the OC pathogenesis in Romanian patients and to contribute in guiding decision-making on their treatment strategies.

Therefore, the complete coding sequences of *BRCA1/2* genes were analyzed by Next Generation Sequencing and Multiplex Ligation-dependent Probe Amplification in 153 Romanian unrelated OC patients, aged between 25–80 years.

We identified pathogenic mutations in the *BRCA1/2* genes in 47 (30.72%) OC patients, including 37 (24.18%) in *BRCA1* and 10 (6.54%) in *BRCA2*. The BRCA1/2 mutation spectrum identified was diverse: 12 frameshifts, 3 missenses, 6 nonsenses, 2 splicing variants and 1 exon deletion. Additionally, 6 VUS (variants with unknown significance) (3.92%) were detected, 2 in *BRCA1* and 4 in *BRCA2*.

The most prevalent mutations were: c.5266dupC p.(Gln1756Profs*74) identified in 14 (9.15%) patients, c.4035delA p.(Glu1346Lysfs*20) in 5 (3.26%) patients and c.3607C>T p.(Arg1203*) in 4 patients (2.62%) in *BRCA1* gene, therefore could be considered founder mutations for Romanian population.

Our study provided valuable molecular information about the spectrum of *BRCA1/2* mutations involved in OC pathogenesis in Romanian patients, contributing both to optimization of therapeutic management of these patients and providing accurate genetic counselling for OC patients and their families.

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P12.028D

A significant percentage of BRCA carriers have coinherited pathogenic mutations in intermediate risk BC genes

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Although multigene panel testing are increasingly used to evaluate patients with a suspected genetic predisposition to breast cancer (BC), most of the published studies using cancer panels are performed on BRCA negative patients. Thus, there are limited data on the coinheritance of BRCA mutations and mutations in other high/intermediate risk BC genes.

With an aim to determine the frequency of multiple heterozygotes of high/intermediate BC genes we have sequenced 94 cancer associated genes in 43 BRCA carriers (15 BRCA1 and 28 BRCA2) using Illumina TruSight Cancer kit on a MiSeq platform.

We identified a high percentage of BRCA carriers (16.3%; 7/43) with pathogenic mutations in ATM, CHEK2, FANCM and RAD51D genes (double heterozygotes). The following double heterozygote genotypes were identified: BRCA1:c.[4356delA];ATM:c.[67C>T], BRCA2:c.[3186_3189delTCAG];ATM:c.[3864delA], BRCA1:c.[181T>G];CHEK2:c.[573+1G>A], BRCA2:c.[8168A>G];CHEK2:c.[599T>C], BRCA2:c.[7879A>T];FANCM:c.[1972C>T], BRCA2:c.[775A>T];FANCM:c.[2953delG] and BRCA1:c.[3700_3704delGTAAA];RAD51D:c.[958C>T].

Double heterozygotes were younger (42+8,4 years) in comparison to simple BRCA carriers (49+12,2 years). ER, PR and HER2 receptor status was available for 37 of the 43 BRCA carriers. The majority of double heterozygotes had ER+/PR+ breast tumors (85.7%; 6/7) compared to 50% (15/30) of the simple BRCA carriers. Only one double heterozygote (14.3%; 1/7) was triple negative compared to 15 simple BRCA carriers (50%; 15/30).

In conclusion, BRCA carriers often have coinherited pathogenic mutation in intermediate risk BC genes; they have earlier onset of the disease and most commonly present with ER+/PR+ breast cancer. The identification of double heterozygotes is important for the patients, but has implication also for the families.

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A next generation sequencing study to detect large rearrangements in BRCA1/2 with high sensitivity in blood and FFPE samples

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Introduction: Identifying germline and somatic mutations in BRCA1 and BRCA2 genes is important to support translational cancer research as these genes are implicated in inherited risk and response to certain therapies. Although small variants (SNV and INDEL) in these genes are commonly detected, large rearrangements (LRs) such as exon level copy number variations are difficult to detect using traditional sequencing approaches and often require additional tests such as multiplex ligation dependent probe amplification (MLPA).

Methods: A total of 219 samples were screened for exon level copy number variations using the Oncomine BRCA Research NGS Assay and MLPA. The NGS assay covers 100% of all exons of BRCA1/2 with 263 amplicons (targeted regions). A comprehensive bioinformatics algorithm was developed to detect both small variants and exon level copy number variations in BRCA1/2.

Results: LRs were detected at high sensitivity from 219 blood and FFPE research samples, even in the absence of control sample(s). Of the 23 samples that were positive for LRs using MLPA, 22 (95.7%) were also detected by the NGS based assay. A range of LRs were detected including heterozygous whole gene deletions, single and multiple exon heterozygous deletions, single and multiple exon duplications, and homozygous multiple exon deletion.

Conclusions: An NGS assay with a comprehensive data analysis approach was developed that is capable of detecting both small mutations and LRs simultaneously in FFPE samples with high sensitivity and is an important advance in BRCA1/2 translational research.

For research use only. Not for use in diagnostic procedures.

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P12.030B

Risk of primary and contralateral breast cancer in BRCA1/2 mutation carriers previously affected with ovarian cancer

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Aim: To evaluate primary and contralateral breast cancer (PBC and CBC) risks in female BRCA1 and BRCA2 mutation carriers with and without ovarian cancer (OC).

Methods: We selected 1457 BRCA1 (219 with previous OC) and 976 BRCA2 mutation carriers (78 with OC) at risk of PBC, and 976 BRCA1 (94 with OC) and 551 BRCA2 mutation carriers (30 with OC) at risk of CBC. We used the Fine and Gray regression model.

Results: PBC was diagnosed in 10 (5%) BRCA1 mutation carriers with OC and in 192 (16%) without OC. For BRCA2 mutation carriers, PBC was diagnosed in 6 (8%) OC patients and in 97 (11%) women without OC. OC diagnosis was associated with a significantly lower PBC risk in BRCA1 mutation carriers (HR, 0.22; 95% CI, 0.11–0.42) and with a non-significantly lower risk in BRCA2 mutation carriers. CBC was diagnosed in 6 (6%) BRCA1 mutation carriers with PBC/OC and in 114 (13%) PBC patients without OC. For BRCA2 mutation carriers, CBC was diagnosed in 3 (10%) PBC/OC patients and in 43 (8%) PBC patients without OC. In BRCA1 mutation carriers, OC diagnosis was associated with a lower CBC risk (HR, 0.39; 95% CI, 0.17–0.89). For BRCA2 mutation carriers the HR was 1.42.

Conclusion: BRCA1 mutation carriers with previous OC have lower risks of PBC and CBC than BRCA1 mutation carriers without OC, which was not observed in BRCA2 mutation carriers. Our data suggest that the current prevention strategies regarding surveillance and mastectomy might be reconsidered for BRCA1-associated OC patients.

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P12.031C

BRCA testing in ovarian tumors initiated by a pathologist: a pre-screen for germline testing and therapy choice

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Introduction: Dutch guidelines recommend germline *BRCA* testing in all epithelial ovarian cancer (EOC)

patients. Approximately 20% of them have tumor DNA *BRCA* mutations of which 75% germline and 25% somatic. Both may benefit from PARP inhibitor therapy.

We aimed to implement **O**varian tumor DNA *BRCA* testing by a **PA**thologist (**OPA**) in all newly diagnosed EOC patients, as a pre-screen for germline *BRCA* testing and to enable timely genotype-based therapeutic choices.

Materials and methods: Pathologists were invited to submit Formalin Fixed Paraffin Embedded (FFPE) samples of newly diagnosed EOCs for tumor DNA *BRCA* testing by single molecule Molecular Inversion Probe (smMIP)-based targeted next generation sequencing of *BRCA1/2* and Multiplex Ligation-dependent Probe Amplification (MLPA) of *BRCA1*. Outcomes were communicated to patients by their gynecologist, who referred patients with tumor DNA *BRCA* mutations for genetic counseling and germline *BRCA* analysis. OPA uptake is evaluated with data from PALGA (Dutch Pathology Registry) and patients and physicians experiences with questionnaires.

Results: From October 2015 to January 2017 OPA was initiated in 199 women. In 195 women with a median age of 65 years (range 21–87), OPA was feasible and 32 tumor DNA *BRCA* mutations were identified (16%). Median turnaround time was 14 days (8–29). Germline testing revealed 11 *BRCA* mutations in 18 women (61%) with a tumor DNA *BRCA* mutation.

Conclusion: OPA provides a feasible pre-screen for genetic testing and PARP inhibitor therapy shortly after EOC diagnosis. If evaluation by patients and physicians is positive, international implementation can be considered.

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P12.032D

A recurrent intragenic *BRCA1* deletion in the Slovene population could be associated with a high ovarian cancer risk

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Introduction: There are several published studies on genotype-phenotype correlations in BRCA-positive families but the data on cancer risks associated with different mutations is currently insufficient to enable individual risk assessment. We present a study of women carrying a deletion of exons 4–9 in *BRCA1*, a recurrent genomic rearrangement in the Slovene hereditary breast and ovarian cancer population.

Materials and Methods: A retrospective analysis of a cohort of 382 BRCA-positive probands with breast or/and ovarian cancer (from 382 different families) tested at our institution was performed to determine the frequency of the *BRCA1* exons 4–9 deletion. Families of deletion carriers were subsequently analyzed to determine the number of breast and ovarian cancer patients. Proven, obligatory and probable carriers were included in our study.

Results: *BRCA1* exons 4–9 deletion was seen in 3.4% (13/382) of our BRCA-positive probands. The deletion was somewhat more common in patients with ovarian cancer than those with breast cancer but the difference was not statistically significant (4.7% vs ~2%; $p = 0.17$). 42 carriers of the deletion were identified in the 13 families studied. Of those, 40.5% were diagnosed with breast cancer, 38% with ovarian cancer and 9.5% with both breast and ovarian cancer; 12% were without cancer. Interestingly, a pair of monozygotic twins developed ovarian cancer aged 48 and 55.

Conclusions: The high number of ovarian cancer patients in families with the deletion of exons 4–9 in the *BRCA1* gene implies that the deletion could be associated with a particularly high risk of developing this disease.

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P12.033A

BRCA1 p.His1673del is a pathogenic mutation associated with a predominant ovarian cancer phenotype

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Background

Unclassified BRCA1/2 variants pose serious problems for counseling and managing patients. Aim of this study was to investigate the significance of the unclassified BRCA1 variant p.His1673del, which has been detected in multiple families from the Emilia-Romagna region of Italy.

Methods

The study involved 14 families carrying the BRCA1 p.His1673del variant, including 20 breast and 23 ovarian cancer cases. The multifactorial likelihood method by Goldgar was used to estimate the probability of the variant being causative; among the factors analyzed, LOH was further investigated in ovarian carcinomas through microsatellite analysis, CGH-array and FISH. Moreover, *in silico* modeling was performed to predict the impact of the deletion on BRCA1 functions.

Results

The multi-likelihood ratio was 2,263,474.04:1 in favor of causality, exceeding the cutoff of 1,000:1 adopted to establish that a variant is pathogenic. The breast to ovarian cancer ratio was 0.87:1, and four out of 14 families displayed site-specific ovarian cancer. All probands shared the same uncommon haplotype at the BRCA1 locus, consistently with a common ancestor. All six ovarian carcinomas and two out of four breast carcinomas available showed LOH at BRCA1 locus, which in three out of four ovarian carcinomas analyzed by FISH was associated with duplication of chromosome 17 containing the variant. *In silico* modeling suggests that His1673-lacking BRCA1 protein may have a decreased binding capability towards BARD1 and other related proteins.

Conclusions

BRCA1 p.His1673del should be regarded as a likely pathogenic mutation conferring a high ovarian cancer risk, probably through impairing BRCA1-BARD1 interaction.

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P12.034B

One in four women with high-grade serous ovarian cancer carries a loss-of-function *BRCA1* & *BRCA2* germline allele

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Introduction: High-grade serous ovarian cancer (HGSOC) patients carrying loss-of-function (LoF) in homologous recombination (HR) genes, and especially in *BRCA1* & *BRCA2*, have improved overall survival and better therapeutic response, exemplified by the increased sensitivity to poly (ADP-ribose) polymerase inhibitors.

Methods: All patients diagnosed with HGSOC from July 2014 and thereafter, unselected for age at diagnosis or family history, were sequenced by Trusight cancer panel. All diagnoses were confirmed by histopathology reports, while detailed pedigrees were constructed. As a first step, *BRCA1* & *BRCA2* germline mutations were evaluated, while analysis for mutations in other HR genes is under way.

Results: In total, 23.7% (64/270) of the 270 patients (mean age at diagnosis 57±11 years) carried LoF *BRCA1* & *BRCA2* mutations; 17.4% *BRCA1* and 6.3% *BRCA2* (mean age at diagnosis 52±9 years). Approximately 10% (6/64) of the mutation carriers had a previous personal history of breast cancer, while in one case ovarian preceded breast cancer diagnosis. Family history of breast and ovarian was seen in 56% (36/64) of the cases. Paternal ancestry, limited family structure, large number of male family relatives and prophylactic hystero-oophorectomy can explain the lack of family history in 20/28 cases with no apparent family history.

Discussion: Our results further support the recommendation to test all patients with HGSOC and have implications for treatment and prevention for them and their families. HGSOC diagnosis itself can be thought as a predictor for HR deficiency, as highlighted by the high frequency of germline *BRCA1* & *BRCA2* mutations.

The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398–1792 for more information.

P12.035C

Linking uterine serous carcinoma to BRCA1/2-associated cancer syndrome: a meta-analysis and case report

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Backgroud: Uterine serous carcinoma (USC) shows greater morphological, clinical and molecular similarities to high-grade ovarian tubal serous carcinoma than to other types of endometrial cancer. As high-grade ovarian tubal serous carcinoma is known to be associated with BRCA1/2 pathogenic germline mutations (PMs), we aimed to explore whether USC is also a constituent of hereditary breast and ovarian cancer syndrome. Methods: Pubmed, EMBASE and Web of Science were searched in July 2016 for articles assessing the association between USC and germline BRCA1/2-PMs. Pooled analysis and comparisons were performed using a random effects logistic model, stratifying for ethnicity (Ashkenazi versus non-Ashkenazi). In addition, tumour tissue from an USC case with a hereditary BRCA1-PM was analysed for loss of heterozygosity at the BRCA1 locus and was functionally analysed for homologous recombination proficiency. **Results:** The search yielded 1893 citations, 10 studies were included describing 345 USC patients. For Ashkenazi Jews, the pooled odds ratio of having a germline BRCA1/2-PM was increased in USC patients compared with the general Ashkenazi population: odds ratio 5.4 (95%confidence interval: 2.2–13.1). In the patient with USC, we identified the known germline BRCA1-PM in the tumour DNA. Furthermore, we showed both loss of heterozygosity of the wild-type allele and a deficiency of homologous recombination. Conclusion: This study suggests that USC may be an overlooked component of BRCA1/2-associated hereditary breast and ovarian cancer syndrome. Screening for germline BRCA1/2-PMs should be considered in patients diagnosed with USC, especially in cases with a positive first-degree family history for breast and/or ovarian cancer.

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P12.036D

NGS-based BRCA1, BRCA2, and PALB2 mutation testing in Iranian population with breast cancer

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Background: Hereditary Breast Cancer (HBC) is a well-characterized syndrome. The cause of this syndrome has been mainly attributed to the mutations in the *BRCA1*, *BRCA2* and *PALB2* genes. In this study, we aimed to estimate the prevalence of these gene mutations in Iranian breast cancer patients. Materials and Methods: Next Generation Sequencing (NGS) was conducted on 424 breast cancer patients comprising 2 groups. The first group consisted of 281 patients who were selected due to presenting the HBC features including age at onset of under 40 years, positive family history of breast/ovarian cancer, male breast cancer or multiple primary tumors in the same individual. The second group was included 143 patients without the aforementioned criteria. **Results:** Overall, 33 pathogenic mutations were found. This was 13 *BRCA1*, 14 *BRCA2* and 1 *PALB2* in 281 selected patients and 4 *BRCA2* and 1 *PALB2* in 143 patients without the inclusion criteria for genetic testing. All mutations were singleton. Only 1 out of 96 patients with only an early onset of breast cancer, 2 out of 10 patients with multiple primary breast cancers and 25 out of 172 patients with family history of breast/ovarian cancer were diagnosed as the pathogenic mutation carriers. No pathogenic mutation was found in male breast cancer patients. Conclusion: Our results indicated that *BRCA1/2* mutations are common among Iranians with breast cancer who reported positive for family history of Breast/Ovarian cancer. Screening of the early onset breast cancer patients for *BRCA1/2* mutations may not be cost-effective among Iranian population.

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P12.037A

Thorough second hit analysis in breast and ovarian cancers from *BRCA1/2* germline mutation carriers reveals different mechanisms in both tumor entities

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Objective: To paint a detailed picture of somatic *BRCA1/2* alterations in breast and ovarian cancers from patients with a germline mutation at these loci. LOH (loss of heterozygosity) is accepted as the general mechanism, however, little is known about the prevalence of other somatic events.

Tumor material and Methods: We included 56 breast (36 *BRCA1*; 20 *BRCA2*) and 16 high grade serous epithelial ovarian (9 *BRCA1;* 7 *BRCA2*) carcinomas. The complete coding region of *BRCA1/2* was analysed in DNA extracted from tumor and blood using next generation sequencing. Exon-spanning deletions/duplications were investigated using MLPA. MS-MLPA was applied to unravel the *BRCA1/2* methylation status.

Results & conclusions: 23/36(64%) *BRCA1*-breast tumors were triple negative, compared to 3/20(15%) *BRCA2*-breast tumors. Differences in somatic changes were established in breast versus ovarian cancers: loss of the wild type allele was observed in 15/16(94%) ovarian but in only 31/56(55%) breast tumors. Surprisingly, loss of the mutant allele was found in 7 breast cancers. Therefore, genetic counseling of VUS in *BRCA1/2*, should not rely on LOH analysis in breast tumors. In several breast tumors somatic truncating point mutations were detected (also in the gene without the germline mutation); this was not observed in the ovarian cancers. Methylation of the *BRCA1/2* promoters was not established. MLPA suggests complete or partial gene deletions in the majority of the samples. The complex second hit mutation spectrum in the breast cancers could potentially help explain why PARP inhibitor treatment is less successful in *BRCA1/2*-associated breast tumors compared to ovarian tumors.

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P12.038B

Transcript analysis redefines the pathogenicity of *BRCA1/2* VUS and reveals the limitations of *in silico* prediction tools

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BRCA1 and *BRCA2* genes are involved in hereditary predisposition to the development of breast and ovarian cancer with high penetrance. The detection of *BRCA* pathogenic variants allows offering appropriate cancer-risk management and has now considerable clinical impact, guiding the surgical and pharmacological approach. In this context, a major drawback is represented by the identification of variants of uncertain significance (VUS), accounting for a relevant fraction of alterations. Many intronic/exonic VUS potentially affect mRNA splicing and transcript analysis is an essential step for the definition of pathogenicity. Here, we assessed the impact on splicing of 17 *BRCA* variants whose biological and clinical effects were unknown until now. Nine variants were demonstrated to impact on the normal splicing process and aberrant transcripts were characterized. Different types of abnormal splicing event were observed, namely exon skipping, intron retention, usage of "de novo" and cryptic splice sites. As a

consequence, transcripts with premature stop codons or in-frame loss of functionally important residues were generated. A possible "leaky" effect was investigated, allowing the classification into classes 3 or 4/5, according to the current guidelines of the ENIGMA consortium. Importantly, this study also highlights that *in silico* tools are limited in the prediction of aberrant isoforms. Experimental analysis revealed that these predictions would have led to erroneous conclusions in 55% of cases. In conclusion, this study allowed re-defining the pathogenicity of *BRCA* variants of uncertain significance and underlines the need of more accurate *in silico* programs, especially for unmasking the usage of alternative or pseudo-splice sites.

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P12.039C

BRCA2 mis-splicing: exons 17 and 18 regulation

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Mutation screening of BRCA2 identifies a large fraction of variants of uncertain clinical significance (VUS). Our goal was to investigate the impact of reported variants of BRCA2 exons 17 and 18 on splicing to assess their role in hereditary breast cancer and to identify critical regulatory elements that may constitute hotspots for spliceogenic variants.

A splicing reporter minigene with BRCA2 exons 14 to-20 (MGBR2_ex14–20) was constructed in the pSAD vector. Fifty-two candidate variants were selected with splicing prediction programs, introduced in MGBR2_ex14–20 by site-directed mutagenesis and assayed in MCF-7 cells. Functional mapping by microdeletions revealed essential sequences for exon recognition on the 3′ end of exon 17 and the 5′ end of exon 18. Thirty out of the 52 selected variants induced anomalous splicing in minigene assays with >16 different aberrant transcripts, where exon skipping was the most common event. According to the biological indicators of pathogenicity, 18 variants could be classified as disease-causing, accounting for 28.6% of all pathogenic variants of exons 17–18 at the BRCA Share database. Aberrant splicing is especially prevalent in BRCA2 exons 17 and 18 due to the presence of active ESEs, which are recognized by splicing factor as SC35 and Tra2β that play an important role in exon recognition. Splicing functional assays with minigenes are a valuable strategy for the interpretation of VUS of any disease-gene.

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P12.040D

Experience of a genetic diagnostic laboratory with a panel of 18 genes involved in gynecologic cancer

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Introduction. Breast cancer is the most commonly diagnosed cancer among women worldwide. In Spain, 500.000 and 65.000 new breast and ovary cancer cases are diagnosed per year. A 5–10% of these are thought to be hereditary. The objective of this work has been to conduct a retrospective analysis of our experience with an NGS panel containing 18 genes involved in gynecologic cancer.

Materials and Methods. Cohort of 194 patients from Europe (the majority), Latin America and Middle East. Samples were sequenced on a Illumina platform using TruSight Cancer kit (Illumina). 18 genes were analyzed: *ATM-BRCA1-BRCA2-BRIP1-CDH1-CHEK2-EPCAM-MLH1-MSH2-MSH6-NBN-PALB2-PMS2-PTEN-RAD51C-RAD51D-STK11-TP53*. MLPA was carried out for *BRCA1, BRCA2* and 3′ end of *EPCAM*. Pathogenic (P) and likely patogenic (LP) mutations are confirmed by sanger sequencing. Variant classification is carried out following ACMG guidelines.

Results. A total of 88 variants were identified, including P (7,2%), LP (2,1%) and variants of unknown significance (VUS) (28,9%). In 122 patients (62,4%), no variants (P, LP or VUS) were identified.

Conclusions. P, LP and VUS percentages are similar to the ones described by Susswein (1) (9% P + LP; 20,4% to 39,7% VUS, depending on the origin). We have observed that VUS are more frequent in (a) genes that have been

more recently described and therefore less studied, and in (b) non-caucasian populations. These results highlight the importance of cosegregation studies and taking part in public databases, with the purpose of decreasing VUS percentage n these kind of studies.

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P12.041A

The 30 kb deletion in the *APOBEC3* cluster decreases *APOBEC3A* and *APOBEC3B* expression and creates transcriptionally active hybrid gene but does not associate with breast cancer in European population

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Introduction: A common CNV in the *APOBEC3* cluster (*APOBEC3B* deletion) was reported to be associated with breast cancer. Our aim was to assess the influence of *APOBEC3B* deletion on the expression of affected genes and to analyze its association with breast cancer in European population.

Materials and Methods: We analyzed structure of *APOBEC3B* deletion with three distinct approaches: Sanger sequencing, and designed by ourselves MLPA and A3B+ PCR assay. Then, we exploited publically available expression data and our own qPCR/ddPCR results for the analysis of a relation between *APOBEC3B* deletion and mRNA levels of *APOBEC3B*, *APOBEC3A* and hybrid *APOBEC3A/APOBEC3B*. Additionally, we performed a large-scale association study in three different cohorts (in total 2972 cases and 3682 controls) using A3B+ PCR assay.

Results: We elucidated the structure of *APOBEC3B* deletion with nucleotide resolution and confirmed it with three different tests. This knowledge allowed us to confirm the presence of transcriptionally active hybrid gene (*APOBEC3A/APOBEC3B; APOBEC3A* with 3′UTR of *APOBEC3B*) and design tests for separate analysis of expression of three highly homologous transcripts: *APOBEC3A*, *APOBEC3B* and *APOBEC3A/APOBEC3B*. The analysis showed that *APOBEC3B* deletion negatively correlates with expression of *APOBEC3A* and *APOBEC3B*, and positively correlates with expression of *APOBEC3A/APOBEC3B*. We observed no association between *APOBEC3B* deletion and breast/ovarian cancer.

Conclusions: We provided a direct evidence that *APOBEC3A/APOBEC3B* hybrid gene is transcriptionally active and confirmed association of *APOBEC3B* deletion with the expression of affected genes. Our large case-control study does not confirm association of *APOBEC3B* deletion with breast cancer.

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P12.042B

Transcriptome wide analysis of natural antisense transcripts shows potential role in breast cancer

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Introduction: In developed countries, breast cancer is the most frequent cancer type in females, accounting for approximately one third of all cancers. It is a complex disease, characterized by genomic instability.

We investigated the potential role of natural antisense transcripts in the oncogenic process of breast cancer.

Materials and Methods: Both tumor and normal breast tissue from 25 female breast cancer patients have been analyzed by transcriptome-wide strand-specific paired-end RNA sequencing.

Differential expression analysis of the antisense transcripts, differential correlation analysis between the antisense transcripts and their paired protein coding genes, and

read counts ratios variation analysis between the antisense transcripts and their paired protein coding genes have been performed.

These three methods yielded candidate antisenses lists for which the association with survival of the corresponding protein coding gene has been tested on an independent cohort of 1060 breast cancer patients from the TCGA.

Results: All three lists showed a higher amount of genes significantly associated with survival than a list of randomly selected protein coding genes of the same size.

Conclusions: The significant difference in terms of association with survival might be indicative of an oncogenic role of the antisense transcripts that have been selected through the three aforementioned methods.

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P12.043C

Downregulation of *Caspase 8* in sporadic human breast tumours in Iranian patients

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Purpose: It is now well known that evading from apoptosis, as a cancer hallmark, can lead to tumour initiation, progression and metastasis. As a result of GWA studies, an initiator protease in this pathway, CASP8, has been found to be an important gene regarding to breast cancer susceptibility. It's expression alterations have been reported in breast cancer cell lines. Given that in previous studies expression analysis of this gene had only been done in breast cancer cell lines, in this study we aimed to evaluate the expression of CASP8 in breast cancer tissues versus adjacent normal tissues, using real-time quantitative method. **Method:** Caspase 8 mRNA expression was quantified using comparative RT-qPCR in 27 fresh frozen breast tumours and 27 adjacent normal tissues. Moreover, relationship between the expression changes of CASP8 in tumour tissue and various clinical and pathological features were evaluated in an Iranian population. **Results:** The present study showed that CASP8 expression was significantly ($p = 0.024$) reduced in tumour tissues compared to neighbouring normal tissues. CASP8 expression was significantly correlated with the status of hormone receptors (ER and PR). **Conclusion:** To the best of our knowledge, this study is the first report on reduced expression of CASP8 in breast cancer versus adjacent normal tissues. Our data support previous results obtained from cell lines, therefore highlights the seminal role of the induction of CASP8 expression, as a novel therapeutic approach, in order to sensitize tumour cells to apoptotic stimuli.

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P12.044D

Beyond *CHEK2* in breast cancer: Search for additional moderately penetrant risk gene variants by analyzing the oligogenic disease course in *CHEK2* mutation carriers

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In one third, breast cancer (BC) shows a familial aggregation suggesting a genetic predisposition. Besides *BRCA1/2*, *CHEK2* displays a relevant risk gene which controls DNA repair. Although a multiplicative nature of risk effects by additional variants has been suggested, additional risk genes in *CHEK2* mutation carriers have not been well studied so far. Here we focused on 168 *BRCA1/2* negative BC cases (average age at onset: 46) from 151 families which are either heterozygous for *CHEK2* founder mutation c.1100delC or delExon9/10. Using targeted NGS, we were able to identify a total of 10 patients (10/168;6%) carrying a deleterious mutation in addition to the known heterozygous *CHEK2* mutation. Deleterious autosomal dominant variants were identified in *ATM* (p.I1581Nfs*5, c.496+5G>A), *CHEK2* (2x p.R117G), *FANCM* (p.Q156*), *MLH1* (p.N64S), *PMS2* (p.D414Rfs*44, *c*.250+3_250+6del), and in the autosomal recessive cancer predisposition genes *MUTYH* (p.G396D) and *XPC* (p.C670*). Our results support the assumption that the increased cancer risk for *CHEK2* mutation carriers with a positive cancer family history is at least partially explainable by an oligogenic disease course. Preliminary data suggest an association of the presence of additional deleterious mutations and an earlier age of onset in *CHEK2* mutation carriers. Further data analysis is mandatory to validate these findings. In addition to the 48 analyzed genes, a total of 318 polygenic risk score (PRS) variants are currently under examination. In summary, we could confirm panel diagnostics as a powerful tool to improve individual risk prediction and clinical care of *CHEK2* mutation carriers. Supported by German Cancer Aid (#110837).

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P12.045A

Chromosomal alterations in patients with breast cancer

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Today, breast cancer (BC) is the most commonly occurring cancer among women. The aim of the present study was to describe the types and frequencies of CAs in BC patients. Fifty-one patients were analysed as part of an ongoing study of the cytogenetics of BC. Cytogenetic analysis of blood samples was conducted by Giemsa-banding in patients with BC. The karyotype results were normal in 68.6% of 51 patients; however, CAs were detected in 31.4% of all patients. In total, 27.5% of patients exhibited structural aberrations (translocations, deletions, inversions, duplications and fragilities) and 3.9% exhibited numerical aberrations. Specifically, the deletions and fragilities among structural aberrations were the most common karyotypes (7.8% each) among the patients. Deletions included del (7p11), del(7q32), del(9p32), del(11q23) and del(11q15). Inversions were present in 3.9% of all patients, and included inv(21)(q11;q21) and inv(11)(p15;q12). Translocations were detected in one patient (2%) and the frequency of other CAs, different to those types already mentioned, was 5.9%. Regarding numerical CAs, two patients had aneuploidies. This study demonstrated that genetic instability existed in 31.4% of BC patients, and emphasizes the importance of combined cytogenetic analysis for the diagnosis of BC. The 7p11, 7q32, 9p32, 11p15, 11q12, 11q23, 21q11, 21q21, 15p11 and 17q23 regions are becoming a model for understanding chromosomal regions in BC. Therefore, the presence of 11q and 7q deletions in breast tumors may suggest that it plays an important role in the development of BC. If so, different chromosome anomalies might have different pathogenetic and prognostic significance.

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P12.046B

NGS-based detection of copy number variations in hereditary breast- and ovarian cancer germline diagnostics

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A cohort of 3,032 consecutive index cases with breast and/or ovarian cancer (BC/OC) was analysed by next generation sequencing (NGS) covering 34 cancer risk genes/candidate genes (TruRisk® gene panel, Agilent SureSelect). Sequencing was performed on Illumina NextSeq/MiSeq devices. For bio-informatic analyses of sequencing date, we

employed the Sophia Genetics DDM pipeline. All index cases fulfill the criteria of the German Consortium for Breast- and Ovarian cancer (GC-HBOC) for germline testing. According to the Sophia Genetics pipeline, 77 CNVs (46 deletions, 31 duplications) were observed in 74 independent cases (2.4%). Of those, a total of 33 patients carry heterozygous CNVs in *BRCA1* (n = 22), *BRCA2* (n = 4) or *CHEK2* (n = 7). CNVs in further established cancer predisposition genes were observed in another 15 index patients (5 × *ATM*, 3×*PMS2*, 2×*RAD51C*, 2×*BRIP1*, 1×*TP53*, 1×*FANCM*, 1×*NBN*). No CNVs were detected in *MLH1*, *MSH2*, *MSH6*, *RAD51D*, *PALB2*, *CDH1*, *NBN*, *PTEN*, *STK11* and *XRCC2*. Among the candidate genes investigated in this study, CNVs were frequently observed in *BARD1* (4×), *MRE11A* (8×), and *ERCC2* (12×). At the time point of the data collection, 40/77 CNVs have been confirmed by either MLPA or aCGH. In summary, bioinformatic analyses of NGS gene panel data robustly detects CNVs. However, it remains to be determined whether sensitivity/specificity meets diagnostic criteria.

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P12.048D

Identification of potential driver or disease associated genomic variants as part of multiomic research in patients with breast cancer

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Breast cancer is the most common cancer in women worldwide. Its genetic basis is comprehensively studied and results from genetic and genomic studies showed potential to be utilized in identification of novel biomarkers valuable in disease diagnostics, prognostics and therapy.

In our study 50 DNA samples isolated from tumor tissue of patients with primary breast cancer were analyzed with custom gene panel containing 56 genes that have been found to be causaly involved in breast cancer promotion, progression or having therapy decision value. Study was performed on samples that were clinically well defined as were previously or simultaneously analyzed for circulating tumor cells (CTC) and RNA expression.

After filtering of identified variants with Ingenuity Variant Analysis using Cancer driver variants filter totaly 363 variants remained to be potential candidates. Of these 51 were found to be listed in COSMIC database simultaneously 21 of these were classified as likely pathogenic or pathogenic although the list of likely pathogenic and pathogenic genomic variants contained 31 variants. Most frequently mutated gene in the patient group was found to be TP53 with 10 likely pathogenic or pathogenic variants, second most mutated gene was found to be PIK3CA with 6 and third most mutated gene was found to be BRCA2 gene with 3 such variants.

Integration of data from performed gene panel resequencing with clinical records, CTC status and RNA expression will be performed with ambition find novel disease specific associations and potentially novel disease biomarkers.

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P12.049A

Antidiabetic exendin-4 activates apoptotic pathway and inhibits growth of breast cancer cells

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Introduction: Exendin-4 is a GLP-1 analog used for the treatment of type 2 diabetes mellitus in its synthetic form. As women with diabetes have higher breast cancer incidence and mortality, we examined the effect of the incretin drug exendin-4 on breast cancer cells. The aim of the study is to investigate anticancer mechanism of exendin-4 in MCF-7 breast cancer cells. Materials and Methods: Cytotoxic effects of exendin-4 were determined by XTT assay. mRNA expressions were evaluated by Real-time PCR. Effects of exendin-4 on cell invasion, colony formation, and cell migration were detected by Matrigel chamber, colony formation assay, and wound-healing assay, respectively. **Results:** IC_{50} dose of exendin-4 in MCF-7 cells were

detected as 5 μM at 48th hour. According to results, *caspase-9, Akt*, and *MMP2* expression was reduced in dose group cells, compared with the control group cells. *p53, caspase-3, caspase-8, caspase-10, BID, DR4, DR5, FADD, TRADD, PARP, PTEN, PUMA, NOXA, APAF, TIMP1*, and *TIMP2* expression was increased in dose group cells, compared with the control group cells. **Conclusions:** To conclude, it is thought that exendin-4 demonstrates anticarcinogenesis activity by effecting apoptosis, invasion, migration, and colony formation in MCF-7 cells. Exendin-4 may be a therapeutic agent for treatment of breast cancer as single or in combination with other agents. More detailed researches are required to define the pathways of GLP-1 effect on breast cancer cells. Because the molecular biology of breast cancer involves a complex network of interconnected signaling pathways that have role in cell growth, survival, and cell invasion.

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P12.050B

Rapid genetic counselling in the primary diagnostic workup for young women with breast cancer as a basis for informed treatment decisions: a prospective cohort study

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Introduction: BRCA screening results and familial risk assessment may guide women with breast cancer in their choice between different risk management strategies. Rapid genetic counselling was introduced in the primary diagnostic workup for women diagnosed with breast cancer before 50 years of age in Central Region Denmark (2013). The purpose was to avoid adjuvant radiation therapy if women chose prophylactic mastectomy after genetic counselling.

Methods: Clinical details recorded for all women included age at diagnosis, pathology, BRCA screening results, and familial risk assessment. Known BRCA carriers were excluded. Data on contralateral prophylactic mastectomy were obtained from a central pathology database. The women consented to anonymous use of their data for research purposes.

Results: 133 women with breast cancer accepted genetic counselling (median age, 36; range, 21–49). We detected 12 BRCA-1 (9.0 %) and 3 BRCA-2 mutations (2.3 %). 41 women (33 %) had triple-negative breast cancer (ER-/HER2-). 10 women (24 %) with triple-negative breast cancer had detectable BRCA mutations vs. 5 women (6.1 %) with non-triple-negative breast cancer. Women with unilateral cancer who chose contralateral prophylactic mastectomy included 13 women (93 %) with BRCA mutations, 8 women (67 %) with high-risk pedigrees but no detectable BRCA mutation, and 18 women (23 %) with moderate or low-risk pedigrees.

Conclusion: The prevalence of contralateral prophylactic mastectomy in young women with breast cancer reflected their familial risk assessment. Most BRCA mutations were found in women with triple-negative breast cancer. Genetic counselling in the primary diagnostic workup provides a basis for informed treatment decisions.

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P12.051C

Major challenge of breast cancer molecular genetics: From association studies to causation and function

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A family history of breast cancer (BC) has long been thought to indicate the presence of inherited genetic events that predispose to this disease. Actually, the major challenge of BC molecular genetics is to identify causal variants. Because of important differences in genetic patterns between ethnicities, association studies and haplotype analysis in different populations can help to overcome this challenge. Our present genome-wide-scanning was applied with Affymetrix-Genome-Wide-Human-SNP-Array-6.0 in order to determine allelic frequencies of the known BC associated variants in 135Tunisians representing the general-population. We constructed and phased haplotypes carried by the studied Tunisian-population. Moreover, we assessed the biological effect of the putative functional variants by conducting eQTL-assays. We also compared BC associated-loci between different HapMap-populations by comparing LD-blocks and by conducting Principle-Component-Analysis. Our haplotype analysis showed that the Tunisian-population is more predisposed to the following at risk loci: 2p24, 4q21, 6q25, 9q31, 10q26, 11q13, 14q32. Four polymorphisms specific to the Tunisian population have also been identified including one on the *ESR1*-gene. Furthermore, we characterized a *BRCA1*-variant that can be considered as a causal polymorphism. In addition, we conducted Whole-Exome-Sequencing in 11BC

patients. Our results showed a family specific BC genetic predisposition in a non-*BRCA* family, and a second Tunisian family with bi-allelic mutations on the *BRCA2* gene, a phenomenon rarely observed in BC families. Therefore, combining association and functional analysis, it is now possible to refine the BC genetic component in Tunisia which will provide a better clinical management of patients. Funding Agency: Qatar National research FoundationGrant ID: NPRP 08-083-3-031

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P12.052D

Familial breast cancer with hereditary mutation in MMR genes: does it exist?

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The use of multi-gene panels in the diagnostic of hereditary cancers has become a standard in cancer genetics. These panels usually include 15–30 cancer susceptibility genes responsible for specific hereditary cancer syndromes. However with the use of these panels we observed the emergence of variations in unexpected genes regarding the clinical pictures.

In this study we reviewed the clinical data and pedigrees of women diagnosed with breast cancer and carrying mutations in DNA mismatch repair genes (MMR) involved in Lynch syndrome (MLH1, MSH2, MSH6 and PMS2). Among 1200 patients tested between March 2016 and December 2016 using a multi-gene panel 736 women with new breast cancer diagnoses were included. Of those women 49 were found to carry mutations in MMR genes with two of them carrying more than one single mutation. This represents 47 missense substitutions of unknown significance (MLH1: 8; MSH2: 9; MSH6: 16; PMS2: 14) and 4 clearly pathogenic mutations (MLH1: 1; MSH2: 1; MSH6: 2).

Clinical histories were then correlated with the presence of Lynch syndrome-related risk. Amsterdam I and II criteria were absent in almost all families whereas few patients entered Bethesda guidelines (6/49). By contrast strong family histories of breast cancer were reported for 32/49 patients.

These data pointed to the detection of mutations without regard to family histories of cancer in women with breast cancer diagnoses. For women with strong family histories of breast cancer, genetic counselling remains complex especially about the risk assessment for cancer sites being part of the Lynch syndrome spectrum.

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P12.053A

The identification of microRNA and genes connected with metastasis among breast cancer patients

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There were investigated of 16 microRNA quantitative expression levels in a sample of breast cancer tumors in relation to the normal breast tissue. All patients of the sample were not subject to radiation, neither to chemotherapy. Three clusters of gene co-expression were identified. One of the clusters was enriched by patients with metastases. The accumulation of patients with metastases in the cluster relative to other clusters was near of statistical significance ($p = 0,07$, $OR = 5$). The differential expression and significant association with metastasis was revealed for MIR34A, MIR125B1, MIR222 and MIR21. In the study of 75 gene expression on the same sample the association of genes ZEB2, PLAUR, MMP9, CSF1R, FN1 with metastases was identified. In analyzes of all genes and microRNAs the expression relationship 9 new negative correlations were found, but not among the metastasis-associated genes and miRNAs. The analysis of interconnection of associated with metastasis miRs and genes by MirOB database found the principal possibility of their combining in the network through intermediaries, but on the basis of known data on breast cancer a single network has not been received. Analysis of GO processes showed that these genes and microRNA integrates participation in the migration and tumor progression. The genes are also involved in the invasion, metastasis and in EMT processes, the microRNAs in the process of MAPK pathway regulation that stimulate of metastasis. Finally, we identified associated with metastasis miRNAs and the genes, which are functionally involved in different processes of metastasis.

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P12.054B

Strategies to predict treatment response & select therapies in metastatic breast cancer patients using a next generation sequencing multi-gene panel

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Introduction: In the last decade, an increasing number of molecularly targeted drugs have been developed for the treatment of metastatic breast cancer (BC). The ability to perform multi-gene testing for a range of recurrent molecular alterations may provide an opportunity to clarify the mechanisms of treatment response, to find the strategies to overcome treatment resistance and thus, to identify patients who may be candidates for matched targeted therapies. The aim of our research is to find prognostic and predictive molecular biomarkers for the management of BC.

Materials and Methods: We used an NGS panel of 25 genes involved in the pathways of resistance to endocrine and targeted treatments, to test the mutational status of 7 primary BCs and matched metastatic sites using a custom Ampliseq® panel and the Ion Torrent PGM sequencer.

Results: Ten different genes (*PTEN, PIK3CA, mTOR, ERBB2, ERBB3, MET, INPP4B, MAP2K1, CDK6, KRAS*) in 6 patients showed possible damaging variants. In 4 patients the mutational status of primary and secondary tumors were identical, and the variants found in primary tumors were able to explain the subsequent treatment resistance. In 1 patient, the metastatic site presented 3 new variants, which could explain the progression of the disease. In 2 patients, some of the mutations expressed by the primary tumor were disappeared in the metastases.

Conclusion: In 5 patients the mutational status of primary tumor predicted treatment resistance and thus relapse; while in 3 patients the mutational status of metastatic site could have guided differently subsequent treatment choices.

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P12.055C

Association of *MUTYH* with familial breast and ovarian cancer

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The *MUTYH* gene as a caretaker of the genome is associated with hereditary cancer. It is a component of a base excision repair (BER) system that protects DNA from oxidative damage. Biallelic mutations in this gene are known to be associated with hereditary colorectal cancer.

In the past different cohorts have been tested for an association of *MUTYH* and breast cancer with controversial results. Here we present the largest case-control-study of German patients concerning this association. In this study, we screened a cohort of 2.051 healthy German controls and 3.324 patients with a family history of breast cancer (BC) and/or ovarian cancer (OC) for pathogenic variants in the *MUTYH* gene. All patients fulfill the inclusion criteria of the German Consortium for Hereditary Breast- and Ovarian Cancer (GC-HBOC) for germline testing. Samples were screened via NGS (TruRisk® gene panel, Agilent SureSelect).

We have detected 53 index cases carrying ten different variants in *MUTYH* that were previously described pathogenic for colorectal cancer. In the cohort of healthy controls 28 individuals were detected to carry a pathogenic *MUTYH* variant. No biallelic pathogenic *MUTYH* mutations were identified. (Odds ratio 1.17; 95% CI, 0.72–1.91; $P = 0.50$). To the time of data-cut our results do not support an association of heterozygous *MUTYH* mutations with breast and ovarian cancer. In order to get more precise evidence further investigations need to be done.

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P12.056D

Next-generation sequencing in familial breast cancer patients from Lebanon

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Background: Familial breast cancer (BC) represents 5 to 10% of all BC cases. Mutations in two high susceptibility *BRCA1* and *BRCA2* genes explain 16–40% of familial BC, while other high, moderate and low susceptibility genes explain up to 20% more of BC families. The Lebanese reported prevalence of *BRCA1* and *BRCA2* deleterious mutations (5.6% and 12.5%) were lower than those reported in the literature.

Methods: In the presented study, 45 Lebanese patients with a reported family history of BC were tested using Whole Exome Sequencing (WES) technique followed by Sanger sequencing validation.

Results: Nineteen pathogenic mutations were identified in this study. These 19 mutations were found in 13 different genes such as: *ABCC12*, *APC*, *ATM*, *BRCA1*, *BRCA2*, *CDH1, ERCC6*, *MSH2*, *POLH*, *PRF1, SLX4*, *STK11* and *TP53*.

Conclusions: In this first application of WES on BC in Lebanon, we detected six *BRCA1* and *BRCA2* deleterious mutations in seven patients, with a total prevalence of 15.5%, a figure that is lower than those reported in the Western literature. The p.C44F mutation in the *BRCA1* gene appeared twice in this study, suggesting a founder effect. Importantly, the overall mutation prevalence was equal to 40%, justifying the urgent need to deploy WES for the identification of genetic variants responsible for familial BC in the Lebanese population.

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P12.057A

Population-based testing for hereditary breast and ovarian cancer predisposing mutations in the Australian community

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Germline mutations in hereditary breast and ovarian cancer (HBOC) genes such as BRCA1 and BRCA2 confer a high lifetime risk of these cancers but importantly these risks are not irreversible. Identification of asymptomatic carriers could significantly reduce the incidence of these diseases. The current model of familial cancer practice involves ascertaining high-risk individuals based on family history but >50% of mutation carriers may not have a family history of cancer. Momentum toward genetic testing of the asymptomatic population is growing but it remains unclear what is the true frequency of actionable mutations in the general western population and the extent to which the public would accept such testing, particularly for those individuals identified with an actionable mutation in the absence of an overt family history.

We sequenced the coding regions of 20 hereditary breast and ovarian cancer (HBOC) genes in 5,500 cancer-free Australian women from the lifepool study (www.lifepool.org). The data was filtered to identify all clinically "actionable" mutations.

Thirty-nine women were identified with actionable mutations in BRCA1 (5 mutations), BRCA2 (18 mutations), PALB2 (14 mutations) or ATM (4 mutations). To date 24/24 women notified of an actionable mutation subsequently accepted an invitation to attend a Familial Cancer Centre and proceeded to formal clinical genetic testing and risk mitigation. In addition, 15 women had pathogenic mutations in potentially actionable genes BRIP1, RAD51C and RAD51D.

Our data directly demonstrates a population carrier frequency of ~1% for pathogenic mutations HBOC genes and that such testing is well accepted by the screened population.

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P12.058B

Next-generation sequencing via TruRisk® genepanel reveal high mutation prevalence in additional risk genes in German *BRCA1/2*-negative breast and ovarian cancer families

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Within the framework of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) 24% of the analyzed familial breast cancer (BC) and/or ovarian cancer (OC) cases are due to pathogenic mutations in *BRCA1/2*. However, the mutation prevalence of non-*BRCA1/2* genes associated with familial BC/OC is largely unknown. Here, we present NGS data generated using the GC-HBOC-designed TruRisk® gene panel. In this study our cohort of 2515 *BRCA1/2* and *CHEK2* c.1100delC negative index cases collected was analyzed which comprises consecutive patients from BC families and BC/OC families complying the inclusion criteria of the GC-HBOC. By focusing on 22 BC/OC associated genes, we identified 115 different deleterious variants in 191 unrelated mutation carriers derived from 2515 BC and BC/OC families (7.6%). Interestingly, we identified a high prevalence of *ATM* mutation carriers (n = 42, 1.7%) in the familial cases. Additionally mutations in *PALB2* (n = 25), *CHEK2* (n = 18), *BARD1* (n = 10), *BRIP1* (n = 10), *RAD51C* (n = 9), *NBN* (n = 8) and *TP53* (n = 7) were frequently observed and we confirmed *FANCM* (n = 19) as a novel BC predisposing gene. No mutations in *MRE11A*, *PTEN*, and *STK11* were identified in our collective. Due to the unexpectedly high mutation prevalence in familial cases, our study highlights the importance of these genes to be included in BC/OC routine diagnostics. In contrast we found low occurrence or absence of mutations for a subset of our gene selection which requires further investigation to optimize the gene panel for diagnostic purposes. Nevertheless this approach confirms the TruRisk® gene panel as a reliable tool for this comprehensive analysis.

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P12.059C

Routing: A response to the saturation of cancer genetic clinical sessions

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Introduction: Cancer genetic sessions reach a saturation point, especially since the development of PARP inhibitors, which requires ensuring fast tracks in order to identify *BRCA1* and *BRCA2* mutation carriers.

Materials and Methods: A new triage procedure has been set up within our Centre for Genomic and Personalized Medicine, covering a population in Normandy of 3.3 M ha: D0, a phone-based appointment (PBA) is fixed within 7 days; D7, trained genetic counsellors or medical residents call the proband for a 20 min PBA, collecting personal and family medical history through a structured questionnaire, in order to establish a pre-genetic session medical file; D10, routing: Pre-genetic session medical files are analyzed by a senior cancer geneticist with 3 possible conclusions: (i) priority face to face genetic session with a medical geneticist (immediate therapeutic impact); (ii) face to face genetic session with a genetic counsellor, or (iii) no clinical session needed (lack of personal or familial indication and/or substitution by a more appropriate index case).

Results: Since 2015 August, in the context of breast and ovarian cancers, 1039 PBA were performed, 38% of PBA did not lead to face to face genetic sessions, the median delay for a cancer genetic session was maintained to 12 weeks and all genetic sessions with therapeutic impact could efficiently be ensured within 4 weeks.

Conclusion: This routing procedure allows to suppress up to 1/3 of unjustified sessions, to prioritize genetic sessions according to therapeutic impacts, satisfies the patients, and optimizes collaborations between genetic counsellors and medical geneticists.

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P12.060D

High resolution of breast tumor heterogeneity enabled with massively parallel single cell transcriptomics

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Introduction: Cellular diversity within breast tumors shape complex cell to cell interactions that drive the tumoral life cycle from initiation to metastasis. Understanding intratumoral heterogeneity is the gateway for discerning key mechanisms of breast cancer evolution and has direct implications for patient prognosis and therapeutic treatments.

Materials and Methods: To assess cellular heterogeneity among breast cancer specimens, we utilized BD™ Resolve to capture the transcriptomes of 62081 single cells from 3 primary breast tumors and 3 lymph node metastases. We employed two strategies for RNA-sequencing library preparation: whole transcriptome amplification (WTA) and targeted library preparation using multiplex PCR with a gene panel consisting of more than 400 genes. While the WTA approach allowed unbiased exploration of gene signatures, the targeted approach provided a focused evaluation of genes known to be correlated with prognosis and breast cancer subtypes, as well as markers for the plethora of cell types found within tumoral microenvironments.

Results and Conclusions: Both whole transcriptome and targeted RNA-sequencing revealed diverse cellular composition in a patient- and sample-dependent manner. We observed distinct sets of cancer-associated fibroblasts, endothelial cells, tumor infiltrating immune cells, and disparate subpopulations of breast cancer cells. Direct comparison of primary and metastatic breast cancer cells derived from a single patient revealed divergent gene signatures that may inform the evolutionary processes of a primary tumor to a metastasis. These data highlight the power single cell RNA-sequencing to discern heterogeneity in tumor samples, with implications for both basic cancer research and translational medicine.

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P12.061A

Evaluation of breast cancer susceptibility loci in Singapore Chinese women

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Aim: Genome-wide association studies (GWAS) have identified common single-nucleotide polymorphisms (SNPs) associated with breast cancer (BC) risk. However, these studies have focused predominantly on Caucasian populations, and the relevance of these SNPs in Asian populations are less known.

Methods: We studied 48 SNPs in 1,233 BC patients and 1,189 healthy controls, which comprised of women of Chinese ancestry accrued at the National Cancer Centre Singapore and KK Women's and Children's Hospital, Singapore. Genotyping was carried out on Fluidigm's 192.24 microfluidics dynamic arrays using TaqMan® SNP Genotyping Assays. Logistic regression analysis was carried out using R SNPassoc package.

Results: Via an additive model, four SNPs rs4784227 (OR=1.37,95%CI=1.21–1.55;P=7.95×10^{-5}), rs3757318 (OR=1.34,95%CI=1.18–1.52;P=3.41×10^{-6}), rs12662670 (OR=1.37,95%CI=1.21–1.55;P=2.80×10^{-7}) and rs11155804 (OR=1.31,95%CI=1.16–1.47;P=7.87×10^{-6}), were found to be significantly associated with increased BC risk at adjusted P value of <1.16 ×10^{-3} after Bonferroni correction. Rs3757318, rs12662670 and rs11155804 are located on 6q25.1. Rs4784227 is within *TOX3*, a gene expressed specifically in ER-positive and PR-positive BC cases. In our study, rs4784227 was found to be significantly

associated with only ER-positive (OR=1.35,95%CI=1.17–1.56;P=2.76×10^{-5}) and PR-positive (OR=1.4,95% CI=1.21–1.62;P=7.77×10^{-6}) BC cases. An additional SNP, rs7297051, was associated with decreased BC risk in cases with a family history of BC (OR=0.68,95%CI=0.54–0.85;P=4.50×10^{-4}).

Conclusion: Four of the 48 SNPs were found to be associated with increased BC risk, with higher ORs in Singaporean Chinese compared to Caucasians. This finding reiterates the notion that variants identified from GWAS in Caucasians might not always be relevant to other populations. This work was supported by grants from the National Medical Research Council of Singapore.

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P12.062B

Analysis of genomic alterations in tumours from breast cancer in women below 40 years old

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Introduction: Breast cancer in young women (BCYW) is considered a distinct entity characterized by poorer prognosis, more aggressive tumor phenotypes, high germline mutation rates in BRCA1/2 genes and some differences in risk factors, being familial history the most important. Identification of copy number alteration (CNA) on BCYW may provide key genes playing causal roles in breast oncogenesis and potential therapeutic targets.

Materials and methods: 58 FFPE tumour from BC in women below 40 years were analyze for CNA with Affymetrix® SNP Arrays and BRCA1/2 mutation status was previously studied in DNA from peripheral blood. Thus allowed us to differentiate 3 groups: BRCA mutation carriers (n = 12) and non-carriers; this one divided in two groups: with and without familial history of breast cancer (FBC+,n = 20; FBC- n = 26). GISTIC software let us analyzed common genes and altered regions. CTLPScanner tool were used to detect chromothripsis like patterns (CTLPs).

Results: Genomic instability was higher in BRCA+ tumours and specific altered regions were found statistically significant in each group (Table) These frequent alterations had not been reported in other publication before. CTLPs were confirmed in 17% (n = 10) of all cases and chromosome 17 (ERBB2, CDK12) was the most altered. The FBC + group was the most affected by chromothripsis (n = 5).

Group	Gains (%) and genes involved*	Loss (%) and genes involved*
BRCA +	6p25(83%), NOQ2	18q23 (83%), GALR1
	5q35 (100%), GNB2L1	
FBC-	8p11 (61%), FGFR1	4q31 (54%), TLR2, SFRP2
FBC+	12q14(57%), CDK4	–

*statiscally significant difference between groups

Conclusion: This analysis provides new insights and differences between these subgroups and it is the first time chromothripsis is reported in BCYW.

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P12.063C

Rising of the c-Myc oncogen expression might be correlated with poor prognosis in CML

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Recent studies show cancer development and progression largely is related to non-coding genes beside well known coding genes. Long non-coding RNAs (lncRNAs) are important players in the complex world of gene expression regulation. Many lncRNAs regulate the expression and stability of main transcription factors in cells. The c-Myc is one of the transcription factors playing a major role in hematopoiesis. Increased expression or aberrant activation of c-Myc plays an important role in leukemia. It has been

proposed that PVT-1 lncRNAs regulates c-Myc gene transcription.

In the present study we aimed to investigate the expression of PVT1 & MYC in APL and CML peripheral blood mononuclear cells. To this end peripheral blood samples were collected from 73 pathologically confirmed CML patients and 17 pathologically confirmed APL patients. Correlation of the *BCR/ABL* translocation and the expressions of c-MYC and PVT-1 has been analyzed.

At result we found that in our enrolled patients including; 36 cases negative for BCR/ABL, 37 samples positive-BCR/ABL. The expression of the *MYC* increased significantly in positive-BCR/ABL samples in comparison to the patients being negative for BCR/ABL (about~20 fold, $p<0.05$).

In conclusion, c-MYC expression increases under BCR/ABL possibly independently from PVT1 expression. Our result shed light on possible impact of c-MYC and PVT-1 on leukemia characterization. These preliminary data need additional investigation to explain somewhat about the clinical use of c-MYC and PVT1 expression in leukemia characterization and therapy processes.

H. Galehdari: None.

P12.064D

Two novel CHEK2 large genomic rearrangements are associated with breast cancer predisposition

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Introduction: *CHEK2* damaging alleles are associated with increased risk of breast cancer, with their type and frequency being variable among populations. *CHEK2* large genomic rearrangements (LGRs) are very rare and up to date two have been described, namely a Czech founder 5.6kb deletion and an Italian 23kb duplication. The aim of the present study is to elucidate the role of *CHEK2* LGRs in Greek breast cancer patients.

Materials and Methods: Analysis for the two *CHEK2* LGRs was performed in 2355 breast cancer patients and 1580 controls using a customized PCR.

Results: Two novel LGRs were identified, namely a ~5.8Kb and a ~7.5kb deletion removing exons 2–3 and exon 6 of the gene, respectively. The exact breakpoints of mutations were defined and further analyzed by a custom PCR. The deletion of exons 2–3 (p.E107_K197fsX0) was rare, detected in only 0.09% (1/1020) of breast cancer cases, while the deletion of exon 6 (p.D308fsX0) was detected in 0.22% (5/2355) of patients and none of the 1580 control subjects. Haplotype analysis of p.D308fsX0, the more frequent LGR, showed that it is a Greek founder mutation with carriers sharing a common haplotype of 1850kb. Although the reading frame is retained, the p.D308fsX0 deletion impairs CHEK2 function, as shown by *in silico* analysis, a *CHEK2*-yeast functional assay and structure-prediction models.

Conclusions: The present study reports the association of rare genomic events with breast cancer susceptibility, which are rarely reported presumably due to incomplete analyses.

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P12.065A

Utility of panel testing in hereditary cancer: a laboratory perspective

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We have developed a fully customisable 155 gene hereditary cancer panel enabling rapid, cost-effective analysis, ideal for investigation of patients with complex personal and/or family histories. To maximise test sensitivity, gene dosage changes and several pathogenic variants falling outside the normal scope of standard commercial kits are also detected e.g. EPCAM deletions, a recurrent MSH2 inversion and BRCA2 Alu insertion, and several deep intronic and promoter mutations. Results for over 2200 patients indicate an overall diagnostic yield of 16%, representing 359 likely pathogenic or pathogenic variants detected in 51 genes, and including patients with mutations in more than one cancer predisposition gene. Dosage anomalies account for >10% of clinically actionable findings. Review and classification of detected variants represents a significant component of service workloads. Detection of variants of uncertain significance (VOUS), which have no immediate clinical utility, is particularly pertinent for recently characterised cancer predisposition

genes e.g. POLE, POLD1, and for genes with low-mid penetrance e.g. CHEK2, ATM. However, through regular review of available evidence, patients will hopefully benefit from these findings in the future as more genotype-phenotype data is collated worldwide. In our cohort, VOUS were reported in only 13% patients overall. This is in part explained by users typically adopting a phenotype-focused subset of genes, which tends to result in greater diagnostic yields e.g. 25% and 19% yield in pheochromocytoma and melanoma sub-panels respectively. However, the use of extended panel testing still attains a diagnostic yield of 12% (VOUS rate 17%) and indicates a valid clinical approach.

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P12.066B

Analysis of mismatch repair genes (MMR) in Greek families with suspected Lynch Syndrome detects a significant proportion of *MSH2* large genomic rearrangements

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Introduction: Lynch syndrome accounts for about 3%-5% of all colorectal cancer (CRC) cases and is caused by germline mutations in the MMR genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*. Most of these loss-of-function (LoF) alleles are single nucleotide substitutions or small insertions/deletions, with large genomic rearrangements (LGRs) being detected with variable frequency among different populations. We aimed to clarify the mutation spectra along with the prevalence of LGRs in the MMR genes in Greek CRC patients.

Materials and Methods: Probands from 168 unrelated families fulfilling the Revised Bethesda criteria were sequenced by Sanger or Next Generation Sequencing, followed by MLPA in MMR genes.

Results: The prevalence of MMR LoF mutations was 20.8% (35/168). Point mutations or small insertions/deletions were detected in 17.8% (30/168), while LGRs were detected in 3% of the cases. Mutations in *MLH1* and *MSH2* were predominantly identified, (18.5%), with LGRs in *MSH2* alone (involving deletions of exons 1–6, 1–7, 9–10 and 7–16) seen in 2.4% of the patients tested. CRC was the most frequent cancer diagnosis (mean age at diagnosis 41.2y, range: 21 – 64 years) among thirty-five probands found to carry a mutation. Targeted mutation testing was uptaken from 46 relatives, with 27 of them testing positive for the family mutation. Furthermore, 5 out of these 27 carriers were diagnosed with cancer.

Conclusion: Overall, 20.8% of the patients fulfilling the Revised Bethesda criteria carried LoF MMR mutations, with *MSH2* LGRs representing a significant proportion, indicating that such phenomena are possibly frequent in patients of Greek descent.

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P12.067C

Performance of a 94-gene panel for cancer predisposition on Greek breast and ovarian cancer patients fulfilling the NCCN criteria for genetic testing

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Introduction: NGS technologies, especially in the form of gene panels, have surely entered the clinical practice for cancer patient management. Among high-risk breast and/or ovarian cancer patients of Greek descent, although five *BRCA1* alleles with founder effect dominate the mutation spectra, additional susceptibility alleles in a number of genes are definitely present.

Materials and Methods: In total, 555 breast and 104 ovarian cancer patients fulfilling NCCN genetic testing criteria have been analyzed by Trusight Cancer panel targeting 94 genes associated with cancer predisposition, in order to identify loss-of-function (LoF) mutations.

Results: LoF mutations have been identified in 22% and 26% of the breast and ovarian cancer group respectively, in 27 genes involved in DNA repair. Of these, 87% and 89% lie in genes with known or suspected associations with breast and/or ovarian cancer predisposition, predominantly *BRCA1* and *BRCA2* (62%; 74%). Mutations in *CHEK2*, *PALB2* and *ATM* were more frequent in the breast cancer series and *RAD51C* in the ovarian cancer series. Of note is the remaining 12% of LoF alleles in non-associated genes to date, which can be either regarded as incidental findings, while putative causality for breast/ovarian cancer cannot be excluded.

Conclusions: Among a high-risk group of breast and ovarian cancer patients of Greek descent, where there are strong founder effects, the mutational spectrum is highly

heterogeneous with respect to both loci and alleles. This approach can shed light on associations with the post-BRCA genes and will provide connections of phenotypes with mutations in additional DNA repair genes.

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P12.068D

Monitoring cancer related mutations in cell-free DNA of lung cancer patients

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Introduction: According to WHO, lung cancer is the most common cause of cancer deaths worldwide. Cancer burden can be reduced by early detection and response-adjusted treatment. It can be achieved by analysis of cell-free DNA (cfDNA) from blood samples. This minimally invasive method can capture the whole tumour heterogeneity in almost real-time setting. Materials and Methods: Our cohort comprises of 106 lung adenocarcinoma patients who have donated pre-therapy cfDNA plasma samples. FFPE tumour samples were available for 70% of subjects. We have set up an amplicon-based multiplex next-generation sequencing (NGS) workflow for 9 lung adenocarcinoma-related genes. To verify our results, FFPE analysis results by certified diagnostic laboratories was initially used. In the later analysis, NGS of FFPE samples served as an internal control for cfDNA. **Results:** Our results showed that cfDNA NGS can detect ~1% mutant allele content. The overall concordance between diagnostic laboratory and cfDNA NGS results was 89%. FFPE analysis provided higher detection sensitivity, whereas cfDNA analysis has higher specificity. We found a resistance mutation in cfDNA of a patient with previous history of long-term EGFR-TKI therapy whose treatment should be changed to third generation EGFR TKIs. Perspectives: Our further aim is to collect longitudinal samples and perform a larger screen of drug susceptibility and resistance mutations in our study cohort, involving detection of copy number variations and DNA methylation changes. We believe that regular cfDNA analysis should become a clinical routine in various cancers. This research was supported by the EU project 2014–2020.4.01.15–0012 and PUT736 personal grant.

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P12.069A

First Evidence Supporting "Genometastasis Hypothesis" in Co-Culture Systems

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Introduction: The main cause of breast cancer death is metastasis to vitally organs such as lungs, liver, brain, lymph nodes, and bones.According to recent research, cf-DNA can cause horizontal gene transfer as name is "Genometastasis Hypothesis" (1).cf-DNA originated from tumor and healthy cells and present in blood (2).In this study,we used MDA-MB-231 (breast cancer cells) and hFOB1.19 (osteoblast cells) for co-culture;so that we aimed to create *in vitro* metastasis model. Subsequently, whether *p53* exon 8 horizontal gene transfers has occurred and affects the transfers or not viability of osteoblast cells evaluated in the study.

Materials and Methods: MDA-MB-231 cells were co-cultured with hFOB1.19 cells for 72 hours.DNA's were isolated from cells and cf-DNA isolated from medium and *p53* exon 8 mutant sequence analyzed by PCR.Viability was evaluated Tripan Blue assay.

Results: We determined that hFOB1.19 cells mutant *p53* exon 8 horizontal gene transfection occurred at the end of 72 hours later.hFOB1.19 cells viability were increased in co-culture system ($p<0,05$).

Conclusions: In this study,MDA-MB-231 cells co-cultured with hFOB1.19 cells.We evaluated for the first time "Genometastasis Hypothesis" in vitro systems and

concluded that mutant *p53* exon 8 horizontal gene transfer to hFOB1.19 from MDA-MB-231 was occurred.

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P12.070B

Frequency and significance of cryptic chromosomal aberrations in childhood T-cell acute lymphoblastic leukemia (T-ALL)

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Introduction: T-ALL represents 15% of newly diagnosed children with ALL and is regarded as a high-risk disease. It is a clinically and genetically heterogeneous malignancy which showed a normal karyotype of bone marrow cells in approximately half of the cases. The aim of the study was to determine a frequency of recurrent cryptic chromosomal aberrations and to correlate the findings with the course of the disease.

Materials and Methods: Karyotypes of all patients were analyzed at the time of diagnosis by G-banding and FISH methods in order to detect the most frequent known chromosomal changes, i.e. rearrangements of *TCR* loci (*TRA*, *TRB*, *TRG*) and *TLX3* gene, deletion of *CDKN2A* and amplification of *ABL*.

Results: 64 children with T-ALL were examined (19 girls, 45 boys, median age 8,25 years). Chromosomal aberrations were detected by conventional cytogenetics in 50% of patients, versus 86% found by molecular cytogenetic methods. Rearrangements of *TCR* loci were found in 17 children: 11×*TRA* and 6×*TRB*. Deletion of *CDKN2A* was proved in 35 patients: 19x homozygous and 16x heterozygous. *TLX3* gene rearrangement was established in 15 patients. 48 patients are living in the first/second complete remission and sixteen children died. In this cohort, rearrangements of *TCR* loci were associated with favorable prognosis.

Conclusion: Using molecular cytogenetic methods cryptic recurrent aberrations were proved in vaste majority of children. However, additional studies of larger cohort of patients are needed to determine their prognostic impact and to understand implications in T-cell oncogenesis.

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P12.071C

Germline translocation affecting ETV6 predisposes to childhood acute lymphoblastic leukemia (ALL)

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Introduction: Studying familial childhood acute lymphoblastic leukemia (ALL) provides a unique opportunity to understand the genetics underlying ALL. Rare germline pathogenic variants in the ETV6 gene have previously been reported in families with thrombocytopenia and predisposition to hematological malignancies, especially ALL. In addition, overrepresentation of high hyperdiploid (HeH) ALL and higher age at onset compared to common HeH ALL was observed in a cohort of childhood ALL cases with ETV6 germline pathogenic variants.

Materials and Methods: We studied a family with two cases of childhood ALL in 2nd degree relatives. All family members were analyzed by karyotyping. Whole genome sequencing (WGS) was performed on one healthy carrier.

Samples from leukemia diagnosis were run on SNP array. Fusion transcripts were Sanger sequenced.

Results: Karyotyping identified t(12;14) in seven family members. Two carriers developed ALL at age 9 and 12 years respectively. SNP-arrays showed leukemias were HeH. WGS confirmed the t(12;14)(p13.2;q23.1) which, according to transcript sequencing, creates two transcribed fusion genes. However, transcript sequences (*ETV6*ex1/*RTN1*ex2; *RTN1*ex1/*ETV6*ex2-4) suggest translation of functional fusion protein is unlikely.

Conclusion: Here we report a unique family with predisposition to ALL carrying a t(12;14)(p13.2;q23.1) affecting *ETV6*. Our finding further supports leukemia susceptibility in patients carrying germline mutations in ETV6. In line with previous reports leukemias were HeH and had higher age at onset, however, no thrombocytopenia was observed. Further, in contrast to previous cases where germline pathogenic variants in ETV6 create mutant ETV6 protein, this translocation causes a heterozygous loss of ETV6.

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P12.072D

Chromothripsis is associated with altered gene expression in chronic lymphocytic leukemia

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Introduction: Chromothripsis represents one-off event causing extensive chromosomal rearrangements. In cancer it is frequently associated with a wt-*TP53* gene loss and consequently with adverse disease course. However, functional consequences of the chromosomal shattering have not been studied extensively yet. Therefore, we performed characterization of chromothripsis in chronic lymphocytic leukemia (CLL), as a model disease, with aim to understand its impact on gene expression and cellular processes.

Materials and methods: Chromothripsis was screened among *TP53*-mutant CLL cases using CytoScanHD arrays (Affymetrix). Transcriptome analysis was performed using TruSeq Stranded Total RNA Library Prep Kit on HiSeq 2000-1T (Illumina) and in parallel using Human Transcriptome Arrays 2.0 (Affymetrix). Obtained data were analyzed with in-house biostatistic and bioinformatic pipelines.

Results: Among 72 CLL samples analyzed using cytogenetic arrays, chromothripsis was identified in 16 of them (22%), affecting recurrently chromosomes 6 (5 cases), 8, 9 and 13 (3 cases each). Ten cases with chromothripsis were subjected to the transcriptome analysis. Upregulation of several cellular pathways (i.e. focal adhesion, PI3K-Akt-mTOR signaling, apoptotic signaling, actin cytoskeleton regulation and others) was observed. Although median of 49 breakpoints (range 24–74) was detected per case, they mostly did not lead to formation of expressed fusion genes. Only in 5/10 cases, aberrant expression of 1–3 fusion transcripts was noted.

Conclusion: Chromothripsis affects approximately one fifth of CLL cases with wt-*TP53* inactivation and is associated with altered regulation of cellular pathways. However, *de novo* fusion transcripts are expressed only to a limited extent.

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P12.073A

Studying the effects of disease associated polymorphism on a transcriptional pathway: A case study in renal cell carcinoma

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Genome-wide association studies (GWAS) are an important way of identifying germline polymorphisms that are associated with a specific trait or disease. However, characterizing the functional effects of disease-associated variants is challenging since many are located in intergenic regions of the genome. We have established a methodological framework to study the functional effects of disease-associated polymorphisms by examining their effect on disease-

specific transcriptional pathways. Specifically, we show that renal cell cancer (RCC) associated polymorphisms co-localize and are statistically enriched at binding sites for the transcriptional enhancer, hypoxia-inducible factor (HIF). HIF is constitutive activated in kidney cancer through loss of the von Hippel-Lindau (VHL) tumor suppressor. Determining HIF-target genes linked to RCC-associated polymorphisms helps identifying HIF-target genes that are driving kidney cancer from those that are simply co-acquired as a result of pathway activation. We show functional effects of RCC-associated polymorphisms, by demonstrating allele-specific HIF binding in heterozygous cells lines and link the polymorphic binding sites to their target genes by examining chromatin looping. Finally, we use eQTL analysis to show the effect of the RCC-associated polymorphism on expression of the target gene and demonstrate the dependence of genotype-expression correlations on HIF. Although focused on the HIF pathway in kidney cancer, this work forms a paradigm for studying the effect of non-coding variants linked to other diseases.

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P12.075C

Integrated analysis of germline and tumor DNA for the identification of new candidate genes involved in familial colorectal cancer

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Introduction: Colorectal cancer (CRC) presents sometimes familial aggregation with an autosomal dominant pattern of inheritance but no alterations are found in the known hereditary genes. The purpose of this study was the identification of novel variants in candidate genes potentially involved in the germline predisposition to familial CRC with an unknown basis. The possible tumor suppressor gene (TSG) role of the selected candidates was evaluated based on the Knudson's two-hit hypothesis.

Materials and Methods: Integrated analysis of germline and tumor whole exome sequencing data was performed in five unrelated familial CRC patients. Deleterious single nucleotide (SNV) and copy number variants (CNV) were assessed as candidates for first germline or second somatic hits. A filtering process considering sequencing quality, frequency, family segregation and pathogenicity was used. Somatic mutational profiling was also performed for all samples.

Results: A series of germline-somatic variant pairs affecting proteins and miRNAs functionally related with CRC were prioritized. Among them, *ADCY8*, *MUC16* and *HEXIM1* were finally selected as the most promising candidates for a putative role as TSGs related to CRC germline predisposition. A hypermutator phenotype was suggested in one of the analyzed tumors, due to the elevated number of variants detected in the somatic profile analysis.

Conclusions: The identification of new potential predisposition genes to familial CRC was achieved by our integrated analysis. Integrative Genomics Viewer and Sanger sequencing validations were performed for SNV, whereas somatic CNV were verified by OncoScan. Further functional studies will be required to confirm the selected candidates.

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P12.076D

Early detection of colorectal cancer with a blood based test; evaluation of methylated *SEPT9* gene in 2272 Spanish population samples

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Introduction. In Spain the population-based CRC screening program offered by the public health system does not achieve the expected participation due to the low acceptance of the common stool blood tests. Today, there are other screening options available, like the "Septin9" test, that consists in detecting the methylation status of SEPT9 gene in plasma. Sensitivity and specificity are 80.6% and 99.3% respectively, and the positive predictive value is 45.7% (data from manufacturer), whilst stool blood test shows a sensitivity and specificity of 61–69% and 91–98%, respectively (1, 2). The objective of this work has been to review the 2272 Septin9 samples studied since 2012. Samples are from asyntomatic and mixed (high and low risk) population.

Materials and Methods. 2272 plasma samples from Spanish population from low and high risk patients, collected from 2012–2016. EpiproColon2.0 test has been carried out following manufacturer protocol, both for technique and analysis (Roche LightCycler480).

Results. From the 2272 samples, 154 (6.8%) have been rejected prior to the study mainly because of a bad sample quality. Of the 2118 studied samples, positivity rate was 7.0% (148), so that 1970 samples were informed as negative for Septin 9 test (93%).

Conclusions. Positivity rate in our mixed cohort (7.0%) was higher than the one described in literature (3,4%) in general population (3); we assume that a 3,6% of samples with CRC and advanced adenomas were detected.

The compliance to CRC screening programs using a blood test is considerably higher, allowing a better performance of the screening (4).

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P12.077A

Exome sequencing data analysis to chracterize rare germline copy number variants involved in colorectal cancer and serrated polyposis syndrome predisposition

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INTRODUCTION: Colorectal cancer (CRC) represents the third most common cancer worldwide. Next generation sequencing (NGS) has permitted to identify germline predisposition genes for this disease. Copy number variants (CNV) can be the mutational event involved in this predisposition, and they can be inferred from NGS data. METHODOLOGY: We analyzed germline DNA whole-exome sequencing (WES) data from 38 families with strong CRC and 16 families with serrated polyposis syndrome (SPS) aggregation without alterations in known hereditary genes. To infer rare candidate CNVs involved in this predisposition, we used ExomeDepth and CoNIFER. Variants shared between family members were compared to Database of Genomic Variants catalog and our in-house database. Selected CNVs were validated and segregation analysis was performed using Comparative Genome Hybridization. In some cases, gene expression arrays, qRT-PCR and immunohistochemistry (IHC) were conducted to check for gene and protein expression alterations. RESULTS: Sixteen candidate CNVs were detected in CRC families. After multiple filtering steps, a duplication in chromosome 1 stood out including *TTF2*, *TRIM45*, *VTCN1* and miR942. Expression studies pointed out *TTF2* and miR942 overexpression in carriers, and tumor IHC showed high levels of TTF2 protein and low levels of the TMEM158 protein, a predicted miR942 target. Six candidate CNV were inferred in SPS families and one duplication in chromosome 3 including *GOLGA3*, *C3orf35* and *ITGA9* was further selected and validated. CONCLUSIONS: WES data can be used as a first approach to identify CNVs. Confirmed duplications may correspond to the initial germline mutational event involved in CRC and SPS predisposition.

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P12.078B

TP53 codon 72 and CCND1 G870A polymorphisms are related to colorectal cancer in an Iranian population

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Introduction: Colorectal cancer is the third most common cancer worldwide. It is a multifactorial disease, with etiology encompassing environmental exposures, inflammatory conditions and genetic factors. TP53 is the most common mutated gene in human cancers. This gene is involved in cell cycle control and apoptosis induction response to DNA damage. The sequence polymorphism at codon 72 of this gene results in either a proline or an arginine. It is hypothesized that two codon 72 variants may induce different functional activities. Cyclin D1 is encoded by CCND1 gene and regulates the cellular proliferation by controlling cell cycle transition from G_1 phase to S phase. A common polymorphism (G870A) at codon 242 in CCND1 may influence an individual's susceptibility to the development of certain cancers.

Materials and Methods: in this study, we investigated TP53 codon 72 and CCND1 G870A polymorphisms in 80 colorectal cancer cases and 120 healthy individual in Northwestern Iran. TP53 codon 72 and CCND1 G870A were genotyped by ARMS-PCR and RFLP-PCR respectively.

Results: There was a significant difference between frequencies for TP53 Proline variant in patients with colorectal cancer (41.6%) and healthy controls (13.2%; P= 0.0001). There was also a significant difference between frequencies for CCND1 AA genotype in patients and healthy groups (30.12% and 10.25%, respectively; P= 0.01).

Conclusions: In conclusion, our findings suggest that genetic polymorphisms in TP53 codon 72 and CCND1 G870A are associated with the risk of colorectal cancer in an Iranian population.

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P12.079C

Search for rare variants in unexplained adenomatous polyposis: Relevance of DNA repair pathways

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Introduction: Patients with colorectal adenomatous polyposis are at high risk of colorectal cancer (CRC). However, the genetic basis underlying the development of multiple adenomas has not yet been fully explained, since approximately 30% of the patients tested negative for germline mutations in *APC* or *MUTYH*.

Materials and Methods: Whole-exome sequencing was performed in 27 patients with unexplained colonic polyposis (>10 adenomas, onset <60 years) from 19 unrelated families. Common variants with MAF_{ExAC}>0.001 (heterozygous variants) or >0.01 (homozygous variants) were excluded. To identify rare causative variants, we focused on all truncating variants and missense of high functional impact. Subsequently, we prioritised genes exhibiting recurrent rare variants, and genes implicated in cancer-

related KEGG pathways or identified through CRC GWAS. Further gene prioritization included cosegregation analysis in families and sequencing in an external validation cohort of 300 unexplained polyposis patients and healthy controls.

Results: Rare potentially pathogenic variants were especially found in genes involved in DNA repair such as Nucleotide Excision Repair, Mismatch Repair and Homologous Recombination pathways. Moreover, genes of the Fanconi Anemia pathway were also enriched with CRC risk variants.

Conclusions: According to previous studies in which the DNA repair genes *NTHL1* and *MSH3* have been proposed as susceptibility genes to multiple colonic adenomas and CRC, these results might suggest the importance of DNA repair pathways in polyposis etiology.

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P12.080D

Identification of functional mutations of CTCF in primary cancers

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CCCTC-binding factor (CTCF) is a highly conserved ubiquitously expressed protein that have 11 Zinc Finger DNA binding domain (DBD). CTCF is able to use combinations of the ZFs to bind different DNA target sequences and proteins. It is directly implicated in cancer development and frequently altered by hemizygous deletion or mutation in cancers. NGS recently led to sequencing of cancer exomes and genomes. 10 CTCF missense mutations were selected from COSMIC database and studied in vitro to identify their functionality in primary cancers. CTCF mutations were generated by site-directed mutagenesis with CTCF cDNA cloned into pET7.1. 10 mutant plasmids were synthesized together with WT-human CTCF. Mutants were used for coupled in vitro transcription/translation to synthesize proteins. These proteins were tested in Electro Mobility Shift Assay (EMSA) to compare with the WT protein. 8 DNA-probes were used for EMSA including cancer related genes, promoters etc. The binding of CTCF mutants was reduced to the probes that were important for the expression of tumor suppressor genes but was not affected to globin insulator. ZF1 mutants showed no reduction of binding to Probe4 (Mus musculus selenoproteinT) compared to WT-CTCF, whereas in linkers' and ZF11 mutants binding is significantly reduced. However, the binding affinity of mutant ZF6 Q418R is markedly increased to Probe4 (~50–100 folds). To further test the functionality of ZF6 Q418R, to define its binding behaviour, in vivo experiments will be performed. Expected results might have translational significance, as they could lead to development of novel diagnostics and treatment of cancers.

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P12.081A

Angiogenic and Lymphangiogenic proteins as prognostic markers in Cutaneous Squamous Cell Carcinoma

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Introduction: Cutaneous squamous cell carcinoma (CSCC) accounts for approximately 20% of skin cancer. Mortality arises secondary to the development of metastases and angiolymphatic invasion (ALI) seems to play a role. ALI is considered a poor prognosis sign by different authors regarding several neoplasias Aim: To asses if expression of angiogenic and lymphangiogenic proteins in primary CSCC correlates to disease relapse or progression. Methods: A preliminary study was designed. We measured *VEGFA*, *VEGFR2* and *VEGFC* gene expression by RT-qPCR using TaqMan probes in FFPE samples corresponding to 29 patients (11 females; 20 males) showing CSCC without ALI by routine techniques. Eleven of them developed disease relapse or progression (to lymph nodes or to viscera) and 18

did not suffer disease progression after at least 60 months of follow-up. *ACTB*, *18S*, *HPRT1*, *TFRC* were analyzed as internal reference control genes. **Results:** No significant differences were evidenced for sex, age or tumour location between groups. Consistent results were obtained when using *ACTB* and *18S* as internal control genes in CSCC tissue. *VEGFC* gene expression was significantly higher for patients with disease progression (p=0.027) compared to those without it. Nor *VEGFA neither VEGFR2* gene expression did differ between the analyzed groups (p>0.05). **Conclusions:** Current results suggest that the expression of the angiogenic and lymphangiogenic protein VEGFC in primary CSCC may be related to disease progression in patients without diagnosis of IAL by routine methods. A larger cohort is needed to confirm its role as a marker of poor prognosis in patients with CSCC.

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P12.082B

Genetics of *DICER1* syndrome: a retrospective study of 204 analyses

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Background. *DICER1* syndrome is an autosomal dominant inherited disorder associated with a large spectrum of malignant and benign tumours including pleuropulmonary blastoma (PPB), cystic nephroma (CN), ovarian Sertoli-Leydig cell tumours (OSLCT) and thyroid multinodular goiter or cancer. The genetic diagnosis relies on the identification of a germline *DICER1* inactivating pathogenic variant. To improve the knowledge of clinical and genetic aspects of this syndrome, we performed a retrospective study of *DICER1* gene analysis on patients diagnosed with lesions suggesting the *DICER1* syndrome.

Methods. This study was conducted on 204 subjects: 101 probands and 103 relatives, from 2012 to 2016. *DICER1* gene analysis was carried out at the Institut Curie, Paris, by Sanger sequencing or Next Generation Sequencing. Genetic counselling was performed in various family cancer clinics and hospitals.

Results. Germline *DICER1* inactivating pathogenic variants were identified in 43 probands (n = 43/101) and 45 relatives (n = 45/103). Most pathogenic variants were identified in patients diagnosed with PPB (n = 27/35), CN (n = 7/12) and OSLCT (n = 10/19). Germline pathogenic variants were inactivating point mutations in *DICER1* coding sequence or exon/intron junctions except two large rearrangements and one deep intronic substitution. Seven pathogenic variants were *de novo*, including a mosaic variant.

Conclusion. This large cohort study confirms that 77% of PPB and 53% of OSLCT are associated with germline *DICER1* pathogenic variants. *DICER1* gene analysis should therefore be proposed to all patients diagnosed with any of these lesions. Tumor-specific *DICER1* variants could explain some sporadic cases. Further studies are needed to identify other genes involved in this syndrome.

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P12.083C

Acute myeloid leukemia in a case with Tatton-Brown-Rahman syndrome: the peculiar *DNMT3A* R882-mutation

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Recently a novel syndromic form of overgrowth with intellectual disability and distinct facial features was identified caused by constitutional mutations in the epigenetic regulator DNA-methyltransferase 3A (*DNMT3A*), referred to as Tatton-Brown-Rahman syndrome (TBRS). Somatically acquired mutations in *DNMT3A* occur in hematological malignancies and are frequently present in acute myeloid leukemia (AML) affecting in the vast majority the arginine residue at position 882 (R882). To date, none of the reported cases with TBRS developed AML.

Here we present the first case of a TBRS patient who developed AML at the age of 15 years. Whole exome sequencing identified a constitutional heterozygous *DNMT3A* R882C-mutation. The patient exhibits macrocephaly, intellectual disability, distinct facial dysmorphisms and other features fitting with the TBRS phenotype. The AML of the myelomonocytic subtype harbored only few additional somatically acquired mutations, i.e. an aberrant karyotype and a recurrent *PTPN11*-mutation. After relatively uncomplicated AML treatment, he has persistent high hemoglobin and thrombocytopenia without enough evidence for polycythemia or myelodysplastic syndrome.

Interestingly, among the constitutional *DNMT3A*-mutations in TBRS, residue R882 is only affected in a minority of patients (4 out of 29, including our case described here), which clearly differs from the somatic mutational spectrum in AML. We speculate that the R882-mutation gives a more potent predisposition for AML in contrast to the other *DNMT3A* mutations.

In conclusion, our case represents the first evidence that TBRS patients might be at increased risk for the development of hematological malignancies in particular AML.

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P12.084D

Early onset breast cancer: differences in risk factors, tumor phenotype and genotype between North African and South European women

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Introduction: This report compares the life factors, tumor phenotype, family history and *BRCA1/BRCA2* genotype of early onset breast cancer (EOBC) between France and North Africa (NA).

Methods: 455 women with invasive EOBC (≤40 years) were prospectively included from 4 centers in France (n = 270) and 4 centers in NA (Algeria, Egypt, Morocco, Tunisia; n = 185).

Results: We found an older age at menarche, a higher number of childbearing, a longer duration of breastfeeding, a higher body mass index, a lower use of oral contraceptives in North African as compared to French women. TNM stage at diagnosis was higher in North African women. Incidence of triple negative and proliferative (Ki 67 index) tumors was lower in NA. There was a lower rate of *BRCA1* mutation in NA (7% vs 15%, P=0.02) but no difference for *BRCA2* mutation (8% vs 7%, P=0.583). The rate of mutations in BRCA1/2 was higher for sporadic EOBC in NA (*BRCA1*: 8% vs 1%, p<0.03 ; BRCA2: 7% vs 1%, p<0.05). Three putative *BRCA1/2* founder mutations were identified in NA.

Conclusions: In EOBC, we found higher incidences of triple negative and proliferative tumors in France as compared to NA. This difference is not fully explained by the higher incidence of *BRCA1* mutations observed in French women. It could be hypothesized that major differences observed in life factors could influence the phenotype of EOBC. The worst prognosis previously reported for EOBC

in NA is more likely due to a higher stage at diagnosis than to a more aggressive phenotype.

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P12.085A

Novel *MSH6* gene mutation in a family with endomerial cancer

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Carcinoma of the uterus is one of the most common invasive malignancy in women worldwide. Most endometrial cancers are adenocarcinomas. The occurence of the endometrial cancers may be connected with the genetic factors in about 1% of cases. Hereditary endometrial cancer is a genetic predisposition to cancer with an autosomal dominant pattern of inheritance caused by mutations in the *MSH6* gene in the most of the cases. Herein, we describe a family diagnosed with endometrial cancer in five women in two generations caused by a novel mutation in the *MSH6* gene. In immunohistochemistry performed on probands endometrial cancer paraffin tissue block is the lack of MSH6 protein expression. This mutation is a p. Thr767Ile (c. 2300C>T) substitution. The novelty of this substitution have been verified in a number of mutation databases as well as large-scale genome sequencing databases. Functional consequences of *MSH6* T767I were predicted by several algoritms using sequence and structural information. *MSH6* T767I was predicted deleterious that likely decreases affinity of MSH6 to MSH2 and decreases affinity of MSH2/MSH6 complex to DNA. Furthermore the mutations cosegregated with the disorder in family. To the best of our knowledge, this is the first description of *MSH6* T767I mutation in the literature that could be coupled with a hereditary endometrial cancer.

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P12.086B

Determination of whole genome expression differences in larynx cancers and clinical significance

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Introduction: Laryngeal cancers which constituting 2% of adult tumors are one of the most common malignant tumors of the head and neck region. The mortality rate of larynx cancers which is responsible for around 7% of male deaths in Turkey is quite high. In our study, it was aimed to detect altered gene expression between primary laryngeal squamous cell carcinoma and adjacent normal tissue.

Materials Methods: During surgery, tumor and normal tissue samples were taken from the 12 patients who diagnosed larynx cancer as clinical and histopathological. Izolated RNA was studied by whole genome expression microarray method (Illumina iScan) and gene were identified which expression significantly increasing and decreasing tumor tissue than in normal tissue.

Results: In study using 47,323 probe, expression differences revealed at 14.294 mRNA. When the FC value is considered to be 2 for the significance of the expression differences, increased expression of 22 genes and decreased expression of two genes were observed. While the cell cycle pathway and extracellular matrix degradation pathway are the most active pathway, electron transport chain pathway is one of the most repressed pathways according to the pathway analysis of affected genes.

Conclusions: 11 new genes have been identified which is differentially expressed in cancerous tissue compared to normal tissue in addition to 13 genes known to be involved in the etiology of laryngeal tumors. These genes which identified for the first time in our study, are thought to be biomarker for early diagnosis, prognosis and targeted therapy on larynx cancer patients.

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P12.088D

Burden and profile of somatic mutation in duodenal adenomas from patients with familial adenomatous and MUTYH-associated polyposis

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Introduction: Duodenal polyposis and cancer are important but poorly understood causes of morbidity and mortality in familial adenomatous polyposis (FAP) and MUTYH-associated polyposis (MAP). This study aimed to characterise somatic genetic changes in duodenal adenomas from patients with FAP and MAP to better understand duodenal tumorigenesis in these disorders.

Materials and methods: Sixty-nine duodenal adenomas were biopsied during upper GI endoscopy in 16 FAP patients and 10 MAP patients. Ten FAP, 10 MAP adenomas and matched blood DNA samples were subjected to whole exome sequencing; 42 further adenomas underwent targeted sequencing and 47 were studied by array comparative genomic hybridisation. Findings in duodenal adenomas were compared to each other and to the reported mutational landscape in FAP and MAP colorectal adenomas.

Results: MAP duodenal adenomas had significantly more protein-changing somatic mutations ($p = 0.018$), truncating mutations ($p = 0.006$) and copy number variants ($p = 0.005$) than FAP adenomas, even though MAP patients had lower Spigelman stage duodenal polyposis. Fifteen genes were mutated significantly more than expected from the background mutation rate. Targeted sequencing of *APC, KRAS, PTCHD2* and *PLCL1* identified further mutations in each of these genes. In contrast to MAP and FAP colorectal adenomas, neither exome nor targeted sequencing identified any *WTX* mutations (P=0.0017).

Conclusions: The mutational landscapes in FAP and MAP duodenal adenomas overlap with, but have significant differences to those reported in colorectal adenomas. The significantly higher burden of somatic mutations in MAP than FAP duodenal adenomas could increase cancer risk in lower Spigelman grade disease.

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P12.089A

High level of co-occurrence of moderate risk alleles in the germline of familial intestinal gastric cancer syndrome

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Introduction: Ten percent of all gastric cancers show familial aggregation. Whilst germline defects were found for "hereditary diffuse gastric cancer" and "gastric adenocarcinoma and proximal polyposis of the stomach", "familial intestinal gastric cancer" (FIGC) remains genetically unexplained. We hypothesised that the rare FIGC syndrome is caused by germline co-occurrence of moderate-risk alleles and represent a polygenic, rather than a classical monogenic disease. Therefore, we dissected the germline and somatic landscapes of the largest FIGC cohort ever studied.

Materials and Methods: Constitutional and tumour DNA from 53 families, fulfilling clinical criteria of FIGC, were screened for 55 candidate gastrointestinal cancer-associated genes with Illumina's MiSeq-platform, and classified according to ACMG. Somatic loss of heterozygosity and promoter methylation at potentially causative genes were also searched for in FIGC tumours.

Results: 25/53 (47%) families carried germline variants, and co-occurrence of germline moderate-risk alleles was found in ten families. 7/10 families harboured one pathogenic/likely pathogenic variant combined with one or more unclassified novel variants. Three families carried clusters of novel unclassified variants. Moderate-risk alleles of BRCA2, MAP3K6, MSH6, MSR1, SDHB and SDHD were the most frequently found in this cohort. Tumours arising in these 10 families were enriched in somatic variants within DNA repair genes and often display microsatellite instability phenotype.

Conclusions: The clinical homogeneity and relatively high number of FIGC families herein studied allowed supporting the hypothesis that FIGC may be a polygenic syndrome caused by moderate-risk alleles in gastrointestinal cancer-associated genes. Grants; Fellowships: NORTE-07-0162-FEDER-000118 and -000067; NSFC Foundation; SFRH/BPD/89764/2012; /86543/2012; /79499/2011.

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P12.090B

Direct evidence for a polygenic etiology in familial multiple myeloma

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While common risk alleles for multiple myeloma (MM) were recently identified, their contribution to familial MM is unknown. Analyzing 38 familial cases identified primarily by linking Swedish nation-wide registries, we demonstrate an enrichment of common MM risk alleles in familial compared to 1,530 sporadic cases (P=4.8×10^{-2} and 6.0×10^{-2} respectively for two different polygenic risk scores) and 10,171 population-based controls (P=1.5×10^{-4} and 1.3×10^{-4} respectively). Using mixture modeling, we estimate that about one third of familial cases result from such enrichments. Our results provide the first direct evidence for a polygenic etiology in a familial hematologic malignancy.

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P12.091C

Improving Appropriateness of Referrals for Familial Breast Cancer in Primary Care

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Abstract Body

Introduction Breast cancer is the commonest cancer in women, with up to 20% significant inherited component. Taking a family history in primary care can help identify those at increased risk requiring specialist assessment. However, this typically occurs in an opportunistic and variable way, and it is unclear whether women are referred appropriately, with implications for best care and use of resources. This study aimed to explore whether an intervention to proactively identify women at risk, and support assessment, in primary care improves appropriateness of referrals.

Methods and Materials Women, aged 30 - 60 years, in four family practices were invited to complete a validated family history questionnaire, providing information for primary care practitioners to use in a familial breast cancer risk assessment tool (FaHRAS), based on English NICE guidelines for risk stratification and specialist referral. Women were followed-up for 16 months to determine referral outcomes.

Results 1137 (16.22%) of 7012 eligible women participated. Among the 1337 women, 122 (10.73%) were assessed at higher than average population risk with referral to secondary care recommended. At 16 months, 69 women had taken up offer of referral, and 59 (85.5%) of these were confirmed appropriate by specialist review, and offered increased cancer surveillance.

Conclusions This observational study suggests systematically identifying familial breast cancer risk in primary care has potential to optimise referral for specialist assessment and reduce breast cancer morbidity through increased targeted surveillance. Further assessment of effectiveness in a randomised trial is now needed.

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P12.092D

Male breast cancer susceptibility due to FANCM mutation: a case report

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Male breast cancer (MBC) is a rare, poorly understood disease. Germline susceptibility to MBC is substantial and mostly is confined to *BRCA2*/*BRCA1* genes mutations, however majority familial MBC has no known genetic basis. Recently, with the broader application of NGS, newer female breast cancer (FBC) susceptibility genes were established. Interestingly, a vast majority of FBC susceptibility genes are related to Fanconi anemia pathway. FANCM was recently identified as a moderate FBC predisposition gene, with the preponderance to triple negative BC. However, the contribution of these genes to MBC is still understudied. **Case report**. 66 yrs old male patient was referred to VULSK Oncogenetic Unit due to MBC diagnosed at the age of 65 (pT1cN0M0, ductal carcinoma G2, ER-98%, PR-90%, HER2(FISH)(-)). Family history showed prostate cancer in paternal uncle (dx 87). As part of routine comprehensive genetic testing, the patient underwent 96 cancer genes TruSight Cancer NGS testing panel on MiSeq (Illumina). Truncating FANCM (NM_020937) gene mutation c.5101C>T (p.Gln1701*) was identified; no other pathogenic/likely pathogenic genes mutations were revealed. During predictive testing, healthy daughter carrier (age 29) was identified. **Discussion:** From the newly identified FBC genes to date, only PALB2 was associated with possible MBC risk. No clear FANCM involvement in MBC susceptibility was demonstrated in published studies; only one MBC case (untested) was noted in a family with FANCM c.5791C>T mutation from Spain. **Conclusion:** FANCM c.5101C>T in MBC patient is reported for the first time, highlighting potential contribution of FANCM to MBC susceptibility. **Funding:** Lithuanian Research Council, SEN18/2015.

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P12.093A

Hereditary leiomyomatosis and renal cell cancer: Description of the families identified at the Catalan Institute of Oncology

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Introduction: Hereditary leiomyomatosis and renal cell cancer (HLCCR) is an autosomal dominant genodermatosis with a very low incidence predisposing to cutaneous and uterine leiomyomas and renal cancer. It's caused by germline mutations in the Fumarate Hydratase (*FH*) gene.

Material and methods: We have reviewed the families visited in our institution from 1998 to the present, identifying in our database 6 families with a *FH* germline test because of their clinical characteristics. 4 were studied with direct sequencing, 2with techniques of massive sequencing.

Results: 5 familes with *FH* mutations (80%) have been identified, these mutations are all distinct. In these families, a total of 11 individuals were studied, with mutations detected in 10 (91%). Among the carriers the average age at time of study was 53 years. 70% women, 30% men. Three cases of renal cancer (30%) have been detected, one of them bilateral, with a mean age at diagnosis of 48 years (histologies: 2 hypernefromas (1 bilateral) and 1 papillary. Cutaneous leiomyomas have been detected in 70% of cases and 6/7 (85%) women have uterine fibroids, with 3 with hysterectomies (42%) at a mean age of 31 years. Only 1/5 families (20%) had no individuals with suspected skin lesions. Among other neoplasms in carriers, metastatic colon cancer stands out at 57 years.

Conclusions: Families with HLCCR are infrequent. Although cutaneous and uterine leiomyomatosis is the most frequent manifestation, about 30% of cases are associated with renal cancer, generally aggressive. Not all families have cutaneous manifestations, which could make diagnosis difficult.

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P12.094B

Deep excavation of gallbladder carcinoma genomes using targeted sequencing

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Gallbladder carcinoma is common carcinoma of the hepatobiliary tract, its late diagnosis makes it fatal type of cancer with very poor prognosis. Unlike other common cancers, GBC remains under-studied as only a few small-scale mutational investigations have been reported yet. This study is the first ever effort to perform ultra-deep sequencing across 409 AmpliSeq comprehensive cancer panel genes, on 11 GBC patients from North-Indian descent. We have identified nonsynonymous somatic and rare germline deleterious and likely deleterious mutations. We detected highest number of somatic mutations in the exonic regions of SYNE1 and NBPF9 genes. Bona-fide cancer associated genes like TP53, SMAD4, APC were also observed to be frequently mutated. In addition, we detected high impact somatic mutations in novel genes (table) in GBC, these genes are known to play a role in carcinogenesis in common cancers. All the early onset cases or hyper-mutated cases harbour mutation(s) in critical DNA repair genes. Our results indicate significance of inherited germline mutations in DNA repair pathway genes in addition to acquired somatic mutations in GB tumorigenesis. Additionally, by considering the entire pool of germline and somatic mutations, possible clinical strategies for each tumour could be assessed. This study highlights importance of comprehensively studying mutation profiles in rare tumour types such as GBC to realistically achieve the goals of precision medicine.

Table: Detected novel genes mutations in GBC.

CASE NUMBER	CHR: COORDINATE	NOVEL GENE WITH MUTATIONS	MUTATIONS IN COSMIC (GB ADENOCARCINOMA
S1	chr14:99641509	BCL11B	0
S8	chr15:99434685	IGF1R	0
S1	chr8:145739015	RECQL4	0
S1	chr7:13978764	ETV1	0
S4	chr3:12475559	PPARG	0
S5	chr17:17127274	FLCN	0
S5	chr4:123374914	IL2	0
S5	chr9:120475302	TLR4	0
S5	chr9:120475602	TLR4	0
S8	chr1:147092612	BCL9	0
S8	chr10:96540292	CYP2C19	0
S10	chr1:147095804	BCL9	0

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P12.095C

CDH1 missense variant disrupts N-glycosylation and promotes gastric cancer

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Introduction: Germline mutations in the *CDH1* tumor suppressor gene are associated to Hereditary Diffuse Gastric Cancer (HDGC), which predisposes to a higher risk to develop diffuse gastric cancer and lobular breast cancer. Prophylactic gastrectomy is usually considered in carriers of *CDH1* pathogenic mutations, but management of patients carrying missense variants is complicated and there is a need to assess the functional impact in the E-cadherin protein.

Material and Methods: Genetic screening of *CDH1* identified three unrelated Spanish families with HDGC that

harbored the same missense variant of uncertain clinical significance. In order to determine the outcome of this variant, we performed genetic studies, together with *in silico* and *in vitro* functional analyses.

Results: The consequent amino acid shift disrupts a consensus NST sequence required for N-glycosylation, a relevant modification for proper E-cadherin status. Consistent with this, every tool used supported a pathogenic effect of the variant. The variant segregated with the disease and was not found in a control cohort, *in silico* analyses predicted the variant as deleterious and *in vitro* approaches revealed an impairment in the protein's ability for cell aggregation, an irregular pattern of E-cadherin expression and an increased invasive ability of mutant cells.

Conclusions: Our work demonstrates the pathogenic effect of the variant found and hence allows us to accomplish an accurate genetic counseling in the three families.

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P12.096D

Exome sequencing method applied for molecular genetics study of gastric cancer

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Gastric cancer (GC) - one of the most common cancers in the world. The aim of this study was to find new candidate genes associated with the risk of gastric cancer. This study enrolled 16 samples of DNA isolated from the tumor and adjacent to it a normal stomach tissue of patients with gastric cancer (GC) with the adenocarcinoma of the stomach. The DNA fragmentation, preparation of libraries and capture exome were performed in accordance with the manufacturer's instructions. Select specific DNA fragments was performed using the SureSelect system with subsequent parallel sequencing on Illumina received library technology on the device HiSeq 2000. Discovered annotated using ANNOVAR program. The analysis has revealed an average 33134 nucleotide sequence changes in normal tissue (including 3.3% have mutations that lead to frame shift reading, 40.0% - nonsynonymous substitutions, 0.3% - mutations that lead to the formation of stop codons) and 38960 - in the tumor tissue (including 3.3% have mutations resulting in a frame shift, 37.1% - nonsynonymous substitutions, 0.4% - mutations that lead to the formation of a stop codon). Each sample showed an average of 7516 somatic mutations. With the highest frequency of mutations identified in genes *TP53AIP1, ARID1A, PDGFRA, SEPT9, CACNA1G, PCDH17, NDRG2, SUPT16H, MLLT10*. Also found pathogenic changes in genes whose role in gastric cancer has not been described previously. To determine the functional significance of the identified options and the involvement of the genes found in the pathogenesis of the disease requires further in-depth analysis of the results.

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P12.097A

Genetic and clinical heterogeneity in families with gastric neuroendocrine tumors

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Type I gastric neuroendocrine tumors (gNETs) classically arise from hypergastrinemia. gNETs cause parietal cells (PC) destruction that are responsible of gastric acid secretion through ATP4A proton pump and correlates with achlorhydria. However, we have described different gNET familial cases with mutations in genes that affect the function and viability of PCs that affect gastric acidification. By NGS, we recently identified a homozygous deleterious mutation in the *ATP4A* gene that explained an aggressive familial form of gNETs. The achlorhydria was described as the causative parameter of the hypergastrinemia that develops gNET. A second family with eleven siblings and three affected members with classic clinic of gNETs plus hypothyroidism and rheumatoid arthritis was also studied and a missense mutation in heterozygosis in *ATP4A* was

identified. Carriers of this variant had low ferritin levels but without gNETs development. By WES, a second heterozygous mutation in a novel gene, *PTH1R* $^{p.E546K}$, was also uncovered. Gastrin also activates PTHLH/PTH1R regulation factor, which is involved in PC development. Activation of PTH/PTH1R, which is upregulated by thyrotropin in thyroid, is also involved in *RANKL* expression to regulate bone homeostasis. Thyrotropin and *RANKL* expression were found deregulated in those members carrying *PTH1R* mutation plus hypothyroidism and rheumatoid arthritis suggesting a link between PTH1R gene and these pathologies and gastric disease. Both mutations in *ATP4A* and *PTH1R* genes suggest a digenic model for this family and contribute to the function and viability of PC and lead to the achlorhydria that drives hypergastrinemia and gNETs instead of classic assumption.

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P12.098B

Use of a custom-designed NGS-hereditary cancer panel increases the diagnostic yield in the clinical setting

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Objectives: Mutation analysis of 122 genes in a cohort of 432 patients with clinical suspicion of hereditary cancer. Evaluation of the diagnostic yield.

Materials and Methods: NGS using a custom panel (1,2) analysed in a two-tiered mode. In a first "diagnostics stage" a specific subset of genes is analyzed depending on the clinical categorization of the patients; in a second "research stage", the whole panel is investigated.

Results: The table below summarizes the number of patients with putative pathogenic mutations. It can be observed that the second stage analysis identified mutations in an additional 10% of the patients.

Clinical Suspicion	n	+ve Diagnostics	Additional +ve Research
HBOC	215	31	27
HNPPC	129	41	13
FAP/AFAP	18	3	3
Others	70	14	2
	432	89 (21%)	45 (10%)

Currently a panoramic view of the variation spectrum landscape is under evaluation in order to investigate the role of genetic modifiers.

In addition, in 10 patients more than one pathogenic mutation was detected (2.3%), consistent with the existence of the so-called MINAS syndrome (Multilocus Inherited Neoplasia Alleles) (see abstract of Stradella et al.).

Conclusions: Our developed NGS-strategy led to an increase of the diagnostics yield in our clinical setting, improving clinical management of our patients as well as providing a better knowledge of the genetic bases of hereditary cancer.

(1,2) Castellanos et al., and Feliubadaló et al., Scientific Reports, 2017-Jan.

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P12.099C

Genetic alterations in benign prostatic hyperplasia patients by FISH

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Introduction: Benign prostate hyperplasia [BPH] is the classical age related disease of prostate, present in 20% of men at the age of 40 years with progression to 70 % by the age of 60 years. BPH is associated with various lower urinary tract symptoms, which affect their day to day life.

Materials and Methods: Our objective was to evaluate the association between HER-2/neu, c-myc, p53, and clinicopathologic variables in 47 patients diagnosed with benign prostatic hyperplasia patients using fluorescence in situ hybridization (FISH). The patients underwent transurethral prostate resection to address their primary urological problem. All patients were evaluated by use of a comprehensive medical history and rectal digital examination. The preoperative evaluation also includes serum prostate-specific antigen (PSA) measurement and ultrasonographic measurement of prostate volume. Prostate cancer was detected in two patients, who were then excluded from the study. The mean age of the 45 patients was 66.8±7.2 years. The mean PSA value of patients was 6.7 ±7.0 ng/mL. The mean prostate volume was 56.6±24.9 mL.

Results: Amplification of HER-2/neu was seen in 4/45 (8.8%) cases and amplification of c-myc was seen in 7 of 45 (15.5%) cases; neither was associated with adverse clinicopathologic variables. Deletion of p53 was seen in 31/45 (68.8%) cases. This study found that p53 deletion is a common genetic alteration in BPH.

Conclusions: In this study, we discuss genetic markers in benign prostatic hyperplasia patients which may, in the future, be used as markers for diagnosis and prognosis, as well as targets for therapeutic intervention.

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P12.100D

MiRNA profiling of gastrointestinal stromal tumors by next generation sequencing

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Introduction: Deregulation of miRNAs has been observed virtually in all major types of cancer, whereas the miRNA signature in GIST is not well characterized yet. In this study the first high-throughput miRNA profiling of GIST was performed and differentially expressed miRNAs, as well as isomiRNAs, were defined. Materials and Methods: miRNA profile of 15 paired GIST and adjacent normal tissue samples was determined using small RNA-seq approach. Highly significantly deregulated miRNAs were selected for validation by Taq-Man low-density array in replication group of 40 paired samples. Validated miRNAs were further subjected to the miRNA set enrichment analysis. Further, we used an integrated analysis of miRNA-mRNA correlations for *KIT* and *PDGFRA* target genes. All statistical analyses were performed using the in-house bioinformatics pipelines and statistical computing environment R. **Results:** 110 miRNAs were identified to be differentially expressed in GIST by small RNA-seq approach and 19 were verified to be associated with GIST after validation (13 novel associations). Enrichment analysis revealed significantly enriched KEGG pathways in the main GIST associated pathways and a significant correlation was found between all of the enriched miRNAs and their target gene *KIT*. Results of the phenotype analysis showed miR-509-3p to be up-regulated in epithelioid and mixed cell types compared to spindle type, whereas miR-215-5p showed negative correlation with risk grade of GIST. **Conclusions:** Our data reveal a detailed miRNA profile of GIST and highlight new candidates that may be important in the development of malignant disease. Supported by the Research Council of Lithuania (Grant No: MIP-006/2014).

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P12.101A

Genotype-phenotype correlations in Gorlin syndrome

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Introduction: Gorlin syndrome (GS) is an autosomal dominant syndrome leading to multiple basal cell carcinomas and an increased risk of jaw cysts and brain tumours. Individuals with GS can manifest a wide range of phenotypic abnormalities, with about 5% of affected individuals developing a medulloblastoma in early life. Gorlin syndrome is associated with germline mutations in components of the Sonic Hedgehog pathway (SHH) including Patch1 (*PTCH1*) and *SUFU*.

Methods: We analysed 182 patients with clinical features of GS and correlated their phenotype with their germline mutation status. A total of 126 patients had a heterozygous pathogenic *PTCH1* variant, 9 had *SUFU* pathogenic variants and 46 had no identified mutation.

Results: Patients with *PTCH1* variants were more likely to be diagnosed earlier (p=0.02), have jaw cysts (p=0.002) and have bifid ribs (p=0.003) or any skeletal abnormality (p=0.003) than patients with no identified mutation. Patients with a missense variant in *PTCH1* were diagnosed later (p=0.03) and were less likely to develop at least 10 BCCs and jaw cysts than those with other pathogenic *PTCH1* variants (p=0.03). Patients with *SUFU* pathogenic variants were significantly more likely than those with *PTCH1* pathogenic variants to develop a medulloblastoma (p=0.009), a meningioma (p=0.02) or an ovarian fibroma (p=0.015), but were less likely to develop a jaw cyst (p=0.0004).

Conclusions: We have identified a number of features that predict the presence of a *PTCH1* or *SUFU* mutation as well as showing that missense mutations in *PTCH1* may be associated with a milder phenotype.

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P12.102B

Germline mutational spectrum of Armenian HBOC patients analyzed with multigene panel

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Background: Genetic counselling and testing of hereditary breast and ovarian cancer (HBOC) are not appropriately provided in Armenia, and no data was available on the genetic background of HBOC in this homogeneous population. Given NGS allows cost-effective testing of a panel of genes with high and intermediate/low risk of HBOC, we studied the spectrum of genetic variations among 39 patients selected according HBOC clinical criteria.

Methods: 14 genes with known hereditary risk on HBOC were screened by NGS analysis with minimum coverage of 100x depth), including *BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53*, ATM, BARD1, BRIP1, CHEK2, MRE11, NBN, and RAD50.

Results: 22 patients had only BC at young age of diagnosis (56%, mean age at diagnosis 36 years) and 17 patients had also a family history of the disease (mean age at diagnosis 40 years). 5 and 4 pathogenic variants were identified in high risk genes BRCA1 and BRCA2, respectively. 12 variants of uncertain significance were detected in 5 genes (*BRIP1, BRCA1, BRCA2, BARD1, CHEK2*), and 18 other benign/likely benign variants were identified in six genes.

Conclusions: A multigene panel testing revealed pathogenic mutations only in high susceptibility genes and contributes in the diagnosis of HBOC patients to benefit of surveillance strategies. However, a proportion of patients with clinical criteria for HBOC had no known germ-line mutations, and another proportion of patients were found with novel and/or variants of uncertain significance in high, moderate and low penetrance genes for which no genetic counselling or management guidelines are available yet.

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P12.103C

The results of BRCA1 and BRCA2 testing in a cohort of patients in the Department of medical genetics in the Teaching hospital of Olomouc

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BRCA1- and BRCA2-associated hereditary breast and ovarian cancer syndrome (HBOC) is the cause of 5–10% breast cancers. Mutation testing should be undertaken following a genetic consultation in eligible patients, either as a diagnostic or predictive test.

We set out to determine if in the 146 patients tested in the lab of the Department of Clinical Genetics of the Teaching Hospital of Olomouc during 2013 the indication criteria were fulfilled, what was the detection rate, which mutations were found, what percentage of mutations would be detected by DTC testing offered in Czechia and what was the sex ratio of predictively tested patients.

The indication criteria were not fulfilled in 14 (9.5%) patients, in none of them was a mutation found. 88 (60%) patients were tested because of a disease occurrence - a mutation was found in 15(17%, 9 in BRCA1, 6 in BRCA2). 18 (12%) patients were tested because of a positive family history and an impossibility of testing the index case - a mutation was found in 1 (5%, BRCA2).

All mutations were unique. DTC kit would detect 3/9 (33%) BRCA1 mutations and 1/7 (14%) BRCA2 mutations. 12/16 (75%) mutations would not be detected.

Out of the 40 predictively tested patients 26(65%) were female and 14(32%) were male.

Our results confirmed good adherence to indication criteria, good sensitivity of indication criteria, increased interest in predictive testing in women compared to men and poor detection rate of the DTC kit.

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P12.104D

Next-Generation Sequencing in uniformly treated Head and Neck Squamous Cell Carcinoma within Spanish population, preliminary results

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common neoplasia in the developed world. Tobacco smoking and alcohol consumption are the most classical risk factors although viral etiology is an established factor too. However, both ways represents a distinct clinical and epidemiological entity.

Next-generation sequencing (NGS) technologies have identified genetic alterations, transforming illness diagnostics and therapeutics. Targeted sequencing has become an easier and cheaper tool in the analysis of those genes previously correlated with HNSCC.

Materials and methods: 162 head and neck squamous cell carcinoma formalin-fixed paraffin-embedded blocks were included in this study. All of them belong to the clinical trial TTCC-2007-01. After deparaffinization and DNA extraction, we performed the TruSight Tumour 26 panel technology. VCF files were read in the Variant Studio Software.

Results: 134 (82.7%) patients carry one or more pathogenic mutations in the 26 genes amplicon-based panel, while 28 (17.3%) do not have any mutations. Although not significant, we found a tendency between 27 (16.7) HPV+ tumours and a lower mutation percentage than mutated ones, p=0.063. Furthermore this distribution seems to be related with sex, where HNSCC in women have less mutations than men, p=0.056. However we do not find any association between mutation status and other clinicopathologic features or response to treatment.

Conclusion: Female sex and HPV+ infection are associated to lower mutations in HNSCC in classical driver genes. This result corroborates different HPV etiology in HNSCC. Studies in larger groups should be done to reply these results.

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P12.105A

Fusion chimeras between HBV and humans in HCC development

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Hepatocellular carcinoma (HCC) is a deadly cancer of the liver due to late detection and the limited interventional methods. Chronic infection by Hepatitis B virus (HBV) is associated with this cancer and it is known that the genome of the virus can integrate into the genome of the host cell during a chronic HBV infection leading to a series of events that can potentially alter host cells leading to tumorigenesis.

Transcriptome profiling of the tumors and adjacent non-tumorous tissues of 25 HBV-HCC patients using Next-Generation Sequencing Technology reveals that differentially expressed transcripts are mainly involved in cell-cycle regulation and several are associated with clinical characteristics. An average of 230 somatic mutations was identified in in each patient. Greater than 90% of the somatic mutations are genic. Greater than one type of HBx-Human chimeric transcripts was observed in each patient with tumor tissue harboring less variety of chimeric transcripts than the adjacent non-tumorous tissues.

A few chimeric HBx-Human transcripts were recapitulated in the non-transformed LO_2 liver cell-line. Interestingly, one chimeric transcript exhibited faster cell proliferation, higher resistance to induced apoptois and was able to transform the non-transformed LO_2 cell-line. Expression profile of this chimeric transcript compared to the wild-type revealed 151 genes to be deregulated. Characterization of these deregulated genes is in progress.

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P12.106B

Assessment of genetic testing criteria in a population-based hereditary cancer clinic

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Introduction: Cancer genetics services for the population of British Columbia, Canada are provided solely by the BC Cancer Agency's Hereditary Cancer Program (HCP). In October 2014, the HCP introduced a multi-gene panel for affected adults meeting specific phenotypic-based testing criteria for at least one syndrome on the panel. Testing criteria for Hereditary Breast and Ovarian Cancer Syndrome (*BRCA1*, *BRCA2*) have shown variable mutation detection rates. Based on preliminary data, the criterion of 3 breast cancer (BrCa) cases in a family with one diagnosed at age 50 years or younger has a mutation detection rate of only 4%.

Materials and Methods: 179 patients met the criterion of 3 BrCa cases with one diagnosed at age 50 or younger, 8 of which tested positive for presumed pathogenic variants in *BRCA1* or *BRCA2*. We calculated BOADICEA and Manchester scores for all mutation positive pedigrees and 10% of uninformative pedigrees.

Results: The mean BOADICEA scores for uninformative versus positive pedigrees were 13.8% and 23.1%, respectively ($p= 0.41$), and the mean Manchester scores were 18 and 24, respectively ($p= 0.21$).

Conclusion: While risk prediction methods BOADICEA and Manchester have higher mean scores for positive versus uninformative pedigrees, the range in scores was large. Our preliminary findings reveal the variability within families that meet this criterion. Further analysis of families with uninformative testing may help clarify the subset of cases for which targeted enrichment within the current criterion could improve mutation detection rates.

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Case-control analysis of truncating mutations in DNA damage response genes connects *TEX15* and *FANCD2* with hereditary breast cancer susceptibility

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Background Several known hereditary breast cancer susceptibility genes encode proteins involved in DNA damage response (DDR) and are characterized by rare loss-of-function mutations. However, these explain less than half of the familial cases. To identify novel susceptibility factors, 39 rare truncating mutations, identified in 189 Northern Finnish hereditary breast cancer patients in parallel sequencing of 796 DDR genes, were studied for disease association.

Methods Mutation screening was performed for Northern Finnish breast cancer cases (n = 578–1565) and controls (n = 337–1228). Four mutations showing potential cancer association were analyzed in additional Finnish cohorts.

Results A duplication leading to premature stop codon in *TEX15*, a gene encoding a DDR factor important in meiosis, associated with hereditary breast cancer (p=0.018, OR=14.6) and likely represents a Northern Finnish founder mutation. The duplication was stable at mRNA level, whereas another studied *TEX15* nonsense mutation produced a null allele and did not show association. A deleterious splice site mutation in the Fanconi anemia gene, *FANCD2*, was over two times more frequent in the combined Finnish hereditary breast cancer cohort compared to controls. A deletion in *RNF168*, involved in BRCA1-mediated DDR and causative for recessive RIDDLE syndrome, had high prevalence in majority of the analyzed cohorts, but did not associate with breast cancer.

Conclusions Protein truncating variants in *TEX15* and *FANCD2* are potential breast cancer risk factors, warranting further investigations in other populations. Furthermore, high frequency of the identified *RNF168* deletion indicates the need for its testing in Finnish patients with RIDDLE syndrome symptoms.

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P12.108D

Novel candidate alleles for hereditary breast cancer identified in Greek families via whole exome sequencing

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Introduction: Approximately ~10% of breast cancer (BC) cases are hereditary (HBC) and are caused by loss-of-function (LoF) mutations in known genes. HBC is

suspected when familial clustering of BC is observed, or when BC is diagnosed at a young age. To date, LoF alleles in over 20 genes have been associated to HBC susceptibility. However, the genetic aetiology for a large fraction of cases remains unexplained and additional causal variants are expected to be found. The aim of this project was to identify novel genetic variants linked to HBC in Greek patients who were negative for mutations in known HBC genes.

Subjects and Methods: Whole exome sequencing (WES) was performed on 52 individuals (33 patients and 19 relatives). Following variant calling, we applied three prioritization strategies to shortlist HBC candidate variants. Variants were filtered further by interrogating BC patient data of The Cancer Genome Atlas (TCGA).

Results: We identified twelve variants (ten missense, one stop gain and one splice variant), as the most likely candidates for HBC susceptibility. Of these, three variants were found in unrelated patients and nine were also present in BC TCGA patients.

Conclusion: Candidate variants detected in Greek families map in genes involved in distinct biological processes. Variants in genes involved in DNA repair (*PARP9*, *ERCC4*) and cell proliferation (*DIS3L2*) are interesting candidates to take forward to functional studies to explore their possible role in cancer pathogenicity. Funding was provided by NSRF 2007–2013 (SYN11_10_19) and the Stavros Niarchos Foundation.

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P12.109A

Hereditary breast/ovarian cancer families: beyond *BRCA1* and *BRCA2*

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Introduction: Many breast/ovarian high risk cancer families have a BRCA-like phenotype in the absence of *BRCA* mutations (BRCAness). Other genes also implicated in DNA repair may be related to the genetic risk in these families. Objective: to understand the contribution of genes besides *BRCA1/2* in high-risk *BRCA* wildtype families. Patients and Methods: All patients selected for this study were *BRCA1/2* wildtype and consented on multigene testing after counseling. Mutation screening was performed by NGS for point mutations and sequencing was carried out in the MiSeq platform (Illumina). **Results:** Since September 2016 multigene testing allowed the identification of 6 pathogenic mutations in 57 *BRCA* wildtype index probands: 2 in *RAD50* gene (3.5%), 2 in *RAD51C* gene (3.5%), 1 in *RAD51D* gene (1.7%), 1 in *CHEK2* gene (1.7%). Previously we had tested 93 patients with a different panel of genes and found 7 pathogenic mutations: 1 in *RAD51C* gene (2%), 2 in *PALB2* gene (2%), 2 in *CHEK2* gene (2%), 1 in *TP53* gene (1%) and 1 in *ATM* gene (1%). The *ATM* mutation segregated with one *PALB2* mutation in the same patient. All carriers of mutations in these genes belong to families with history of breast and ovarian cancer except for the carrier of the *RAD51D* mutation (only ovarian cancer) and the *TP53* mutation carrier who belongs to a family with only breast cancer in 3 consecutive generations. Conclusion: Taken together, these preliminary results suggest the contribution of *RAD51C*, *RAD50* and *CHEK2* genes in our *BRCA* wildtype families.

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P12.110B

Absence of large genomic rearrangements of cancer predisposition genes in Romanian patients

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Germline mutations in BRCA1/2 genes confers up to 90% lifetime risk to familial breast and ovarian cancer (HBOC), while carriers of mutations in MMR genes (MSH2, MLH1, MSH6, PMS2) are mainly predisposed to familial colorectal cancer (Lynch syndrome). Although the majority of germline defects represent point substitutions or short frameshift alterations, deletions and duplications of complete exons were also observed in HBOC or Lynch families, with proportions variating from 3% to 40% in different populations. Common oncogenetic diagnostic, mainly focusing on entire gene sequencing, is missing such alterations, which can be detected by Multiplex Ligation-dependent Probe Amplification (MLPA). Oncogenetic diagnostic in Romania was

implemented 10 years ago, and up to date no large genomic rearrangement has been reported. In order to obtain a wider overview of Romanian population, we performed an extended investigation by MLPA in familial and non-familial cancer patients, using MRC-Holland kits P002, P077, P045, P003, P008, P072 and P248. We screened overall 50 familial HBOC and 20 familial Lynch cases, as well as sporadic young cases of breast(50), ovarian(50) and colorectal(50) cancer patients. After investigating all 220 samples, no large genomic rearrangement was observed in BRCA or MMR genes. Although previous results showed a more important proportion of hereditary alterations comparing to other populations, this striking result is questioning the opportunity to implement MLPA procedures in local oncogenetic diagnostic. This report is the first extensive investigation of large genomic rearrangements in cancer predisposition genes in Romanian patients. Acknowledgement: Research Grant PN-II-RU-TE-2014-4-2257, UEFISCSDI.

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P12.111C

Panel gene analysis allows the identification of multiple mutations in hereditary cancer patients

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The analysis of 432 patients with clinical suspicion of hereditary cancer using our custom 122-gene panel I2HCP (1,2) (see abstract of del Valle et al.) identified 10 patients carrying more than one putative pathogenic mutation (see Table).

Patient/Family Criteria	First Mutation	Additional Pathogenic Mutations (Dominant genes)
Hereditary Breast and Ovarian Cancer	***BRCA1***	***EXO1; XPA***
Hereditary Breast and Ovarian Cancer	***BRCA2***	***LZTR1***
Reed"s syndrome	***FH***	***BARD1***
HNPCC-Bethesda	***MSH2***	***KLLN***
HNPCC-Amsterdam, Multiple Endocrine Neoplasia-1	***MEN1***	***MLH1***
HNPCC-Bethesda	***MSH6***	***PRSS1***
Familial Adenomatous Polyposis	***APC***	***BRCA1***
Tuberous Sclerosis	***TSC2***	***RAD51D***
		Additional Pathogenic Mutations (Recessive Genes)
Hereditary Breast and Ovarian Cancer	***CDH1***	***MUTYH***
HNPCC-Bethesda	***MSH6***	***MSH3***

Although we are still working in collecting clinical and genotype data of patients and relatives of each family, our first glance analysis was unable to identify a clear correlation between the molecular results and the clinical phenotype. In most of the cases the patient has clinical criteria associated with the first identified mutation but not for the other. However, in a few families, clinical characteristics of the two mutated genes are observed (such as *MEN1-MLH1; MSH6-PRSS1*). Several considerations have to be taken into account when analyzing these results: incomplete penetrance, possible *de novo* mutations, etc. Note the importance of identified unexpected pathogenic mutations in high risk genes for predictive testing and clinical surveillance of other carriers in the family.

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P12.112D

Spectrum of mutations in hereditary cancer syndromes associated genes in patients at high risk of breast and ovarian cancer

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Introduction: Breast cancer is among the most common cancers in women and in the Czech Republic. Carcinomas of the ovaries and fallopian tubes represent approximately 15% of all malignant tumors in women. Hereditary breast and ovarian cancer syndrome (HBOC) concerns 5 - 10% of all breast cancer cases and in majority is caused by congenital mutations in the *BRCA1* and *BRCA2* genes. However, actually there is also *PALB2* gene recommended for HBOC testing in the Czech population, because mutations in this gene seem to be the third most frequent DNA predisposition events in our population.

Materials and Methods: For HBOC patients we performed massive parallel sequencing on MiSeq (Illumina) using sequence capture approach on the gene panel (ROCHE, own design including 48 genes), followed by Sanger sequencing and MLPA analysis.

Results: Altogether, we have tested 720 patients using own-designed Oncogene panel and detected germline deleterious alterations within *BRCA1* and *BRCA2*, and *PALB2* gene. We have also identified pathogenic or potential pathogenic variations in other genes included in the Oncogene panel. These results demonstrate the efficiency of gene panel approach in performing molecular diagnosis of HBOC. Detection of several mutations within other genes than *BRCA1/2*, *PALB2* highlights the genetic heterogeneity of HBOC.

Conclusions: We have tested patients for presence of pathogenic mutations in *BRCA1/2* and *PALB2* gene. Using own-designed panel of associated HBOC genes we were able to identify several pathogenic mutations in genes related also to other malignancies (e.g. colorectal, gastric, pancreatic, gallbladder, bile duct cancer and malignant melanoma).

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P12.113A

Exploring germline variation in known cancer predisposing genes amongst *CDH1*-negative hereditary diffuse gastric cancer families

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Introduction: Gastric cancer is the fourth most common cancer globally. Between 1–3% of these cases, of which the majority are of the diffuse type, arise from predisposition syndromes. Within hereditary diffuse gastric cancer (HDGC) families, 25%-30% of cases can be attributed to a germline mutation in *CDH1*, of which over 100 have been described.

Materials and Methods: Whole exome sequencing (WES) was performed on DNA extracted from blood obtained as part of the Familial Gastric Cancer Study. Analysis was performed over 37 individuals, both affected and unaffected, from 21 *CDH1*-negative families with a strong family history of HDGC. Genes with loss-of-function variants were prioritised using prior biological knowledge to identify those that could be involved in HDGC predisposition.

Results: High quality sequencing data was generated from 11 unaffected and 26 diffuse gastric cancer cases. Germline protein affecting variants were identified in known cancer predisposition genes in 4 HDGC families. A frameshift deletion within *PALB2* was found in a family

with a history of gastric and breast cancer. Two *MSH2* variants were identified, one frameshift insertion and one previously described start loss, in unrelated affected individuals. The *BRCA2* polymorphic stop codon variant (c.9976A>T) was identified in an individual with DGC and Lobular breast cancer.

Conclusions: *PALB2*, *MSH2* and *BRCA2* variants have been previously described in diffuse gastric cancer families however they are more commonly associated with other cancers. This study suggests that more work should be done to understand their role in HDGC predisposition.

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1st Slovak germline deletion in CDH1 gene concurrently with BRCA2 mutation in patient with hereditary diffuse gastric cancer

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Introduction: Hereditary diffuse gastric cancer (HDGC) is an inherited condition associated with an increased risk of diffuse gastric and lobular breast cancer (LBC). *CDH1* genomic rearrangements are not frequent but present in HDGC. We screened germline DNA from mutation negative probands for large genomic rearrangements. Materials and Methods: We performed Sanger sequencing analysis of *CDH1* gene in 82 samples with HDGC or LBC families fulfilled revised International Gastric Cancer Linkage Consortium criteria. Subsequently, we selected 43 mutation negative probands for MLPA analysis. **Results:** We identified 2 truncating mutations out of 82 samples (2.5%) in *CDH1* gene. Series of 43 probands, negative for *CDH1* germline point mutations, were tested for the presence of large genomic alterations at the *CDH1* locus using MLPA. We found that 1 out of 43 samples (2.3%) showed signal reduction of exons 15–16. Meanwhile, clinical genetics identified distant kindred with truncating *BRCA2* mutation carrier. Our proband share this *BRCA2* mutation with her cousin. Conversely, cousin of our proband carried only *BRCA2* mutation without *CDH1* deletion. Young daughter of our proband displayed the same deletion exons 15–16 without *BRCA2* mutation, so far in age 37 is asymptomatic. **Conclusions:** In our study we identified two germline mutations, and one *CDH1* genomic rearrangement in patient who harbors germline *BRCA2* truncating mutation simultaneously. This publication is the result of the project implementation: 'Establishment of competence centre for research and development in the field of molecular medicine'. Supported by the Research & Development Operational Programme funded by the ERDF (ITMS 26240220071).

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P12.115C

Diagnostic yield of a comprehensive gene panel for hereditary tumor syndromes

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Background: In several patients with suspected hereditary tumour syndromes (HTS), no germline mutation can be identified in the most likely affected genes. Here, we analysed the diagnostic yield and clinical utility of an extensive gene panel including all known genes relevant for solid HTS.

Methods: The Illumina TruSight™Cancer Sequencing Panel of 94 known genes for HTS was extended by 54 genes. For validation, 64 HTS patients with various known germline mutations were included. Additionally, 173 patients with suspected but unexplained HTS were analysed.

Results: In addition to the known mutations, 192 rare, potentially pathogenic variants were identified. The proportion of rare variants was similar in both patient groups including variants in the moderate penetrant genes *CHEK2* and *ATM*. After further filtering, potential causative mutations were identified in 22% of patients with known mutation (eight truncating, six missense and one stoploss). The most interesting finding was a *NF1* nonsense mutation in a case with known *TP53* frameshift mutation. In 17% of patients without known mutation, we identified 20 truncating mutations, one startloss and 15 missense variants. In

three patients (2%), the mutations are very likely causative (*PMS2*, *PTEN, POLD1*). In both groups, presumptive predictive information could be revealed (truncating mutations in *SDHA*, *EXT1, RAD51C*).

Conclusions: We demonstrate that a comprehensive gene panel can identify the etiology in some prescreened patients and provide predictive incidental findings. However, our findings also show that some patients harbour predicted pathogenic mutations in more than one established cancer gene which makes the interpretation even more challenging.

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Down regulation of human telomerase reverse transcriptase *(hTERT)* expression by BIBR1532 in human glioblastoma LN18 cells

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Introduction: Increased telomerase activity can be blocked by targeting the hTERT activity at both RNA and catalytic subunits. Various inhibitors have been used to regulate hTERT activity in glioblastoma cell lines and showed conflicting results. The present study shows that the BIBR1532 effectively down-regulate the telomerase activity in LN18 glioblastoma cell line.

Methods: LN18 glioblastoma cell line was treated with different concentrations of BIBR1532 at different time intervals. MTT assay was performed to determine cell viability after BIBR1532 treatment. *hTERT* mRNA and protein expressions were quantified using qRT-PCR and western blotting. Flow cytometry assay and TRAP assay was performed to detect the rate of apoptosis and telomerase activity in treated and control.

Results: LN18 cells showed a significant dose dependent cytotoxic effect after treatment with BIBR1532. The level of hTERT mRNA expression in cells treated with 25 μM, 100 μM and 200 μM BIBR1532 treated groups was decreased ~ 21% and ~ 61.2% and ~ 77% respectively with a $p<0.05$. We also observed that, BIBR1532 treatment reduce the expression levels of hTERT protein in LN18 cells in a dose dependent manner. The Flow cytometry data showed that, the drug induce significant increase in the total percentage of apoptotic cells with 200 μM concentration of BIBR1532 in all time points.BIBR1532 exhibited potent inhibition of telomerase activity in a dose-dependent manner in LN18cells.

Conclusion: BIBR1532 could induce apoptosis in LN18 glioblastoma cells through the down-regulation of telomerase activity at transcriptional and translational level.

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P12.117A

Juvenile polyposis syndrome in a family suspected of familial adenomatous polyposis - a case report

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Introduction: Juvenile Polyposis Syndrome (JPS) and Familial Adenomatous Polyposis (FAP) are histologically characterized by distinct polyps with different ability of malignant transformation. JPS: a few to more than a hundred hamartomatous polyps, mainly benign but malignant transformation might occur. FAP: hundreds to thousands precancerous adenomatous polyps, malignancy is almost inevitable. Autosomal dominant inheritance of both syndromes.

Patients and Methods: We report a family that for a quarter of a century was clinically suspected of having FAP. The proband is a 50-year-old female who had a total colectomy with ileorectal anastomosis performed by the age of 24 years due to suspicion of FAP, because her father was diagnosed with polyposis and she was beginning to develop polyps. Her paternal grandmother was diagnosed with sigmoid colon cancer at the age of 50 and died shortly after.

Results: Using a predesigned cancer gene panel targeted next generation sequencing was performed. Due to the clinical diagnosis of FAP, first the *APC* data was analyzed, the result was normal. Then the subpanel of the polyposis genes were analyzed and a paternally inherited known pathogenic, *BMPR1A* variant, (c.1328G>A; p.Arg443His) was identified, confirming the diagnosis of JPS. We present the detailed clinical data of the three family members in comparison with cases reported in medical literature.

Conclusions: Colectomy is advised for FAP but not necessarily for JPS. This case illustrates the importance of molecular genetic screening, as management and surveillance of at-risk family members and affected individuals is very different depending on the syndrome.

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P12.118B

Examination of effects of daylight on melatonin hormone, kisspeptin protein, skin cancer formation and metastasis in mice

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Introduction: Relationship of skin cancer with constant redundancy of melatonin is known. Kisspeptin's cellular level is inversely proportional with melatonin level in blood and it is mostly synthesized in hypothalamus. Kisspeptin functions as a metastasis suppressor in melanomas. This study aimed to determine the relationship between daylight, melatonin levels in blood and kisspeptin levels in hypothalamus. In addition, skin cancer is formed on mice to examine relationship between cancer formation, metastasis, daylight, melatonin levels and kisspeptin levels. Materials and Methods: New born mice are raised for 17 weeks as blind group (24 hours dark, $n = 45$) and daylight group (12 hours light/12 hours dark, $n = 42$). At the end of 11th week, melanoma cell lines are inoculated and tumor growth is observed for 6 weeks. At the end of the experiment, melatonin levels are measured from blood serum, kisspeptin levels and *KISS1* gene expressions are measured from hypothalamus tissue. **Results:** Blind group is found to have higher melatonin levels and lower *KISS1* levels with respect to daylight group. Melatonin levels are found to be inversely proportional and *KISS1* levels are found to be directly proportional with tumor volumes. Tumor growth speed is found to be lower in blind group than in daylight group. **Conclusions:** Melatonin and kisspeptin are shown to be important tumor suppressors who are highly affected by daylight. Levels of these suppressors are found to be affected by each other, however, this relationship is speculated to be complicated and affected by other factors too instead of being simple and direct.

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P12.119C

Detection of KRAS mutations in metastatic colorectal cancers

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The KRAS oncogene that encodes p21 protein, regulates critical cellular processes. The most frequent mutations of KRAS gene occur in codons 12 and 13. Activating point mutations in codons 12 and 13 of KRAS gene are predictive biomarker for resistance to anti-EGFR therapies in mCRC. The present study showed the frequencies and types of KRAS mutations in the tumor samples of 63 patients with mCRC.

Genomic DNAs were extracted from FFPE sections that included at least 60% tumor cells of the samples. The pyrosequencing technique was used in determining the KRAS mutational status. The gDNAs were amplified by TheraScreen KRAS assays and evaluated by using Pyro-Mark software.

Of 63 mCRC samples, KRAS codon 12 mutations were detected in 38% and codon 13 mutations were in 15%. The most common mutations were G-A transitions (61 %) followed by a G-T transversions (32%) (Table). The relations between the mutation types and clinical features of the cases were compared.

Our results showed that the frequency and type of mutations were in accordance with other studies. In agreement with previous data of the literature, the highest frequency of KRAS mutation was detected in codon 12. We concluded that pyrosequencing is highly specific and can productively be used in the detection of hot spot KRAS mutations in mCRC samples for the establishment of the effective therapy.

Table1. KRAS mutation results

Codon	Mutations	Frequence (n / %)
12	c.35G>A p.G12D	10 /22%
12	c.35G>T p.G12V	9 /20%
12	c.34G>T p.G12C	2 /4%
12	c.34G>A p.G12S	1 / 2%
12	c.35G>C p.G12A	2 / 4%
12	c.34G>C p.G12R	–
13	p.G13D c.38G>A	10 / 22%

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P12.120D

Mutational screening of KRAS, NRAS and BRAF genes in Romanian colorectal patients

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Colorectal cancer (CRC) is a heterogeneous disease, exhibiting multiple genetic alterations. Screening for KRAS, NRAS and BRAF mutations becomes increasingly important for clinical assessment, activating mutations in these genes being strong predictors of resistance to EGFR-targeted agents.

Aim: screening of KRAS, NRAS and BRAF genes in CRC patients for tailoring their specific therapeutic strategy.

Our on-going study has included 60 unrelated Caucasians patients, between 38–85 years old, with histologically-proven diagnosis of CRC in advanced stages. Genomic DNA was isolated from FFPE primary (34) or secondary (26) tumors samples, with 2–96% tumor cells content. Mutational status of KRAS, NRAS and BRAF genes was analyzed by Real-Time PCR and capillary sequencing.

Mutations in KRAS, NRAS and BRAF genes are mutually exclusive and confer resistance to anti-EGFR therapy in CRC patients. In our study, KRAS activating mutations were detected in 27 samples (45%), distributed in exon 2 (codon 12- 70.37%; codon 13- 22.23%), exon 3 (3.7%) and exon 4 (p.Ala146Thr- 3.7%). NRAS activating mutations were identified in 3 samples (5%), distributed in exon 2 (p.Gly12Ser- 66.7%) and exon 3 (p.Gln61Leu-33.3%). Five K-/NRAS wild-type CRC patients underwent screening of BRAF V600 locus without any mutation identified.

Nowadays, screening for activating mutations in the KRAS, NRAS and BRAF genes has became a routine analysis for CRC Romanian patients, contributing in this way to their effective selection prior to anti-EGFR targeted-therapy initiation. Also, our results, in accordance with international data, have shown that mutational screening is essential for tailoring of specific therapeutic strategy in CRC patients.

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P12.121A

The silenced potassium channels Kv1.3 and Kv10.1 affects miR-126 in breast cancer cells

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Introduction: It has been shown that non-coding regulatory RNAs, especially play a role in formation and progression of cancer. miR-126 is markedly downregulated in human breast cancer tissues. Various studies indicate that some voltage gated potassium channels are also overexpressed in the tumor. Kv1.3, a member of the voltage-gated potassium channel family is overexpressed in cancerous breast tissues than in normal breast tissues. Another protein, Kv10.1 is overexpressed in many human tumors, including breast cancer and it has oncogenic properties. Ether à go-go 1 (Eag1) channel is overexpressed in a variety of cancers and plays important roles in cancer progression. However, the association between with voltage gated potassium channels and miR-126 is unclear. In our study, we aimed to show whether there is association between miR-126/miR-126* and Kv1.3 or Kv10.1 in non-invasive estrogen positive MCF-7 and invasive estrogen negative MDA-MB-231 human BC cells. Materials and Methods: MCF-7 and MDA-MB-231 cells were transfected with Kv1.3 and Kv10.1 specific siRNA. miR-126 and miR-126* expression was determined in total RNA isolated from the cells. All samples were normalized to the internal controls, and fold changes were calculated through relative quantification ($2^{-\Delta\Delta Ct}$). **Results:** The results of our research indicate that there is a strong inverse relationship with the expression levels of *miR-126* and potassium channels. **Conclusions:** The miR-126/126* expressions increased in the MDA-MB-231 cells and decreased in the MCF-7 cells by using siRNA against potassium channels Kv1.3 and Kv10.1.

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P12.122B

Germline *TP53* Mutations Result into a Constitutive Defect of p53 DNA Binding and Transcriptional Response to DNA Damage

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Introduction: Li-Fraumeni Syndrome (LFS) results from heterozygous germline mutations of *TP53*, encoding a key transcriptional factor activated in response to DNA damage.

Materials and Methods: We performed the p53 functional assay, based on the p53 transcriptional response to DNA damage, in lymphocytes from 56 *TP53* mutation carriers harboring 35 distinct alterations, then chromatin immunoprecipitation-sequencing (ChIP-Seq) in lymphocytes exposed to doxorubicin in order to compare the impact of the different types of germline *TP53* mutations on DNA binding.

Results: The p53 functional assay shows that germline dominant-negative missense mutations alter the transcriptional response to DNA damage more drastically than null mutations, indicating that this defect constitutes an endophenotype of germline *TP53* mutation severity. ChIP-Seq performed in wild-type *TP53* control lymphocytes accurately mapped 1287 p53-binding sites. New p53-binding sites were validated using a functional assay in yeast. ChIP-Seq analysis of LFS lymphocytes carrying the *TP53* dominant-negative missense mutations p.R273H or p.R248W revealed only 310 and 143 p53-binding sites, respectively, and the depths of the corresponding ChIP-Seq peaks were drastically reduced. In contrast, analysis of LFS lymphocytes with *TP53* null mutations (p.P152Rfs*18 or complete deletion) or with the low penetrant "Brazilian" p.R337H mutation revealed a moderate decrease of p53-binding sites (949, 580 and 620, respectively) and of peak depths.

Conclusions: Altogether, our results show that *TP53* mutation carriers exhibit a constitutive defect of the transcriptional response to DNA damage and that the clinical severity of *TP53* dominant-negative missense mutations is explained by a massive and global alteration of p53 DNA binding.

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P12.123C

Highly reliable single cell whole exome sequencing of circulating tumor cells (CTCs)

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Whole exome sequencing (WXS) allows to determine the mutational landscape in cancer at the single-nucleotide-level. Together with whole genome amplification the full potential of the method is applicable down to the single cell level to investigate individual heterogeneity. For several reasons we combined the single cell genome amplification method *Ampli*1^{TM} WGA with the whole exome enrichment technology SureSelect XT for WXS of circulating tumor cells (CTCs) in liquid biopsy samples from metastatic cancer patients: (1) *Ampli*1^{TM} WGA excludes random priming thereby eliminating introduction of priming-derived sequence alterations; (2) the deterministic nature of this technology ensures that the genomic amplicon population is amplified equally in all single cells of all patients thus facilitating sample quality control (QC) implementation; and (3) Agilent SureSelect XT has shown the best performance in coverage of clinically interesting mutations. The assessment of the combination of both technologies on WGA samples derived from single peripheral blood lymphocytes (PBLs) isolated from a healthy human donor revealed a high reproducibility and reliability of the optimized workflow, almost equivalent to routine diagnostic pathology. In applying the established approach on single CTCs of metastatic breast cancer patients from DETECT III, a multicentric phase III study, the newly developed sample QC permits to identify high-quality amplified CTC samples. The comparison of the identified mutation profiles with matched PBLs allowed to eliminate vastly amplification-induced sequence alterations. By developing a bioinformatics tool fine-tuned for *Ampli*1^{TM} WGA, single cell WXS data sets can be additionally used for reliable CNV analysis in CTCs.

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P12.124D

Dysregulation of the long non-coding RNA, PRNCR1, in breast cancer

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Breast cancer (BC) is one of the most common cancers worldwide. Long non-coding RNAs (lncRNAs) have been approved to play a critical regulatory role in various cellular activities such as carcinogenesis, which indicates that

lncRNAs are therapeutic targets and potential biomarkers for cancer detection. Prostate cancer associated non-coding RNA1 (PRNCR1) that is located in 8q24.21 is a long non-coding RNA that deregulate in some cancers. In our study, we investigated the expression level and clinical significance of PRNCR1 in BC.

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression level of PRNCR1 in BC tissues. In a cohort of 25 patients, PRNCR1 was significantly overexpressed in BC tissues compared with the expression in adjacent tissues, with an average fold increase of 6.26 (P=0.004). Additionally, a high level of PRNCR1 was associated with large tumor size and lymph node metastasis (P<0.05). Based on receiver operating characteristic curve (ROC), we found that the area under the curve (AUC) of PRNCR1 was 0.76 indicating that PRNCR1 could be a sensitive diagnostic biomarker of BC.

In conclusion, our study demonstrated that PRNCR1 is an oncogene of breast cancer and dysregulation of PRNCR1 could be a potential target for gene therapy in breast cancer.

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P12.125A

Clinical and biological significance of Y chromosome loss in a series of 2,423 male patients with Myelodysplastic syndromes and Chronic Myelomonocytic Leukemia

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Isolate loss of chromosome Y (-Y) in myelodysplastic syndromes (MDS) is associated to a better outcome but it is also well described as an age-related phenomenon. In this study we aimed to analyze the prognostic impact of -Y in the context of the IPSS-R cytogenetic classification, evaluate the clinical significance of the percentage of metaphases with isolated -Y, and test whether finding -Y may predispose to over-diagnose MDS in patients with borderline morphological features. We evaluated 3,581 male patients from the Spanish MDS Registry with a diagnosis of MDS or chronic myelomonocytic leukemia (CMML). -Y was identified in 177 patients (4.9%). Compared with the 2,246 male patients with normal karyotype, -Y group showed a reduced risk of leukemic transformation that did not translate into a survival advantage. The overall survival and the risk of leukemic transformation were not influenced by the percentage of metaphases with -Y. The -Y group was not enriched in patients with minor morphologic traits of dysplasia, suggesting that the better outcome in the -Y group cannot be explained by enrichment in cases misdiagnosed as MDS. In conclusion, our results support the current recommendation of classifying patients with -Y within the very good risk category of the IPSS-R for MDS and rule out a selection bias as a possible explanation of this better outcome. An analysis of the molecular basis of MDS with isolated -Y would be of interest as it may provide a biological basis of protection against progression to acute leukemia.

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P12.126B

The Use of Molecular Identifiers (MIDs) for Improved Low Frequency Mutation Detection in Ovarian Cancer

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Improving the sensitivity of next generation sequencing (NGS) technologies to detect low frequency variants involves removal of PCR duplicates to achieve accurate coverage. By uniquely labeling every DNA molecule within an NGS library, more confident detection of low frequency mutations and other genetic variants is possible. Molecular ID (MID) labeling of unique library molecules enables detection and removal of PCR duplicates while preserving fragmentation and complementary strand duplicates. Prior to removal, the MID can also be used to generate a consensus sequence from PCR duplicates, reducing sequencing and PCR-induced errors. Here MID technology was combined with Swift Biosciences' Accel-NGS® 2S Hyb DNA Library Kit to evaluate the effect on data analysis. Libraries were prepared with DNA from a series of tumors from a stage 3B ovarian carcinosarcoma patient and circulating cfDNA from blood plasma collected at the time of surgery. We determined data retention after de-duplication using either standard Picard tools without using MIDs or UMI-tools (Fulcrum Genomics) that utilize MIDs. We observed an increase in data retention for both Covaris-sheared gDNA and cfDNA libraries that lead to a 1.7 to 2.9 fold increase in coverage using UMI-tools over Picard de-duplication under the conditions tested and found this effect was maximized by lower DNA input quantities and higher depth of sequencing. These libraries were further evaluated for variant calling using oncology-related gene panels. We identified low frequency variants (as low as 0.5%) present across all tumor samples and within cfDNA, as well as variants unique to each tumor sample.

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P12.127C

Integrated DNA methylation and copy-number profiling in adults with lower-grade brain gliomas

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Lower-grade gliomas (LGG, grade II-III) represent heterogeneous group of brain tumors that comprise astrocytomas (A), oligodendrogliomas (O) and oligoastrocytomas (OA). Patients with LGG have highly variable clinical behavior not adequately predicted on the basis of histological classification. Assessment of the genome aberrations by integrated molecular-cytogenetic techniques allows robust and prognostically relevant classification of these tumors.

To investigate the genetic and epigenetic profile of tumor cells, genome-wide analyses of 111 samples of LGG (76xA, 28xO, 7xOA) was performed. The tissues taken from primary tumors were analyzed to detect recurrent chromosomal aberrations, copy number variations, *IDH* mutations and hypermethylation of *MGMT* and MMR genes promoters using I-FISH (Abbott), SNP array (Illumina), and MLPA (MRC-Holland).

Mutation of *IDH* genes was detected in 75.7% LGG. A total of 30.6% patients with mutated *IDH* had simultaneous co-deletion 1p/19q, which is considered the predictive marker of better response to radio-chemotherapy. In 35.3% astrocytomas, shattering of different chromosomes (chromothripsis) always in combination with complex changes was observed by SNP array. Copy-neutral LOH17p, often associated with *TP53* mutation, was proved in 56.6% of astrocytomas. In 37.8% LGG hypermethylation of *MLH3* gene promoter was detected. Patients with hypermethylated *MLH3* had significantly better overall survival ($p=0.001$).

At present, sub-classification of highly heterogeneous diffuse LGG based on genetic and epigenetic profile of tumor cells and its continuous monitoring provides better genetically defined patient's sets with narrowed variability

of clinical outcomes. In the future it will facilitate more personalized treatment.

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Famale NSCLC genes' mutation screening with Ion AmpliSeq™ colon and lung cancer research panel

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Objectives: Molecular testing of cancer is increasingly critical to medicine. Next-generation sequencing (NGS) provides comprehensive, unbiased, and inexpensive mutation analysis of multiple genes with a single test. The aim of our study was to assess the clinical usefulness of the Ion AmpliSeq™ colon and lung cancer's research panel on Ion Torrent™ PGM, which allows sequencing 22 genes and detects mutations of lung cancer DNA.

Methods: NGS was performed with DNA from frozen and formalin-fixed, paraffin-embedded NSCLC tumor samples obtained from 16 non-smoking women using Ion AmpliSeq colon and lung cancer research panel, ThermoFisher. Twenty-two oncogenes: *KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MAP2K1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBX7, FGFR3, NOTCH1, ERBB4, EGFR1* and *FGFR2* have been sequenced with this panel.

Results: 16 DNA samples from NSCLC female tumors were sequenced and mutations were detected in 12 oncogenes. Mutations in *KRAS* gene were found for 4 patients, *BRAF* – 1 patient, P*IK3CA* – 2 patients, *TP53* – 8 patients, *EGFR* – 2 patients and *ERBB2* – 1 patient. Furthermore, for 4 cases mutations were detected in two different genes, for 7 cases mutations were detected in one gene and in one case gene with two different mutations was identified.

Conclusion: The the Ion AmpliSeq™ colon and lung cancer's research panel exhibited good clinical performance, strongly supporting the implementation of the NGS assay in routine clinical use to facilitate therapeutic decision-making for lung cancer patients.

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P12.129A

Monitoring lung cancer therapy via liquid biopsy

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Introduction: Liquid biopsy is a non-invasive sample source that can be utilized to assess cancer response to drug therapy by measuring the tumor- derived fraction of circulating cell-free DNA (cfDNA) in plasma. The purpose of this study was to evaluate the clinical significance of liquid biopsy by monitoring the treatment outcome of a patient with lung cancer (NSCLC).

Materials/Methods: Both FFPE and cfDNA samples of this patient were subjected to Accel-Amplicon 56G Oncology Panel which offers comprehensive and hotspot coverage of 56 clinically-relevant oncology-related genes. The runs were conducted on MiSeq platform (Illumina) with MiSeq- 300V2 kit. Liquid biopsy was performed after one week and then after a month of the beginning of a third generation EGFR-TKI treatment (with rociletinib).

Results: The mutation T790M of the EGFR gene was detected in both FFPE and in first cfDNA samples. The T790M mutation levels were slightly decreased after one week and much further after one month of therapy. Therefore, therapy seems to be effective.

Conclusion: Tissue biopsy still represents the gold standard for characterizing the initial tumor sample, but liquid biopsy seems to be a valuable tool for monitoring patients subjected to therapy.

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P12.130B

"Omic" approach in non-smokers with adenocarcinoma pinpoints to germline susceptibility and personalized medicine

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A cohort of four never-smoker patients with primary *EGFR*/*KRAS*/*ALK*-negative lung adenocarcinoma and their normal sibs were subjected to a novel integrative "omic" approach using a pedigree-based model for discovering genetic factors leading to cancer in the absence of well-known environmental trigger. A first-step whole-exome sequencing on tumor and normal tissue did not identify mutations in known/candidate driver genes. Building on the idea of a germline oligogenic origin of lung cancer, we performed whole-exome sequencing of DNA from patients' peripheral blood and their unaffected sibs. Filtering for rare variants with CADD>25 and potentially damaging effect, we identified a mean of 100 potentially deleterious variants per patient, 20% of which mapping in genes previously associated with cancer. The majority of the variants were exclusively identified in patients with few exceptions being present also in the healthy sib. Transcriptome profiling on both tumor and normal lung tissues revealed that among the selected mutated genes, a mean of 10 genes per patient were either down- or up-regulated in cancer specimens. Among the down-regulated genes, 80–90% of reads carried the mutated allele suggesting a loss of heterozygosity. Notably, the group of mutated genes was unique for each patient pinpointing to a "private" oligogenic germline signature. Noteworthy, at least 2 mutated genes per patient turned to be druggable because related to known drivers, such as NOTCH1 and YAP1. In the era of precision medicine, this report emphasizes the importance of an "omic" approach to uncover oligogenic germline signature underlying cancer development and identify suitable therapeutic targets.

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P12.131C

Histological and molecular hallmarks of PMS2 associated CRC

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Background Lynch syndrome is caused by germline mutations in one of the mismatch repair genes, including PMS2. This study aims to unravel for the first time the histological and molecular hallmarks of PMS2 associated CRC.

Methods Histological hallmarks in 20 PMS2 deficient tumours were scored by an experienced pathologist. The Ampliseq Cancer Hotspot panel was used to analyse the somatic tumour spectrum, which covers 50 genes (~2800 COSMIC mutations), including KRAS, APC and TP53. Results were compared with (hypermethylated and mutated) MLH1 deficient and sporadic CRCs.

Results PMS2 associated CRCs showed a number of LS associated hallmarks: 81% were right-sided, 43% had Crohn's like infiltrate and 81% showed microsatellite instability. However, a majority (63%) hardly had any tumour infiltrating lymphocytes, a well-known characteristic of Lynch associated tumours. We identified a high percentage of a specific FBXW7 mutation (c.1393C>T, p.Arg465Cys). Notably, 5/20 CRCs had this transition, where the sporadic controls had none. We also found a relatively rare KRAS mutation in exon 4 (c.436G>A, p.Ala146Thr) occurring three times in the PMS2 cohort but not in the control cohort and once in MLH1 associated CRCs. Strikingly we found CTNNB1 mutations in 14/25 (60%) of MLH1 associated CRCs, but none in the PMS2 cohort.

Discussion Results from this study indicate the possibility of a specific route to tumorigenesis in PMS2 carriers, which may have consequences for detection and treatment. Moreover, these observations might also contribute to tailor-made surveillance guidelines for PMS2 mutation carriers. Supported by the Dutch Cancer Society

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P12.132D

Universal screening for Lynch syndrome in patients with endometrial carcinoma

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Introduction After colorectal cancer, endometrial cancer (EC) is the second most prevalent cancer in Lynch syndrome, and EC is the first cancer in approximately 50% of women with Lynch syndrome. We present a study with consecutive screening for Lynch syndrome using immunohistochemistry (IHC) of the mismatch-repair (MMR) proteins in patients diagnosed with EC.

Material and Methods All incident EC cases in the Northern Region of Denmark diagnosed between 2013 and 2016 were screened with IHC of the four MMR proteins pMLH1, pMSH2, pMSH6, and pPMS2 and methylation analysis of the *MLH1* promotor in tumors with loss of pMLH1/pPMS2. Patients with loss of pMLH1/pPMS2 and no *MLH1* promotor methylation, loss of pMSH2/pMSH6, or isolated loss of one MMR protein were offered screening with Sanger sequencing of the MMR genes.

Results Out of 246 EC patients, IHC revealed 65 patients with loss of MMR proteins. 46 patients had loss of pMLH1/pPMS2 and *MLH1* promotor methylation. Nineteen patients were offered genetic counselling. Sequencing of the MMR genes in 11 of these patients revealed six patients with a pathogenic variant (one *MSH2* and five *MSH6*) and one patient with a variant of unknown significance in *PMS2*.

Conclusion Lynch syndrome was identified in 2.4% of the EC patients. None of the five patients with *MSH6* mutations would have been diagnosed with Lynch syndrome if only family based criteria had been used. Universal screening with IHC of the MMR proteins seems as relevant in patients diagnosed with EC as it is in colorectal cancer cases.

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P12.133A

Routine molecular analysis for Lynch syndrome in patients with advanced adenoma or colorectal cancer within a national screening program for colorectal cancer

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Aim To assess the diagnostic yield of immunohistochemistry (IHC) for Lynch syndrome (LS) in patients with advanced adenomas or CRC within a national colorectal cancer (CRC) screening program.

Methods We included participants of the national CRC screening program, referred for colonoscopy from December 2013 onwards. IHC was performed on advanced adenomas (adenomas with villous component, high-grade dysplasia or ≥ 10mm in size) and CRCs. Also, in cases with ≥4 non-advanced adenomas, IHC was performed on the largest adenoma. *MLH1* hypermethylation analysis was performed if indicated. Patients suspect for LS were offered germline mutation analysis. If no pathogenic mutation was found, we performed somatic mutation analysis.

Results We included 913 patients (53% male; mean age 66 years (±6 years)). At colonoscopy, 345 (38%) patients (63% male; mean age 67 years (±6 years) were eligible for IHC. In total, 316 adenoma patients were analyzed. None had aberrant IHC. Of these adenomas, 148 (47%) had villous component and/or high-grade dysplasia. Out of 44 CRC patients, 6 (15%) showed loss of MLH1 and PMS2 protein expression. Four cases had *MLH1* promoter hypermethylation. In the remaining two patients no germline *MLH1* mutation was found and somatic mutation analysis showed that both had a likely sporadic tumor. **Conclusion** Our results indicate that routine LS screening by IHC in patients with advanced and multiple adenoma within a national CRC screening program is not an effective strategy. The diagnostic yield of LS screening in younger adenoma patients should be assessed.

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P12.134B

FANCM germline mutations in *BRCA1/2*-negative male breast cancer cases from Italy

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Introduction: Male breast cancer (MBC) is a rare disease. Inherited mutations in *BRCA1*, *BRCA2* and *PALB2* accounts for 10–15% of all cases. Thus, a fraction of MBCs is expected to be caused by genetic risk factors yet to be identified.

FANCM has been suggested as a novel breast cancer (BC) susceptibility gene. Particularly, two *FANCM* mutations, c.5101C>T (rs147021911) and c.5791C>T (rs144567652) have been associated with female BC risk.

Materials and methods: Aimed at investigating the genetic component of MBC, *FANCM* c.5101C>T and c.5791C>T mutations were genotyped in 506 *BRCA1/2* mutation negative MBCs and 854 male controls from the Italian Multicenter Study on MBC using TaqMan assays. Furthermore, the entire coding region of *FANCM* was screened in 286 MBCs, by a custom gene panel using NGS technologies.

Results: Case-control study showed that *FANCM* c.5101C>T was not present in any of the samples analyzed, and *FANCM* c.5791C>T was found in two controls (0.23%). Screening of the whole gene revealed two *FANCM* truncating mutations, c.1432C>T (p.Arg478Ter) and c.1972C>T (p.Arg658Ter) in two (0.7%) young MBC patients (42 and 55 years old, respectively).

Conclusions: *FANCM* c.5101C>T and c.5791C>T mutations may not have a major role in MBC susceptibility. Nevertheless, other rare *FANCM* truncating mutations may be associated with MBC risk. We suggest the inclusion of *FANCM* in gene panel testing for MBC cases, so that the identification of a higher number of mutation carriers will help estimate MBC risk associated with *FANCM* mutations.

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P12.135C

Characterisation of myelodysplastic syndromes in an Asian population by conventional cytogenetics

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Introduction: Myelodysplastic syndromes (MDS) is a neoplastic bone marrow disorder characterised by dyspoiesis of the hematopoietic cells of all lineages. Hence, MDS patients are often cytopenic. The aim of this study was to corroborate and charaterise the karyotypic aberrations of MDS among our Singapore General Hospital (SGH) patients by conventional cytogenetics (CC).

Materials and Methods: A review was done on patients in our laboratory with MDS between January 2010 and December 2014.

Results: A total of 340 patients with MDS was identified, comprising 140 females and 200 males, with a ratio of 1 female:1.4 male. The ages of the patients ranged between 15 and 92 years, with a median age of 67 years. CC was performed on 332 patients, with 166 patients presenting chromosomal abnormalities.The detection rate of MDS by CC was therefore 50.5%. Eighty-three patients presented

with a single aberration, with trisomy 8 (+8) being the most common sole abnormality, followed by a loss the Y (-Y) chromosome, deletion of the q-arms of chromosome 20 (del20q) and chromosome 5 (del5q). Twenty-eight patients had 2 aberrations, with trisomy 1 (+1) as the most common additional aberration, followed by a loss/partial loss of the q-arm of chromosome 7 (-7/del7q) and + 8 as the second most common additional aberration. Ten and 50 patients had 3 and >3 chromosomal aberrations, respectively.

Conclusions: MDS more often presents with a sole abnormality and more often affects the elderly. The aberrations commonly but not exclusively include +8, -Y, del20q, del5q, +1 and -7/del7q, in decreasing order.

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P12.136D

Detecting EGFR exon 19 deletions and L858R in patients with non small cell lung cancer

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Background: Non-small-cell lung cancer (NSCLC) is the leading cause of death in Bulgaria for both men and women. NSCLC patients usually have activating *EGFR* mutations which leads to a very good response when is treated with EGFR-tyrosine kinase inhibitors (TKIs). We evaluate the presence of active *EGFR* mutations in a large sample of NSCLCs. Methods: DNA was extracted from formalin-fixed paraffin-embedded NSCLC tumor samples and was performed by QIAamp DNA FFPE Tissue Kit. The samples were analysed by real-time PCR, using *therascreen EGFR* assay. We determined the types and frequency of *EGFR* mutations and their association with smoking status, sex and histology. **Results:** We tested 600 lung cancer samples for the presence of *EGFR* exon 19 deletions, L858R in exon 21 and another 9 somatic mutations. *EGFR* sensitive mutations were found in 57 NSCLC samples (9.5%). The frequency of *EGFR* mutations was greater for females versus males (6.6% versus 3%, P < .001). The frequency of *EGFR* mutations is greater in never smokers (5.5%) after that is the frequency of ever smokers (3.83%) at the last is the smokers (0.16%) with P < .001, and the frequency for adenocarcinomas versus other histological types (squamous cell carcinoma and other) are 7% versus 2.5%; P < .001. Conclusion: Oure results show that activating *EGFR* mutations are more frequent in females than in males, in adenocarcinoma than squamous cell carcinoma and large cell carcinoma. *EGFR* mutations are more frequent in non-smokers than smokers. Acknowledgements: Astra Zeneca

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P12.137A

Novel germline variants in melanoma families

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Germline mutations in *CDKN2A* account for susceptibility in ~40% of cutaneous melanoma (CM) families, while mutations in the *CDK4*, *MITF*, *TERT*, *POT1*, *ACD* and *TERF2IP* genes each occur in <1–2% of CM families. Mutations in *BAP1* have been associated with susceptibility to both CM and uveal melanoma (UM), as well as mesothelioma and a range of other cancers. *BAP1* is the only known high penetrance UM gene, responsible for susceptibility in ~5% of CM families with single cases of UM. Thus the genetic mutations underlying predisposition in the majority of CM and UM families is unknown. To identify new familial melanoma genes we have used whole genome sequencing (WGS) or whole exome sequencing (WES) of multiple affected members of >176 CM and/or UM families. Each family was prescreened and shown to be wildtype for all of the known melanoma predisposition genes. WGS/WES was performed by the Wellcome Trust Sanger Institute (UK) or Macrogen (Korea) on the Illumina Hiseq 2000 or X Ten platforms. Variants were filtered for stringency using quality score of >70 and alternate read counts of >2 and >20% of all reads at a given position. Novel or rare (frequency < 0.001 in the ExAC Consortium population of 60,706 individuals) protein-changing variants that lead to premature truncation of the protein were prioritized as likely pathogenic variants. Based on known biology several of these genes are plausible candidates for novel familial melanoma genes. Follow-up studies are underway to accrue additional support.

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Analysis of mutations in melanocytic nevus and melanoma samples by next-generation sequencing

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Melanoma remains one of the most aggressive types of cancer despite advancements in our understanding of the molecular mechanisms underlying malignization, disease progression, and therapeutic response. Identification of mutations predicts the progression of disease and determines the choice of therapy, providing a personalized approach to treating melanoma. In addition, the search for new mutations that can cause tumor response to target inhibitors is of great interest. In present study we used targeted next-generation sequencing (NGS) for the analysis of mutations in 6 melanocytic nevus and 29 melanoma samples. Sequencing was performed using a platform GS Junior (454/Roche). The Nimblegen technology (Roche) was used for target regions enrichment including *NRAS, PDGFRA, KIT, RASA1, RAC1, MET, BRAF, PTEN, AKT1, MAP2K1, MAP2K2, TP53* and *TERT* genes. In melanocytic nevus somatic mutations in *BRAF* (p.Val600Glu) (16%), and germline mutations in *CDKN2A* (p.Ala148Thr, p. Gly63Arg) (33%), *PDGFRA* (p.Ser478Pro) (33%) were found. In melanoma samples different types of somatic mutations in *NRAS* (p.Gly13Arg, p.Gln61His) (10%), *BRAF* (p.Val600Glu, p.Val600Lys) (45%), *MAP2K1* (p. Pro124Ser) (3%), *TP53* (p.Gln331Ter, p.Glu224Lys) (7%), *PDGFRA* (p.Glu571Lys) (3%) and germline mutations in *PDGFRA* (p.Leu221Phe) (3%), *KIT* (p.Met541Leu, p. Val50Leu) (20%), *RASA1* (p.Ala99Val) (14%) were discovered. The somatic/germline nature of the mutations was determined by Sanger sequencing of normal tissue from the same patients. Further investigations using NGS may help in searching of new pathogenic mutations on different stages of tumor development. This work was supported by the Russian Science Foundation (grant # 14−35-00107).

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P12.139C

The molecular differences between tumor and its liver metastases in colorectal cancer

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Presence of malignant cells with different biological characteristics in one tumor is an intratumoral heterogeneity. Formation and development of tumor metastases of colorectal cancer in distant organs are observe in 50% cases. It is known there are tumors and metastases cells with different aggressive grade. The aim of our study was to analyze genes expression (*ZEB1*, *ZEB2*, *VIM*, *CDH1*, *SFRP2*, *FOXQ1*, *TNC*, *MACC1*, *PLS3*, *CFTR*, *FLNA*, *MUC2*, *TFF3* и *RARRES3*) and genes somatic mutations (*KRAS*, *NRAS*, *BRAF*, *PIK3CA*) in the primary tumor and liver metastasis. In this study, using PCR real time and sequencing, were investigated samples of synchronic metastatic CRC from 40 patients. In 120 cases, tissue, tumor and nodes of the liver metastases were available. Data expression genes of tumors and its liver metastases have been concurred for 55% samples. Epithelial-mesenchymal transition was found in one tumor and 7 liver metastases from 8 patients. *NRAS* and *BRAF* mutations were not found. Overall frequency of *KRAS, PIK3CA* mutations of tumors was 18/40 (45%), 9/40 (23%), respectively and had been correspond to with metastases. Our study revealed that gene expression profile in the primary tumor and metastasis is different for half cases, whereas there are full concordance between tumor and metastasis for somatic mutations.

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P12.141A

Themurine miR-302 host gene structure and its potential use as cancer stem cellmarker

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Stem cells have unique properties including self-renewal and multipotency that are shared with cancer stem cells. MicroRNAs are important regulators of stemness state of the cells. MiR-302s/367 is a polycistronic miRNA cluster including miR-302b/c/a/d and miR-367 which is located in the intron of a non-coding host gene. MiR-302s have been shown to repress mRNAs required for differentiation to induce pluripotency in somatic cells. The stem cell specific transcription factors Oct4, Sox2 and Nanog drive miR-302s expression. We found mmiR-302 expression in CJ7 murine ES cells, teratomas, and PymT induced mammary gland tumors. Of these, teratomas showed the highest miR302 expression. 'Rapid Amplification of cDNA Ends' (RACE) for the 5′ and 3′ ends of the miR-302 host gene showed that this RNA is capped and polyadenylated. Sequencing of ES cell and teratoma derived cDNAs showed alternative splicing and polyadenylation which differs in ES and teratoma derived cells. Cell fractionation and qPCR showed that the host RNA of mmiR-302 is exported to the cytoplasm. Utilizing luciferase-based reporter vectors we found the regulatory sequences to extend at least 2 kb upstream of the transcription start site. This regulatory region contains several conserved binding sites for both transcriptional activators and suppressors. Reporter constructs with different upstream regions result in differential expression in ES and teratomas and might therefore explain the differential expression of miR-302 in different types of stem cells. Moreover, as a proof of principle, cancer stem-like cells were selected from teratomas utilizing the NEO resistance gene driven by the mmiR-302 promoter.

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P12.142B

The microRNA 221: Biogenesis, Function and signatures in Human Cancers

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MicroRNAs (miRNAs) are small non-coding RNAs of (18–25 nucleotides) that control gene expression in post transcriptional level through binding to the 3´-UTR of mRNAs and block mRNA transcription and/or adversely regulate its resistance. Much research indicates that dysregulation of miRNA is a sign of cancer. The expression of miRNA are frequently abberant and their function is relevant to the regulation of oncogenes and/or tumor suppressor genes involved in cell signaling pathway. MiR-221 and miR-222 are two extremely homologous microRNAs, which high expression has been commonly indicated in various types of human cancers. The function of miR-221/miR-222 have been investigated as oncogenes or tumor suppressors, related to tumor system. Here we review the role of miR-221/miR-222 in various types of cancer progression and development: controling proliferative signaling pathways, avoiding cell death from tumor suppressors, monitoring angiogenesis and even supporting epithelial-mesenchymal transition. We discuss that miR-221/miR-222 act as promising biomarkers for prognosis in various types of cancer and they would suggest a new pathway in molecular targeting cancer treatment.

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P12.143C

microRNA-31 explicitly down-regulated in PTEN low gastric cancers

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Introduction: Gastric cancer is one of the most common world-wide cancers where early diagnosis is essential due to its poor prognosis. MicroRNAs (miRNAs) are small (~22 nucleotide) non-coding RNAs that gained great impact in the last decade owing to their critical roles in regulating gene expressions through post-transcriptional suppression or translational repression. Recent studies have linked deregulation of miR-31 to gastric cancer; however its precise mechanism remains to be elucidated.

Materials/Methods: We immunohistochemically evaluated SMAD4, PTEN and microsatellite instability biomarkers of MLH1, MSH2, MSH6 and PMS2 expressions within 115 Gastric adenocarcinoma (GA) and 20 control gastric tissue samples with prognostic significance of clinicopathological factors. Moreover, 15 miRNA expressions were investigated within subgroups of GA according to SMAD4 and PTEN expressions. miRNAs were isolated

from paraffin-embedded tissue specimens and expressions were carried out by Real Time PCR using miScript miRNA Primer Assays (Qiagen, USA) and analysed by online analysis tool (www.qiagen.com). **Results:** miR-31 expressions were significantly 6.87 (p<0,001), 4.25 (p = 0,048) and 4.28 (p=0,012) fold down-regulated in PTEN low GA subgroups as all low (SMAD4, PTEN, MLH1, MSH2, MSH6 and PMS2), only PTEN low and both PTEN and SMAD4 low, respectively. **Conclusion:** Not only miR-21 by up-regulation, but also miR-31 by down-regulation might have role on apoptosis pathway through PTEN and p53 in gastric cancer. Hence, miR-31 might be served as tumor suppressor biomarker for gastric cancer. Grant: Research Fund of Istanbul Medeniyet University; Project # TSA-2013-401 and TSG-2013-333 Reference: Wang H et al. 2016. PMID: 27174918

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P12.144D

HTG EdgeSeq miRNA Platform Performance Metrics Utilizing The MicroRNA (miRQC) Study Specimens

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Introduction: miRNAs are short, ~22nt RNA sequences that modulate gene transcription and downstream cell behavior. There are several platforms used for quantitation of low abundance miRNAs found in circulating human bodily fluids, the miRQC manuscript included 12 such platforms from quantitative PCR (PCR), hybridization (HYB), and sequencing (SEQ) based technologies. The HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA) is a technology that enables users to measure the expression of human miRNA transcripts using NGS.

Methods: We obtained control samples used in the miRQC study (Mastdagh, et. al., Nature, 2014) to measure performance of our miRNA assay using the methods described in the Nature publication. Sixteen mandatory and 4 optional human serum RNA control samples were included in the miRQC study. These were prepared using HTG protocols; including RNA at 25ng. These control samples were constructed to evaluate a series of metrics measuring platform performance: reproducibility, detection rate sensitivity, accuracy, specificity, and differential expression.

Results: The HTG assay demonstrated excellent reproducibility with an area to the left of the cumulative distribution curve (ALC) of 0.108, ranking 2nd across platforms. Accuracy, measured by the median deviation from the expected ratio, was 22% (ranking 5th). Specificity (cross-reactivity) was moderate at 29.2%. The 14% false positive rate was equivalent to that seen in two other platforms PCR and SEQ based technologies with only 4 platforms performing better.

Conclusions: With the lowest RNA input of all 12 comparator platforms the HTG EdgeSeq miRNA WTA produced reproducible, accurate, and sensitive results using the miRQC study samples.

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P12.145A

MiRNAs and MAPK signaling pathway in pediatric acute lymphoblastic leukemia susceptibility

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Background

Recently, several Genome wide associations studies (GWAS) have found genetic variants associated with pediatric acute lymphoblastic leukemia (ALL) risk. These studies were mainly focused in coding regions. Nowadays, it is known that more than 40% of significant variants associated with cancer risk are situated in non-coding regions, where non-coding RNAs are located. MicroRNAs (miRNAs) are non-coding RNA molecules dysregulated in ALL, suggesting a role in ALL risk. Despite miRNA SNPs interfere with miRNA levels or function, only 3 studies in ALL susceptibility were done, showing significant results. Therefore, variants in miRNAs could contribute to childhood B-ALL predisposition. Nowadays, a large number of new miRNAS were annotated. Therefore, the aim of this study was to determine if any of SNPs in these new miRNAs are involved in B-ALL susceptibility.

Material and Methods

Blood samples of 217 pediatric patients with B-cell ALL in complete remission and 330 healthy controls of Spanish origin were analyzed. We selected all the SNPs described in pre-miRNAs with a MAF > 1% (213 SNPs in 206 miRNAs). VeraCode GoldenGate platform was used. MirWalk, RNAFold web server and Consensus Path Databases were used to performed bioinformatic analysis.

Results

The SNPs rs12402181 in mir3117 and rs62571442 in mir3689d2 were associated with B-ALL risk possibly through its effect on MAPK signaling pathway.

Conclusion

Therefore, in this study we found rs12402181 in mir3117 and rs62571442 in mir3689 associated with B-ALL risk. These SNPs could be novel markers for B-ALL susceptibility.

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P12.146B

Frequency of germline mutations in MMR genes among Russian patients with Lynch syndrome

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Introduction: Lynch syndrome is one of the most frequent hereditary colorectal cancer syndromes. The syndrome is caused by mutation in one of the mismatch repair (MMR) genes: mainly *MLH1, MSH*2, *MSH6*, *PMS2* and *PMS1*. The aim of this investigation was to study frequency and spectrum of germline mutations of MMR genes among Russian patients. Materials and Methods: Microsatellite instability was studied in tumor samples of probands who corresponded to next criteria: age ≤ 45 and/or family history of colorectal cancer. Germline mutations in MMR genes of patients with MSI-H (high level) tumors were detected by PCR, conformation-sensitive electrophoresis, Sanger sequencing and NGS. **Results:** Microsatellite instability of high level was found in 88 tumor samples. Thirty nine out of 88 patients had germline mutations in MMR genes. Seventeen mutations were found in *MLH1*, 17 mutations - in *MSH2*, 3 mutations - in *MSH6*, 1 mutation - in *PMS2* and 1 - in *PMS1*. Eighteen of these mutations were frameshift, 10 - nonsense, 5 - splice sites and 6 pathogenic missense mutations. Conclusion: Frequency of germline mutations in *MLH1, MSH2, MSH6, PMS2* and *PMS1* genes among Russian patients with Lynch syndrome was 43,6%, 43,6%, 7,6%, 2,6% and 2,6% respectively.

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P12.147C

Cowden syndrome caused by low level mosaicism of PTEN

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Introduction: Cowden syndrome (CS) is part of the PTEN cancer predisposition syndrome, characterized by an increased risk for benign or malignant thyroid tumors, breast, endometrial and renal cancer. Individuals with CS may have macrocephaly, trichilemmomas and papillomatous papules. The main gene known to cause CS is *PTEN*.

Description: A 47 years old woman was referred to our Onco-genetics clinic by oral surgery clinic for mucosal papillomatosis and the possibility of CS. A tongue biopsy revealed multiple fragments of epithelial hyperplasia. Recently she was diagnosed with a follicular variant of papillary thyroid carcinoma. An endometrial polyp was diagnosed as complex hyperplasia with moderate atypia. Colonoscopy detected several polyps, one of them adenoma with low grade dysplasia and gastroscopy revealed glycogenic acanthosis. Head circumference measured 59 cm (> 98th percentile). Her family history included her mother with breast cancer at age 68, not a carrier of the 3 Ashkenazi mutations in the *BRCA1/2* genes. Her father had CLL at age 78.

Methods: A cascade genetic testing approach was applied on DNA extracted from blood; Sanger sequencing and MLPA analysis for duplication/deletion mutations in the *PTEN* gene and promotor sequencing were followed by deep next generation sequencing.

Results: Only deep next generation sequencing revealed a pathogenic mutation. The pathogenic variant p.Q149X; c.445C>T in the *PTEN* gene was detected in 22/646 (3%) reads - indicating mosaicism.

Conclusion: We report low level mosaicism causing classic CS. Mosaicism should be evaluated by deep sequencing when the phenotype fits clinical criteria, even with normal Sanger sequencing.

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P12.148D

Prevalence of multilocus inherited neoplasia alleles syndrome among lynch syndrome patients

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Introduction: Multilocus Inherited Neoplasia Alleles Syndrome (MINAS) has been recently described as the presence of two or more inherited cancer predisposition alleles in the same individual. This condition could explain a part of the large phenotypic variability in Lynch syndrome (LS). We aimed to study the prevalence of MINAS in genetically diagnosed LS patients.

Materials and Methods: Eighty-four LS probands with characterized pathogenic variants in MMR genes were included in this study. All patients were treated through the Hereditary Cancer Program, Comunidad Valenciana (Spain). DNA from PBL was sequenced by NGS for 94 hereditary cancer related genes (MiSeq and TrueSight Cancer Panel, Illumina). Standard pipeline for genetic variant analysis and recommended international guidelines for clinical variant classification were applied. All pathogenic (class 5) and probably pathogenic (class 4) variants were confirmed by Sanger sequencing.

Results: The average coverage at 20X of the 255Kb target sequence was 96.2%. Besides the known mutations in LS genes, we found 6 pathogenic/probably pathogenic variants in 6 patients associated to 3 hereditary cancer syndromes: hereditary breast cancer (*ATM, NBN, FANCA, FANCI*), neurofibromatosis type 1 (*NF1*) and hereditary leiomyomatosis (*FH*). These results show that the prevalence of MINAS is about 7% (6/84).

Conclusions: The prevalence of MINAS among LS patients is relevant. The underlying genetic landscape in LS becomes more complex than expected. Further analyses are needed to clarify the clinical impact of these findings.

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P12.149A

Profiling cancer-related gene alterations in Japanese multiple myeloma patients by targeted amplicon sequencing

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Introduction: Multiple myeloma (MM) is an incurable and refractory hematological malignancy even after introduction of the novel treatments. The comprehensive analysis of genetic alterations in tumor by next generation sequencing can allow for the prediction of drug resistance and facilitate improvements in the treatment of MM. We tried to define genetic changes specific for each MM sample using targeted semiconductor sequencing technology. Materials and Methods: DNA was extracted from five MM cell lines and magnetic bead-enriched bone marrow CD138-positive malignant plasma cells from 11 cases of MM, and CD138-negative cells were used as matched non-tumor cells. 40ng of DNA were used for multiplex PCR amplification with an Ion Ampliseq Comprehensive Cancer Panel that offers targeted coverage of all exons in 409 tumor supressor genes and oncogenes frequently cited and mutated in human cancers. **Results:** The average numbers of non-synonymous mutations and copy number variations (CNVs) detected were 6.1 (range 3-11) and 15.2 (3–27) per patient, and 16.8 (13–26) and 59.8 (18–154) per cell lines. Somatic mutations and CNVs were found in known MM-associated genes, including *TP53*, *NRAS* and *CCND1*. We also identified numbers of novel recurrent alterations. Pathway assessment has shown that somatic aberrations within MM genomes are mainly involved in several important pathways, including cell cycle regulation, RTK-MAPK-PI3K and NF-kB. **Conclusions:** We performed targeted next-generation sequencing for rapid (2 days), standardized, and cost-effective gene analysis of MM patients. This targeted next generation sequencing may allow clinically oriented variant screening in MM.

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P12.150B

Whole genome sequencing in patients with multiple primary tumors

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Introduction

The risk of developing three or more different primary tumors is very low in the population, but not uncommon in hereditary cancer syndromes e.g. Lynch syndrome. It is thus reasonable to believe that there is a genetic predisposition to cancer in individuals with multiple tumors.

Materials and Methods

DNA from peripheral blood from twelve patients with three or more primary tumors was analyzed with exome/genome sequencing on the Illumina platform. Variant triage was performed in two steps. Variants fulfilling initial criteria (in a gene associated with cancer, minor allele frequency <2%, previously reported in mutation databases or affecting the RNA/protein structure) were classified according to the ACMG criteria.

Results

So far, six patients have been analyzed. The age-range of first cancer diagnosis was 27–60 years. Five patients had one or more variants classified as either pathogenic or probably pathogenic. All patients also had three or more variants of unknown significance. Two patients had a *CHEK2*-variant. One had 1100delC, pathogenic in hereditary breast cancer, and she had tumors in the ovaries, endometrium, colon and lymphoma. The other, with tumors in the ovaries, breast and rectum, had a missense variant (p. Arg224Cys), previously described in prostate cancer syndrome.

Conclusion

Whole genome sequencing may identify predisposing pathogenic variants in individuals with more than three primary tumors. More research is needed to evaluate the complex interplay between these different genetic variants in order to determine if genetic testing can improve the follow-up and clinical care of these patients.

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P12.151C

Complex chromosome translocations as a driving event of *MYC* upregulation in multiple myeloma

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Introduction: Activation of *MYC* is a late progressional event in multiple myeloma (MM), which negatively affects patients' survival. Here, we focused on the characterization of *MYC* rearrangements and their association with *MYC* upregulation.

Materials and Methods: In total, 48 MM patients were analyzed using a custom targeted-capture of a 4.5Mb region surrounding *MYC*. Copy-number abnormalities were analyzed by normalized tumor/germline depth ratio. Manta and Strelka were used for structural and single nucleotide variants, respectively. *MYC* expression level was determined by gene expression arrays.

Results: *MYC* rearrangements including translocations, gains/tandem duplications, deletions and/or inversions were detected in 52% (25/48) of samples. 92% (23/25) of abnormal cases had a gain and/or translocation which associated with higher *MYC* expression (P<0.0001), hyperdiploidy (P<0.001) and *NRAS* mutation (P<0.01). We considered translocations as drivers of *MYC* activation as 1) they were detected in 88% (22/25) of abnormal cases; 2) they defined a subgroup of MM with significantly higher *MYC* expression compared to cases with other *MYC* rearrangements (P<0.01); and 3) most (68%, 15/22) involved known super-enhancers near *IGH*, *IGK*, *IGL*, *FAM46C* and *TXNDC5* loci. A minimally gained region of 59kb was present in 44% (11/25) of abnormal cases and included the *MYC* locus. Translocations co-occurred with gain of *MYC* in 10 cases identifying a group with highest *MYC* expression. Complex rearrangements were identified which will be resolved by phased-read whole-genome sequencing.

Conclusions: 8q24.21 is a hotspot for heterogeneous rearrangements in MM, however, *MYC* translocations are the most important events as they result in significant *MYC* upregulation.

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P12.152D

Evaluation of antifolate drug in head and neck carcinoma and preneoplastic lesions

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Introduction: There are subpopulations of tumor cells identified by biomarkers such as CD44, which have been considered as cancer stem cells (CSCs). These cells are responsible for sustaining tumor growth, resistance to conventional therapies and disease recurrence. The aim of this study was evaluated the treatment response of 5-Fluorouracil in a subpopulation CD44+ cells of head and

neck carcinoma and preneoplastic lesions (dysplastic and hyperplastic epithelium). **Material and Methods:** HEP-2 (laryngeal carcinoma) and HN13 (oral cavity carcinoma) cell lines, primary oral tumor and preneoplastic lesions were incubated at 37°C in 5% CO_2. CD44+ phenotype cells were isolated by fluorescence-activated cell sorting (FACS) equipment (BD Becton Dickinson). CD44+ subpopulation was considered as CSCs and it was cultured and exposed to chemotherapeutic treatment with 0.37 mg/ml of 5-Fluorouracil for 24 hours. CD44+ cells untreated were used as control. Trypan blue staining was performed to identify viable cells. Student t test was used for statistical analyzes and the significance level was 0.05. **Results:** The percentages of viable tumor cells after 5-fluorouracil treatment compared to non-treated cells were 45.05%. The treatment of preneoplastic lesions resulted in 54.31% of viable cells. No significant difference was shown between the number of viable cells after the treatment and without treatment ($p>0.05$). Conclusion: The 5-fluorouracil treatment appears to have no effect on elimination of CD44+ subpopulation in head and neck carcinoma and preneoplastic lesions. The evaluation of CSCs-targeting agents is essential for the development of therapies that can avoid cancer eradication. Grants: FAPESP (2015/04403-8) and CAPES.

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P12.153A

Treatment response with Docetaxel and Paclitaxel in head and neck cancer stem cells

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Introduction: Docetaxel and Paclitaxel chemotherapies treatment in head and neck cancer has presented failure. The fact may be explained due to presence of cancer stem cells (CSC) that have high capacity of tumorigenic potential, self-renewal properties and slow growth cycle. CSC can be detected with specific biomarkers such as CD44 associated with cell aggregation, proliferation, invasion. This study evaluated the treatment response with Docetaxel and Paclitaxel drugs in CD44+ stem cells of head and neck cancer.

Material and Methods: Primary oral tumor, HEP-2 (laryngeal carcinoma) and HN13 (oral cavity carcinoma) cell lines were cultured and maintained in a 5% CO2 humidified incubator. CSC were identified by cell sorting with anti-CD44-PE-conjugated using FACSAria Fusion Citometry (BD Biosciences). CD44+ subpopulation were cultured and treated with 3.7 mg/ml of Docetaxel and 0.05 mg/ml of Paclitaxel during 24 hours, separately. CD44+ cells without treatment were used as control group. Living cells was identified by trypan blue staining. Test t Student was utilized for statistical analysis. $P<0.05$ was considered significant.

Results: The number of living CD44+ cells in primary tumor and cell lines was lower after the treatment with Docetaxel ($p=0.03$) and Paclitaxel ($p=0.009$) when compared with non-treated living CD44+ cells.

Conclusion: The treatment with Docetaxel and Paclitaxel reduced the number of living CSC of head and neck. The use of drugs targeting CSC is a promising therapeutic strategy in cancer eradication so further studies are required before their clinical application in the future.

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a novel risk SNP in the colorectal-cancer risk locus on chromosome 9q22

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Introduction: Heritable factors are well known to increase the risk of cancer in families. Our linkage analysis in 2006 suggested a region on chromosome 9q22. to be linked to an increased risk of adenomas and cancer in one large study and the same region was also published by others. A few years later we performed another linkage study in familial colorectal cancer and again could replicate the region on chromosome 9q22.

Aims: We set up to identify the genetic mutation within this locus.

Materials and Methods: We used a combination of exome sequencing, targeted sequencing and association study. The cases are Swedish familial and consecutive colorectal cancer samples. 500 genotypes in the 9q region were selected from an ongoing genome-wide association study in Swedish colorectal cancer to search for a founder effect in the 9q.

Results: In the exome sequencing study, there was no mutations suggested to be disease causing in the coding region. In the association study using 2709 colorectal cancer cases and 4782 controls, one SNP, rs6477733, suggested a risk with odds ratio 1.53 and p-value 0.000192.

Conclusions: We proposed a novel SNP, rs6477733, as a moderate risk factor to colorectal cancer. Further studies will show how the risk SNP influence the risk of colorectal cancer.

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NGS analysis of BRCA negative hereditary ovarian cancer patients: aimed on repair genes

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Ovarian cancer represents the leading cause of cancer deaths among gynecological malignancies. The lack of effective early detection strategies is associated with the advanced stage at diagnosis and poor prognosis. As a retrospective study, NGS panel (enrichment/hybridization approach) targeting main cancer predisposition genes, double-strand breaks repair as well as other repair pathway genes (MMR, BER, NER) was used to analyze genomic DNA of 100 high risk ovarian cancer patients with significant personal and family history of ovarian/breast cancers without previously identified mutation in BRCA genes. All deleterious mutations detected by NGS technology have been confirmed by Sanger sequencing. In about 1/3 of tested individuals at least one deleterious mutation was detected. Several mutations were detected in known ovarian cancer predisposing genes in DSBs repair genes as RAD51C, BRIP1, RAD51D. NGS analysis revealed also cancer syndromes including Cowden (PTEN), Von Hippel-Lindau (VHL) and Lynch syndrome (MSH2) in relation to ovarian cancer. Majority of deleterious mutations were detected in genes where association with ovarian cancer remains unclear: ATM, CHEK2, BARD1, NBS1, FANCD2, RAD52, MUTYH, MSH5, EXO1, ERCC3, ERCC5, TELO2, RECQL, BUB1B, GRB7 and others. Panel genetic testing in high risk individuals may provide valuable information for potential personalized therapy and extend the possibilities of predictive testing in families and specialized management. Supported by Czech Ministry of Health: grant NV15-27695A and DRO: MMCI 00209805

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High resolution copy number variant and mutation analysis identify novel candidate genes in optic nerve hypoplasia

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Introduction: Optic nerve hypoplasia (ONH) is a congenital ocular malformation and a common cause of visual impairment in children. Patients with ONH often have coexisting pituitary hormone deficiencies, intellectual disability, autism, and neurological impairments. In the majority of cases the etiology is unknown, but both environmental factors (e.g. prenatal alcohol exposure, congenital infections) and genetic causes have been described. This study was performed to establish the genetic contribution to ONH and to identify novel candidate genes.

Materials and Methods: We have performed a systematic investigation of genetic variants in a population-based cross-sectional cohort of children and adolescents with ONH. DNA from 37 patients was available for genetic analysis. To identify both single nucleotide changes as well as gene dose alterations we used a combination of massive parallel sequencing of 56 candidate genes and a custom design array-comparative genomic hybridization able to

detect both large copy number variants (CNVs) and small intragenic variants in 2000 target genes.

Results: The CNV analysis revealed 5 deletions and 6 duplications in 10 ONH cases, affecting 11 genes. The sequencing of candidate genes in 17 cases identified candidate variants in one third, of which three individuals had a pathogenic variant in *COL4A1*.

Conclusions: *COL4A1* is a major genetic contributor to ONH with mutations found in 3/17 (18%) cases studied here. Our data also highlights several novel candidate genes involved in different developmental pathways.

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Role of 14q32 miRNA cluster in Osteosarcoma susceptibility

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Background

Recent genome wide association study (GWAS) in osteosarcoma risk showed that several significant results were located in intergenic positions. These results suggest that non-coding regions could play an important role in the risk of osteosarcoma. One of the most studied non-coding molecules are microRNAs (miRNAs), whose deregulation has been associated with osteosarcoma evolution. Therefore, genetic variants affecting miRNA function could contribute to the risk of the disease. To date, only three polymorphisms in miRNAs have been analyzed in relation to the risk of osteosarcoma, and two of them showed significant association. In this context, this study aimed to evaluate the involvement of all genetic variants in pre-miRNAs in the risk of osteosarcoma.

Material and methods

We selected all SNPs described in miRNAs with a MAF>0.1. A total of 213 SNPs in 206 pre-miRNAs were analyzed in a cohort of patients (n = 74) and their corresponding controls (n = 160) using Goldengate Veracode technology. χ^2 or Fisher and FDR were used.

Results

The most remarkably finding was the association detected between 4 SNPs located at 14q32 miRNA cluster. Interestingly, miRNAs in this cluster have been associated with MYC deregulation. Therefore, genetic variations in these miRNAs could lead to their downregulation, which in turn would lead to the overexpression of MYC. These results suggest that this region is a hotspot for the development of the disease.

Conclusion

In conclusion, different variants in 14q32 miRNA cluster could be implicated in osteosarcoma susceptibility.

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P12.158B

BRCA1 and *BRCA2* tumoral mutation detection in Nantes's molecular genetics laboratory

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Ovarian cancer has an estimated incidence of 225.000 women per year worldwide and remains a significant cause of mortality (140.000 deaths per year). The mean probability of finding a germline *BRCA1* or *BRCA2* mutation in epithelial ovarian cancer is 12.7%. Somatic mutations of the *BRCA* genes have been identified in approximately 6% of serous ovarian carcinomas. It has been shown that tumors with *BRCA1* or *BRCA2* pathogenic mutations respond to drugs which inhibit PARP. It's becoming increasingly apparent that knowledge of *BRCA* status has prognostic utility that can affect treatment decisions and may improve survival.

In the molecular genetics laboratory of Nantes, we analyzed formalin-fixed paraffin-embedded ovarian carcinoma of 160 patients, from April 2016 to January 2017. Tumoral mutations of *BRCA1* and *BRCA2* were screened by high-throughput sequencing. Pathogenic mutations were found in 18/160 (11.25%) tumors ; 11/160 (6.8%) were only somatic.

Recent studies have suggested that homologous recombination deficiency in epithelial ovarian cancer can be due to *BRCA1* and *BRCA2* mutations (included large

rearrangements), epigenetic silencing of *BRCA1*, and loss of function of other genes involved in homologous recombination. Reduced *BRCA1* expression as a result of promoter methylation has been reported in 11.5% high-grade serous ovarian adenocarcinomas.

We therefore assessed complete *BRCA* status of some of these ovarian cancer specimens by analyzing tumoral *BRCA1* and *BRCA2* loss of heterozygosity, *BRCA1* promoter hypermethylation and BRCA1 protein expression. Here, we present the results of these analyses.

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P12.159C

Single nucleotide polymorphisms of genes immune response and ovarian cancer pathogenesis

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Ovarian cancer (OC) is the third most common gynecological malignancy following uterine corpus cancer and cervix cancer. Annually in the world registered more than 238 000 new cases of the disease, half of which are fatal. The situation is similar in Russia. The immune system is thought to be an important mediator of ovarian cancer.

We analyzed the associations of the 2 single nucleotide polymorphisms (c.C1438T in *ITIH2* gene and c.G481A in gene *AGBL2*) with the risk of ovarian cancer in cases (n = 286) and controls (n = 223) from Bashkortostan. Polymerase chain reaction - restriction fragment length polymorphism was used for genotyping.

We identified an association of allele T for the c.C1438T polymorphism in *ITIH2* gene and decreased ovarian cancer risk, OR=0,04, 95% CI:(0,01–0,15), p=0,0005. ITIH2 involved in inflammation and immune response, also inhibits tumor metastasis and interacts with proteins such as the EGFR and FN1. According to some studies, breast cancer observed expression loss *ITIH2* gene.

Also we identified an association polymorphism c. G481A in *AGBL2* gene with the risk of OC in Russian, OR=0.11, 95% CI:(0.04–0.3), p=0,0005. AGBL2 participates in the regulation of proliferation of tumor cells, epithelial-mesenchymal transition and resistance to chemotherapy.

Our data indicate that c.C1438T and c.G481A polymorphisms are associated with ovarian cancer risk in Bashkortostan.

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Cell-free miRNAs as potential biomarkers in the diagnosis of ovarian cancer

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Background: Ovarian cancer is the fifth leading cause of cancer mortality among women. There is no early diagnostic tool for the detection of this type of aggressive malignancy. Cell-free microRNAs seem to be a promising possible biomarker in the diagnosis.

Materials and methods: We involved 11 ovarian cancer patients and 11 controls in the study. 15–16 ml EDTA blood was drawn; the microRNA was isolated with miRNeasy Mini kit (Qiagen, Germany). cDNA was synthesised and miR-103; miR-125, miR-193b and miR-200b expression were determined by using miRCURY Universal RT microRNA PCR Starter kit (Exiqon, USA). miRNA-93 was used as reference gene. Student t-test was applied for the statistical calculations of the results.

Results: The isolated microRNA was suitable for cDNA synthesis. We found significant difference in the expression of miR-103 (p=0.001), miR-193b (p=0.0236), miR-125 (p=0.029) and miR-200b (p=0.0273) among the cancer patients and controls.

Conclusion: We determined the concentration of four cell-free microRNAs from the plasma of healthy controls and ovarian cancer patients. There was significant difference in the expression of the studied microRNAs. We extend our study on higher number of cases as these microRNAs seem to be promising biomarkers for the diagnosis of ovarian cancer.

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Polymorphisms associated with breast cancer susceptibility in patients with ovarian cancer

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Ovarian cancer is the third most frequent gynecological tumor and the fifth cause of women cancer deaths in Spain. Only 5–10% of the cases of ovarian cancer have an inherited genetic condition. Therefore, a diagnosis of hereditary Ovarian Cancer Syndrome should be considered in families with multiples cases of breast cancer. Two genes have been associated with familiar breast and ovarian cancer: *BRCA1* and *BRCA2*. For this reasons, the aim of our study was to characterize whether polymorphisms in genes associated with breast cancer susceptibility would modify the risk of developing ovarian cancer. We have studied BRCA1 rs799917, BRCA1 rs16941, BRCA1 rs16942, TP53 rs1042522, miR146 rs2910164, FGFR2 rs2981582, 8q24 region rs13281615 polymorphisms in a cohort of 117 Spanish patients and 116 healthy subjects.

Our results show that being a carrier of the allele C of TP53 rs1042522 polymorphism is associated with reduced risk of developing ovarian cancer. Non-significantly differences were found in the analysis of the BRCA1, miR146, FGFR2 and 8q24 region polymorphisms in our cohort of patients.

In conclusion, there seems to be no relation between studied polymorphisms associated with breast cancer and susceptibility to ovarian cancer, with the exception of the allele C of TP53 rs1042522.

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Multi-level genomic profiling of heterogeneous FFPE tumors with low tumor cellularity sorted by DEPArray™ technology

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Introduction: The main objective of precision medicine is finding the right therapy for the right patient. In oncology, this goal is particularly challenging also due to poor tissue biopsies and low-tumor content, precluding in most cases the study of tumor complexity. Here we describe a multi-level approach that allows an accurate tumor characterization in FFPE samples with low tumor-cellularity.

Methods: FFPE sections from pancreas ductal adenocarcinoma, with <20% tumor content, were processed by DEPArray™ sorting protocol. Pools with different number of cells (range=1–142) from 100%-pure stromal and tumor populations were recovered and lysed. Single-cell DNA was amplified using Ampli1™ WGA kit. DNA libraries were prepared for low-pass Whole Genome Sequencing on MiSeq. At the same time, two libraries from a stromal (n = 142) and tumor (n = 129) pool of cells were enriched with Agilent SureSelectXT capture kit for Whole Exome Sequencing (WES) on HiSeq. Moreover, cell pools were used as input for the DEPArray™ OncoSeek amplicon-based panel.

Results: Paired analysis of low-pass copy-number profiles and WES data identified several Loss-of-Heterozigosity (LOH) as well as absolute copy-number alterations in sorted pure-tumor populations, both undetectable in bulk DNA from unsorted cells. Moreover, this paired approach allowed to unambiguously detect a homozygous loss of PTPRD onco-suppressor gene. Inter-cell heterogeneity was observed through the analysis of single-cell copy-number profiles. Finally, most non-synonymous somatic variants detected, all confirmed by panel results, are relevant in pancreatic cancer.

Conclusions: Our results demonstrate the value of DEPArray™ technology for the characterization of cancer genomes in low-cellularity FFPE tumor samples.

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P12.163C

BRAF V600E mutational analysis of metastatic lymph nodes from two Tunisian females with papillary thyroid carcinoma

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BRAF V600E mutation, the most potent activator of the mitogen-activated protein kinase pathway, plays a central role in the regulation of cell growth, division, and proliferation. To date, BRAF V600E analysis has been widely adopted in the management of papillary thyroid carcinoma (PTC) patients. Here, we explore the correlation and the clinical value of B-RAF V600E mutation detection in PTC and regional lymph node metastasis (LNM). Two Tunisian female patients P1 and P2 (aged 22 and 32 years respectively), suffering from PTC, underwent total thyroidectomy to remove the primary tumor as well as a routine cervical lymphadenectomy. Real Time Polymerase Chain Reaction was used to detect BRAF V600E mutation using fixed paraffin-embedded tumor samples from both, primary tumor and LNM detected at histological evaluation. The BRAF mutation was present in the two primary tumors. However, only LNM of P2 was positive for the V600E mutation. These two patients were followed up for 47 months with no disease recurrence, no lymph or distant metastasis, no radioactive iodine resistance and no death. Growing number of evidence has shown that BRAF mutation is associated with a higher risk of recurrence and mortality in PTC, but the correlations with parameters for aggressiveness such as tumor size, multifocality and metastasis remains controversial. A more controversial topic is whether routine central lymph node dissection should be performed in PT microcarcinoma (PTMC) patients without evident LNM. However, limited data are available on the analysis of somatic mutations in lymph nodes in adult patients with PTC and PTMC.

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P12.164D

Missed Opportunities for Genetic Testing in the Clinical Managemeng of Pheochromocytoma and Paraganglioma in Singapore

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Background: Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumours of the adrenal glands and the sympathetic and parasympathetic paraganglia. They can occur sporadically or as a part of different hereditary tumour syndromes. About 30% of PCCs and PGLs are currently believed to be caused by germline mutations and several novel susceptibility genes have recently been discovered. The clinical presentation, including localization, malignant potential, and age of onset, varies depending on the genetic background of the tumours. Genetic testing for PPGLs is not well studied in

Asia. We review our clinical management of PPGLs in Singapore and highlight current gaps in clinical practice.

Methods. Medical records of patients with PPGLs between 2005 - 2016 were reviewed. Diagnosis was confirmed histologically. Patients were stratified into; familial/ syndromic (FS), those with positive family history and/or syndromic presentation, or sporadic.

Results. Twenty-seven (21.8%) patients were referred to the Cancer Genetics Service (CGS). Incidence of FS PPGLs (18.5%) and extra-adrenal PPGLs (58.1%) were higher than previous studies. Genetic referrals were much lower for sporadic PPGLs patients compared to FS PPGLs patients (3.7% vs 100%). Genetic referrals were highest at age of diagnosis <20years old (80%) and decreases with increasing age; ≥20-<40years old (32.1%), ≥40-<60years old (10.6%). Genetic testing uptake was 12/27 (44.4%) patients of which 7/12 (58.3%, *3 SDHB, 2 SDHD, 2 VHL*) had germline mutations.

Conclusion. Missed opportunities for genetic testing due to low referral rates in patients with SPR PPGLs and those presenting at age ≥20-<40years old and ≥40-<60years.

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P12.165A

The effect of decreased expression of the mismatch repair gene *PMS2* on DNA mismatch repair efficiency

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Introduction: Lynch syndrome (LS), the most common inherited colon cancer syndrome, is associated with DNA mismatch repair (MMR) malfunction. While ~50% expression decrease (mutation carrier level) of the main LS susceptibility genes *MLH1*, *MSH2* and *MSH6* has been shown to affect MMR capability, the influence of reduced *PMS2* expression is not known. Here, the repair efficiency of knockdown (KD) cell lines retaining varying levels of *PMS2* mRNA expression is assessed in a functional MMR assay.

Materials and Methods: Four different *PMS2*-specific shRNA targets were used for stable transfection in human fibroblasts. RNA was reverse transcribed into cDNA for quantitative PCR carried out using Taqman® assays and *GAPDH*, *HPRT1* and *ACTB* as reference genes. Nuclear proteins were extracted from selected KD cell lines and the repair efficiencies were studied in the *in vitro* MMR assay.

Results: KD clones retaining 18.5%, 33% and 52.5% of *PMS2* expression were selected for functional analysis. A prerequisite for clone selection was that the KD cell line would retain 50% of *PMS2* expression or less. All selected clones were shown to be MMR proficient, although clones retaining 18.5% or 33% of normal expression repaired less efficiently than their respective controls. Further tests are ongoing.

Conclusions: *PMS2* mutations have been difficult to detect and interpret due to several pseudogenes in the human genome, low mutation penetrance, and/or complex disease phenotype. Our preliminary findings suggest that already reduced level of *PMS2* mRNA expression results in decreased MMR efficiency, which may help to elucidate the variant pathogenicity.

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P12.166B

Germline mutation c.1089C>A of POLE could be associated with an increased risk of colorectal cancer and glioblastoma

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Germline mutations of *POLE* are responsible for polymerase proofreading-associated polyposis syndrome (PPAP). These mutations were hypothesized to predispose to extra-digestive tumors (ovarian, endometrial, brain) but this association was not confirmed so far. We report a family with an autosomal dominant inheritance of PPAP

due to a mutation of *POLE* c.1089C>A; p.(Asn363Lys) in the proofreading exonuclease domain. 11 patients with colorectal tumor onset and three patients with polyposis are indexed in this family. Two carriers (siblings) developed glioblastoma (at 55 and 42 respectively) and another member (not tested) presented glioblastoma at 37 years old. This is the second family reported with such a mutation. In the first described, one patient among 12 mutation carriers displayed a glioblastoma at 28 years old and his grandfather (not tested) developed a glioblastoma at 68 years old. All mutations of *POLE* seem not to be equally associated with extra-digestive tumors. These cases suggest that *POLE* germline mutation c.1089C>A could be involved in an increased risk of brain cancer (incidence: 15 % (3/20) in mutation carriers combining the two families). More cases are needed to support this hypothesis. Although carriers of a mutation responsible for PPAP syndrome should benefit from screening for colorectal and uterine cancer, due to the rapid evolution of glioblastoma, neurological and brain imaging screening remains questionable. Nevertheless, taking into account the limited standard therapy for glioblastoma, mutation status could be useful for targeting therapy and to adapt radiotherapy procedures. The biological mechanism linking *POLE* mutation to glioblastoma is still to be demonstrated.

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Mutations in ion and transporter genes in aldosterone producing adenoma

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Mutations in ion and transporter genes in aldosterone producing adenoma.

Introduction: Primary aldosteronism is the most common form of secondary hypertension and affects 8–13% of patients with hypertension. Genes encoding for either cell membrane ion channels or ion exchanger have been found to be mutated in about 60% of patients with aldosterone producing adenomas (APAs). The aim of this study was to identify the mutation rates of *KCNJ5, CACNA1D, ATP2B3, ATP1A1* and *CTNNB1* in our cohort. We also compared the genome wide expression of tumors with either *KCNJ5* or *CACNA1D* mutation.

Material and Method: 35 APAs were analyzed by Sanger sequencing for mutations in the hot spot regions of the genes. Genome wide expression analysis was performed on *KCNJ5* and *CACNA1D* mutant adenomas.

Result: We identified 11 (31%) mutations in *KCNJ5*, 2 (6%) in *ATP1A1*, 3 (9%) in *ATP2B3*, 3 (9%) in *CACNA1D* and 1 (3%) in *CTNNB2*. *CACNA1D* mutations were exclusively found in males, patients with younger in age, smaller tumors and patients with higher aldosterone levels. In contrast, *KCNJ5* mutations were more prevalent in females, larger tumors, older patients and patients with lower aldosterone levels.

The top pathways found to be enriched were Wnt/β-Catenin pathway, *GNRHR* pathway, angiogenesis, cadherin and integrin signaling pathways. *CACNA1D* mutant tumors had higher expressions of *CYP11B2, GNRHR, PRKCQ*, *NPNT* and lower expressions of *CYP17A1, CYP11B1, KLHL4* compared to *KCNJ5* mutant tumors.

Conclusion: In our cohort, we found mutations in almost 60% of APAs. Patient with *CACNA1D* mutations had a more aggressive phenotype.

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P12.169A

Mutation and Polymorphism Analysis of *Androgen Receptor* Gene in Prostate Cancer Patients in Indonesia

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Prostate cancer (PCa) is non-cutaneus malignancy diagnosed in men, especially in the elderly. The development, growth and function of the prostate gland depend on the Androgen signalling. Mutations or polymorphisms on Androgen Receptor (AR) gene had been known to play a role in the carcinogenesis of the prostate tissue. The aim of this study was to performed AR mutations/polymorphism analysis in PCa patients in Indonesian population.

Thirty eight PCa patients from Javanese ethnic in Indonesia group enrolled in this study. The diagnosis of PCa was based on histopathology of prostate tissue using Gleason Score criteria. All exons and exon-intron boundaries of AR gene was amplified using Polymerase Chain Reaction and followed by Sanger sequencing.

The age of PCa patients who were enrolled in this study were between 54 to 89 years old. Two types of

polymorphism were identified in the exon 1, those are p. P214E and CAG repeat sequences (CAGn) with the length of the repeats between 13 to 34. Two rare variants in exon 1 were identified in one patient. Those are novel missense mutation (p. P146Q) and deletion of GCA sequence (p. Q91del). Hence, the frequency of AR mutations in PCa patients from Javanese population is 2.6% (2/76 alleles).

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P12.170B

Towards optimization of *PTEN* mutation testing criteria: 20 years of experience since its discovery

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Introduction: PTEN hamartoma tumour syndrome (PHTS) is a severe genetic tumour risk syndrome predisposing to multiple types of malignancies and non-malignant syndromic signs. Timely detection mutation carriers is important for optimal cancer risk management. We aimed to evaluate the trends in detection and clinical presentation of PTHS patients.

Materials and Methods: A consecutive cohort study including nationally referred probands (N = 2.384) and their relatives (N = 504) undergoing PTEN germline testing at our centre over the last 20 years (1997–2016). The mutation detection rates and demographics of probands only and of all mutation carriers were assessed using descriptive statistics and logistic regression analyses.

Results: In total 202(8.5%) probands and 138(27.4%) relatives were tested positive for a pathogenic PTEN mutation. The median annual detection rates were 10.5% (range 3.5–50%) and 30% (range 0–80%), respectively. Summarized per 5 years (1997–2001,2002–2006,2007–2016), the number of mutation positive probands were 19 (21%), 35 (11%), 89 (8%) and 57 (6%), and the median age of the probands 41, 23, 31 and 44 years. The annual detection rates showed a decreasing trend ($p<0.001$), and the age genetic testing an increasing trend ($p<0.001$). The median age of female and male probands was 46 (range 0–95) and 9 (range 0–82, $p<0.001$), and detection rates 7% (N = 103) and 11% (N = 98, $p=0.006$), respectively.

Conclusions: Annual PTEN mutation detection rates decrease, but number of detected mutation carriers increase. The effect of year, age and gender on detection rates likely reflect less stringent referral e.g. female malignancies. Though, further research should address optimization of testing criteria.

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P12.171C

A functionally null RAD51D missense mutation is strongly associated with ovarian carcinoma

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Introduction: RAD51D is a key player in DNA repair by homologous recombination (HR) and *RAD51D* truncating mutation carriers have an increased risk for ovarian cancer (OC). The contribution of non-truncating *RAD51D* variants to cancer predisposition remains uncertain.

Methods and **Results:** A panel screening identified the *RAD51D* -c.620C>T;p.S207L variant in two French Canadian (FC) kindred affected with familial High Grade Serous Cancer (HGSC) of the ovary. Through a case-control genotyping study, we showed that the missense *RAD51D* variant c.620C>T;p.S207L is over-represented in the French Canadian population affected by HGSC of the ovary (3.8% cases vs 0.002% controls; $p < 0.0001$).The frequency of the p.S207L variant did not differ from that of controls in breast, endometrial, pancreas and colorectal adenocarcinomas. A common haplotype shared by all the carriers suggested a founder origin for c.620C>T;p.S207L mutation. Whole exome sequencing (WES) analysis of *RAD51D* associated tumor profiles revealed the presence of signature 3, known to be associated with HR defects. RAD51 foci formation, CRISPR-Cas9-stimulated gene targeting assay and a DR-GFP assay confirmed HR impairment activity of

RAD51D-c.620C>T;p.S207L mutated cells. By co-immunoprecipitation and *in-vivo* single cell colocalization assays we show that this mutation impairs HR by disrupting the RAD51D-XRCC2 interaction. Sensitivity to PARP inhibitors (PARPi) was confirmed in RAD51D-c.620C>T; p.S207L mutant cells .

Conclusions: This work identifies *RAD51D*-c.620C>Tp. S207L as the first *bona fide* pathogenic missense susceptibility allele for HGSC of the ovary and supports the use of targeted PARPi therapies in OC patients carrying missense *RAD51D* mutations.

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Extended RAS minor variant detection by Sanger sequencing from FFPE samples to a 5% Limit of Detection

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RAS mutational testing is frequently performed by clinical researchers because of a demonstrated strong correlation between RAS mutational profiles of colorectal cancers and their anti-EGFR response. Sanger sequencing is an ideal choice for detection of RAS mutations due to its high accuracy, fast turnaround time, simple workflow and cost-effectiveness. When combined with Minor Variant Finder software, Sanger sequencing enables high sensitivity with variant detection down to a 5% Limit of Detection.

We have developed an extended RAS Sanger sequencing panel targeting eight hot-spot regions of KRAS and NRAS genes (codons 12–13, 59–61, 117 and 146). Besides these hotspot codons, the panel is capable of detecting any variants along the entire amplicons. The panel was optimized for low amount of FFPE DNA input, down to 1 ng/reaction.

To further streamline the workflow for the user, we designed and developed 96-well plates pre-loaded with the eight hot-spot primer pairs so only the PCR mix and templates need to be added. The same plate migrates through the entire workflow, minimizing risk of sample mix-up or contamination.

The 96-well plates were tested using hot-spot positive FFPE control DNAs, and Minor Variant Finder successfully identified the expected 5% variant allele frequency. Additionally, variants from numerous FFPE DNAs derived from colon cancer biopsies with variant allele frequencies ranging from approximately 5% to 80% were also successfully identified.

These extended RAS plates performed well on all models of Applied Biosystems capillary electrophoresis instruments that were tested.

For Research Use only. Not for use in diagnostic procedures.

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P12.173A

Expression profiles of miRNA genes in clear cell renal cell carcinoma patients

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Introduction: Renal cell carcinoma (RCC) constitutes about 3% of all human cancers. Clear cell renal cell carcinoma (ccRCC) is the most common subtype at diagnosis and accounts for 75–88% of RCCs. MicroRNAs (miRNAs) are small noncoding RNAs that play a key role in cancer pathogenesis and are involved in several human cancers, including ccRCC.

Materials and Methods: A global miRNA profiling study to identify a specific miRNA signature characterizing ccRCC was performed at 18 samples of tumor and 6 samples of normal kidney tissue of ccRCC patients using OpenArray technology at QuantStudio 12K Flex Real-Time PCR System. In total, 758 miRNA genes were analyzed using TaqMan OpenArray MicroRNA Panel. Validation study was performed using TaqMan qRT-PCR assays at 48 ccRCC samples.

Results: Microarray analysis identified two over-expressed microRNA genes in tumor kidney tissue - miR-210 (FDR p-value=0.033) and miR-642 (FDR p-value=0.021). After validation only miR-210 showed statistically significant expression levels between normal and tumor kidney tissue (p-value=0.0134). There is a large amount of data that miR-210 is the most ubiquitously

upregulated miRNA in different cancers including renal cancer. Recently a few studies showed that changing of the microRNA-210 expression levels is directly related to hypoxia and HIF1α activity, key components of tumorigenesis.

Conclusions: Our results suggest that microRNA gene expression alteration may contribute to the genetic predisposition for kidney cancer and may serve a diagnostic marker of the disease. The work was supported by RFBR grant №14-04-97083.

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P12.174B

Germline nonsense mutations of the *SMARCB1* gene in Russian patients with rhabdoid renal tumors

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The malignant rhabdoid tumor (RT) is one of the most aggressive childhood neoplasms. RTs are characterized by the presence of inactivating mutations in the *SMARCB1* (*hSNF5/INI1/BAF47*) gene - a tumor suppressor localized in 22q11.2. The *SMARCB1* gene is involved in chromatin remodeling. Up to 30% of RTs are caused by germline mutations of this gene, to date those cases are considered as a manifestation of the rhabdoid tumor predisposition syndrome type 1 (RTPS1). We have analyzed the *SMARCB1* mutations for improving of genetic laboratory diagnostics of the RTPS1, as well as searching of genotype-phenotype correlations in this disease. Genomic DNA was isolated from blood samples of 18 patients with RT in different localizations. Then *SMARCB1* exons 2–9 were amplified by PCR and subsequent Sanger sequencing with the 3500xl ABI capillary genetic analyzer was performed. Three patients had *de novo* nonsense mutations c.157C→T (p. R53*), c.669_670del (p.C223*) and c.843G→A (p. W281*), confirming RTPS1, which were associated with RT in the kidney, early age at diagnosis (median 2.6 months) and poor prognosis. High frequency of the nonsense mutations corresponds to data obtained by other authors in the studies of renal RTs, but not of RTs in other localizations. This study is a first experience of *SMARCB1* mutation testing in Russian population to our knowledge. Identification of germline *SMARCB1* mutations in the patients with RTs is essential to assess the risk of metachronous tumors and for genetic counseling of other family members.

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P12.175C

RET-gene rearrangements in papillary thyroid carcinoma subtypes

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RET gene rearrangement is considered the second most important mutational event involved in the occurrence of papillary thyroid carcinoma (PTC). Aim of the study was to investigate the occurrence of RET gene rearrangements in tumor cells from different PTC subtypes: classic PTC (cPTC), follicular variant (fvPTC) and aggressive forms (AGR). Patients and methods: Paraffin-embedded tumor tissues from 57 PTC patients were examined for RET rearrangements using fluorescence *in situ* hybridization (FISH) technique: 12 cPTC cases, 31 fvPTC cases and 14 aggressive PTC cases. FISH was performed with the ZytoLight SPEC RET Dual Color break-apart probe (ZytoVision GmbH, Germany). At least 100 non-overlapping nuclei were taken into account and were analyzed with Leica CW4000 CytoFISH program to indicate the percent of positive RET-split cells. **Results:** The percent of positive RET-split cells ranged from 5% to 95%. We established 10% RET-split positive cells as the cutoff level to consider this event as a clonally one. In cPTC cases the positivity was registered in 8 cases (66.67%), in 13 fvPTC cases (41.94 %), and in 6 AGR cases (42.86%). Overall, RET gene rearrangements was encountered in 47.37% of cases, with 52.63% of tumors having positive RET-split cells below 10%. Split level over 20% is correlated with the invasiveness of tumor. Conclusion: The frequency of RET gene rearrangements in this study is similar with those

reported in other European populations without important differences between PTC subtypes. This study was funded by UEFISCDI grant no.135/2012

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P12.176D

Incidence of second primary cancers in heritable and non-heritable retinoblastoma: Results from a Danish population based study with more than 70 years follow-up

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Purpose

To determine the incidence of second primary cancer in the 323 patients diagnosed with retinoblastoma in Denmark from 1943–2013.

Methods

Data was retrieved from the Danish Retinoblastoma Database, a national population database complete since 1943. Heritable retinoblastoma is defined by bilateral or multifocal disease, familial presentation, and/or identification of an *RB1* mutation. Data on second primary cancers and standardized incidence rates (SIR) were extracted from the Danish Cancer Registry.

Results

For the 323 retinoblastoma patients diagnosed from 1943–2013 in Denmark, data on secondary primary cancers was divided in 133 heritable retinoblastoma (her-RB, 41%) and 190 non-heritable retinoblastoma (non-her-RB, 59%). 25 patients with her-RB (19%) and 13 patients with non-her-RB (7%) were diagnosed with a second primary cancer ($p=0.001$). In her-RB the most common second cancer diagnosis were sarcoma (56%) and malignant melanoma (24%). The overall SIR for her-RB is 11.4 (95% CI: 7.4–16.8) and 1.5 (0.8–2.6) for non-her. The cumulative incidence rate of a second primary cancer at 60 years of age is 51% for her-RB and 13% for non-her-RB ($p<0.001$). The hazard ratio is 5.0 (2.5–10.2). There is no indication of an association between risk of second primary cancer and the use of external radiotherapy in the treatment of her-RB.

Conclusion

The incidence of second primary cancer is significantly higher in heritable retinoblastoma versus non-heritable retinoblastoma. The most common types in heritable retinoblastoma are sarcoma and malignant melanoma. Unique for this study are the completeness of our nationwide collected data and the extent of long-term follow-up.

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P12.177A

Correlation between *RB1*germline mutation and second primary malignancies in hereditary retinoblastoma patients treated with external beam radiotherapy

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Retinoblastoma (Rb) results from biallelic inactivation of the *RB1* gene. Hereditary Rb patients *i.e* germline carriers of a *RB1* mutation also have a risk of developing subsequent malignant neoplasms (SMN) such as osteosarcomas. This SMN risk is maximized by external beam radiotherapy treatments (EBRT) which is why these treatments are now avoided. Nevertheless, EBRT is still a matter of great concern, as EBRT-treated patients are in their adulthood and SMNs remain the major cause of death for patients. To decipher the relationship between *RB1* genotype and SMN development in EBRT treated patients, we conducted a retrospective study in a cohort of 160 irradiated hereditary Rbs with fully resolved *RB1* mutational status. Among these 160 Rb patients, 120 did not develop any SMN (75%) and 40 developed SMNs (25%). We didn't find any difference in *RB1* mutation type between patients with or without SMN, neither could we detect any linkage between mutation type and SMN location, SMN type and age at diagnosis. Fisher's test for *RB1* mutation type and SMN showed that they are not independent variables ($p=0.016$) but the series is too small to point out a specific mutation type. Interestingly, among 8 carriers of a *RB1* low penetrance

mutation, 3 of them developed sarcomas, a rare tumor that cannot be attributed to the general population. Our study cannot explain why a *RB1* mutation leads or not to a SMN but demonstrated that EBRT patients with a low penetrance mutation remain at risk of SMN and should be cautiously monitored.

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P12.178B

Identification of a *SMAD4* retroduplication event using NGS targeted gene panel

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Introduction: We report a 69 y/o male patient, who presented at the age of 53 with 9 adenoma treated by total colectomy.

Materials and methods: We performed molecular analysis of genes involved in hereditary colorectal cancer and polyposis using Next Generation Sequencing (NGS) and copy number assessment by Multiplex Ligation Probe Annealing (MLPA).

Results: MLPA and NGS revealed the duplication of the whole coding region of the *SMAD4* gene, while the introns and other non coding regions were present in two copies. NGS depth profile was unusual and several aberrant exon-exon sequences were identified. These findings, together with consistent Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) brought the hypothesis of the insertion within the genome of a retrotranscribed copy of *SMAD4* mRNA. Successful amplification of the full length cDNA sequence from genomic DNA validated this hypothesis. NGS capture data allowed the identification of the insertion breakpoints, located in a deep intronic region of the *SCAI* gene, on chromosome 9.

Discussion: This so-called retroduplication variant (RDV) with no obvious biological or clinical significance is unlikely to be responsible for this patient's polyposis. But the identification of this particular event highlights the benefit of our NGS targeted panel which includes not only coding regions of genes but also introns, and thus allows detection of genomic structural variants.

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Comparative analysis of a RNA sequencing panel for detection of genetic alterations in haematological malignancies

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Introduction: Recurrent chromosome aberrations resulting in gene fusions or altered expression are of particular prognostic significance in patients diagnosed with acute lymphoblastic leukaemia, acute myeloid leukaemia and multiple myeloma. Interrogation of mRNA has the capacity to detect altered transcripts and expression, in addition to gene mutations. Retrospective analyses of patients with these neoplasms were carried out using RNAseq next generation sequencing and compared with results from traditional cytogenetic methods (karyotyping and FISH). Materials and Methods: RNA from 20 diagnostic patient samples and 2 normal controls extracted from blood, marrow or CD138+ separated cells were obtained from an ethically approved biobank. The patient samples selected had chromosome rearrangements, complex karyotypes or other aberrations of significance. All samples were enriched using the 1,385 gene Illumina TruSight® RNA Pan-Cancer panel and sequenced using the Illumina MiSeq. Bioinformatics tools used included TopHat-Bowtie, Star and Cuffflinks. **Results:** Fusion transcripts were detected in all 9 patients with known gene fusions. Of the 4 patients with rearrangements known to cause altered expression, only 1 had statistically significant differential expression. In total 13 patients had significantly altered expression of a number of clinically relevant genes associated with haematological neoplasms including *MECOM, FLT3* and *PAX5*. Discussion Our results have shown that RNAseq panels have the ability to detect gene fusions and altered expression. However, further work is needed to improve expression analysis to enable implementation into diagnostic service. This technology represents one of the first significant steps away

from karyotyping and FISH analysis for haematological malignancies.

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Clinical aspects of *SDHA*-related pheochromocytoma and paraganglioma; a nationwide study

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Introduction: Pheochromocytoma and paraganglioma (PPGL) carry the highest degree of heritability among human neoplasms. Germline mutations in five succinate dehydrogenase (SDH) genes, predispose heterozygous carriers for the development of PPGL. So far, clinical information of germline *SDHA* mutation carriers is limited to case reports and small series.

Subjects and Methods: This retrospective nationwide study included 393 genetically unexplained PPGL-patients referred for germline *SDH* mutation analysis. Relatives of *SDHA* mutation carriers were tested through cascade screening.

Results: Germline *SDHA* mutations were identified in 30 of the 393 index-patients (7.6%), exerting head and neck PGL (n = 22), pheochromocytoma (n = 4) and sympathetic PGL (n = 4). The mean age (± SD) at diagnosis in index *SDHA* mutation carriers was 43 ± 16 years compared to 52 ± 15 years in non-mutation carriers (p=0.002). Three index-patients had malignant PPGL, and one had furthermore a SDHA-related pituitary adenoma. Two index-patients had each one relative with a *SDHA*-associated tumour, one with head and neck PGL and one with gastrointestinal stromal tumor. The *SDHA*-associated disease penetrance was 6% in 51 identified non-index *SDHA* mutation carriers, with a mean age (± SD) of 56±20 years.

Conclusion: Germline *SDHA* mutations can be frequently found in genetically unexplained PPGL patients. The majority of identified index-patients presented with an apparently sporadic solitary PPGL. In the largest *SDHA* series assembled so far, we found the lowest penetrance of all major PPGL predisposition genes. This suggests that recommendations for genetic counselling of at risk relatives and stringency of surveillance for *SDHA* mutation carriers might need to be reassessed.

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Exome sequencing identified potential candidate genes for serrated polyposis syndrome

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Background: Serrated polyposis syndrome (SPS) is a still poorly defined colorectal cancer predisposition syndrome characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. To date, only few molecular signatures have been described and the etiology of the syndrome has not been identified in the vast majority of patients.

Methods: To uncover causative mutations, the exomes of 31 SPS patients have been sequenced (Illumina HiSeq) using leukocyte DNA. The germline variants were filtered for rare (MAF: biallelic ≤ 1%, heterozygous 0.1% according to dbSNP, EVS, and ExAC), truncating, and missense

variants (predicted to be pathogenic by ≥ 2/3 prediction tools). For data analysis and filtering, the GATK software and the Cartagenia Bench Lab NGS Software were applied.

Results: After stringent filtering steps, potentially biallelic variants were found in 60 genes, some of which are recurrently mutated or functioning in tumorigenesis-associated pathways. Most interesting, seven genes were affected by biallelic truncating mutations with two patients carrying a homozygous nonsense mutation in one specific gene. Heterozygous variants in at least two patients were found in 334 genes. These encompass 40 cancer genes or genes of cancer-associated pathways. Here, the most interesting finding was a heterozygous *RNF43* splice-site mutation identified in an index patient and his affected daughter.

Conclusions: The data indicate that exome sequencing might identify causative variants for SPS. The current work-up includes testing of relatives to determine the zygosity of assumed biallelic variants, analyzing the segregation with the phenotype, and functional analyses of the most interesting variants.

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Improved differential diagnostic information on soft tissue tumors by genomic array analysis of pre-operative core needle biopsies

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Introduction: The most important pre-operative information in the management of patients with bone and soft tissue tumors (BSTT) concerns histologic subtype and malignancy grade; increasingly, such tumor classification is based on cells obtained through core needle biopsies (CNB). Several BSTTs have characteristic genetic changes and chromosome banding analysis was often used as a diagnostic adjunct, but turned out have a low success rate in CNB. Therefore, the aim of this study was to evaluate the use of genomic array analysis as a diagnostic tool for CNB from BSTT.

Materials and methods: All CNB obtained for diagnostic purposes from 47 patients with suspected BSTT from March 2016 to January 2017 were included. QIAamp Fast DNA Tissue Kit (Qiagen) was used to extract DNA and CytoScan HD genomic array (ThermoFisher/Affymetrix), including ChAS 3.1 software, was applied for detecting aberrations. When applicable, these findings were compared with genetic aberrations detected in the surgically removed tumor.

Results: DNA could be extracted from all 47 samples, 28 (60%) of which showed an abnormal array profile. All 10 samples that turned out to be non-neoplastic and 6/7 benign lesions had normal profiles. Only 3/30 (10%) samples from BSTT that typically show imbalances lacked aberrations. A specific diagnosis could be suggested in 4 cases. Abnormal array profiles in CNB were largely identical to those in post-operative samples in all 8 evaluable cases.

Conclusions: By replacing chromosome banding analysis with genomic array as the gold standard for CNBs from BSTTs the success rate increased from <50% to 90%.

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Application of subtractive hybridization approach for somatic retroelement insertions detection in human cancer cells

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Introduction: Retroelement (RE) transpositional activity is one of the important cause of the human genome instability. Previous studies revealed that new somatic RE insertions occur in different type of tumor cells especially in tumors of epithelial origin. Modern approaches for somatic RE detection are based on high-throughput sequencing of amplicons containing DNA fragments adjacent to RE insertions in target and reference samples. However, existing methods have a relatively low level of enrichment by target molecules. This fact significantly limits the depth of somatic RE detection and the number of analyzed samples. Here we describe a new cost-effective approach allowing to perform specific enrichment for somatic RE insertions.

Materials and Methods: Libraries for high-throughput sequencing were generated from DNA of 1000 acute myeloid leukemia cells (target) and 1000 normal white blood cells (control) from the same patient. Target and control amplicons for subtractive hybridization were prepared by selective amplification with AluYa5-specific primers and than were mixed in a ration 1:2000. Duplex-

specific nuclease from Kamchatka Crab was used for homo- and hetero-duplex DNA elimination.

Results: The utilization of subtractive hybridization reduce amount of sequences flanking fixed (not somatic) RE insertions by 5-fold in average and thus increase a portion of somatic RE insertion flanking molecules in the sequencing library by 40-fold.

Conclusions: The advantages of developed method significantly increase the efficiency of somatic RE insertions detection in different type of tumor cells.

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Detection of *KRAS* oncogene mutations (codon 12 and 13) in stool DNA of patients with sporadic colorectal cancer

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Introduction: colorectal cancer is one of the most common cancer types and leading causes of cancer death worldwide and it is curable if diagnosed at an early stage. Since *KRAS* mutations occur early in colorectal cancer tumorigenesis and happen in 30–50% of all CRC cases, the aim of this study was to detect *KRAS* mutations in stool samples as a non-invasive screening method among patients with colorectal cancer. Materials and Methods: DNA was extracted from stool samples of 50 individuals with colorectal cancer using a Qiagene kit. *KRAS* mutations were identified using polymerase chain reaction method and direct sequence analysis. **Results:** sequencing of all PCR products showed no mutations in *KRAS* codons 12 and 13 of the cases. Conclusion: our results revealed no mutations in common *KRAS* mutated codons. These observations may reveal the association of different etiological factors in the occurrence of colorectal cancer of which *KRAS* mutation might not appear in all populations.

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Telomere length, ATM mutation status and cancer risk in Ataxia-Telangiectasia families

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Recent studies have linked constitutive telomere length (TL) to aging-related diseases including cancer at different sites. ATM participates in the signaling of telomere erosion, and inherited mutations in *ATM* have been associated with increased risk of cancer, particularly breast cancer. The goal of this study was to investigate whether carriage of an *ATM* mutation and TL interplay to modify cancer risk in ataxia-telangiectasia (A-T) families. The study population consisted of 284 heterozygous *ATM* mutation carriers (HetAT) and 174 non-carriers (non-HetAT) from 103 A-T families. Forty-eight HetAT and 14 non-HetAT individuals had cancer, among them 25 HetAT and 6 non-HetAT were diagnosed after blood sample collection. We measured mean TL using a quantitative PCR assay and genotyped 7 single-nucleotide polymorphisms (SNPs) recurrently associated with TL in large population-based studies. HetAT individuals were at increased risk of cancer (OR=2.3, 95% CI=1.2–4.4, P=0.01), and particularly of breast cancer for women (OR=2.9, 95%CI=1.2–7.1, P=0.02), in comparison to their non-HetAT relatives. HetAT individuals had longer telomeres than non-HetAT individuals (P=0.0008) but TL was not associated with cancer risk, and no significant interaction was observed between *ATM* mutation status and TL. Furthermore, rs9257445 (*ZNF311)* was associated with TL in HetAT subjects and rs6060627 (*BCL2L1)* modified cancer risk in HetAT and non-HetAT women. Our findings suggest that carriage of an *ATM* mutation impacts on the age-related TL shortening and that TL *per se* is not related to cancer risk in *ATM* carriers. TL measurement alone is not a good marker for predicting cancer risk in A-T families.

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P12.186B

TaqMan Rare Mutation Assays targeting the TERT promoter region<!--EndFragment-->

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The enzyme Telomerase maintains telomeres at the ends of chromosomes. The Telomerase Reverse Transcriptase (TERT) gene codes for the enzyme's catalytic domain and is not expressed in normal somatic cells. As a consequence, normal cells acquire senescence by shortening of their telomeres during cell division and eventually undergo apoptosis. In contrast to normal somatic cells, expression of TERT is reinstated in cancer cells causing escape from senescence and apoptosis by maintaining the telomeres. It has recently been shown that mutations in the TERT promoter region play a key role in regulating and reinstating TERT expression. 90% of cancers carry a mutation in the TERT promoter region. Mutations like C228T and C250T create a new binding site for the E26 transformation-specific (ETS) transcription factor that regulates TERT expression. Experimental evidence showed that the ETS factor GA-binding protein, alpha subunit (GABPA) binds to the de novo ETS motif and activates TERT transcription in cancer cells.

We undertook a project designing TaqMan Rare Mutation assays addressing mutations in the TERT promoter. These assays are TaqMan SNP Genotyping assays optimized for use in digital PCR with the Applied Biosystems QuantStudio 3D. Digital PCR facilitates detection and quantification of rare mutant alleles. TaqMan SNP Genotyping Assays ensure reliable discrimination of mutant and wild-type allele. This will enable easy and sensitive detection of TERT promoter mutations in cancer research samples. These assays are suitable for detection in liquid biopsy applications with cell free DNA (cfDNA).

For Research Use Only. Not for use in diagnostic procedures.

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P12.187C

Genetic risk factors for sporadic testicular germ cell tumors

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Testicular germ cell tumors (TGCTs) derive from embryonic stem cells and directly related to the formation of male gametes. Genes *KITLG, SPRY4, BAK1* have an effect on testis development, their change results in a significant increase in the risk TGCTs. We have developed the system for detecting and investigated the frequency of the alleles and genotypes of *KITLG* (rs995030, rs1508595), *SPRY4* (rs4624820, rs6897876) and *BAK1* (rs210138) in 90 fertile men and 73 patients with TGCTs (34 seminomas and 39 non-seminomas).

Significant association with the development of TGCTs was identified in *KITLG* (rs1508595: p = 0.0003 for G, p = 0.0014 for GG), rs995030: p = 0.003 for GG). When comparing patients with seminomas and fertile men we revealed significant differences in *SPRY4* (rs4624820: p = 0.0226 for A), in *KITLG* (rs995030: p = 0.037 for G and p = 0.028 for GG), rs1508595: p = 0.03 for G) in *BAK1* (rs210138: p = 0.032 for G and p = 0.02 for GG). When comparing patients with non-seminomas and fertile men we revealed significant differences only in *KITLG* (rs1508595: p = 0.0005 for G and p = 0.002 for GG).

We have shown that the combination of three genes genotypes increases the risk of TGCTs in 6.5 times (p = 0.0005; OR: 6.526 [2.078–20.5]), and the risk of seminoma in 12 times (p <0.0001; OR: 12.68 [3.73–43.11]).

The study of genotypes associated with TGCTs can be used to identify of the individuals with an increased risk of TGCTs.

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P12.188D

A novel homozygous germline mutation in *TP53* presenting with aggressive synchronous tumours in infancy

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Introduction: Li-Fraumeni Syndrome (LFS) is a rare autosomal dominant cancer predisposition syndrome. Heterozygous germline mutations in the *TP53* tumour suppressor gene are causative in ~80% of cases; furthermore, somatic *TP53* mutations are frequent in human cancers. Intact p53 function is fundamental to maintenance of genomic integrity, cell cycle regulation, DNA repair and apoptosis. Homozygous germline *TP53* mutations are rare, only previously described in individuals with a low penetrance founder mutation (Arg337His).

Materials and Methods: We describe a consanguineous family in which the infant proband presented with two synchronous tumours; family history includes several relatives with cancer. SNP microarray was performed on DNA derived from lymphocytes, and both tumours. Sanger sequencing of the *TP53* gene was performed.

Results: Tumour histopathology confirmed two distinct malignancies, a periorbital embryonal rhabdomyosarcoma and choroid plexus carcinoma; in addition, the proband's pregnancy was complicated by metastatic choriocarcinoma. The family meet revised Chompret criteria. SNP microarray identified several long continuous stretches of homozygosity, encompassing ~5% of the genome, including the *TP53* locus. We identified a novel homozygous germline *TP53* mutation in exon 2 (c.[52delA]+[52delA]: p.Thr18Hisfs*26), This mutation is predicted cause protein truncation and a null product. Predictive testing confirmed parents and younger sibling are heterozygous for this mutation.

Conclusions: We report a consanguineous family in which the proband is homozygous for a novel *TP53* mutation, predicted to produce loss of p53 function. This case demonstrates that bi-allelic loss of *TP53* is compatible with human development, but appears associated with an aggressive, early onset cancer phenotype.

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Molecular characteristics of novel germline mutations in *TP53* in Sweden

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Introduction: Germline *TP53* mutations predispose to a wide range of early onset cancers most commonly breast cancer, soft tissue sarcoma, osteosarcoma, brain tumors and adrenocortical carcinoma. p53 is a transcription factor that binds to DNA after tetramerization and regulates several pro-apoptotic and cell cycle regulating proteins like Mdm-2, p21, Bax, and PUMA. **Materials and Methods:** We have generated cDNA expression constructs for all novel germline *TP53* mutants in the Swedish cohort and investigated the activity and molecular effects of these mutations on downstream target proteins by immunoblotting, immunofluorescent and flowcytometry. **Results:** In this cohort of 32 families, we found 25 different mutations in the *TP53* gene. Nine have not been reported previously as germline mutations according to the IARC databases. Five of these have not even been reported as somatic mutations in the IARC or cosmic databases and are thus truly novel. We showed that some mutations have activities close to wildtype while others have undetectable activities. We also reported that some mutations can increase expression of PUMA and Bax in p53-null cell lines which lead to caspase 3 cleavage and apoptosis. Cellular localizations of the mutated p53 proteins were also investigated and varied among the mutants. **Conclusions:** We showed that all novel mutations were stably express in p53-null cell lines and reduced spontaneous apoptosis as a result of less expression of pro-apoptotic proteins. The nuclear translocation ability of the different mutants varied. These data indicate that affected individuals are susceptible to development of neoplasia. **Grant:** BRECT

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P12.190B

TRIM8 in the pathogenesis of glioma: a possible role in the mitotic spindle regulation

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Introduction: Our data showed a down regulation of the E3 ubiquitin ligase TRIM8 in glioma, a lethal brain tumor, and its role in controlling tumor cell growth. We hypothesized that TRIM8 activity impairment might alter the glioma-related proteome, perturbing specific normal pathways and favoring tumorigenesis. This study explored TRIM8 role in gliomagenesis by identifying TRIM8-associated proteins and biological pathways in embryonic neural stem cells (ENSC), the healthy counterpart of glioma

Materials and methods: We profiled transcriptome and proteome of ENSC overexpressing Trim8 by using RNAseq and LC-MS/MS, respectively

Results: Gene-set enrichment analysis of RNAseq data highlighted pathways related to neurological functions, cell death, and inflammation. Proteomic analysis followed by co-immunoprecipitation assays identified as TRIM8 interactors four members of the kinesin-like protein family, including KIF11 and KIFC1, known to be involved in mitotic spindle dynamic. Based on our data, we explored the effect of TRIM8 expression on mitotic spindle organization by performing immunofluorescence assays in glioma cells depleted for TRIM8. The absence of binucleated cells, chromosomal bridges, and micronuclei in TRIM8 interfered cells excludes cytokinesis defects. However, an accumulation of monopolar spindles in TRIM8 depleted metaphase cells suggested its role in centrosome maturation and separation, a phenotype also observed in cells with a perturbed expression of KIF11 or KIFC1

Conclusions: TRIM8 might play a crucial role in controlling the mitotic process and contribute to the glioma development through an aberrant regulation of key mitotic factors. Further studies will elucidate a possible role of TRIM8 in the organization of the microtubule dynamic

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Four novel pathogenic variants in TSC2 gene of Turkish patients with tuberous sclerosis complex

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Introduction: Tuberous sclerosis complex (TSC) involves multiple abnormalities in different tissue and organ systems of effected patients like skin, brain, kidney, heart and lungs. Heterozygous pathogenic variations of *TSC2(OMIM* * 191092) gene are responsible from ~73% of Tuberous Sclerosis Complex (TSC, OMIM # 613254) patients. The aim of this study to summarize novel pathogenic TSC2 variants found in Turkish patients who were directed to Trakya University Genetic Diagnosis Center with a suspicion of TSC. **Materials and Methods:** Genomic DNA samples isolated from peripheral blood of patients were used. Two different Next Generation Sequencing (NGS) platforms [Ion Torrent PGM (Thermo Fisher) and MiSeq (Illumina)] were used to investigate the genetic basis of TSC in four patients (4 female aged between 5–30). *TSC2* gene coding regions were amplified using Ion AmpliSeq™ Library Kit 2.0 before enrichement by OT2 200 Kit and sequenced on the Ion Personal Genome Machine. TruSight Cancer Kit (Illumina) was used to determine the variations on the MiSeq System (Illumina). Ion Reporter v4 (Thermo Fisher) and Base Space (Illumina) softwares and IGV 2.3.36 (Broad Institute) were used to define the variations of TSC2 gene. **Results:** Pathogenic variants defined with Ion Torrent were mosaic c.996_998delGGT(p.Val333del) and heterozygous c.5192_5193insA(N1731Kfs*44). Other pathogenic variants found with MiSeq system were mosaic c.4006-4_4010deletionCTAGTCGTC (S1336Lfs*76) and heterozygous c.4252_4253insC(p.Gln1419fs). **Conclusions:** NGS is a powerfull tool for determining pathogenic variants in *TSC2* gene and allows to determine low mosaic variations even in DNA samples extracted from peripheral blood of patients.

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P12.192D

Increased *TSPY* copy number represents a risk factor for prostate and testicular cancers

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The human *TSPY* is a multi copy gene family (20–40 copies) located on the Y chromosome and expressed in spermatogonia and early spermatocytes, where it is involved in sperm differentiation and proliferation. It is abundantly expressed in germ cell cancers and gonadoblastoma. Ectopic expression of *TSPY* has been demonstrated in some somatic cancers such as melanoma, prostate and liver cancer. The aim of our study was to investigate the possible association of *TSPY* gene copy number with the occurrence of male specific cancers. To overcome possible population stratification bias the distribution of *TSPY* copy numbers in different Y chromosome haplogroups was also analyzed. We have analyzed a total of 125 cancer patients: 92 prostate cancer (mean age 68.7±7.47) and 33 testicular cancer patients (33.6±13.43) as well as 79 healthy controls (64.4±14.85). *TSPY* gene copy number measurement was based on real-time qPCR (ddCt method) using a sample with known *TSPY* copy number as a reference. Single copy *SRY* gene was used for data normalization. Y chromosome haplogroups were predicted using 17 Y-STR markers. Our results showed statistically significant higher mean *TSPY* copy number among cancer patients (38.75±16.14, p=0.0006, Student's t-test) than controls (30.87±15.24). The mean values were higher in cancer patients than controls in all haplogroups except J1, thus favoring unbiased statistical significance. In conclusion, *TSPY* copy number represents a risk factor for prostate and testicular cancers. Further studies are needed to clarify the association of *TSPY* copy number variation with *TSPY* mRNA expression levels.

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P12.193A

PCR Recombinants, Off-target Alignments and their Effect on the Genetic Diagnosis of Tumour Tissue

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We describe an effective diagnostic tool for the analysis of low mass tumour DNA samples by NGS with a limit of detection of 1%.

Routine genetic analysis of tumours is becoming a standard of care required for the effective delivery of targeted therapy, for example the use of EGFR TKIs, such as

afatinib, gefitinib or erlotinib
in non-small cell lung carcinoma (NSCLC). The majority of NSCLC patients present with advanced disease, cytology and small biopsy specimens can pose challenges due to low tumor content. Multiple usage of the same sample for single amplicon tests soon depletes the tissue, yet typical NGS sequencing libraries require 100ng to 1ug of tissue.

Here we report on the use of a multiplex PCR amplicon panel that can produce sequencing libraries from as little as 10ng DNA. We observed a success rate of >95% with an unselected series of clinical samples. Our analytical pathway, AmpliVar (https://github.com/LordGenome/amplivar) permits robust identification of variants down to a selectable sensitivity, typically 1%. Since sequence reads are hashed at an early stage in the analysis, it is possible to see output prior to alignment and filtering. Especially at low DNA mass, we saw an increasing percentage of PCR recombinants and off-target products were generated in the course of amplification.

As well as genome-wide alignment of the sequence reads, AmpliVar includes an orthogonal genotyping method matches known variants to a look-up table, obviating the necessity for confirmatory Sanger sequencing.

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P12.194B

A novel *RHBDF2* mutation in a Black African family with tylosis and squamous cell carcinoma of the oesophagus

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Introduction: Tylosis with squamous cell carcinoma of the esophagus (TOC; OMIM 148500) is a rare autosomal dominant disorder. The condition is characterized by focal non-epidermolytic palmoplantar keratoderma and a high risk for squamous cell carcinoma of the oesophagus. Three pathogenic missense mutations in the *RHBDF2*-gene (p. Ile186Thr, p.Asp188Asn and p.Pro189Leu) were recently described to be associated with TOC in four families (one each from the UK, USA, Germany and Finland). To the best of our knowledge TOC has not been reported in patients of African descent. We here describe the first African family, with tylosis and oesophageal cancer.

Patients and methods: This four generation African family had a total of six members presenting with palmoplantar keratosis. None exhibited the extreme fissuring of the palmoplantar skin or oral leukokeratosis that is usually associated with TOC. Two of the family members also developed oesophageal cancer. After written informed consent was obtained from the family members, blood samples were taken for *RHBDF2*-gene analysis. Genomic DNA was extracted and the *RHBDF2*-gene (NC_000017.10), was amplified and subjected to Sanger sequencing using Life Technologies BigDyeV3.1 chemistry.

Results: A novel missense mutation (c.562 G>T, p. Asp188Tyr) of RHBDF2 (NM_024599.5) was detected in all affected members of the African family and was absent in unaffected family members. Interestingly, this mutation alters the same codon as that in the family from Finland (p. Asp188Asn).

Conclusions: These results further validate *RHBDF2* mutations as the cause of TOC. In addition, this family can now proactively manage their cancer risks through regular clinical screening.

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P12.195C

Von Hippel Lindau Syndrome - Multidisciplinary Clinics in Western Denmark

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Background: Von Hippel Lindau syndrome (vHL) is a genetic condition with an increased risk of especially renal cell cancer and abnormal growth of blood vessels in many organs. Surveillance programs are crucial for early detection. Until recently, these patients spent several days a year attending various surveillance programs at the departments of neurosurgery, ophthalmology, urology, endocrinology, audiology, and radiology, respectively, potentially limiting self-esteem, causing stress as well as causing lost earnings.

By establishing multidisciplinary clinics, patients may substantially reduce time spent on transport and hospital visits. Compliance may increase and in addition, the specialists will gain easy access to multidisciplinary discussions, optimizing patient treatment and care.

Methods: In 2015, a multidisciplinary team was set with representatives from the involved medical departments. Surveillance is scheduled for two days starting with all

diagnostic imaging, blood and audiometric tests on day 1. Day 2 is initiated by a one-hour conference with specialists in neurosurgery, ophthalmology, urology, endocrinology, audiology, radiology, hepatology, pediatrics, and genetics. Each patient is discussed. Next, each patient is seen by specialists in neurosurgery, opthalmology, endocrinology, and genetics. If tumors needing treatment are identified, the treatment is planned instantaneously.

Results**:** In 2015, 12 patients underwent surveillance in the multidisciplinary vHL clinic and in 2016 the total number of patients was 22.

Conclusion: The increasing number of patients choosing the multidisciplinary clinic indicates that process optimization is beneficial for the patient as well as for the healthcare system.

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P12.196D

Lower penetrance of von Hippel-Lindau disease (vHL) influences risk assessment in predisposed families

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von Hippel-Lindau disease (vHL) is a multisystemic tumor syndrome; carriers of germline *VHL* mutations should undergo prophylactic surveillance due to risk of multiple tumors. Before genetic testing was available, vHL was estimated to be fully penetrant at age 60.

In a national cohort of all Danish *VHL* mutation-carriers, we evaluated how and when families were diagnosed and assessed vHL penetrance.

Through interviews and hospital records, we evaluated 34 families with *VHL* mutations (97 mutation-carriers and 262 first-degree relatives). In the majority of familial cases (74%, 58 of 78), the vHL diagnosis was made pre-symptomatically or at the time of their first manifestation. In the remaining 31 patients diagnosed while alive, there was a median delay of 10 years (range: 2–34 years) from their first manifestation to vHL diagnosis.

Remarkably, 35% (46 of 131) of the living first-degree relatives were not genetically tested (median age when their family's *VHL* mutation was found: 52.5 years (range: 0 - 85 years)). Especially siblings (61%) and parents (20%) were not tested, and relatives over 40 years at the time the familial *VHL* mutation was found, were significantly less likely to be genetically tested ($p<0.0001$).

vHL penetrance was 87% at age 60. When considering only patients without pre-symptomatic surveillance, 20% were asymptomatic at age 60. This is important in relation to genetic counselling when assessing vHL risk in asymptomatic adult first-degree relatives, who not often genetically tested. Funding: the Lundbeck Foundation, the Danish Cancer Society, and the Danish Association for vHL association and their relatives.

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von Hippel-Lindau development in children and adolescents

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The autosomal dominant von Hippel-Lindau disease (vHL) is associated with a high risk of tumour development throughout life, especially retinal and CNS hemangioblastomas, pheochromocytoma, and renal cell carcinoma. Knowledge of paediatric vHL development is limited, and current Danish surveillance guidelines are based on best clinical judgement. We aimed to describe the course of vHL manifestation development in children and adolescents, focusing on age at first manifestation, manifestation frequencies, and types.

The prevalence of the vHL diagnosis, as well as manifestations in childhood, were evaluated based on 99 vHL patients, who had started surveillance before 18 years: 37 Danish patients from the national vHL research database and 62 international patients reported in 15 scientific articles.

Overall, 70% (69 of 99) developed manifestations before 18 years (median age at first manifestation: 12 years (range: 6–17 years)). Thirty per cent (30 of 99) had developed more than one manifestation type; the most frequent were retinal (34%) and CNS (30%) hemangioblastomas. Among the 37 Danish patients, 25 (68%) had manifestations and 85% (97 of 116) of their tumours were asymptomatic.

We found that childhood manifestations of vHL were more frequent than previously reported. We recommend surveillance ophthalmoscopies from early childhood to detect retinal hemangioblastomas. Further, we suggest that surveillance MRIs of the CNS should be initiated around the age of eight years, which contrasts most international guidelines that recommend MRI initiation between 15–16 years.

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P12.198B

No influence of XPC gene polymorphisms on FLT3 or DNMT3Amutations in acute myeloidleukemiapatients

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Background: Recently, it was reported thatXPC gene, involved in the nucleotide excisions repair system, confersusceptibility to developingacute myeloid leukemia (AML). We aimed to assess the impact of XPC C499Tgene polymorphism on developing AML and to investigate the correlation between XPC C499T and FLT3 and DNMT3A mutations. Material and methods: This study included 253 subjects divided in two groups: AML group with 123 patients and the control group with 130 heathy individuals. XPC C499T polymorphism was investigated in all subjects by RFLP-PCR technique byusing Cfr42I digestion enzyme. FLT3 (ITD, D835) and DNMT3A mutations were investigated in AML patients. **Results:** For XPC C499T polymorphism wild type genotype (CC) was identified in 26 patients and 59 controls; heterozygous genotype (CT) in 91 cases and 59 controls,while thevariant homozygous genotype in 6 patients and 17 controls.FLT3 (ITD, D835) was found in 28 patients, DNMT3A mutation in 16 cases, while both genes mutation was observed in 7 AML patients Variant genotype CT+TT XPC C499T was associated with the risk of developing AML (p=0.0001, OR=2.89 and 95% CI:1.67–5.02) but no withthe presence of FLT3/DNMT3A mutations. Conclusion: The XPC C499Tgene polymorphism is a risk factor for AML in this population. The presence of variant genotype was not a predisposing factor for the presence of FLT3 or DNMT3A mutations. Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2016-1076 WITHIN PNCDI III, contract no 147 PED/2017.

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P13 Basic mechanisms in molecular and cytogenetics

P13.01A

Molecular genetic and functional analysis indicate novel genes involvement in retinopathy of prematurity

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Retinopathy of prematurity (ROP) is one of the major complications of premature birth which causes abnormal retinal vascular development leading to severe visual impairment. The underlying molecular mechanisms behind ROP pathogenesis is still unclear. The present study is focused on unravelling the factors responsible for the progression of ROP to severe stage among only 10–15% of preterm infants with initial symptoms despite of similar and timely antenatal care. A comprehensive analysis of candidate genes in premature infants with (n = 200) and without ROP (n = 200) was undertaken to identify genetic variants conferring disease susceptibility. Subsequently, the functional analysis of identified genetic associations were undertaken at vitreous and cellular levels. Of 384 variants evaluated, significant associations of few variants in *CFH, C2/BF, ANGPT2, FBLN5, CETP, CXCR4* and *MMP9* were observed with ROP. At protein level, also, MMP9, CFH, C3, C4, APOA1, APOC3, VEGF and proinflammatory cytokines exhibited significant elevations in ROP vitreous as compared to the controls. Western blotting and zymography further provided interesting insights on the activation of complement components and matrix metalloproteinases in ROP pathogenesis. LC-MS/MS analysis after in-gel digestion of proteins in vitreous further validated the complement activation in the patients. Parallelly, we also found that macrophages/microglia might have a major contribution in the progression of ROP. Summarily, the present study demonstrated that along with genetic predisposition, hypoxia driven complement activation mediated by retinal macrophage/ microglia could eventually cause neovascularization and lead to the progression of disease in premature infants. Grant Support from Department of Biotechnology to IK

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P13.02B

Cryptic complexity and disease candidate genes identified in *de novo* apparently balanced translocations using whole-genome mate-pair sequencing

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The majority of apparently balanced translocation (ABT) carriers are phenotypically normal; however phenotype association is present in 6–10% *de novo* cases. Detailed ABT characterization is crucial for precisely reconstructing derivative chromosomes, as well as identifying disease candidate genes in affected individuals and making accurate pregnancy risk estimations in non-affected individuals, especially in those with complex translocations.

In the current study, whole-genome mate-pair sequencing (WG-MPS) was applied to characterize five *de novo* ABT carriers with neurodevelopmental or other abnormalities and three non-affected individuals with recurrent miscarriages. All findings were validated with Sanger sequencing and real-time PCR.

WG-MPS precisely mapped all ABT breakpoints. Based on their molecular characterization, microhomology-mediated or non-homologous end-joining mechanisms are suggestive for ABT generation. Gene disruption occurred in 4/8 cases while cryptic complexity and imbalances were detected in 2/8 cases. Specifically, an additional breakpoint was detected in a phenotypically normal t(6;7;10) carrier thus increasing the percentage of unbalanced gametes and future pregnancy risk. In a t(6;7;8;12) carrier with intellectual disability, a chromothripsis rearrangement was detected with thirty-eight translocation breakpoints and a heterozygous deletion disrupting *SOX5*, a dominant nervous system development gene previously reported in similar patients. Finally, in a t(1;X) carrier with hearing loss, the chrX breakpoint mapped ~90kb upstream *POU3F4*, an X-linked non-syndromic deafness gene, indicative of an underlying position effect mechanism.

In conclusion, we demonstrated that WG-MPS can accurately characterize *de novo* translocation breakpoints, reveal ABTs' full complexity, and suggest underlying mechanisms for phenotype association, thus supporting its potential introduction in the clinical investigation of ABTs.

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P13.03C

Nested inversion polymorphisms predispose for chromosome 22q11.2 rearrangements

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Inversion polymorphisms between low copy repeats (LCRs) often predispose to meiotic non-allelic homologous recombination (NAHR) events causing genomic disorders. However, for the 22q11.2 deletion syndrome (22q11.2DS), the most common genomic disorder, no such inversions have been uncovered as of yet. Using fiber-FISH, we demonstrate that parents transmitting the de novo 3 Mb LCR22A-D 22q11.2 deletion, the reciprocal duplication, and the smaller 1.5 Mb LCR22A-B 22q11.2 deletion, carry inversions between LCR22B or LCR22C and LCR22D. Hence, the inversions predispose to meiotic 22q11.2 rearrangements and increase the individual risk over two-fold for transmitting rearrangements when compared to the population. Interestingly, the inversions are nested or flanking rather than coinciding with the deletion/duplication sizes. This finding raises the possibility that inversions are a prerequisite not only for 22q11.2 rearrangements but for all NAHR mediated genomic disorders. Additionally, the lack of normal individuals homozygous or patients hemizygous for the inversion suggests negative selection.

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P13.04D

Alpha-1 antitrypsin gene expression in liver organoids

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Introduction: In vitro organoids are three dimensional (3D) cell culture systems, in which adult stem cells can self-organize and differentiate into structures that show realistic micro-anatomy. This new cell culture tool facilitates modeling of diseases, study of biological processes and testing of new therapies. Alpha-1 antitrypsin (AAT) is largely secreted by the liver, and its accumulation into hepatocytes causes AAT deficiency in individuals with Z mutation (Glu342Lys) in SERPINA1 gene. Nevertheless, since liver biopsies are difficult to obtain, organoids might be a good alternative to study molecular processes associated with AAT deficiency.

Materials and methods: We have determined optimal culture conditions for generation of liver organoids from healthy donors and patients with AAT deficiency. We have evaluated AAT gene and protein expression in organoids and its secretion into the medium. To characterize the expression of SERPINA1 gene in liver cells, expression of the alternative transcripts of SERPINA1 has been investigated by RT-PCR and QT-PCR.

Results: We have analyzed the expression of transcripts 1A, 1B and 1C in liver organoids and obtained the specific profile of the alternative transcripts of SERPINA1 in liver cells. We are evaluating the ability of organoids to reproduce AAT deficiency in vitro, by detection of AAT protein aggregates.

Conclusions: Generation of liver organoids might be a useful tool to investigate the factors contributing to hepatic disease in AAT deficiency.

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P13.05A

Alpha-thalassemia due to novel deletions and complex rearrangements in the subtelomeric region of chromosome 16p

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Introduction: Inherited deletions removing the α-globin genes and/or their upstream regulatory elements (MCSs) give rise to alpha-thalassemia, one of the most common genetic recessive disorders worldwide. The pathology is characterized by microcytic hypochromic anemia due to reduction of the α-globin chain synthesis, which are essential for hemoglobin tetramerization.

Material and Methods: In order to clarify the suggestive α-thalassemia phenotype in eleven patients, we performed Multiplex Ligation-dependent Probe Amplification with commercial and synthetic probes, gap-PCR, and

Sanger sequencing to search for deletions in the subtelomeric region of chromosome 16p.

Results: We have identified six distinct large deletions, three of them novel, and one indel. The deletions range from approximately 3.3 to 323 kb, and i) remove the whole α-globin cluster; or ii) remove exclusively the upstream regulatory elements leaving the α-globin genes structurally intact. The indel consists in the loss of MCS-R2 (HS-40), which is the most important distal regulatory element for the α-globin gene expression, and the insertion of 39 nt, seemingly resulting from a complex rearrangement involving two DNA segments (probably from chromosome 3q), bridging the deletion breakpoints with a CC-bp orphan sequence in between. Finally, in one patient no α-globin deletion or point mutation were found. This patient revealed to have acquired alpha-thalassemia associated with a myelodysplastic syndrome.

Conclusions: Our study widens the spectrum of molecular lesions by which α-thalassemia may occur and emphasizes the importance of diagnosing large α^0-deletions to provide patients with appropriate genetic counseling.

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P13.06B

Splice-site variant validation by NGS based amplicon sequencing of rtPCR products in short stature

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The application of next generation sequencing technologies has been successful in unraveling the causes of genetic disorders, but simultaneously increased the number of variants potentially affecting the splice-sites. To identify the genetic origins of idiopathic short stature by exome sequencing in more than 200 patients, we observed 44 variants possibly affecting splicing. Computational methods to calculate their potential effect were partly inconclusive. Therefore we performed standard rtPCR analyses comprising Sanger sequencing. In case of suspected alternative splicing the evaluation of the distinct resulting isoform, though, was often hampered by the overlap of sequences. This was especially severe when multiple alternative transcripts were present. To circumvent these issues, we established a deep sequencing approach (targeted rtPCR-Seq) to identify all present isoforms in patients and controls. For this method the 44 rtPCR fragments were normalized to generate an equimolar mixture. We generated libraries by ligation of distinct index adaptors for both amplicon pools and subsequently sequenced on an Illumina MiSeq platform. The resulting reads were aligned and visualized as sashimi plots. Observed splice-junctions in patients were compared to the controls and public databases. Whereas the computational approach was able to imply potential effects on splicing only rtPCR results gave conclusive information. The specific effect was more obviously ascertained by the NGS based approach. rtPCR-Seq was able to unambiguously separate multiple co-occurring isoforms. Thus, we established a simple amplicon based deep sequencing approach for standard rtPCR fragments enabling a fast, comfortable and precise interpretation of the effect of a specific variant.

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P13.07C

Sequencing analysis of the *ISL1* gene in human bladder exstrophy

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Bladder exstrophy is a rare congenital closure defect of the urinary bladder with profound impact on morbidity. A

genetic background of the malformation is supported by an increased recurrence risk in offspring and siblings, higher concordance rates in monozygotic twins and associated chromosomal aberrations including chromosome 22q11 duplications. Recently, *ISL1* was presented as a candidate gene in a genome wide association study for bladder exstrophy. Here, we assessed the frequency of single nucleotide variations in exons of *ISL1* using DNA from 125 Swedish bladder exstrophy patients. In addition, we assessed *ISL1* expression levels in human foetal bladder tissue during the critical time period of bladder development.

In total 17 genetic variants in *ISL1* were identified including one splice variant, c.479-4G>A, and one novel missense variant, c.137C>G p.(Ala46Gly). The c.137C>G variant is predicted to be possible pathogenic/possible damaging by M-CAP and PANTHER, respectively, however the pathogenicity is currently uncertain. Both variants were inherited from a healthy mother. The existence of these variants in healthy parents may not exclude causation in accordance with the previously reported 22q11 duplication carriers. Furthermore, we detected *ISL1* mRNA expression during week 5–10 of foetal bladder development.

Our results suggest that mutations in *ISL1* may be a rare mechanism behind bladder exstrophy. Further, functional studies of ISL1 mutations and gene expression profiles during bladder development could identify key genes important for bladder development.

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P13.08D

Sequencing of synthetic long reads to elucidate structure and mechanisms for formation in patients with genomic structural alterations

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Introduction: Genomic rearrangements are structural alterations in the DNA molecule. For identification of its mechanisms, a base-pair level analysis is necessary. Next Generation Sequencing (NGS) is an option, but it generally uses short reads, which make it difficult to identify the actual position of a determined fragment in a chromosome. Recently, techniques have been developed that synthesize long reads by adding barcodes in long fragments before performing the fragmentation. Materials and Methods:In this sense, we used the new 10x Chromium protocol, followed by exome sequencing by Illumina HiSeq, in order to understand the structure of rearranged chromosomes and propose, in an unprecedented way, mechanisms for formation. We performed Chromium analysis in samples of two patients previously identified with structural alterations by karyotype.

Results: The analysis was successful in detecting the translocations revealed by karyotype and, furthermore, delineated breakpoints more accurately. Patient 1 presented a karyotype 46,XY,t(7;14)(q34;q21.2) which was redefined as 46,XY,t(7;14)(7pter->q34::14q21.2->qter) with breakpoints precisely at chr7:138.399.664 and chr14:46.624.191. Patient 2 presented a karyotype 46,XY,der(9)t(9;18)(p22;q12.2) which was redefined as 46,XY,der(9)t(9;18)(9qter->p24.1::18q12.3->qter) with breakpoints precisely at chr9:4.368.704 and chr18:39.163.156. Since there are no other alterations in each patient, nor LCRs (Low-Copy Repeat) nearby, we could infer that NHEJ (Non- Homologous End Joining) was the mechanism that generated both rearrangements.

Conclusions: These results show the efficiency of the 10x Chromium method for identifying structural rearrangements and its importance in the investigation of breakpoint mechanisms. Grant reference: CNPq

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P13.09A

A tale of two anomalies. A paternal duplication and a maternal deletion of 15q13

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A male newborn presented with an antenatally detected cardiac malformation. Postnatal examinations revealed a double chambered left ventricle as single malformation.

Array-CGH molecular karyotyping identified a chromosomal anomaly of the 15q13.2q13.3 region (hg19, 30,954,726–31,972,705;1018kb) including 21 genes, among others TRPM1, MTMR10, OTUD7A, KLF13, ARHGAP11B and FAN1.

Familial analyses revealed that his mother carried a deletion overlapping the same region (30,954,726–32,509,926; 1555 kb) while his father carried a duplication of the same chromosomal region (32,065,000–35,509,926; 445kb). His father presented with a congenital cataract and a congenital deafness, both attributed to neonatal asphyxia. His mother presented with a hypophysary microadenoma. Both parents have a normal intelligence. A healthy sister with a normal development at the age of 3 presented the same heterozygous anomaly as his brother.

A double chambered left ventricle has never been described previously in patients with 15q13 deletion. However, other congenital cardiac anomalies have been described possibly associated with haploinsufficiency of KLF13. In our case, we postulate that the co-occurrence of two chromosomal anomalies of the same 15q13 region has contributed to the occurrence of this malformation. Chromosomal anomalies are risk factors for malformations with a wide variability of phenotype, even including the absence of symptoms, thereby explaining the sister normal phenotype.

The possibility of a co-occurrence of a parentally inherited duplication on one allele with a complementary deletion of the allele inherited from the other parent has been postulated in the literature, but according to our knowledge has never been described yet.

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P13.10B

Interpretation of NGS-mapped chromosomal breakpoints: The importance of healthy controls

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Introduction: Next-generation sequencing (NGS) has revolutionised the mapping of balanced chromosomal rearrangements (BCRs), linking truncated genes to specific diseases, and pinpointing the functional importance of the genomic organisation into topologically associating domains (TADs). However, we lack data from healthy BCR-carriers.

Methods and Materials: We present the first data from the International Breakpoint Mapping Consortium (IBMC), a world-wide effort which includes mapping of normal carriers. We compared breakpoints from simple two-way BCRs in affected vs. controls using truncation of genes, including known autosomal recessive (AR) and dominant (AD) disease genes and loss-of-function (LOF)-constrained genes. Breakpoints potentially associated with long range position effects (LRPE) are reported separately.

Results: We increased the number of NGS-mapped healthy BCR carriers manyfold, and expanded the number of affected carriers (Table).

Conclusion: The proportion of truncated genes are similar in unaffected and affected carriers, with a significant excess of truncated AD disease and LOF-constrained genes in the latter. Truncation of known AD genes in apparently healthy carriers does occur, e.g. *ABCC9*, *DCC*, *CACNB4* and *TGFB2*. Strikingly, 23% of the truncated genes in healthy carriers are LOF-constrained, at odds with exome sequencing data. Our study illustrates the importance of NGS-based mapping of BCRs irrespective of phenotype, and the potential of IBMC to confirm known disease genes and identify novel candidate disease genes and LRPE-regions.

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Number of mapped BCRs and breakpoints truncating genes

Phenotype	Total number of BPs truncating						Novel candidate disease genes in IBMC
	BCR carriers (published)	#BPs	genes (%)	2 genes (%)	AD genes	AR genes	LOF-genes (%)	
Affected	87 (198)	570	231 (40)	44 (15)	39	20	95 (41)	57
Healthy	117 (8)	250	110 (44)	27 (22)	4	13	26 (23)	
	204 (206)	830		p=0.0024			p=0.0016	

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P13.11C

EP300 CNVs in individuals exhibiting genomic/chromosomal instability

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Introduction: Genomic/chromosomal instability (GIN/CIN) can be a consequence of alterations to DNA repair, cell cycle and/or apoptosis pathways and GIN/CIN can be associated with debilitating cancerous and neurodegenerative conditions. Here, we have evaluated CNVs of genes implicated in DNA repair machinery, cell cycle and apoptosis pathways in individuals with GIN/CIN manifested as chromothripsis.

Materials and methods: We have evaluated the presence of copy number variations (CNV) in genes involved in cell cycle pathway (according to KEGG database) in 433 children with intellectual disability, autism, epilepsy, and/or congenital malformations addressed by SNP-array molecular karyotyping. Among them, children exhibiting GIN/CIN manifested as chromothripsis were further addressed bioinformatically.

Results: We found a full loss of the distal half of N-terminal exon of the *EP300* in 4 patients with GIN/CIN manifested as chromothripsis. In addition to chromothripsis, these patients also exhibited dramatically increased CNV burden (50–100 times more than an average number of CNV per genome). This gene encodes an acetyltransferase p300 involved in transcriptional regulation by both chromatin remodeling and activation of various transcription factors.

Conclusions: Terminal exon of *EP300* encodes zinc finger domain is essential for protein activity and a regulatory binding site. *EP300* terminal exon loss is likely to alter cell cycle pathways and contribute to GIN/CIN in these patients. Our study allows speculations that genetic defects in *EP300* is involved in generation of GIN/CIN manifested as chromothripsis. Supported by the Russian Science Foundation (project #14-35-00060).

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P13.12D

Analysing the expression profiles of human *DES* orthologous *desma* and *desmb* by using knockout zebrafish models

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Desmin, a basic intermediate filament protein specific to muscle tissue, facilitates the transmission of force during muscle contraction and relaxation by forming a large network structure. In zebrafish, there are two *DES* orthologous gene (*desma* and *desmb*) and for modeling of *DES*-related diseases, the tissues in which these genes are expressed need to be analysed.

In this study, mRNA and protein expressions of *desma* and *desmb* was compared among wild type AB, *desma* KO (-/-) and *desmb* KO (-/-) zebrafish. mRNA expression was determined by qRT-PCR and whole mount *in situ* hybridization. Protein expression was observed by whole mount immunofluorescence staining technique.

In qRT-PCR study, 99% of *desma* mRNA expression in *desma* KO (-/-) zebrafish and 98% of *desmb* mRNA expression in *desmb* KO (-/-) zebrafish were found to decrease relative to the wild type AB zebrafish. *In situ* hybridization and immunofluorescence staining studies have shown that, in wild-type AB zebrafish, *desma* and *desmb* have similar somite and heart expression profile until 70 hpf; but after 70 hpf, it was observed that the *desma* was expressed predominantly in somites and heart, while *desmb* was expressed mainly in the gut. In *desmb* KO (-/-) zebrafish *desma* is also expressed in the gut. However, in *desma* KO (-/-) zebrafish, no expression occurs in somites or heart.

In conclusion, the *DES* ortologous genes' expression has been analysed in zebrafish and in the future it will remove the barriers of modeling *DES*-related diseases in zebrafish.

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P13.13A

High diagnostic yield through a gene-agnostic trio exome sequencing strategy that identifies mutations in new and old rare disease genes

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Exome sequencing has accelerated novel disease gene discovery from ~2700 in 2010 to ~6000 today. Over the past year ~85 OMIM phenotypes with a newly discovered molecular basis were discovered per month but most targeted exome captures ("clinical exomes") were designed at least 3 years ago. We developed a bioinformatics pipeline to identify SNV, indel and CNVs in unaffected parent-affected child trios using the Agilent (v5/6) whole exome capture. Patients with a high prior probability of a monogenic disorder were selected for testing through multi-disciplinary assessment. Most had already undergone extensive genetic testing. In 21 of the first 50 consecutive trios we identified the likely cause of the patient's presenting phenotype . Variants were classified as pathogenic or likely pathogenic according to the ACMG guidelines and after assessment by a multi-disciplinary team. De novo, AR and X-L variants accounted for 9, 10 and 2 of the cases respectively. A definitive genetic diagnosis was obtained for 6 of 13 cases during an on-going pregnancy. In 5 of these the result was communicated within 3 weeks from sample submission and facilitated prenatal testing decisions. In one family the gene-agnostic approach identified an incidental genetic diagnosis that changed the family's clinical management. For 3 of the 21 cases, the disease gene was discovered in the past 3 years. We conclude that a combination of case selection and whole exome trio-based approach enables a high diagnostic yield (42%) in the clinical setting through a gene-agnostic, hypothesis-free approach that includes all new disease genes.

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P13.14B

Investigation of role of some glaucoma risk factors in the disease pathogenesis

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Introduction: Glaucoma is an optic neuropathy frequently associated with elevated intraocular pressure (IOP) and extracellular matrix (ECM) remodeling in the trabecular meshwork (TM). The pathogenesis is multifactorial and complex, but role of transforming growth factor-β (TGF-β) has been shown in the process. Patients with glaucoma have elevated levels of TGF-β2 in their aqueous humor, and TGF-β has been shown to increase TM ECM production. The bone morphogenetic proteins (BMPs) are other members of TGF-β superfamily that antagonize TGF-β signaling in TM. Gremlin, a BMPs antagonist, is increased in glaucomatous aqueous humor. Mutations in *LTBP2* that encodes latent TGF-β binding protein 2 cause primary congenial glaucoma. On the other hand, many studies have confirmed oxidative stress biomarkers in glaucomatous tissues. Materials and Methods: Cultured human TM cells were treated with TGF-β2, gremlin, *LTBP2* siRNA, H2O2 and N-Acetyl-L-cysteine (NAC) + H2O2. NAC is an antioxidant that serves as negative control for oxidative stress events. Effects of all treatments on activation of TGF-β and BMP pathways and expression of several ECM related genes were evaluated by Western blot and real time PCR, respectively. **Results:** *LTBP2* knock down and oxidative stress significantly increased activation of TGF-β signaling pathway, concomitant with decreased activation of BMP pathway. TGF-β2 and gremlin serve as positive controls of these events. All treatments also increased expression of some ECM genes. **Conclusions:** These finding in cultured TM cells may exert the same effects on the TM of glaucoma patients, and these may contribute to decreased aqueous humor outflow and increased IOP.

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P13.15C

Difficulties obtaining iPSCs from scratch

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Lysosomal storage disorders (LSDs) are a group of genetic diseases characterised by lysosomal dysfunction. Some of the commonest LSDs are currently treated by enzyme replacement therapy. However, particularly in cases of advanced disease or late onset, results are discouraging. The lack of good ex vivo models hinders R&D and delays the understanding of the human pathophysiologic mechanisms. Thus, using iPSCs methods to generate the cell-targets to reproduce the disease, might help create ideal models for studying pathogenic mechanisms and to find new or more effective therapeutic strategies. iPSCs generated from somatic cells from patients are a necessary source for patient-specific studies since they maintain the patient's genetic background.

Material and Methods: Using commercially obtained skin fibroblasts, as a control, guarantees better consistency in technical conditions. In this study we used two different methods to achieve forced expression of Oct4, Sox2, Klf4 and c-Myc: a non-integrative polycistronic plasmid vector and the Sendai virus method Transformation conditions with different vehicles of delivery were tested: different reagents, concentration ratios and timings were compared. Posterior validation of cells pluripotent state is currently underway.

Results: Fibroblasts are very difficult to transform but colonies were observed at around three weeks post-transfection using plasmid DNA. The Sendai virus method proved to be easier and faster.

Aims: Currently we are generating iPSCs from human skin fibroblasts and intend to obtain a good cellular model for LSDs.

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P13.16D

Identification of sSMCs (small Supernumerary Marker Chromosomes) by array methods

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Introduction: sSMC (small supernumerary marker chromosome) is a structurally abnormal chromosome that cannot be exactly identified or characterized by classical cytogenetics (karyotyping, FISH) alone. According to definition is equal or smaller in size than a chromosome 20 of the same metaphase spread. It may be formed by non-coding heterochromatine only, but also can contain coding euchromatine and could have a deleterious effect for carriers. With respect to this fact, identification of sSMC is an important task for molecular cytogenetics.

Material and Methods: Identification of sSMCs was performed by SNP array HumanCytoSNP 12v2.1, Illumina or SurePrint G3 CGH ISCAv2, 8×6K, Agilent (GRCh37/hg19). The majority of sSMCs were originally detected by karyotyping, but two sSMCs were detected by the „first choice regime" of oligoarray examination. We present results of 10 prenatal and 11 postnatal cases.

Results: Out of 21 cases, 10 sSMCs were in mosaic form (48%). One third (33%) of all cases came from acrocentric chromosomes. For 12 (8 post- and 4 prenatal) carriers (57%) the presence of sSMC in genome were clinically

relevant. One sSMC detected in karyotype was not identified either by FISH or array. In one case an inconclusive array result was amended by FISH (chromosome Y origin).

Conclusion: It was confirmed that not only SNP array, but also oligoCGH technology is suitable for mosaic sSMC identification in case of higher percentage of aberrant cells. In the average the lowest number of genes for phenotypical abnormities was 3, the size of a duplicated region was about 3 Mb.

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P13.17A

Impaired balance of mitochondrial fission and fusion in an in-vitro model of mitochondrial unfolded protein response

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Introduction: Mitochondrial function is vital for cellular health and mitochondrial dysfunction/dysmorphology has been recognized as a key pathological hallmark of several neuromuscular disorders. In order to maintain mitochondrial quality, several control pathways that act on the molecular, organelle and cellular level have evolved. Mitochondrial fusion and fission are recognized to be an important constituent of quality control mechanisms at the organelle level. The counterbalance of these two mechanisms regulates and maintains function and distribution of organelle, as well as cellular homeostasis. However, the relationship between these mechanisms and mitochondrial unfolded protein response (UPRmt), which is activated in response to different types and levels of stress, especially in conditions where unfolded/misfolded mitochondrial proteins accumulate and aggregate, has not been investigated yet.

Materials and Methods: Key proteins involved in mitochondrial fusion (Mfn1, Mfn2, OPA1) and fission (Drp1, Fis1, Mff) were analyzed by western blot and immunofluorescence staining in C2C12 myogenic cells in which UPRmt is activated by treating cells with an appropriate non-toxic conditions of antimycin A. **Results:** UPRmt activation in myogenic cells results in a reduction in the fusion GTPase Mitofusins (Mfn1 and Mfn2) in outer membrane and long form of OPA1 (L-OPA1) in inner membrane and affects mitochondrial morphology, cellular respiratory and bioenergetic functions due to the impairment of the mitochondrial dynamics.

Conclusion: Identification of the relationship between mitochondrial quality control pathways has opened new directions for exploring mitochondrial biology and novel therapeutic targets for neuromuscular diseases related to mitochondrial failure. This study was supported by TÜBİTAK (Project no:114S876).

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P13.18B

Unexpected mosaicism for two unbalanced derivative chromosomes without normal cell line

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Mosaicism is the presence of genetically different cell lines in one individual derived from a single zygote. We well known mosaicism for a full aneuploidy or for a structural rearrangement like a marker or ring but rarely with unbalanced reciprocal translocation. In this latter case, the mosaicism involves both normal cells and cells carrying an unbalanced reciprocal translocation (Pater et al 2003, Gijsbers 2011Robberecht 2012).

We report an unique case of mosaicism of two unbalanced cell lines from a probable malsegregation from a reciprocal translocation without normal cell.

A young child was referred at 1 year of age to our hospital for isolated developmental delay. She was the third child born to healthy unrelated parents. SNPa analysis detected a terminal duplication of 34Mb of 5p13.2pter and a 90% mosaic deletion of 2.2Mb of 8p23.2pter. Chromosome analysis on cultured peripheral lymphocyte revealed a 46 chromosomes number with two cell lines: a major one with a derivative of chromosome 5 and a very smaller one with one mitosis out of 26 with a derivative of chromosome 8. FISH analysis confirmed the mosaicism state with 5% of der(8).The karyotype of our patient is :46,XX,der(5)t(5;8)(p13.2;p23.2)[47],der(8) t(5;8)(p13.2;p23.2)[3]. The parents' karyotypes were normal.

We discuss the possible mechanisms for obtaining such an unusual karyotype and review the literature.

Our case appears to be unique in showing reciprocity for two derivatives products from malsegregation of a likely (5;8) translocation.

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P13.20D

Development of harmatomas is associated with constitutive activated mTORC1 in Tuberous Sclerosis complex

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Introduction: *TSC1* and *TSC2* are genes mutated in the syndrome TSC (tuberous sclerosis complex). The gene products of *TSC1*/2 form a complex which at energy limiting states, down-regulates the activity of the regulator of protein synthesis, the mammalian target of rapamycin complex1 (mTORC1).

Materials and Methods: We describe a 3-generation family with 17 affected members, all presenting classic TSC features except renal manifestations. The index was screened for mutations in TSC1 and TSC2. The effect of the identified mutation was investigated by RT-PCR on RNA isolated from fibroblasts. Activity of mTOR was investigated in cultured fibroblast.

Results*:* The disease segregates with a silent substitution in *TSC2*, c.4149C>T, p.(Ser1838Ser), which leads to the formation of an active donor splice site, resulting in three shorter alternatively spliced transcripts with premature stop codons. A small amount of normal spliced transcript is apparently produced from the mutated allele, which might explain the milder phenotype. Interestingly, in contrast to cultured control fibroblasts, starvation of cultured patient fibroblasts obtained from lesional skin (harmatoma) did not lead to repression of mTORC1, whereas partial repression was observed in patient fibroblasts obtained from non-lesional skin.

Conclusion: The findings indicate that the development of harmatomas is associated with constitutive activated mTORC1, whereas mild deregulation of mTORC1 allows the maintenance of normal skin. Furthermore, the finding emphasizes the need for awareness when interpreting silent substitutions in general.

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P13.21A

The mechanism through which nonsense mutations are recognized as premature translation-termination codons

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About one third of the gene mutations found in human genetic disorders, including cancer, result in premature termination codons (PTCs) and the rapid degradation of their mRNAs by nonsense-mediated decay (NMD). NMD controls the quality of eukaryotic gene expression. The strength of the NMD response appears to reflect multiple determinants on a target mRNA. We have reported that human mRNAs with a PTC in close proximity to the translation initiation codon (AUG-proximal PTC), and thus, with a short open reading frame, can substantially escape NMD. Our data support a model in which cytoplasmic poly (A)-binding protein 1 (PABPC1) is brought into close proximity with an AUG-proximal PTC *via* interactions with the translation initiation complexes. This proximity of PABPC1 to the AUG-proximal PTC allows PABPC1 to interact with eRF3 with a consequent enhancement of the release reaction and repression of the NMD response. Here, we provide strong evidence that the eIF3 is involved in delivering eIF4G-associated PABPC1 into the vicinity of the AUG-proximal PTC. In addition, we dissect the biochemical interactions of the eIF3 subunits in bridging PABPC1/eIF4G complex to the 40S ribosomal subunit. Together, our data provide a framework for understanding the mechanistic details of PTC definition and mRNA translation initiation.

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P13.22B

Intronic PAH gene mutations cause a splicing defect by a novel mechanism involving U1snRNP binding

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In the phenylalanine hydroxylase (PAH) gene, responsible for phenylketonuria (PKU), one of the most common inherited diseases of amino acid metabolism, exon 11 was

recently identified as a vulnerable exon due to a weak 3′ splice site. Different exonic mutations affected exon 11 splicing by altering exonic splicing regulatory elements. In this work, we report novel intron 11 regulatory elements that are involved in exon 11 splicing, as revealed by the investigated pathogenic effect of variants c.1199+17g>a and c.1199+20g>c, identified in PKU patients. Both mutations cause exon 11 skipping in a minigene system and RNA binding assays indicate that binding of U1snRNP70 to this region is disrupted, concomitant with a slightly increased binding of inhibitors hnRNPA1/H. We have performed deletion and point mutagenesis and over-expression of adapted U1snRNP to identify critical motifs involved in the regulation of correct splicing at the natural 5′ splice site. The results indicate that U1snRNP binding at the intronic region is determinant for efficient exon 11 splicing. In this work we expand the role of intronic U1 snRNP binding as a splicing enhancer for PAH exon 11, describing a novel mechanism by which intronic mutations cause exon skipping. Notably, our results provide a rationale to explain the reported therapeutic effect of exon specific U1 snRNP (ExSpeU1) for splicing mutations in different diseases. Supported by grants from Fundación Ramón Areces (to L.R.D).

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P13.23C

Altered Expression of Perineuronal Net Elements in SMN Knockdown Cells

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Introduction: The extracellular matrix surronding the nervous system, which is called as perineuronal nets, play roles in the control of neuronal plasticity, development and function. It also has a neuroprotective property. The major components of the perineuronal net are chondroitin sulfate proteoglycans (aggrecan), hyaluronan, link proteins (HAPLN1) and tenascin-R. In some neurodegenerative disorders like Alzheimer's disease, multiple sclerosis, stroke, amyotrophic lateral sclerosis loss of perineuronal net elements and also degradation in perineuronal net morphology is detected. However there is no data available about the role of perineuronal net elements in spinal muscular atrophy, one of the most common childhood onset neurodegenerative genetic disorders.Materials and Methods: In this study we investigated the expression of perineuronal net components in an in-vitro model established by survival motor neuron (SMN) gene down-regulation by RNA interference. PC12 rat pheochromocytoma cell line was differentiated into neurons and was transfected with 50 pmol SMN siRNA (Ambion). Aggrecan, HAPLN1, tenascin-R mRNA and protein levels were analyzed by qPCR and Western blot.

Results: PC12 cell lines was differentiated and at day 3, HAPLN1 mRNA levels increased 2 fold whereas HAPLN1 protein levels showed 50% decrease in SMN depleted cells. TNR and ACAN mRNA/ protein levels did not change compared to negative control siRNA.

Conclusions: Detecting perineuronal net loss and degradation also in SMA mouse models, will clarify novel candidate proteins and pathways. Identification of molecules that target perineuronal net will help to discover novel therapeutic approaches. (TUBITAK 114S914)

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P13.24D

Identification of *NIN* missense mutations in a boy with Seckel syndrome

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Microcephalic primordial dwarfism (MPD), a group of disorders characterized by prenatal growth retardation with microcephaly, includes Seckel syndrome, microcephalic osetodysplastic primordial dwarfism, and Meier-Gorlin syndrome. Seckel syndrome is clinically unique from other MPDs due to the presence of developmental delays and intellectual disability. MPDs are known to be associated with mutations in genes important for DNA replication and

cell cycle regulation. Here we report a boy with MPD, consistent with Seckel syndrome, who was found to have compound heterozygous *NIN* mutations by whole exome sequencing, the second case with a MPD phenotype.The patient was the first child of a healthy 26-year-old non-consanguineous parents without relevant family history of genetic disorders or birth defects. Severe intrauterine growth retardation was first noted around 25 weeks of gestation. He was born at 39 weeks, 4 days of gestation. His birth weight was 1444 g (−4.9 SD), length was 35.0cm (−7.6 SD), and occipital-frontal circumference (OFC) was 26.5 cm (−5.2SD). At birth, severe microcephaly, severe growth retardation, micrognathia and facial dysmorphisms (narrow forehead, hypertelorism, down-slanting palpebral fissures, epicanthus, and prominent nose) were noted. We performed whole exome sequencing for the patient with MPD, phenotypically resembling Seckel syndrome, and found compound heterozygous *NIN* mutations, the second reported case of MPD due to mutations in this gene. *NIN* encodes the protein ninein, which is involved in microtubule anchoring and centrosomal structure, and was recently proposed as a novel gene related to MPD. Our report further supports the notion that *NIN* mutations cause MPD phenotype.

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P13.25A

Differences in the *BMPR2* gene expression in B Lymphocytes inherent to Epstein-Barr virus immortalization

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BMPR2, whose expression is describes as ubiquitous, is the main gene implied in Pulmonary Arterial Hypertension (PAH), a rare disease characterized by the increase of vascular resistance. The aim was to functionally characterize the c.419-43delT mutation in *BMPR2*, present in 18% of the patients analyzed.

Minigene assay for mutant sequence and wild type was performed using the pSPL3 vector in order to detect alterations in the splicing process. Peripheral blood samples were drawn from patients (with deletion, without it and controls) and B lymphocytes were subsequently isolated and cultured. Later, immortalization using Epstein-Barr Virus was performed on them. The immortalization process was confirmed by flow cytometry. Lymphoblastoids were then separated according to their cell cycle phase by cell sorting. *BMPR2* gene expression was analyzed by qPCR using commercial Taqman® assays.

No splicing alterations were detected. *BMPR2* expression was not detected in B lymphocytes from peripheral blood, neither after its culture, even though control genes (*GADPH* and *ACTB*) were detected. After immortalization, *BMPR2* and control genes expression were detected. Normalized expression showed increased expression in phase S between patients and controls, and a decrease in phase G_2/M. Patients without deletion showed intermediate values.

BMPR2 expression in lymphoblastoids may be triggered by the immortalization process. Expression differences in *BMPR2* between patients with deletion, without it and controls, suggest that this mutation may influence in its expression regulation. Although, further studies will be needed to corroborate this hypothesis.

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P13.26B

Recurrent 10q11.2 deletions unmasking *CHAT* or *SLC18A3* recessive mutations can lead to congenital myasthenic syndrome

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Recurrent deletions localized in 10q11.21-q21.1 have been associated with variable phenotypes, and can be found in asymptomatic individuals as well as in patients showing intellectual disability, sometimes associated with various symptoms (hypotonia, sleep apnea, chronic constipation,

epilepsy, cerebellar syndrome, dysphagia or ptosis), thus suggesting the role of modifying genetic factors.

Several genes are contained in this chromosomal region, such as the Choline Acetyltransferase gene (*CHAT; MIM# 118490)*, which catalyzes pre-synaptic synthesis of acetylcholine (ACh). Interestingly, *CHATs* first intron contains a small gene, *SLC18A3*, which encodes the highly conserved vesicular ACh transporter (VAChT). Compound heterozygous or homozygous *CHAT* mutations have been identified in patients suffering from congenital myasthenic syndrome (CMS) with episodic apnea. These patients are at risk of lethal episodes of acute respiratory distress and bulbar weakness. These life-threatening events can be reduced with a specific treatment.

Recently, recessive mutations of *SLC18A3* have been involved in a similar congenital myasthenic syndrome.

Here, we report cases of two unrelated children carrying a 10q11.2 deletion and who displayed signs of CMS with episodic apnea. In both cases, the 10q11.2 deletion unmasked a recessive mutation, in *CHAT* gene for patient 1 and in *SLC18A3* gene for patient 2.

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P13.27C

Somatic reversion assists in identification of *RPL4* as a novel disease gene for a condition resembling Diamond Blackfan Anemia

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Revertant mosaicism is caused by the spontaneous correction of a mutation followed by clonal expansion of the revertant cell, resulting in diminished or altered disease symptoms. A cause of reversion in dominant disorders is mitotic recombination, recognized by aquired uniparental disomy (UPD). We report about a girl with intellectual disability, short stature, a cleft lip, hearing loss and toe anomalies. SNP array analysis on blood DNA revealed mosaic 15q22-tel UPD. We hypothesized that this clonal UPD resulted from selective advantage of the homozygous 15q cells. Indeed analysis on DNA taken on earlier and later timepoints revealed a shift from normal heterozygosity patterns towards complete 15q homozygosity. We confirmed that this UPD is confined to blood by testing muscle DNA. The fact that UPD of 15q provoked a selective advantage to blood cells suggests the presence of a haematological condition, caused by a mutation distal from the most telomeric recombination breakpoint. Indeed the girl had transient anemia early in life. We performed whole exome sequencing on muscle DNA and identified a *de novo* c.176-7A>G mutation in *RPL4* on 15q22.31 (r.175_176insUUUUAG; p.(Ala58_Gly59insValLeu)). *RPL4* fits in the list of ribosomal protein genes causative for Diamond Blackfan anemia, and the features of this condition indeed overlap with the phenotype in our patient. Remarkably, in 20% of patients with DBA anemia goes in remission. In conclusion, we report mitotic recombination-based somatic reversion in a girl with a Diamond Blackfan anemia-like phenotype, which directed us to the identifation of *RPL4* as a novel DBA-like disease gene.

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P13.29A

Single-cell RNA-seq reveals distinct dynamic behavior of sex chromosomes during human early embryogenesis

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Introduction: In mammals, various studies have been focused on the differences in sex-chromosome constitution between males (XY) and females (XX). However, limited data exists to investigate whether sex differences are already present during human early embryogenesis.

Methods: We performed global expression analysis between males and females using RNA-sequencing data of 1644 individual cells from 117 human preimplantation embryos, covering development stages from zygote to late blastocyst.

Results: Consistent chromosome-wide transcriptomic level of autosomes was observed in all stages, while sex chromosomes showed significant differences after embryonic genome activation (EGA). The distinction of Y chromosome was due to specific genes, *RPS4Y1* and *DDX3Y*, with abundant expression in all male cells. For X chromosomes in female, over one hundred significantly higher expressed genes were defined in 8-cell stage and both alleles of most heterozygous sites could be detected in our allelic analysis. Contrary to constant pattern of Y, their expression distinctly dropped at late blastocyst stage,

especially in trophectoderm cells, revealing the exact time point and details of dosage compensation.

Conclusion: We conclude that sex chromosomes are activated immediately after EGA, for specific genes and broad region of Y and X chromosome, respectively, with a following dosage compensation firstly occurring in female trophectoderm cells. The two Y-linked genes we find might be novel expression markers to distinguish gender of early embryos for clinical trial. We predict broad utility of such dynamic behavior of sex chromosomes in future studies on human development and sex-specific disease.

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P13.30B

Deep phenotyping and exome sequencing in patients with short stature

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In short stature the underlying cause often remains elusive due its heterogeneity. We deep phenotyped 565 patients and randomly selected 200 for whole exome sequencing. Using WES we identified mutations in 27 known short stature associated genes in 17% of patients with only part of the symptomatology precluding an early clinical diagnosis. Heterozygous carriers of recessive skeletal dysplasia alleles (*ACAN*, *NPR2*) were a frequent cause of idiopathic short stature (3.5%). 54 % of the affected proteins are involved in the main functional categories cartilage formation, chromatin modification and Ras-MAPK signaling. We identified 37 further strong candidate genes, of which seven had deleterious mutations in at least two families. 48% of these candidate genes are involved in the 10 main functional categories already identified for the known short stature associated genes further supporting their pathogenicity. Finally, in 16% of the 200 sequenced individuals our findings were of significant clinical relevance regarding preventive measures, symptomatic or even targeted treatment. Besides evaluation for orthopedic or developmental issues especially screening for neoplasias (*TRIM37*, *PTPN11*, *NF1*), symptomatic treatment for chronic kidney disease (*CLCN5*) and targeted treatment for severe hypertension (*PDE3A*) were of clinical relevance for the affected individuals. These results demonstrated that deep phenotyping combined with targeted genetic testing and whole exome sequencing is able to increase the diagnostic yield in short stature up to 31% with concomitant improvement in treatment and prevention. Rigorous variant analysis considering phenotypic data further led us to the identification of further 37 probable novel candidate genes.

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P13.31C

Genomic and biochemical characterization of sialic acid acetyl esterase (siae) in zebrafish

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Sialic acid acetyl esterase (SIAE) removes acetyl moieties from the carbon 9 and 4 hydroxyl groups of sialic acid and recently a debate has been opened on its association to autoimmunity. Trying to get new insights on this intriguing enzyme we have studied siae in zebrafish (*Danio rerio*). In

this teleost *siae* encodes for a polypeptide with a high degree of sequence identity to human and mouse counterparts. Zebrafish siae behaviour upon transient expression in COS7 cells is comparable to human enzyme concerning pH optimum of enzyme activity, subcellular localization and glycosylation. In addition, and as already observed in case of human SIAE, the glycosylated form of the enzyme from zebrafish is released into the culture media. During embryogenesis, *in situ* hybridization experiments demonstrate that *siae* transcript is always detectable during development, with a more specific expression in the central nervous system, in pronephric ducts and liver in the more advanced stages of the embryo development. In adult fish *siae* mRNA is mainly detectable in muscle, ovary and brain. These results provide novel information about siae and point out zebrafish as animal model to better understand the biological role of this rather puzzling enzyme in vertebrates, regarding immune system function and the development of central nervous system.

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P13.32D

Enhancer swapping: dual loss and gains of enhancers by balanced translocations and inversions between highly conserved regulatory domains

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Introduction: Balanced chromosomal translocations/inversions (BCR) may cause disease by long range position effects (LRPE) due to rearrangements of regulatory landscapes. One consequence of this may be enhancer adoption, where the ectopic action of an enhancer dysregulates another gene. We have defined >400 Topological Domains (TAD) and TAD clusters enriched for evolutionary conserved non-genic elements (CNEs) as high-risk candidates for LRPE. These CNE-TADs might also be high-risk regions for enhancer adoption and even more complex regulatory disturbances.

Materials and Methods: We compared published cases of enhancer adoption with CNE-TADs, and looked for double involvement of CNE-TADs in published and unpublished affected and healthy carriers of two-way BCRs. We exemplify the deleterious nature of this by a 46,XX,t(2;13)(p25;q32)dn associated with severe intellectual disability/microcephaly, analysed by mate-pair sequencing and 4C-Seq analysis.

Results: Ten/eleven cases with proven enhancer adoption involved one or two CNE-TADs. Furthermore, 13/283 affected BCR carriers (4.6%) had breakpoints involving two CNE-TADs, vs. 0/125 healthy BCR carriers (p=0.012). The t(2;13)-breakpoints were located within the regulatory domains of *SOX*11 on chr2, and *SOX21* on chr13. 4C-seq analysis of t(2;13)-fibroblasts showed ectopic interactions between the *SOX11* promoter and the translocated *SOX21*-domain segment, and between the *SOX21* promoter and the translocated *SOX11*-domain segment.

Conclusion: CNE-TADs are high-risk domains for enhancer adoption events, and dual exchanges between CNE-TADs are high-risk mutations. We term this enhancer swapping, due to the simultaneous removal of CNEs and gain of ectopic CNEs in both domains, with potentially unpredictable phenotypic consequences.

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P13.33A

Interruption of the AG-exclusion zone is the major mechanisms of 90 non-canonical NF1 3′ splice-site mutations: lessons to learn for intronic unclassified variants

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Introduction: Two thirds of the NF1 splice mutations do not involve the canonical dinucleotides GT-AG of the splice sites. Hence, this gene is highly suited to elucidate mechanisms of action of non-canonical splice mutations.

Methods and Results: Through a series of mini-gene experiments we studied the mode of action of NF1:c.1722-11T>G, and found that this pyrimidine (Y) to purine (R) transversion, although expected to weaken the polypyrimidine tract (PPT), caused exon skipping primarily by introducing a novel AG between the natural 3′-splice site and the branch point, interrupting the AG-exclusion zone (AGEZ). Evaluating 90 non-canonical intronic NF1 3′-splice-site mutations, with known effect on splicing through

RNA analysis, showed that 49/55 single-nucleotide variants located between positions -35 and -4 created a novel AG dinucleotide in the AGEZ. Two further motifs identified in this cohort were: Y>R transversion at position -3 and removal of ≥2 Ys in the AGEZ. 90% (81/90) of the non-canonical NF1 3′-splice-site mutations follow one of these three motifs. To test whether the three identified motifs can distinguish spliceogenic from splice-neutral variants also in different genes, we analyzed a cohort of 49 BRCA1/2 variants, with already known splicing outcome. The predictions in this cohort reached 85.7% accuracy with 78% positive and 97.5% negative predictive values.

Conclusions: Comparing this three-motifs strategy to a previously suggested strategy using splice-site prediction tools indicated a comparable sensitivity with the three-motifs strategy however offering the advantage of being applicable also to variants located at further distance from the natural 3′ splice site.

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P13.34B

Correction of *IKBKAP* exon 20 splicing by splice switching oligonucleotides

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Introduction: Familial dysautonomia (FD) is a severe recessive disease affecting the sensory and autonomic nervous system. FD is frequent in Ashkenazi Jews with an estimated carrier frequency of 1/30. Almost all patients with FD are homozygous for an IVS20+6T>C mutation in *IKBKAP*, which decreases the 5′ splice site strength of exon 20 thereby causing exon 20 skipping and an NMD-sensitive transcript. Correct splicing of exons with weak splice sites depends on a tight balance between positive and negative splicing regulatory elements (SREs), which are bound by various splicing regulatory proteins (SRPs). **Results:** Previously we identified in vivo binding sites for the major negative SRP hnRNP A1 by iCLIP. An hnRNP A1 binding site in *IKBKAP* intron 20 close to the 5′ splice site of exon 20 was identified, and the binding of hnRNP A1 to this site was validated by in vitro studies. Furthermore, knockdown of hnRNP A1 in FD patient fibroblasts suggested that hnRNP A1 represses the inclusion of *IKBKAP* exon 20. Therefore, we designed a splice switching oligonucleotide (SSO) blocking the hnRNP A1 binding site in intron 20. Transfection of this SSO into FD patient fibroblasts fully restores *IKBKAP* exon 20 splicing and increases the amount of full-length IKBKAP transcript. **Conclusion:** SSO-based blocking of hnRNP A1 binding may thus be a promising approach for future treatment of FD. Funding: The Novo Nordisk Foundation and Natur og Univers, Det Frie Forskningsråd

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P13.35C

Functional analysis of splicing-affecting genomic variants in hereditary diseases

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Introduction: NGS technology greatly promoted the efficiency of DNA diagnostics of hereditary diseases and understanding their pathogenesis. However, the functional interpretation of the pathogenicity of genomic variants is a very difficult task. Splicing-affecting genomic variants are one of the interesting cases that require functional analysis.

Materials and methods: Functional analysis of splicing mutations was performed by two approaches. If gene of interest is expressed in blood, we used RT-PCR from patient's blood samples. If gene of interest is not expressed in blood, we used expression system in model cell line HEK293N. For this aim we constructed expression vectors containing the studied genome region with or without analyzed genomic variant. The obtained plasmids were transfected into HEK293N cell line. After RNA isolation and cDNA synthesis, we carried out expression analysis of target gene isoforms.

Results: For intron variant (c.732+4A>G) in *B4GALT7* gene in patient with Ehlers-Danlos syndrome we used RT-PCR from blood sample and showed isoform with exon skipping. For dozen of genome variants in SPTB, PAX6 and MYH7 genes in patients with spherocytosis, congenital aniridia, early-onset myopathy respectively we used

expression system and showed their pathogenicity which leads to different splicing scenario.

Conclusions: Splicing-affecting genomic variants may occur in exonic or intronic splicing regulatory elements. Prediction of pathogenicity of these mutations is difficult due to an incomplete understanding of the multiple factors that may be responsible. Functional analysis allows us not only to establish their pathogenicity, but also helps to understand the mechanisms of splicing of investigated genes.

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P13.36D

SRSF10 effects splicing of *SMN2* exon 7

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Introduction: Isoform specificity within the SR protein family is poorly understood. The unusual splicing regulatory factor SRSF10 is highly expressed in neuronal tissue. However, there are two isoforms of SRSF10, which could potentially have different targets or different effects.

Results: Using iTRAQ MS/MS, we found that both isoforms of SRSF10 bind to the ISS-N1 splicing regulatory motif in SMN. ISS-N1 has previously been shown to bind the ubiquitously expressed splicing repressor hnRNPA1. Protein binding to ISS-N1 is currently being blocked by an antisense oligonucleotide in the recently approved and very successful treatment of Spinal Muscular Atrophy (SMA). We show that only the long isoform of SRSF10 is able to repress splicing of *SMN2* exon 7. We hypothesize that SRSF10 could contribute to SMA pathogenesis by repressing the inclusion of *SMN2* exon 7 in neuronal tissue. Furthermore, we demonstrate that splice shifting oligonucleotides can be used to switch the ratio between the long and the short isoform of SRSF10 making it possible to investigate the role of each isoform independently. We have performed RNA-seq analysis to investigate isoform specific regulation of alternative splicing. This showed that the long SRSF10 isoform regulates the largest number of events, but that there are both common and unique targets for the two SRSF10 isoforms.

Conclusions: We conclude that alternative splicing of SRSF10 produces functionally different isoforms and that this is important to consider when investigating its role in alternative splicing.

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P13.37A

Unravelling structural chromosomal rearrangements by whole genome sequencing: results of the ANI project, a French collaborative study including 55 patients with intellectual disability and/or congenital malformations

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Introduction: Apparently balanced chromosomal rearrangements (ABCR) associated with abnormal phenotype are rare events, but may be challenging for genetic counseling. Abnormal phenotype may be explained either by cryptic genomic imbalances detectable by array-CGH or by gene disruption or position effect. However, breakpoint cloning using conventional methods is laborious and not performed routinely. Recently, Whole Genome Sequencing (WGS) proved to be a powerful and rapid technique to characterize ABCR breakpoints at the molecular level.

Material and methods: The ANI project is a French collaborative study that aims at characterizing ABCR in patients presenting with intellectual disability and/or congenital anomalies. We included 55 patients (41 reciprocal translocations, 4 inversions, 2 insertions, 8 complex chromosomal rearrangements). Array-CGH showed no pathogenic imbalance. Breakpoints were characterized by paired-end WGS and confirmed by Sanger sequencing. Expression studies of disrupted and neighboring genes were performed on blood cells.

Results: At this time, 45 patients were analyzed by WGS. Breakpoints were characterized for 39 patients (86%). The rearrangements showed unexpected complexity, since 186 breakpoints were identified against 97 breakpoints according to karyotype and included 6 cases of chromoanagenesis. Sixty-seven breakpoints disrupted a gene (36%). In 14/39 patients, WGS allowed a diagnosis, either by gene disruption (11) or by position effect (3), thus a diagnostic rate of 35%.

Conclusion: These preliminary results showed the diagnostic relevance of this approach. We will present the complete results of this study, including molecular characteristics of the breakpoints and expression studies.

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P13.38B

Peptide drug Semax affects neurotransmitter systems in the rat brain in conditions of transient focal ischemia

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Introduction: Synthetic peptide Semax, consisting of a fragment ACTH(4–7) (Met-Glu-His-Phe) and C-terminal tripeptide Pro-Gly-Pro, has established itself as an effective drug for the treatment of patients with severe disorders of cerebral circulation. Nevertheless, many aspects of its neuroprotective effect remain unclear. To elucidate the molecular mechanisms of peptide action we used the analysis of the brain cells' transcriptome in the experimental ischemia model.

Materials and Methods: The model of transient focal ischemia in rats, high-throughput RNA sequencing (RNA-Seq), real-time RT-PCR, bioinformatics.

Results: Using RNA-Seq, the genes with statistically significant change of mRNA level were identified in the subcortical brain structures of ischemic rats treated with Semax 24 h after occlusion. The functional annotation of these differentially expressed genes using DAVID bioinformatic resource has allowed to identify groups of genes, proteins of which participate in the work of GABAergic, Cholinergic, Dopaminergic, Glutamatergic, Serotoninergic synapses, and in Retrograde endocannabinoid signaling. A group of Neuroactive ligand-receptor interaction was made up by the genes, most of which encode membrane receptors, including cholinergic (Chrm1; Chrm4; Chrna3), dopamine (Drd1; Drd2), GABA type A (Gabrb1, Gabra2; Gabra5), and glutamate ionotropic and metabotropic (Gria3; Grin2b; Grik5; Grm3 and Grm5).

Conclusions: In the subcortical structures of rat brains damaged by ischemia, Semax has effects on the regulation of neurotransmitter systems that agrees with nootropic properties of peptide and efficient recovery of cognitive functions in patients with stroke.

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P13.39C

Segmental uniparental disomy of chromosome 1 causing Leber congenital amaurosis and hereditary nonspherocytic hemolytic anemia

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Introduction: The severe generalized retinal dystrophy, Leber congenital amaurosis (LCA) and the hereditary nonspherocytic hemolytic anemia (HNSHA) caused by pyruvate kinase deficiency are two autosomal recessive disorders caused by mutations in the RPE65 and PKLR gene both located on chromosome 1.

Methods: Here we used Whole exome sequencing (WES) to unravel the underlying genetic cause in a 17 year old boy suffering from both LCA and HNSHA. The retinal dystrophy was clinically characterized by ophthalmological examinations, fundus imaging, Goldmann perimetry and electroretinogram (ERG). The clinical diagnosis of HNSHA was established by a broad spectrum of metabolic and hematologic laboratory tests.

Results: Clinically the boy had suffered from severe haemolytic anemia from birth and received a bone-marrow transplant from his father at the age of 4 years. His generalized retinal dystrophy was characterized by a progressive loss of rod and cone photoreceptors leading to night blindness, reduced visual acuity and a progressive visual field loss. The boy was born to consanguineous parents. Using WES, we identified known homozygous pathogenic variants in the RPE65 and PKLR genes, shown to be caused by paternal segmental uniparental disomy of chromosome 1 (UPD1).

Conclusions: In a young boy with both LCA and HNSHA we found segmental UPD1 that resulted in homozygosity of two recessive mutations. The detection of RPE65 mutations might have a therapeutic consequence, since this gene is the subject of several promising clinical treatment trials.

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P14 New diagnostic approaches, technical aspects & quality control

P14.001A

A next-generation sequencing approach targeting the highly repetitive ORF15 region of RPGR improves molecular diagnostics of X-linked retinitis pigmentosa

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Purpose: Retinitis Pigmentosa (RP) represents the most frequent inherited retinal dystrophy, with a prevalence of ~1 in 4000. The most severe form of RP, X-linked RP, can be explained by mutations in the *RPGR* and *RP2* genes, accounting for 75–80% and 20% of XLRP cases, respectively. *RPGR* contains a large exon, *RPGR* open reading frame 15 (ORF15), harboring 60% of the *RPGR* mutations. Because of its highly repetitive and purine-rich regions, conventional Sanger and next-generation sequencing (NGS) of ORF15 is challenging and difficult to implement in a clinical context. Here, it was our goal to develop a NGS-based test targeting the XLRP genes, including the highly repetitive and purine-rich regions of ORF15. Methods and results: To improve the coverage depth of ORF15, we used a customized targeted resequencing workflow, based on an optimized singleplex PCR for enrichment of ORF15 with in house designed primers, followed by a modified Nextera XT protocol for the library preparation, and NGS on a MiSeq instrument, as described by De Leeneer et al. (2015). This allowed us to identify novel sequence variations in ORF15, which could be validated by Sanger sequencing.

Conclusions: We propose an optimized test targeting low-depth regions such as ORF15, without the need for complementary approaches such as Sanger sequencing. As ORF15 represents a mutational hotspot, this will improve molecular diagnostics of XLRP. Finally, our approach can likely be extrapolated to other repeat-rich regions of Mendelian disease genes.

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P14.002B

Tracing the dark matter: investigating the prevalence of exonic copy number and structural variants in Mendelian disorders

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Exonic copy number variants and large structural variants (CNVs/SVs) contribute to disease but their prevalence is not well understood. We applied a next-generation sequencing method to simultaneously detect single nucleotide variants or small indels (SNVs) as well as intragenic CNVs/SVs in a large population undergoing clinical testing for hereditary cancer, cardiac, neurological, or pediatric disorders. From testing subsets of 981 genes in over 58,000 unrelated individuals (equivalent of 1.6 million single gene tests), we identified 1050 CNVs/SVs, or large indels in 386 genes. These included 606 deletions, 373 duplications, and 71 other large variants, representing 591 unique variants. While CNVs/SVs were observed in 1.3% of all patients and accounted for 2.6% of all types of reported events, they represented 7.8% of variants in patients with any clinically significant finding. 95% of deletions were pathogenic whereas only 43% of duplications were. Nearly a quarter of unique CNVs encompassed an entire gene and some were likely larger cytogenetic variants. CNVs/SVs accounted for 6.8% of pathogenic findings in pediatric and rare disorders, 26% in neurological disorders, 4.6% in cardiac disorders, and 8.1% in cancer disorders. CNVs/SVs variants were frequently (71.8%) pathogenic, at a rate far greater than SNVs. In 67 individuals a CNV was compound heterozygous with an SNV, and in 26 individuals the two variants constituted a molecular diagnosis for a recessive disorder. Our data suggest that universal exon-level CNV testing is valuable in genetic testing, and provides clinicians a better view into CNV/SV prevalence and their disproportionate frequency among pathogenic variants.

S. Aradhya: A. Employment (full or part-time); Significant; Invitae. **R. Truty:** A. Employment (full or part-time); Significant; Invitae. **J. Paul:** A. Employment (full or part-time); Significant; Invitae. **M. Kennemer:** A. Employment (full or part-time); Significant; Invitae. **E. Gafni:** A. Employment (full or part-time); Significant; Invitae. **S. Fay:** A. Employment (full or part-time); Significant; Invitae. **S. Lincoln:** A. Employment (full or part-time); Significant; Invitae. **R. Nussbaum:** A. Employment (full or part-time); Significant; Invitae.

P14.003C

Next generation phenotyping in Emanuel and Pallister Killian syndrome using computer-aided facial dysmorphology analysis of 2D photos

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Forms of Next-Generation-Phenotyping (NGP) are needed to increase further the value of traditional and high throughput genetic diagnostics. As NGP we used in this study the Facial-Dysmorphology-Novel-Analysis (FDNA) technology to automatically identify facial phenotypes associated with Emanuel (ES) and Pallister-Killian Syndrome (PKS) from 2D facial photographs. ES and PKS have in common that they are characterized both cytogenetically by a small supernumerary marker chromosome (sSMC). 81 frontal images of children with molecularly diagnosed ES and 92 images from PKS were analyzed and compared to 2 control groups: facial images of unaffected children ($n = 1{,}000$) and of children affected with one of 100 other syndromes characterized by dysmorphic facial phenotypes ($n = 1{,}000$) collected from public image collections and medical publications. A comparison between ES or PKS and normal individuals expressed a full separation between these cohorts. A slightly lower discrimination was possible when comparing between ES or PKS and individuals affected with other syndromes. Applying the FDNA technology we were able to choose the correct syndrome with a mean accuracy of 89.6%. This result is more than 3 times higher than the random chance of 25%. Our results show that computer aided facial recognition is able to help in the clinic and could possibly reduce the time patients spent in the diagnostic odyssey. It may also help differentiate ES or PKS from other patients with sSMC, especially in countries with no access to more sophisticated genetic approaches apart from banding cytogenetics. The continuous support of Nicole Fleischer (FDNA) is kindly acknowledged.

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P14.004D

A rapid genotyping research assay for ABO RhD blood types

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Bone marrow registries require high resolution HLA genotyping data to match donor specimens with their recipients. For optimal outcome, it is also preferable to use bone marrow of identical or compatible blood types between donor and recipient. To aid research in this area, we have developed an assay that utilizes buccal swab DNA from potential donors to determine the ABO and Rh-antigen genotypes. The assay also detects a 32 bp deletion in the CCR5 gene. Homozygous carriers of the deletion are resistant to HIV-1 infection, and thus could be valuable stem cell donors for HIV-infected recipients. This research assay is based on a multiplex-PCR reaction with 5 fluorescently-labeled and 12 allele-specific primers followed by capillary electrophoresis on an Applied Biosytems Genetic Analyzer. The primers for the human ABO gene target 4 SNPs allowing the determination of the basic A, A2, B, O1,2 and O3 alleles. Rh-antigen genotyping is determined by targeting a small deletion that differs between the RhD and RhCE genes. The peak pattern is analyzed by GeneMapper® software, and the resulting peak/genotype table is translated into a genotype/phenotype report using a standard spreadsheet. The assay was verified by analyzing a panel of DNA samples with known blood group antigens and CCR5 gene types, and found to be 100% accurate. This easy to use, rapid research assay may prove useful for future development of bone marrow donor identification assays, and other areas of public health studies. For research use only. Not for use in diagnostic procedures.

E. Schreiber: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Higdon:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **T. Nong:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **J. Lee:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific.

P14.005A

Easy diagnosis of Farber disease in dried blood spots using a specific biomarker confirmed by ASAH1 gene sequencing

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Farber disease is an autosomal recessive, extremely rare disease caused and characterized by a deficient acid ceramidase activity encoded by ASAH1 gene. Low ceramidase activity is resulting in accumulation of fatty substances, mainly ceramides. At clinical level, Farber disease is manifesting through hallmark symptoms such as: periarticular nodules, lipogranulomas, swollen and painful joints and a hoarse voice or a weak cry; in addition to these, also hepatosplenomegaly, rapid neurological deterioration or developmental delay are reported. Seven different Farber types were described, with phenotypes varying from mild cases with a longer life expectancy to very severe cases, where the patients do not survive past their first year of life. We present here a new method of diagnosis of Farber disease by determining the concentration of C26 ceramide isoforms using LC/MRM-MS and C25 ceramide as internal standard followed by ASAH1 gene sequencing. We can demonstrate that cis-isomer of the C26 ceramide is a specific biomarker for Farber disease, with pathological values in a range of 39.2–150.0 nmol/L blood (normal range 13.6–23.4 nmol/L blood, N = 192, healthy individuals). The new biomarker can be determined directly in the dried blood spot extract with low sample consumption, easy sample preparation, high reproducibility and it presents the possibility of being used in high throughput screenings.

M. Iurascu: A. Employment (full or part-time); Significant; Centogene AG. **C. Cozma:** A. Employment (full or part-time); Significant; Centogene AG. **S. Zielke:** None. **J. Lukas:** None. **A. Rolfs:** A. Employment (full or part-time); Significant; Centogene AG.

P14.006B

Comparison of blood microbiome using 16S rRNA amplicon and unmapped reads from WGS

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Introduction: Blood has been generally considered a sterile environment, however, microbiome has been consistently detected in blood in healthy individuals. Identifying the microbiome composition from blood affords opportunity to study the causative relationships between the host microbiome and disease. However, this is challenging due the low abundance of microbial DNA relative to the host. Materials and Methods: The study was conducted with blood DNA of

200 healthy individuals. We performed a whole genome sequencing (WGS) of 30x coverage as well as a 16S rRNA gene sequencing (16S) and quantitative polymerase chain reaction (qPCR) assay to analyze the blood microbiome. About 99% of whole genome sequencing were mapped to the human reference genome. The remaining unmapped reads are then assigned to taxonomic labels.

Results: We observed differences in the relative abundances of blood microbiome in between 16S and WGS. The Proteobacteria was the most abundant phylum in 16S and WGS, but the abundance was <50% and >95% in 16S and WGS, respectively. After the Proteobacteria, the Actinobacteria phylum showed relative abundance of >2.5% in WGS, while it was rare in 16S. The quantification by qPCR assay did not prove the relative abundance of 16S nor WGS.

Conclusions: The blood microbiome, even when present in very low abundance, may have important physiologic role. Proving existence of microbiome in healthy blood could be implicated in non-infectious diseases. Grants: NRF-2016R1A6A3A11932719, NRF-2014R1A2A2A04006291, HI14C1234, and intramural research support program of Ewha Womans University School of Medicine. It was also supported by GSDC and KREONET in KISTI.

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P14.008D

Integrated diagnostic solution for routine testing of mutations in *BRCA1* and *BRCA2* with the BRCA MASTR plus Dx assay

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Targeted NGS has tremendous potential in clinical diagnostics. Tumor tissue oncogenetic profiling allows to steer targeted therapy. In addition, NGS has been implemented in routine testing for germline mutations causing hereditary cancers. To address this dual intended use within clinical setting, Multiplicom's BRCA MASTR Plus Dx assay has been validated for the identification of germline mutations in blood derived DNA and of somatic mutations in formalin fixed paraffin embedded (FFPE)-derived tumor DNA. Despite many benefits, NGS poses challenges such as the proper, assay specific bioinformatics analysis of raw NGS data. Multiplicom now offers an integrated diagnostic solution by adding its proprietary MASTR Reporter software as data analysis to the diagnostic workflow. Next to SNVs, the software permits reliable CNV identification. MASTR Reporter not only provides high standard data analysis, but also a unique Quality Control dashboard, allowing customers to monitor their sequencing workflow in real time. BRCA MASTR Plus Dx on blood derived DNA in combination with Illumina sequencing (MiSeq, MiniSeq, NextSeq) and MASTR Reporter software showed uniformity of amplification of 100% and target specificity of 97.7%. Diagnostic accuracy was 100% [95% CI ≥99.99%] (100% sensitivity [95% CI ≥99.12%], 100% specificity [95% CI ≥99.99%]). Both repeatability and reproducibility were 100% [95% CI ≥99.99%]. Summarized, the BRCA MASTR Plus Dx solution, including the integrated MASTR Reporter software, can be routinely applied in identifying germline mutations in *BRCA1/2* causative for hereditary breast and ovarian cancer.

Products described above are CE-IVD and are not available for sale in the US and other geographies.

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P14.009A

Evaluation of methods for BRCA1 and BRCA2 mutation screening of FFPE-DNA

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Background: Mutation screening of *BRCA1* and *BRCA2* has recently gained importance as germline and somatic BRCA mutations are now treatment predictive for PARP-inhibitors in patients suffering from recurrent ovarian cancer. DNA from formalin-fixed, paraffin-embedded (FFPE) is often the only material available for screening. This brings additional challenges as lower DNA quantity and quality can introduce artifacts and allelic drop-outs, besides the general difficulties of detecting somatic low frequency variants.

Methods: We evaluated two methods and three designs, all including *BRCA1* and *BRCA2* as targets, on DNA from FFPE. Illumina TruSeq Amplicon (TSA) is a strand-specific amplicon based method. Both TSA designs were dual-stranded (separate assays targeting each strand). The first included only *BRCA1* and *BRCA2* (design optimized by Illumina). The other was a custom 6-gene design. Qiagen Generead DNAseq Targeted Panel v2 is based on multiplex-PCR. The design included only *BRCA1* and *BRCA2*. All kits include a qPCR to evaluate the DNA

quality and identify potential failing samples. Libraries from 10 FFPE-DNA samples were prepared using each kit and sequenced on the Illumina Miseq. Evaluated parameters include design (theoretical coverage and amplicon size), coverage (% on target, uniformity and drop-outs) and variant sensitivity and specificity.

Results and conclusion: All kits perform well in terms of coverage and uniformity. The dual-stranded design of the TSA kits reduces the number of FFPE artifacts. The Generead kit targets complete coding region and splice sites of *BRCA1* and *BRCA2*.

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P14.010B

Comparison of buccal specimens collected with FLOQSwabs™ to saliva and blood for genetic investigations

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Introduction: Buccal swab collection is not invasive, easy to use, less expensive and better accepted by donors with respect to blood and saliva sample collection. Copan is producing human DNA free FLOQSwabs™(FS), certified human DNA, DNase and RNase free. The objective of this study was to compare quantity and quality of human DNA (hDNA) obtained FS to saliva in Oragene (OR), ORAcollect (OC) by DNA Genotek, and blood.Materials and Methods: FS, OR and OC from 20 volunteers were collected. FS were stored in dry tubes, while saliva was collected in OR and OC collection devices+medium. Blood samples were collected from 2 donors. DNA was extracted from 200 ul of each sample type using QIAamp® DNA Mini Kit. hDNA was quantified by PCR (Quantifiler® Trio kit), Quality was measure by agarose gel electrophoresis and by NanoDrop. (Thermo Fisher). **Results:** FS gave an average of 5000 ng hDNA/swab (lowest/ highest concentration: 1200ng/ 13000ng), OR gave an average of 4700 ng/200ul (lowest/ highest concentration: 780ng/14600ng) of liquid medium, OC gave an average of 3500ng /sponge (lowest /highest concentration: 1900ng/5900ng), while blood gave an average of 2800 ng/200ul. Absorbance's values at 260/280 and at 260/230 were within acceptability limits for all the samples analyzed (that means good purity of extracted hDNA), and no DNA degradation was detected on agarose gel with all samples. **Conclusions:** Data obtained demonstrated that Copan hDNA free FLOQSwabs™ provide good quantity and quality of hDNA compared to saliva and blood and are less expensive and easy to use.

A. Squassina: A. Employment (full or part-time); Significant; Copan Italia. **S. Castriciano:** A. Employment (full or part-time); Significant; Copan Italia.

P14.011C

C9orf72 testing in a diagnostic laboratory using the Asuragen AmplideX® PCR/CE C9orf72 kit

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Introduction: The pathogenic expanded hexanucleotide repeat element (G_4C_2) in intron 1 of the Chromosome 9 open reading frame 72 gene (*C9orf72*) is the most prevalent genetic cause of the neurodegenerative disorders frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Here we describe our experiences in using the newly introduced Asuragen AmplideX® PCR/CE *C9orf72* assay compared to our home-made long range and repeat-primed PCR tests for the detection of *C9orf72* repeats. Our home-made tests are limited to detection of up to ~60 *C9orf72* repeats.

Materials and Methods: DNA samples isolated from EDTA blood (n = 23) were tested for *C9orf72* using the Asuragen AmplideX® PCR/CE *C9orf72* kit. Amplicons were sized on an ABI 3730xl Genetic Analyzer and analyzed using GeneMarker software. All samples were previously tested using long range and repeat-primed PCR tests. For purposes of this study, normal repeats were categorized as <30 repeats and pathogenic repeats >30 repeats.

Results: All samples previously scored as pathogenic using the home-made tests also showed a pathogenic repeat expansion of at least 145 repeats using the new Asuragen test. No repeats were sized in the range 60–145. Repeats in the normal range were sized exactly the same. DNA concentrations of 50ng/μl (our standard lab dilution) and 25ng/μl showed similar results. A dilution (including a second 30s injection on the ABI) of the PCR products was needed to overcome the saturated normal allele peaks in the undiluted PCR products.

Conclusions: The Asuragen AmplideX® PCR/CE *C9orf72* kit is an effective method for the detection of *C9orf72* repeats.

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P14.012D

Implementation of a laboratory workflow for the parallel analysis of somatic and germline samples in the routine diagnostic

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Molecular profiling of tumor and blood specimens can help to identify somatic mutations that arise in cancer cells and are relevant for patient treatment. Next-generation sequencing (NGS) offers high sensitivity and accurate data quality for identifying somatic variants in cancer. However, laboratory protocols for analysis of paraffin-embedded (FFPE) tumor tissue are still challenging and often blood and tumor specimens cannot be processed with the same protocol. Here we present a single sample preparation and sequencing protocol for the parallel analysis of blood and FFPE based on targeted exon capture. This approach comprises paraffin removal and tissue rehydration using the Covaris AFA method. Sample preparation combines TruSeq Exome Illumina protocol and TruSight Cancer Panel enrichment followed by sequencing in a NextSeq500. Adapted non-equimolar pooling of blood vs. tumor libraries allowed sequencing all samples in the same run. Evaluation of quality criteria with FASTQC showed similar results for blood and tumor samples: DNA OD260/280 >1.8 in all samples, sequencing quality Q30>90% (150-cycle, paired-end), error rate <1%, but tumor samples produced more duplicates (20–50%) compared to blood (17–28%) and mapped filtered reads were also lower in tumor samples. However, sequencing depth of the coding region of BRCA1, BRCA2, MLH1, MSH2, MSH6 and PMS2 genes ranged from 30-fold to 2500-fold for tumor samples which resulted in sufficient coverage for the detection of variant mosaicism down to 10%. Overall, around 90% of the evaluated samples resulted in successful libraries and passed sequencing and mapping quality criteria.

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P14.013A

Optimising efficiency and cost-effectiveness in a clinical genetics laboratory

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Most clinical testing laboratories are facing increasing pressures to expand testing volume whilst reducing costs and turnaround times. TGLclinical is a medical genetic testing laboratory, accredited to ISO15189, that delivers cancer predisposition gene tests to cancer and genetic services. Over the last 4 years we have changed from using Sanger+MLPA to NGS testing with the TruSight Cancer Panel (Illumina). Through strategic restructuring of activities and development of automated pipelines and bespoke databases this change has allowed us to double the number of tests we perform, within the same testing budget. The key difference in cost was the ratio of personnel to consumables which is 80:20 for Sanger+MLPA, and 50:50 for NGS. The cost of NGS analyses is much more volume-dependent than Sanger+MLPA; the affordability of our tests is dependent on performing 96 TruSight Cancer tests every week. Our highly automated data analysis and interpretation pipelines allow a fast turnaround time, median 18 days, whilst requiring only a modest workforce. Although we have been able to achieve efficiency savings and cost-effectiveness in many areas, there are factors that impact on effectiveness that are currently beyond our control. For example, 20% of all test requests received in TGLclinical require some detail to be queried with the referring clinician. This is time-consuming and has to be done manually. We are currently monitoring and evaluating these residual inefficiencies and devising strategies to try to reduce them.

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P14.014B

A direct from plasma approach to non-invasive prenatal fetal sex determination and RhesusD genotyping by quantitative PCR

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Introduction: cell free fetal DNA (cffDNA) analysis by quantitative PCR (qPCR) is commonly used to perform non-invasive prenatal fetal sex determination in pregnancies where the fetus is at risk of inheriting a sex-linked disorder; and fetal RhesusD (RhD) genotyping in pregnant women at risk of developing Hemolytic Disease of the Fetus and Newborn. Currently, cffDNA extraction is required prior to qPCR testing. In this study we demonstrate the performance, time and cost advantages of performing qPCR directly from plasma.

Materials & Methods: plasma from 100 RhD(-) and 50 normal pregnant women was prepared for qPCR using Cell3^{TM}Direct technology (Nonacus Ltd). Testing for fetal RhD status was conducted by targeting exons 5, 7 and 10 of the RhD gene; while fetal sex determination was performed by detecting the SRY, TSPY and DAZ genes located on chromosome Y.

Results: initial testing on genomic DNA proved that the multi-target qPCR approach could detect down to 3 genomic equivalents of the RhD and chromosome Y-located genes. Validation on plasma samples yielded highly accurate and consistent results for both assays. Sensitivity and specificity for fetal RhD genotyping was 100%, with repeat and inconclusive rates of 4% and 2% respectively. For fetal sex determination, sensitivity and specificity were also 100%, with repeat and inconclusive rates of 2% and 0% respectively.

Conclusions: use of a multi-target qPCR approach coupled with direct from plasma testing delivers highly accurate non-invasive diagnosis of fetal RhD status and fetal sex determination, whilst providing a streamlined and more cost effective service.

M. Parks: A. Employment (full or part-time); Significant; Nonacus Ltd.

P14.015C

Creation of a novel site-specific CHO-K1 platform cell line using Jump-In™TI™ Technology and genetic analyzing by QF-PCR and ST-PCR

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Introduction: Chinese Hamster Ovary cell lines, due to their ability in post-translational modification, are preferred systems for biopharmaceutical protein production, but selection of stable cell lines remains a challenge due to random location of integration, since it is a very laborious and time consuming approach. Also, due to positional effects, it results in low and unpredictable yields of expression in the genome.Methods: The CHO-K1 adherent cell line was maintained in DMEM-F12 medium and 10% FBS. Then, pJTI^{TM}/ZEO plasmid (Invitrogen) and PhiC31/Int plasmids were co-transfected to cells, using Lipofectamine 2000. After 48 h, cells were selected with 600 µg/mL of hygromycin B for 3 weeks. Single cells were isolated from the resistant pool. Individual clones were expanded and their genomic DNA was isolated. Using 5 markers, Quantitive Florescent PCR was done to find vector copy number integrated in each clone. Semi-ransom Two-step PCR was done to determine site of integration.**Results:** 28single clones were isolated. QF-PCR results showed that 13 clones have obtained a single copy of vector and 15 have 2 copies of integrated vector. Using ST-PCR, genomic site of integration was determined, which was chromosome 1, contig2404 for 3 clones. **Conclusion:** Using Jump-In™ TI™ technology, we successfully generated platform CHO-K1 cell lines containing R4 attP site and analyzed it with QF-PCR and ST-PCR, which is a novel strategy tobe employed in cell line development. These platform cell lines are stable and because of single copy, site-specific R4 attP site, they can be used for recombinant protein production and drug discovery.

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P14.016D

Circulating free DNA digestion in microfluidic devices based on lab-on-a-chip in Non-invasive prenatal testing

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Introduction: Circulating free DNA (cfDNA) digestion with methylation-sensitive restriction enzymes constitutes an important diagnostic tool for differentiating methylated from non-methylated DNA sequences. In the context of prenatal analysis, this is used to differentiate fetal from maternal DNA. RASSF1A gene is one the most reliable fetal markers detected in maternal plasma because it is methylated in fetal DNA and unmethylated in maternal DNA. In a previous work, we optimized a short digestion protocol of 3.5 hours with multiple enzymes(1). In this work, we present the implementation of this 3.5-hour

digestion protocol in miniaturized device based on lab-on-a-chip technology with freeze-dried enzymes.

Materials and methods: Plasma samples from pregnant and non-pregnant controls were collected after informed consent. The cfDNA extraction and digestion process was performed in lab-on-a-chip as previously reported(2). For digestion, BstUI, HhaI and BstYI enzymes were freeze-dried, and transferred to the digestion chamber in the chip. Digestion products were collected from the chip and *RASSF1A*, *ACTB* and *GAPDH* genes were further analysed by qPCR.

Results: *GAPDH* was detected in all samples as cfDNA extraction control. *RASSF1A* gene was detected in samples from pregnant women and not detected in non-pregnant control samples. No detection of *ACTB* gene was observed in digested samples.

Conclusions: To the best of our knowledge, this is the first report of cfDNA extraction and digestion process in a microfluidic device based on lab-on-a-chip technology.

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(2) M.Agirregabiria, et al., μTAS 2015. KOREA.

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P14.017A

High diagnostic yield using Whole Exome Sequencing and a multidisciplinary team approach

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We have implemented a Clincial WES Service at Guy's Genetic Centre that uses virtual panels to interrogate exome data, restricting the investigations to clinically relevant genes and reducing incidental findings, whilst providing flexibility to add new disease genes to panels. Using WES rather than fixed gene panels allows the use of alternative virtual panels if new phenotypic information becomes available. Virtual panels are designed by scientists and clinical geneticists, while 100K Genomes "PanelApp" panels are also available for selection during the flexible user-driven ordering process. Additionally, clinicians can add genes to virtual panel(s) allowing for patients with heterogenous clinical presentation to have custom built panels. We have developed an integrated laboratory and bioinformatics platform in which Agilent CRE libraries are sequenced on a NextSeq with automatic data transfer to DNAnexus for alignment and variant calling using BWA and GATK. The generated VCF files are uploaded into QIAGEN IVA for variant assessment. This cloud-based data processing is efficient and fast and provides for future proofing. Collaborative efforts between scientists and clinicians involving MDT meetings where putative pathogenic variants are discussed before actions are determined have provided a high diagnostic yield. We have issued reports for 189 cases with variants identified in 82 (43%). Using ACMG guidelines ~35% were class 4/5 variants, while ~8% were novel variants that were consistent with the patient's clinical phenotype but require further investigation. Our results demonstrate that this WES service is an effective diagnostic approach for uncovering genetic defects in patients who may otherwise remain undiagnosed.

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P14.018B

Lessons from the clinical exome: description of three cases

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Introduction: The molecular diagnosis of complex genetic diseases with clinical exome is an increasingly frequent approach in diagnostic laboratories. We present three cases, from which we can extract a lesson to apply in future cases.

Materials and Methods: Samples were sequenced using TruSight One kit (Illumina), in Illumina platform. Results were filtered using Variant Studio, GeneGrid and phenotype.

Results: Female patient, 17 years of age. Started with difficulty to achieve a standing position at 16 months old, and progressed with cognitive delay, loss of speech, spastic paraparesia and basal ganglia calcification. We identified p. Gly1007Arg in *ADAR*, in heterozygous state. This is the fourth described case of autosomal dominant Aicardi-Goutières syndrome due to mutations in *ADAR*. Our patient also presents pubertal delay and delayed bone age, non-previously reported clinical features.

Female patient, 2 years of age. Clinically diagnosed as malignant infantile osteopetrosis. We identified p. Thr360Pro and p.Arg444His in *TCIRG1*. Based in the molecular diagnosis, hematopoietic stem cell transplantation was performed.

Male patient, 52 years of age with albinism, pulmonary fibrosis, Crohn-disease, and bleeding tendency, and 49-year-old brother with albinism. We identified p.Asn16IlefsTer in *HPS4*, in homozygous state in both siblings. They were diagnosed with Hermansky-Pudlak syndrome, illustrating the intra-familial variability of clinical expression of the disease.

Conclusions: In these cases, clinical exome analysis has allowed us to: (a)define an infrequent molecular diagnosis and expand the phenotypic characteristics; (b)impact therapeutic decisions in a prompt way; (c)diagnose two siblings with a disease with a high degree of genetic heterogeneity and variable expressivity.

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P14.019C

Data from Leipzig recommends exome sequencing based on trio analysis and detailed clinical information as an early diagnostic step

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Introduction: We present our experience in exome sequencing in diagnostic setting to review its reported need at an early diagnostic stage.

Materials and methods: Whole exome sequencing was performed by Centogene. We used the annotation software Varvis™. We studied 83 cases, 93% were trios. Of all cases, 89% had a neurodevelopmental disorder.

Results and Discussion: A reliable clinical genetic diagnosis was made in 40% of the cases. In further 7% we found a variant of an unknown significance that indicated further investigations. Moreover, in additional 7% we identified convincing *de novo* variants in candidate genes for neurodevelopmental disorders (*GRIA4*, *GABRB2*, *ACTL6B*, *TOB1*, *TUBA1B*, and *CUX1*). In the 31 solved trio-exomes with non-consanguineous parents, we surprisingly found 8 compound-heterozygous variants and one homozygous variant. In total, we reported the finding of a variant in 52% of the cases. Reducing the available clinical information to solely the indication allowed the identification of the correct variant in only 43% of the cases, and led to a wrong diagnosis in additional 38%. As we have reported a variant in 53% of the trio cases, excluding the exome data of the parents allowed identifying the correct variant in only 43% of the cases and led to wrong diagnosis in 27%.

Conclusion: The high diagnostic yield strongly indicates exome sequencing as an early diagnostic step. Also, our data emphasizes the importance of running the analysis in a trio constellation based on as much clinical information as possible to avoid significant loss of sensitivity and specificity.

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P14.020D

Semi-automated generation of custom clinical genomic reports for rare disease

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Next-generation sequencing provides a higher molecular diagnostic yield than traditional molecular diagnostic methods. In fact, whole genome/exome sequencing is being increasingly applied to the molecular diagnosis of individuals with rare genetic disorders. However, this new technology also brings challenges in terms of data management, analysis, quality metrics, interpretation of results and the generation of comprehensive, and human readable, clinical reports. As part of a collaboration between the ELIXIR-EXCELERATE Rare Disease Use Case (www.elixir-europe.org) and RD-Connect (www.rd-connect.eu), we are developing a software module to overcome these challenges and facilitate the generation of reports by the clinical geneticists themselves. The module enables the generation of custom clinical reports following current regulations and recommendations made by European projects and professional societies such as EuroGentest and ACMG. These reports can be generated from data analysed using platforms such as RD-Connect and may include several sections, including scope and design of the test (applicability, type of sample, technology, reference genome and annotation, targeted genes, etc.), quality metrics (amount of data generated, sequencing coverage, non-tested regions, etc.), relevant variants associated to a clinical condition, incidental findings in actionable genes and pharmacogenomics associations. The baseline of these reports is written in a style that is clear and informative to the clinician and does not require in-depth knowledge of molecular genetics. Therefore, the semi-automated generation of reports through already existing rare genetic data analysis platforms will facilitate the clinical interpretation of genomic data for the whole scientific/medical community.

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P14.021A

Detection of copy number variations with next generation sequencing: laboratory validation

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Introduction: Copy number variations (CNVs) have been recognized as pathogenic alterations for many years, where single or multiple exons are deleted or duplicated, respectively. CNVs are a common feature in pathogenicity of sporadic or hereditary cancer syndromes. MLPA is golden standard for detection of large deletions and/or insertions. However, NGS technology offers detection of single nucleotide variants as well as CNV analysis in one workflow.

Materials and Methods 131 whole intravenous blood samples were sequenced using Illumina TruSight Cancer Panel kit, in order to detect copy number variations. Study included 7 MLPA-positive samples, 6 samples harboring a large deletion and 1 sample harboring a large duplication. The *in silico* CNV analysis was performed using SeqPilot software (CNV module) (JSI Medical Systems, Germany). All samples were also validated by MLPA. **Results:** All known MLPA-positive samples were successfully detected using *in silico* CNV analysis. *In silico* CNV analysis was consistent with MLPA in 97 % cases, false positive samples were detected in 3% cases. Importantly, no false negative samples were detected.

Conclusions: To conclude, *in silico* CNV analysis using SeqPilot software (CNV detection module) could be used for detection of CNVs. However, due to false positive results all samples must be confirmed by MLPA.Abel, H.J., Duncavage, E.J. (2013). Cancer Genet. *206*, 432–440. Stuppia, L., Antonucci, I., Palka, G., and Gatta, V. (2012). Int. J. Mol. Sci. *13*, 3245–3276.

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P14.022B

Extra diagnostic yield gained by CNV detection in WES, clinical exome sequencing and targeted NGS panels

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Chromosome abnormalities and copy number variations (CNVs) are a well-studied and important type of genetic variation that is missed by traditional NGS data analysis. Algorithms have been developed that are able to detect CNVs in exome-level data by comparing relative coverage between samples; however, their routine clinical use is mostly reported on whole-exome level only.

In 2015–2016 we performed 2376 NGS-based tests for patients with suspected monogenic disorders using Illumina NextSeq 500 and in-house analysis software. 1710 samples were sequenced using Illumina TruSightOne enrichment ("clinical exome", ~4800 genes); 447 samples using our custom 2000-gene platform focused on neuromuscular disorders and epilepsy; 68 samples using smaller panels; 151 samples by whole-exome sequencing. A definitive molecular diagnosis could be established in 837 cases (35.2%), with another 387 (16.3%) having a possible diagnosis based on variants of unknown significance with a need for additional evidence. In addition to standard variant analysis, CNV detection was performed in all the samples. This allowed us to report CNVs that we believe to be causative or significant in 80 cases, ranging from 1-exon homozygous deletions to a whole-chromosome duplication, with many microdeletions identified including Wolf-Hirschhorn, Angelman and Miller-Dieker syndromes; the diagnostic yield increase was 3.4% of all cases, or 9.6% of the ones with an established cause. CNVs were detected in all enrichment panel types. In all cases where CMA was available and its resolution was enough, the alterations were confirmed. A major challenge remains in confirming medium-sized CNVs that require MLPA as a method of choice.

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P14.023C

The ICR96 exon CNV validation series: a resource for orthogonal assessment of exon CNV calling

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Detection of deletions and duplications of whole exons (exon CNVs) is a key requirement of genetic testing. Calling this class of variation, particularly those involving only a single exon, is especially challenging in targeted next-generation sequencing (NGS) data which typically does not provide sequence information at the breakpoints. Exon CNV calling methods are often validated based on simulated or in-house data due to a lack of publicly-available datasets with orthogonally generated results. This hinders comparisons of different tools, transparency and reproducibility. To provide a vital community resource for assessment of exon CNV calling methods in targeted NGS data, we present the ICR96 exon CNV validation series. The dataset includes high-quality sequencing data from the TruSight Cancer Panel together with MLPA and digital MLPA results for 96 independent samples; 66 samples with at least one validated exon CNV and 30 samples with validated negative results for 26 genes. The validated exon CNVs include 22 single exon CNVs with excellent representation of the cancer predisposition genes most frequently tested in clinical practice. The series includes 45 exon CNVs in *BRCA1*, *BRCA2*, *MLH1, MSH2, MSH6, PMS2, EPCAM, PTEN*, and *TP53*. The ICR96 exon CNV validation series dataset will be available from the European Genome-phenome archive (EGA), further empowering development, validation and comparison of exon CNV detection tools for the genetic community.

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P14.024D

Identification of mismatch repair deficient tumours using a molecular inversion probe based sequencing assay of short homopolymer repeats

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Introduction: Recent draft guidelines from the UK's NICE recommends that microsatellite instability (MSI) testing should be performed in all patients with colorectal cancer to

identify Lynch syndrome patients. Current techniques for mismatch repair (MMR) status evaluation- immunohistochemistry and fragment analysis, require trained staff for result interpretation and are not amenable to high throughput diagnostics. We aim to develop an automatable, robust and high throughput MSI assay based on next generation sequencing.

Methods and Results: We have developed a sequencing based assay using 17 short intragenic homopolymer repeats (7–12 bp), which are selected from the CGAP database. These are less prone to technical artefact than the longer repeats used in fragment analysis. The repeats used are linked to an informative SNP; to facilitate separation of genuine mutations which display allelic imbalance from PCR artefacts generated from both alleles. Using fragment analysis as the reference technique, we have demonstrated >97% sensitivity and specificity using over 100 residual samples from diagnostic laboratories in Newcastle, Edinburgh and Pamplona. Further enhancement of laboratory procedure involved implementation of a two-step multiplexed target capture and library preparation method which utilised molecular inversion probes (MIPs). We demonstrate that MIPs allow multiplexing of the 17 markers plus inclusion of *BRAF* mutation testing for minimal additional cost. We will present results of assay sensitivity, robustness and reproducibility against fragment analysis, using DNA from fresh and FFPE tissues.

Conclusion: Our novel assay has the potential to streamline diagnostic pathology services by providing a quick and automatable assay for MMR-deficient tumour diagnosis.

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P14.025A

Introducing genetics training and competency assessment into day to day working practice

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The requirement for laboratories to work to ISO 15189 has introduced the need to evidence the training and competency of staff for all tasks performed. The demonstration of staff following testing protocols has been widely adopted, however providing evidence for training and competency of routine genetic laboratory duties is problematic.UK National External Quality Assessment Service (NEQAS) for Molecular Genetics has developed an online Genetics Training Assessment and Competency Tool (G-TACT) in conjunction with Certus Technology, to provide assessment of the ability of individuals to perform both routine and non-routine tasks. These currently include tasks that are undertaken by Sample Receptionists, Duty Scientists, Data Analysts, and Report Authorisers e.g. logging samples into a Laboratory Information Management System, activating/prioritising requests from service users, analysing and interpreting genomic data and authorising clinical reports. Participants enter a virtual genetic laboratory and navigate between workstations to complete randomly generated tasks. Errors/problems are introduced to expose the participant to more challenging scenarios, e.g. handling inappropriate samples, analysing and reporting sub-optimal results. The system assesses the participant's handling of the scenario. A manager is assigned locally so the outcomes can be integrated into the appraisal system and training needs identified. The modular format enables new workstations, tasks and roles to be introduced as the laboratory environment changes. G-TACT offers, for the first time, a consistent approach to ensure individuals at all levels across genetic laboratories are appropriately trained and competent to perform their assigned tasks to a high quality standard.

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P14.026B

Comprehensive next-generation sequencing panel for detection of mutations and large deletions in congenital disorders of glycosylation

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Introduction: Congenital disorders of glycosylation (CDG) comprise a group of >100 conditions affecting glycosylation. CDG can present at any age with a broad spectrum of clinical symptoms, affecting multiple organ systems. Recently, many new genes have been identified by whole exome sequencing testing (WES), utilizing next generation sequencing (NGS) technology. Materials/Methods: We developed targeted NGS panel which includes 116 genes associated with CDG utilizing custom probe mix (SureSelect^{XT}, Agilent). CLC Bio Genomics Server was used for read alignment and variant calling. PatternCNV (in house developed algorithm) was used for copy number variant (CNV) detection. Accuracy was assessed by testing 19 patient samples, 21 samples with large deletions or duplications, 3 controls from Coriell and 2 samples with WES data.

Results: On average, 78.9% of the reads were mapped to target with depth of coverage of 2427. We observed 100% concordance in the 19 samples with previously identified CDG mutations and 21 samples with previously identified large deletions or duplications; and >99.4% concordance with the control set. In addition to all variants reported in samples with WES data, we detected additional 169 variants of unknown significance (43% exonic), demonstrating the higher sensitivity of targeted panels.

Conclusions: An approach, combining biochemical screening and targeted analysis of genes known to be associated with different CDG can facilitate the diagnosis in a significant fraction of patients with suggestive clinical presentation. Bioinformatics analysis of NGS data allows reliable screening for large genomic rearrangements and is a viable alternative to methods such as aCGH and MLPA.

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P14.027C

Validation of low-coverage whole genome sequencing assay for detection of copy number aberrations in inherited disorders

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Detection of copy number aberrations in clinical diagnostics has been limited to few genes in MLPA and low resolution in microarrays. We have developed and validated a low-coverage whole genome sequencing assay for genome-wide and high-resolution detection of copy number aberrations (CNAs) from inherited disorders. We used Illumina NextSeq500 sequencing system to generate paired-end 40 base reads. 28 reference samples with 34 confirmed chromosomal aberrations and the golden standard reference sample (NA12878) were applied in the validation. Reads were divided into 5 kb bins and difficult to sequence regions (bins representing 5.8% of the genome) were filtered out. Read counts were corrected for GC content and average mappability of each bin. Segmentation and calling algorithms (ODNAseq, DNAcopy and CGHcall) were applied to detect CNAs. The CNA calls from the reference samples were compared to Affymetrix Genome-Wide Human SNP Array 6.0, G-banded karyotyping analysis and fluorescence in situ hybridization (FISH) results. The assay's sensitivity to detect >100kb deletions and duplications was 0.97. We utilized a golden standard reference sample containing high-quality copy number variation (CNV) calls of variable sizes to demonstrate the assay's sensitivity to detect smaller changes. The analytical sensitivity was 0.765 for detecting CNVs of 25–50kb in size and 0.990 for detecting CNVs of over 50kb in size. The smallest detected deletion was 10kb. Our results show the validity of the low-coverage whole genome sequencing assay for diagnostic analysis of CNAs in inherited disorders.

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P14.030B

Monitoring Guide RNA Synthesis for CRISPR-Cas9 Genome Editing Workflow

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Bacterial clustered regularly interspaced short palindromic repeats (CRISPR) - associated protein 9 (Cas9) system has increased in popularity as a genome editing tool for targeted mutations, insertions, deletions and gene knock-out studies. CRISPR genome editing has also proved superior to Zinc-finger nucleases (ZFN) and transcription activator- like effector nucleases (TALENs) due to its simplicity and easy programmability. In CRISPR, a guide RNA (gRNA) is used to recognize and introduce a double standard break (DSB) in a target DNA. The DNA repair mechanism triggered after the break is then exploited to introduce an insertion-deletion (indel) in the case of non-homologous end joining (NHEJ), or precise genetic modification if a homology-directed repair (HDR) pathway is triggered.

A critical part of the CRISPR-Cas9 tool is the design and synthesis of the gRNA that comprises T7 promoter sequence, target sequence, and protospacer adjacent motifs (PAM). Monitoring the transcription of the gRNA is critical to the workflow to ensure successful gene editing. Here we present an automated electrophoresis approach for monitoring the synthesis, integrity, and functional activity of gRNAs created for a CRISPR-Cas workflow.

M. Liu: A. Employment (full or part-time); Significant; Agilent Technologies. **J. Molitor:** A. Employment (full or part-time); Significant; Agilent Technologies. **A. Padmanaban:** A. Employment (full or part-time); Significant; Agilent Technologies. **D. Marjenberg:** None.

P14.031C

RNaseH2-dependent PCR (rhPCR) and universal reporter system enables quantitative and multiplex genotyping

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Introduction: rhPCR genotyping assays combine a unique two enzyme system with DNA-RNA hybrid primers to detect genetic variations including SNPs and InDels. Allele specific primers (ASPs) contain a 5′ universal tail, a single RNA base targeting the SNP, and a terminal blocking group. The thermostable RNase H2 cleaves the primer at the RNA base, releases the blocking group only upon hybridization to its perfectly matched target, and hence activates the primer which can be then extended by DNA polymerase. This enables rhPCR genotyping assays to detect different ratios of allele input or quantitative genotyping. A universal reporter system provides cost-effective genotyping, and enables multiple fluorophore detection of multi-allelic variants in a single reaction.

Materials and Methods: Assays were designed to target SNPs on human CYP2D6 genes known to have copy number variation. Assays were tested with synthetic templates at various ratios of allele input and >90 Coriell gDNA samples from three populations. Multiplex genotyping was performed by combining two assays together in the same reaction and using four fluorescence dyes, one for each allele, for detection.

Results: Genotyping results on mixed synthetic templates showed the assays can detect 10% alternative allele in end-point analysis output. Human gDNA data showed the assays are specific and quantitative to measure allelic copy number ratio. Multiplex genotyping was successful with high fluorescent signal and good cluster separation.

Conclusion: New rhPCR genotyping technology provides highly specific genotyping for quantitative and multiplex allelic detection. This technology is applicable for genomics research and molecular diagnostics.

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P14.032D

Novel droplet digital PCR for Non-invasive prenatal testing rhesus genotyping

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Introduction: The development of new more sensitive and easy handling technologies with affordable equipment is a challenge. In the frame of EU 7th Framework ANGELAB project a digital droplet PCR on disk was developed (1). In this work, we present the application of this novel approach in the context of NIPT rhesus genotyping.

Materials and methods: Plasma samples from RhD positive and RhD negative pregnant women were collected after informed consent. cfDNA extraction and digestion was performed in lab-on-a-chip as previously reported (2) with modifications in digestion protocol and enzymes composition. Digestion products were collected from the chip and subsequently analyzed by ddPCR. Droplets generation was performed as previously reported (1) adapted to slide format. Detection of RASSF1A, RHD exon 5, and RHD exon 7 was performed using a microarray scanner and a fluorescence microscope. Samples were also analyzed by qPCR.

Results: Positive detection of the three targets analysed was achieved in samples from Rh positive pregnant women. Fetal rhesus genotyping in Rh negative pregnant women was a challenge due to low gene copy numbers and adjustments in the methodology were needed. A correlation with qPCR was also established.

Conclusions: This novel ddPCR approach was proved to be suitable for rhesus genotyping. Its use for fetal rhesus genotyping will constitute a sensitive and easy handling technology.

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P14.033A

Diagnostic yield of whole exome sequencing in a highly consanguineous population

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Whole exome sequencing (WES) is increasingly used and is fast becoming the tool of choice in establishing an accurate diagnosis in patients with a suspicion of inherited Mendelian diseases.

In this study, the results of 105 consecutive patients with undiagnosed complex disorders at the Genetics and Developmental Clinic of Sultan Qaboos University Hospital from September 2013 to September 2015 are presented. Whole exome sequencing was performed on all patients. The most common referral indication was intellectual disability (47%). Analysis of the WES generated data was first limited to a predetermined disease gene panel whose selection was based on the patient's clinical presentation. In the absence of a pathogenic or likely pathogenic mutation, sequence data analysis was then extended to the entire exome.

A pathogenic or likely pathogenic mutation was identified in 33 patients. Consistent with the relatively high rate of consanguinity in our population, the mutations observed were inherited in autosomal recessive manner (28/33). A total of 6 patients had a combination of 2 single gene disorders. Variants of unknown significance (VUS) were frequently observed, in about 23% of our cases.

The overall detection rate of pathogenic / likely pathogenic mutations achieved in this cohort is consistent with the expected yield in the context of proband-only WES analysis. Challenges remain with the identification of VUS and highlights the importance of further studies, segregation and/or functional.

A. Alsaegh: None.

P14.034B

Back-tracing of leukemia clones in childhood B-cell progenitor acute lymphocytic leukemia using digital PCR

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Introduction: The time of onset of childhood leukemia, especially t(12;21)(p13;q22)[ETV6-RUNX1]-positive ALL, has been explored using nested and real-time PCR in dried blood spots (DBSs) and cord bloods, and contradictory results have been reported. Our main goal is to develop a sensitive and quantitative method to trace leukemic clones at DNA level in DBSs. We used digital PCR (dPCR) to five patients with different aberrations.

Materials and Methods: We collected five 3-mm DBS from 5 BCP-ALL patients (1 MLL, 2 t(12;21) and 2 dic (9;20)). We designed patient specific in-house assays targeting the breakpoints. Following DNA isolation, dPCR mixes were run in 4–8 chips, each containing ~50 ng DNA. Chips were scanned in QuantStudio 3D Digital PCR System and analysed in QuantStudio 3D AnalysisSuite Cloud Software v3.1.

Results: Among DBSs, we detected leukemic clone, 3,44%, in one *KMT2A/MLL*-positive patient. We were not able to detect any leukemic clone in the rest of the DNA samples isolated from the DBSs (Table 1).

Table 1. Summary of dPCR results of five ALL patients

Sample	Chromosomal aberrration	Age of leukemia onset	Ratio of target to total at diagnosis	Amount of DNA obtained from DBS (ng)	Ratio of target to total at birth	Calculated sensitivity of the test	Lowest detectable level achieved	Average number of false positives in the assay
P1	MLL rearrangement	1 mo	25,3%	290	3,44%	0,0946%	0,25%	3,47(±2,87)
P2	t(12;21)	1 y 8 mo	28,4%	250	0%	0,048%	0,09%	1,25(±1,88)
P3	t(12;21)	3 y	29,9%	312	0%	0,033%	0,2%	0,9(±1,1)
P4	dic(9;20)	1 y 1 mo	29,5%	312	0%	0,06%	0,17%	1,5(±1,17)
P5	dic(9;20)	3 y 3 mo	16,2%	183	0%	0,049%	0,15%	1,4(±1,91)

Conclusion: dPCR enables accurate and sensitive detection and absolute quantification of low-prevalence target sequences. Our study confirms the presence of the *MLL* rearrangement at birth but not

t(12;21) and dic(9;20). This could be explained by levels of preleukemic clones below the detection level.

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P14.035C

Detection of oxidative base damage in DNA with Fpg treatment and Northern Lights Analysis

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Introduction: Northern Lights Assay (NLA) is a versatile technique for comprehensive detection of DNA damage based on changes in conformation or structure of DNA molecules. We tested if oxidised base lesions could also be detected by pretreatment of samples with Fpg, an N-glycosylase and AP-lyase acting with a broad substrate specificity for oxidized bases.

Materials and Methods: NLA is based on Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE), a technique of nucleic acid separation based on size, strandness, and conformation changes. NLA was done in microgels to improve sensitivity and speed of analysis. Each DNA specimen was analyzed in sample pairs of non-digested DNA to detect single- and double-stranded breaks and *Mbo*I-digested DNA to detect other lesions. In addition, isolated DNA samples were treated for different incubation times with the Fenton reaction and digested with different amounts of Fpg enzyme.

Results: NLA detected single-stranded breaks, double-stranded breaks, interstrand and intrastrand DNA crosslinks, single-stranded DNA and bulky lesions. Conditions were established were NLA with Fpg pretreatment detected oxidized bases in a specific and semiquantitative manner as increased single-stranded breaks. In addition, we detected interstrand crosslinks in samples treated with the Fenton reaction.

Conclusions: We extend the application of NLA to detection of oxidised bases in DNA as increased single-stranded breaks after Fpg enzyme treatment. Important potential application of NLA-Fpg is analysis of cfDNA, an advantage over the Comet assay, which can only be used on whole cells. We also demonstrated conclusively that he Fenton reaction can induce formation of interstrand crosslinks.

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(stock, stock options, patent or other intellectual property); Significant; LIfeinda ehf..

P14.036D

An approach to detect chromosomal aberrations, absence of heterozigosity and single nucleotide variants from exome sequencing in clinical setting

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The aim of the present study was to evaluate the performance of a new exome sequencing kit that consists of backbones probes for detection and mapping of DNA copy number variations (CNVs), and, ultimately, substitute the use of chromosomal microarray testing and exome sequencing for a single test. A total of 14 DNA samples with known alterations ranging from Megabase-scale CNVs to single base modifications were used as positive controls for exome sequencing data analysis. In the DNA panel were included copy number changes (n = 11) of variable sizes (25 kb to 27 Mb), uniparental disomy (UPD) (n = 1), and single point mutations (n = 2). Our results show that all DNA mutations were correctly identified by exome sequencing, except for two CNVs, which had no coverage in the current platform, showing that CNVs of at least 25 kb can be properly detected. Also, the calculated size of the genomics imbalances detected by microarrays and exome sequencing are virtually the same, suggesting that this approach presents a resolution and sensitivity similar at least to DNA microarrays. Accordingly, our data show that the combination of a sequencing platform comprising exome and whole genome backbone, with the right algorithm is very promising for simultaneous detection of CNVs and single nucleotide variants (SNVs), and should enable a cost-effective and efficient solution for detecting genomic aberrations and SNVs detection simultaneously.

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P14.037A

Utility of exome sequencing in elucidating molecular etiology of monogenic disorders in consanguineous and non-consanguineous Indian families

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Introduction: Consanguinity which is observed in upto 40% of some Indian groups helps in analysis of exome data. The experience of our centre in identifying causative variations in cases with monogenic or possibly monogenic phenotypes is presented.

Materials and Methods: Patients with monogenic disorders like osteogenesis imperfecta, Sotos syndrome, intellectual disability, limb malformations and other malformation syndromes, etc. have been included. Exome sequencing was outsourced (Illumina Nextera Rapid Capture Exome). The annotated data was analysed for identification of disease causing sequence variations.

Results: In total, the data of 66 cases has been analysed, out of which causative sequence variations in known genes were identified in 49% of cases. These included 14 heterozygous pathogenic variations in genes causing autosomal dominant disorders and 17 homozygous or compound heterozygous mutations in genes for autosomal recessive disorders. Out of the total sequence variations labelled as causative, 14 were known pathogenic and 19 were novel. In addition, in a case from consanguineous family, possibly pathogenic mutations in novel genes have been identified and needs further work up. Out of the rest 12 cases from consanguineous families, causative homozygous mutations were identified in 11 cases. In 4 out of the 6 cases from non-consanguineous families with autosomal recessive disorder, the identified sequence variations were homozygous. **Conclusions:** The mutation detection yield increases to more than 90% in cases with consanguinity. In significant proportion of non-consanguineous families with rare autosomal recessive disorders, the mutations occur in homozygous form suggesting hidden consanguinity or inbreeding.

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P14.038B

The added value of rapid exome sequencing in critical clinical situations

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For critical clinical situations, turnaround times (TATs) of exome sequencing need to be fast in order to have an impact on clinical decision making. We therefore set out to develop a fast exome sequencing approach (max 14 days). Urgent exomes are preferably sequenced as trios to enable *de novo* analysis and assist data interpretation. A total of hundred samples (37 families, mostly trios) have been sequenced until now: Six trios were used for experimental setup, in 14 families (possible) pathogenic SNVs were identified, some of which still need follow up, and 17 families remained negative after inspection of SNVs and small indels. A trio-based reference-free CNV approach is still under development, but preliminary data show that all control CNVs (53kb-6Mb) are detected correctly. Shorter TATs were already beneficial for some patients, i.e. an adult male suffering from myelofibrosis and autoinflammatory symptoms. A STING-like phenotype was suspected, with possible involvement of the JAK/STAT pathway. Urgent exome sequencing was performed and results were available within 9 days. Interestingly, both a somatic variant in *MPL* (=trombopoetine receptor > myelofibrose) and a heterozygous variant in *ACP5* (*TRAP*, known immune dysregulation disorder) were identified, both fitting to the patients phenotype. Based on these results the medication of the patient was changed, resulting in a substantial improvement of the patients constitution. In conclusion, we have implemented a rapid exome sequencing workflow for urgent cases. The rapid identification of pathogenic variants already had implications on patient treatment, underlying the added value of a fast genetic diagnosis.

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P14.039C

Comprehensive use of extended exome analysis improves diagnostic yield in heterogeneous rare disease - a retrospective survey in 1.087 cases

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Background Next-generation sequencing, especially with exome sequencing, has become a cornerstone of diagnostics in rare genetic disorders. Because exome sequencing is primarily aimed at detection of single nucleotide and indel variants in coding regions of genes, it may fail to detect a considerable proportion of causative genetic variation. Recently, several studies have shown that information content of exome sequencing data allows for the expansion of the scope of detectable variation, which may be interrogated with extended bioinformatic analyses. **Methods** We retrospectively analysed the results of genetic testing in 1.087 distinct cases referred for exome sequencing to our institution. In these, we routinely employed extended exome analysis approaches in addition to basic variant analysis, including (1) copy number variation (CNV) detection, (2) genomic breakpoint detection, (3) non-consensus splice defect detection, (4) homozygosity mapping and (5) mitochondrial variant analysis. **Results** Combined use of selected extended exome analysis approaches assisted in identification of causative genetic variant in 47 cases. This represented an 4.3% increase in diagnosed cases, raising the overall diagnostic yield from 35.6% to 39.9%. The greatest increase of diagnostic yield was associated with CNV detection (1.7%) and splice variant prediction (1.5%), whereas the remaining approaches contributed an additional 1.1% to the diagnostic yield. **Conclusions** In conclusion, we show that routine use of extended exome analysis approaches improves genetic diagnosis of heterogeneous genetic disorders and results in considerable increase of diagnostic yield of exome sequencing.

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P14.040D

Simultaneous extraction of DNA, RNA and protein for genomic and proteomic applications from fat tissue

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Parallel sets of genome, transcriptome and proteome data offer powerful means to define the molecular basis of common diseases, such as obesity. However, adipose tissue poses a particular problem for biomolecule extraction given its high lipid and low protein content. The goal of this study was to compare 11 current simultaneous extraction methods for comprehensive downstream omics analyses of adipose tissue. We used adipose tissue collected from panniculectomy ($n = 5$) in triplicates with 20–100 mg of input tissue according to the manufacturer's protocol (kits) or established published protocols (Trizol). DNA and RNA concentrations and quality was assessed using UV spectrophotometry. Protein concentration was measured using the Bradford assay. The Trizol-based method yielded highly variable amounts of nucleic acids (0.5–90 μg DNA, 1–40 μg RNA) and protein (4–120 μg). The quality of DNA was poor ($OD_{260/280}$<1.0), but RNA was good ($OD_{260/280}$=1.6–2.0). The Allprep protocol provided very low yields and quality of nucleic acids, and variable yield of protein (6–140 μg). The Zymo protocol, which extracts nucleic acids but not proteins, yielded variable amounts (0.4–7 μg) but good quality ($OD_{260/280}$=1.5–2.0) of DNA and low amounts (0.3–3 μg) and quality ($OD_{260/280}$=1.8–2.8) of RNA. Similar to previous studies, our results suggest variability in both quantity and quality among the different methods, as well as considerable within-subject variability, likely due to difficulty in obtaining precise amounts tissue and the heterogeneous nature of the tissue samples. Existing protocols will need to be modified to optimize the consistency, purity and yield of biomolecules from adipose tissue. Grant HL093093

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P14.041A

the utility of computer-assisted facial recognition in the etiologic diagnosis of patients with global developmental delay & intellectual disability

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Introduction: global developmental delay (GDD) and intellectual disability (ID) are the most frequent reason for consultation in the neuro-pediatrics outpatients, with a prevalence of 1–10% of cases. Of these, 50–80% of patients do not have a set etiologic diagnosis. The aim of this study is to evaluate the efficiency of the computer program Face2Gene (FDNA Inc, USA) as a diagnostic aid in clinical practice, for cases of GDD and ID followed in a tertiary hospital.

Material and Methods: Double blinded prospective observational study. Face2Gene is a search and reference tool designed for the exclusive use of medical staff. Through the analysis of clinical findings and automated recognition of facial traits, the program suggests 30 possible diagnosis per patient. Our study correlates these proposed syndrome matches with genomic data of the patients attending our clinic, after adding the frontal photo and the clinical features of the patient.

Results: 91 patients, ages 6 month to 20 years, have been uploaded to Face2Gene, of which 21 have a molecular diagnosis. Face2Gene recognized 7 of these. For 70 patients we are waiting for molecular results. We will consider Face2Gene a useful tool if in at least 10% of the patients, one of the suggested syndrome matches does coincide with the molecular diagnosis of the patient.

Conclusion: If the results are positive at the end of this study, this could be considered a shortening of the diagnostic odyssey of the patient as well as an increase in the rate of etiologic diagnostics.

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P14.042B

Clinical Application of a Facial Dysmorphology Tool: a performance analysis

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Diagnosis of genetic syndromes associated with facial dysmorphology in children is a real challenge. The rarer the syndrome, the hardest reaching the diagnose. Computer-aided dysmorphology analysis enables to benefit from the cumulative knowledge of geneticists worldwide. Face2-Gene (FDNA Inc. Boston, MA) is an analytic tool that utilizes the Facial Dysmorphology Novel Analysis technology to identify facial patterns associated with genetic syndromes analyzing two-dimensional facial photos. For each case, Face2Gene provides a ranked list of up to 30 possible syndrome matches based on anthropometric measurements, phenotypic features and frontal facial photos submitted. In this study, we aimed to measure the tools performance with patients followed at Clinical Genetics in the University of Siena. Frontal and often lateral pictures of 444 cases were uploaded, among which sixty cases with clinical and/or molecular diagnosis (syndromes diagnosed were 6% Nicolaides-Baraitser, 16% Rett, 10% Pitt-Hopkins, 6% Coffin-Siris, 6% Cohen, 6% Kabuki, 16% other). F2G matched correct diagnosis as first hypothesis in 33,3%; as first 5 hypotheses in 41,6%;as first 10 hypotheses in 46,6% of cases. Although these results do not prove the systematic efficacy of F2G tools usage in the clinical practice, it should be taken into account that in some cases, pictures quality and the lack of anthropometric measurements due to F2G continue updates could have affected the results of dysmorphology analysis. Therefore, although F2G database still lacks some syndromes, we envision that in the future it can be improved to help the clinicians reaching a diagnose validating his idea about a clinical case.

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P14.043C

An approach for determination of copy number variation using short-read next-generation sequencing

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Introduction: The ability to determine Copy Number Variation (CNV) from short-read Next Generation Sequencing (NGS) data would enable labs to determine both CNV and Single Nucleotide Variations (SNVs) simultaneously in one assay. We show here an approach to detecting intragenic CNVs using Next-Generation Sequencing and Bioinformatics. This is demonstrated with examples including *LDLR*, involved in Familial Hypercholesterolaemia; and *DMD* on the X chromosome, involved in Muscular Dystrophy.

Material and Methods: The NGS libraries were prepared by fragmentation of DNA, followed by end-repair and ligation of adaptors. The genes of interest were enriched using a bait-capture hybridisation process and following PCR the libraries were loaded onto an Illumina MiSeq instrument. Array CGH was carried out using high resolution microarrays specifically designed with a high density of probes within the genes of interest.

Results: Intragenic CNVs were detected using the NGS assay and confirmed with Array CGH. The concordance was 100%.

Conclusions: A combined Next Generation Sequencing and bioinformatics approach can be reliably used to determine CNVs in *DMD* and *LDLR* with potential for use in other applications.

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P14.044D

Archival tissue use in NGS applications- Evaluation of DNA extraction methods

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Archival tissues represent a rich resource for clinical genomic studies, particularly when coupled with a comprehensive medical record. The Mayo Clinic has tissues from many decades of clinical trials, and use of these with NGS applications is a priority. Nine FFPE DNA extraction methods were evaluated using twelve FFPE samples of varying tissue types. Quality assessment included the total yield, percent ds DNA, fragment analysis and multiplex PCR. After assessment, three tissue types from four FFPE DNA methods were selected for NGS downstream

evaluation. All samples were prepared using two different low input library protocols, and enriched with Agilent's SureSelect XT Target Enrichment System V5 + UTR. For additional examination, breast tumor samples extracted using the same four methods were prepared using QIAGEN's QIAseq™ Targeted DNA Human Breast Cancer panel. All samples were sequenced on an Illumina HiSeq 4000, PEln 150 bp. Bioinformatics analysis revealed the average coverage across the target regions for the Agilent SureSelect Target Enrichment was approximately 130X for all four FFPE DNA methods. For the targeted panel, the highest molecular tag coverage of ~1600X was obtained with the Kingfisher FFPE extraction method, while three other methods yielded coverage of ~700–800×. Variant comparison between the whole exome and the targeted panel reveals a higher number of called variants with the targeted panel as the result of its higher depths of coverage. The genotype concordance was 99% for the commonly called variant positions between all 4 extraction methods with the targeted PCR NGS panel and 96% with WES.

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P14.045A

Oligoprobes as personalised tools for transformative medicine in FISH

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Introduction: Fluorescence *in situ* hybridisation (FISH) is a widely-used technique in cytogenetic diagnostics. Whilst typical hybridisation for commercial probes occurs in 16 hours, using short oligoprobes (~100 bp), results in faster hybridisation kinetics which could allow much quicker diagnosis. These probes are designed synthetically, increasing flexibility and personalisation.

Methods: Our probes were labelled using methyltransferases. These enzymes recognise specific DNA sequences and facilitate the transfer of a methyl group from their cofactor to the target. This technology utilises synthetic cofactors that allow transfer of fluorophores, as opposed to methyl groups, to the specific DNA sequence. By incorporating methyltransferase recognition sites into the probe design, we can direct fluorophore labelling to the probe.

Results: As proof-of-concept we have explored the use of oligoprobes in cases of acute lymphocytic leukaemia (ALL). Hypodiploidy of chromosome 1, 7 or 17 can be indicative of ALL, a faster hybridisation could result in rapid diagnosis for patients. We have successfully designed oligoprobes for the centromere of chromosome 17 which have proven to hybridise to the complementary target in just 15 minutes, significantly quicker than the traditionally used methods.

Conclusion: Using methyltransferase directed labelling of FISH probes will allow more control over the labelling density and therefore sensitivity of probes, as well as increased specificity, and at a fraction of the cost compared to commercial probes. This is conducive to a range of potential applications including rapid detection of rare or private mutations.

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P14.046B

Pitfall in molecular diagnosis of Friedreich Ataxia

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Freidreich ataxia (FRDA) is the most common hereditary ataxia, nearly 98% of patients presenting homozygous GAA expansions in intron 1 of the *FXN* gene. The remaining patients are compound heterozygotes for an expansion and a point mutation, or an exonic deletion. Molecular screening for *FXN* expansion is therefore focused on (GAA)n expansion analysis, commonly performed by triplet repeat primed PCR (PT-PCR).

We report on an initial pitfall in the molecular characterization of a 15 year-old girl with FRDA who carried a rare deletion in intron 1 of the FXN gene, resulting in a TP-PCR failure of the expanded allele. This exceptional configuration induced misinterpretation of the molecular defect in this patient, who was first reported as having no *FXN* expansion. NGS analysis of a panel of 215 genes involved in nuclear mitochondrial disorders further revealed an intragenic deletion encompassing exons 4–5 of the *FXN* gene. Modified TP-PCR analysis confirmed the presence of a classical (GAA)n expansion located in trans. This case points out the possible pitfalls in molecular diagnosis of FRDA in affected patients and their relatives: detection of the *FXN* expansion may be impaired by several non-pathological or pathological variants around the *FXN* (GAA)n repeat. We propose a new molecular strategy to accurately detect expansion by TP-PCR in FRDA patients.

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P14.047C

Use of epilepsy gene panels for early diagnosis of epilepsy in children 2–4 years of age: expert considerations on current and future practices in Europe

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Introduction: The epilepsy gene panel is a molecular test increasingly used in clinical practice for the genetic diagnosis of epilepsy. A delay from clinical onset to molecular diagnosis may occur due to variability in referral criteria, regional variability in gene panel content, test availability/awareness and cost. Methods: In December 2016, eight European experts in the genetic diagnosis of epilepsy completed a survey and met to discuss current and future application of epilepsy gene panels in the diagnosis of children aged 2–4 years. The meeting was sponsored by BioMarin Pharmaceutical Inc.

Results: Upon first presentation of unprovoked seizures in children aged 2–4 years, molecular testing is usually ordered only after several clinical examinations (e.g. metabolic investigations, EEG, MRI), seizure worsening, manifestation of additional symptoms, and/or resistance to anti-epileptic drugs (AEDs). Key considerations offered: i) To shorten the time to genetic diagnosis of epilepsy, a gene panel test performed during initiation of AEDs may be warranted before worsening of symptoms, when an additional symptom is present; ii) all epilepsy gene panels should include a core set of genes linked to epilepsy syndromes/diseases presenting with epilepsy and with clinically actionable potential; iii) a gene panel is the first-tier choice in contrast to whole-exome/genome, due to higher gene coverage and lower cost.

Conclusions: Early use of an epilepsy gene panel provides a cost-effective diagnostic approach for timely identification of genetic causes of pediatric epilepsy which informs: clinical management, genetic counseling, prognosis and, when available, targeted therapy.

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P14.048D

Usefulness of the genetic risk score to identify phenocopies in families with Autosomal Dominant Hypercholesterolemia?

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Autosomal Dominant Hypercholesterolemia (ADH) is caused by mutations in *LDLR*, *APOB, APOE* or *PCSK9* gene in 80% cases. It has been supposed that polygenic forms of hypercholesterolemia may be present among ADH patients with no identified mutation. Such phenocopies within ADH-families are obstacles in linkage analyses which are not identifiable by clinico-biological criteria.

To address whether polygenic forms may explain phenocopies reported in ADH-families with an identified mutation, we calculated the genetic risk score (GRS), corresponding to the weighted sum of six LDL-C raising SNPs, in affected members from six French ADH families.

In the family with the *APOE*-p.Leu167del, the two affected members with no mutation present a high GRS suggesting a polygenic form for these two phenocopies.

In the family with the large *LDLR* rearrangement, the eight affected carriers present a low GRS consistent with the monogenic form of the disease.

In the family with the *LDLR*-p.Glu228Lys, the two affected members with no mutation present a high GRS revealing the possible polygenic origin for their hypercholesterolemia. However, the three affected carrier also all present a high GRS which can increase the disease severity. Indeed, two of them present higher LDL-C levels than the other affected relatives.

In the three remaining families (*APOB* or *PCSK9* mutations) all fourteen affected member are mutation carriers, but eight of them present a high GRS which is not systematically associated with higher LDLC-levels.

To conclude, this six families study is not in favor of the use of the GRS to identify phenocopies within ADH-families.

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P14.049A

Prioritizing causal variants for rare, inherited syndromes, using patient phenotypes

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A key challenge in using genome and exome sequencing, to identify disease causing variants in patients with multiple congenital abnormalities and rare, undiagnosed genetic disorders, is finding the true causal variant among the hundreds of rare, functional (coding and regulatory) variants. Here we present a patient phenotype driven sorting algorithm that ranks variants using phenotype-disease associations to facilitate improved diagnosis and causal variant discovery. To enable the phenotype-driven ranking, users enter phenotype terms or HPO identifiers that are mapped to the Ingenuity Knowledge Base (IKB). The syndrome prioritization algorithm connect genes and associated diseases with patients' phenotypes, using a directed network built from gene/disease relationships, disease/phenotype relationships, as well as the process hierarchy (ontology) that relates more specific terms of diseases and phenotypes to more general terms in a hierarchical manner. For each gene/disease combination a score that measures how many disease phenotypes can be explained by the disease, while also taking into account phenotype prevalence among all diseases represented in the IKB, along with the confidence of connecting phenotypes to diseases, when traversing the process hierarchy (path weight), is calculated. The specificity is the likelihood of the phenotype being specific to the disease it characterizes and path weight of a shortest path from a phenotype to a disease in the network. The total score for a given gene/disease combination is then computed as the sum over all phenotypes connected to the disease through at least one path. Preliminary benchmarking shows successful prioritization and causal variant discovery in 75% cases.

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P14.050B

Genetic and viral characterization by high throughput mass spectrometry in kidney transplant recipients

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Introduction: The infections are one of the most important risk factors for renal allograft survival. Polyomaviruses (PyVs), a group of small and circular dsDNA viruses, mediate a broad spectrum of diseases in immunocompromised patients. The NF-κB is a key regulator of immune and inflammatory processes and the -94ins/delATTG (rs28362491) polymorphism in the gene promoter has been widely investigated for clinical associations. To date, it has been associated with systemic lupus erythematosus,

rheumatoid arthritis, inflammatory bowel disease and recently renal rejection.

Materials and Methods: We developed a high-throughput mass spectrometry (MS)-based method to detect the rs28362491 and 18 PyVs types. Primer pairs of MS assay were designed within the specific large T antigen genes. Viral and human DNA were extracted from blood samples of 43 kidney transplant recipients, before and after transplantation.

Results: We analysed the correlation among PyVs infections, rs28362491 genotype and post transplant follow up. 5 out of the 18 viral types tested were found in the specimens analysed: BKV, JCV, Merkel cell PyV, Human PyV6 and SV12. In our cohort, 14 patients showed SV12 infection: 10 cases were -94ins/-94ins, 4 were -94ins/-94del. All the patients with the NF-kB -94del/-94del genotype were characterized by the absence of SV12 strain. No correlation between genotype and viral infection was observed for the other viral types.

Conclusions: Our MS assay improved the PyVs typing and allowed to drive towards the identification of novel biomarkers for the infective management of transplanted patients. The genetic background might modulate the viral infection susceptibility.

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P14.051C

A cloud-based high-resolution melting analysis application capable of processing genotyping and copy number variation data from different thermocyclers

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Introduction: PCR followed by high-resolution melting (HRM) is a fast, reliable, and cost-effective method for DNA genotyping and copy number variation (CNV) assessment. Each thermocycler has specific accompanying software, which hinders users from uniformly analyzing their data across thermocyclers. We developed a cloud-based HRM analysis application with comprehensive algorithms capable of analyzing data from different thermocyclers and well plate formats. A cloud-based application does not require installation, and updates for improved functionality and performance are automatically available.

Methods: Relevant, successive steps of curve processing are displayed to provide fast and accurate genotyping and CNV assessment. These steps include performing curve normalization based on nucleic acid thermodynamics and reaction model theory, calculating the negative derivatives, and performing curve comparison through differencing with a baseline curve and data clustering. We collected HRM data from three different, commercially available thermocyclers to assess the application's reliability in curve processing.

Results: For 20 small-amplicon, 20 unlabeled-probe, and 4 CNV assays, the sample melt curves were imported into our software. The data were reviewed by an expert in HRM analysis. Regardless of the source data or assay type, the result determined by the expert was in congruence with the original result.

Conclusion: Our cloud-based software application can reliably process HRM data from different thermocyclers, allowing users to make accurate genotyping and copy number variation calls.

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P14.052D

Using Human Phenotype Ontology (HPO) to direct diagnostic testing

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Diagnostic next-generation sequencing for genetically heterogeneous disorders is now widespread in clinical practice. Clinically curated fixed gene-panels have a clear and demonstrable clinical utility. However, for indications such as learning disability (LD), where genotype/phenotype relationships are still emerging, the flexibility of this approach may be limiting. Exome sequencing overcomes this, however it identifies large numbers of variants in singletons, which is costly in analysis time and can reveal unsolicited findings. We therefore wanted to investigate the potential of using human phenotype ontology (HPO) to improve gene-prioritisation, in an attempt to maximise diagnostic sensitivity in this clinical group through the development of virtual gene-panels.

Using 100 patients who have received a confirmed molecular diagnosis from whole exome sequencing through the Deciphering Developmental Disorders study, we carried out a blinded retrospective comparison of gene-prioritisation methods using HPO terms submitted at the time of patient recruitment by means of the following:

(1) A clique-based tool using HPO-gene relationships.

(2) A simple Phenotype-to-Gene-List tool, selecting genes output that matched 60% of HPO terms.

(3) A manually curated LD diagnostic panel (currently used in practice).

The curated LD gene-panel offered the highest sensitivity (78%) versus Phenotype-To-Gene-List (33%) and clique-based analysis (24%). Precision-recall analysis showed that clique-based analysis had the highest precision over Phenotype-to-Gene-List or the curated LD gene-panel. We suggest that whilst gene-panels are currently the most effective method for detecting disease-associated genes, that clique-based analysis using HPO-gene relationships may provide a useful first approach, reducing costly analysis time.

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P14.053A

Towards a standardised diagnostic procedure for 11p15-associated imprinting disorders - experiences from three rounds of External Quality Assessment (EQA) organised by EMQN

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The chromosome 11p15-associated imprinting disorders (IDs), Silver-Russell and Beckwith-Wiedemann syndrome (SRS, BWS), are characterized by heterogeneous molecular alterations affecting the imprinting control regions 1 and 2 (ICR1, 2), including aberrant methylation, copy number variations (CNVs), uniparental disomies (UPD) and point mutations). In SRS, ICR1 hypomethylation is the predominant finding, whereas most BWS patients show ICR2 hypomethylation or paternal UPD. Methylation testing of these loci is recommended for first-line diagnosis (see EMQN best practice guidelines for SRS and BWS). In case of exclusion, other genomic loci should be considered in the diagnostic workup. The European Molcular Genetics Quality Network (EMQN) has developed an external quality assessment (EQA) scheme for 11p15-associated IDs. Here we discuss the results of three rounds of this scheme. So far, 38 laboratories from 24 countries have participated in the scheme. Methylation-sensitive (MS)-MLPA is used by almost all laboratories to detect aberrant methylation and copy number variants, however, data interpretation is often challenging. Unrequested testing was an occasional problem but most reports were satisfactory. The quality of diagnostic testing improved over three years of EQA scheme provision, especially in interpretation and reporting of test results. We present a detailed summary of our findings, including comments on appropriate testing and genetic counselling in affected families, and reflect on differential diagnoses.

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P14.054B

Global diagnosis of 270 Morquio A patients based on a dry blood spot assay: a two years study

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Mucopolysaccharidosis 4a (MPS IVA; Morquio A disease) is an autosomal recessive disease caused and characterized by an impaired activity of galactosamine-(N-acetyl)-6-sulfate-sulfatase (GALNS), resulting in keratan sulfate and chondroitin-6-sulfate accumulation in tissues and secondary organ damage. Enzyme replacement therapy, currently in clinical trials, renders the identification of MPS IVa patients in a rapid and facile manner of out-most importance. We developed for the early and easy diagnosis of suspected patients an enzymatic assay in dry blood spots (DBS) for the stabile and reproducible detection of GALNS deficiency (Cozma et al, 2015). The material extracted from DBS was incubated with a 4-methylumbelliferyl-β-D-galactopyranoside-6-sulfate as a specific synthetic substrate. Final enzymatic product, 4-methylumbelliferone, obtained after adding exogenous beta-galactosidase, was quantified by LC/MRM-MS (liquid-chromatography/multiple-reaction-monitoring mass-spectrometry). 4-propyl-5-hydroxy-7-methyl-2H-chromen-2-one was used as internal standard, a compound with similar molecular structure and fragmentation pattern in negative ion mode as 4-methylumbelliferone. The assay yielded a positive and negative predictive value of 1.0 for genetically confirmed MPS IVa patients (with a GALNS activity of 0.35 ± 0.21 μmol/L/h) compared with controls (normal GALNS activity 23.1 ± 5.3 μmol/L /h). We present here the results of a global screening study of over 2 years. The samples with low GALNS activity were subjected further to GALNS gene sequencing to confirm MPS 4a. The study so far lead to the identification of 270 Morquio A individuals, from which are 186 MPS 4a patients and 85 carriers. In total 121 unique mutations have been found where 44% were not been described before.

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P14.055C

ACMG classification of variants in *LDLR* gene found in patients with a clinical diagnosis of Familial Hypercholesterolaemia

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Familial Hypercholesterolaemia (FH) is an autosomal disorder of lipid metabolism presenting with increased cardiovascular risk due to life long exposure to high LDL values. *LDLR* mutations are the cause of disease in 90% of the cases but proof of pathogenicity has only been obtained for about 10%. The aim of this study was to classify all *LDLR* variants found in patients with a clinical diagnosis of FH. A database with all variants reported in public databases and in literature review (2005–2016) was constructed. All variants were classified using the recent American College of Medical Genetics and Genomics (ACMG) guidelines. A total of 1924 *LDLR* variants have been identified. The majority were classified as variants of unknown significance (VUS, 804), followed by pathogenic (709), likely pathogenic (398), benign (7) and likely benign (6). The majority of VUS are classified as such due to lack of evidence on its functional effect, but some variants with functional studies are still wrongly classified as VUS or Likely Pathogenic. Additionally, 99 null variants are classified as VUS. Although the ACMG algorithm is a valid toll for variant interpretation it must be adapted to FH to overcome the gaps encountered. For now we recommend that a positive FH molecular diagnosis should only be reported to the clinician if (1) solid functional evidence exists towards the pathogenicity of a variant, (2) ACMG algorithm classifies the variant as pathogenic or likely pathogenic or (3) the variant found produces a null protein. This will avoid misdiagnosis.

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P14.057A

Lyso-SM-509 is an easy-measurable and highly sensitive biomarker for Niemann-Pick disease: a two year study

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Niemann Pick Type C (NPC) disease is an autosomal recessive disease caused by mutations in NPC1 or NPC2 genes translated in defects of the lysosomes cholesterol transport system leading to abnormal accumulation of cholesterol and glycolipids in the lysosome. We present data from a 2 year global cohort of Niemann Pick patients

using lyso-SM-509 biomarker determination, followed by sequencing of NPC1/2 genes. The levels of lyso-SM-509 in blood reflect the burden of the NP disease and it can be used for the easy diagnosis of NPC patients and for the monitoring of the disease progression. Determination of lyso-SM-509 is performed by LC/MRM-MS in plasma, serum, EDTA blood and dried blood spots (DBS). We identified in a world-wide study using lyso-SM-509 as primary screening in DBS samples 268 NPC and 147 SMPD1 affected patients .The diagnosis was confirmed by sequencing of the NPC1/NPC2 genes. In NPC1/2 sequencing negative patients with increased lyso-SM-509 concentrations the sequencing of sphingomyelinase (SMPD1) gene was done. Lyso-SM-509 has a sensitivity of 100 % and specificity of 99.15 % for NPC1/2. Most of the NPC cases were diagnosed in the age of3 to 10 years (30.65 %). We could identify over 700 pathological alleles in 425 different NPC cases. Only 42% of the unique variants identified in this study were previously published. Most NPC cases were linked with detailed clinical information; the most common symptoms were: hepatomegaly (63.6%), splenomegaly (55.8%), neurodevelopment delay (53%), ataxia (35.6%), psychopathology (34.6%), brain atrophy (30.4%), ophthalmoplegia (30.4%), spasticity (30%)Dystonia (24.4%), seizures (20.7%).

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P14.058B

Targeted next-generation sequencing for clinical of genomic profiles of idiopathic infertile men and a comparison of variant calling pipelines

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The improvement of the knowledge on the molecular basis of spermatogenesis failure allows gaining insights into the interplay of causative disease-associated genes and genetic variants changes of the genome in male infertility.

We redesigned the previous AmpliSeq panel to capture the exons and introns of 135 genes to identify the genetic variants that could be associated with male infertility based on a targeted sequencing protocol onto Ion Torrent PGM platform

Here, we analyzed 42 out of 120 patients with idiopathic infertility that presented no Y chromosome microdeletions. In addition, for a systematic comparison we assessed the impact of different variant calling pipelines comparing the same read aligner, BWA-MEM, and four variant callers GATK HaplotypeCaller, SAMtools (the vcfutils.pl script), Platypus and FreeBayes. Veen diagrams using the R package were constructed to illustrate the concordance among all variant-calling pipelines.

We identified 63 possible causative variants in 42 different genes, excluding common polymorphisms, and a higher concordance among the GATK, SAMtools and Platypus (72%) variant calling pipelines compared to a lower concordance with the FreeBayes variant caller.

Our data found an essential variation between the analyzed variant-calling pipelines and suggests the standardization of the available bioinformatics pipelines to reduce the implication on analyzing the next-generation sequencing data (financial support CNCS-UEFISCDI grant PN-II-RU-TE-2014-4-0527).

F. Raicu: None. **R. Cocoş:** None.

P14.059C

Whole exome sequencing or small panels for developmental disorders? Read between

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Second generation of sequencing technologies is now widely used for genetic diagnosis. Nevertheless, whole genome or exome sequencing are still challenging, mainly because of their expensive cost and the broad amount of data they provide. In this study, we present another diagnostic tool: the medical exome. Its analysis is restricted to OMIM genes of clinical interest.

219 patients with developmental disorders were recruited from two French genetic centers, the Groupe Hospitalier Pitié-Salpêtrière and the Centre Hospitalier Universitaire of

Rennes. Their clinical features correspond to our day-to-day practice i) patients with developmental disorders but no precise diagnoses, ii) patients with well-defined clinical hypotheses implying Sanger sequencing with high expenditures. Each case was screened for fragile X syndrome when appropriate and for chromosomal microarray before inclusion. Samples were prepared with the Illumina TruSight One kit. After sequencing, every pathogenic variation was confirmed by Sanger Sequencing.

We had a global diagnosis yield of 26%. Clinical exome was particularly efficient to identify a pathogenic mutation in patients with neurological disorders including epilepsy, intellectual disability, micro or macrocephaly and neurodegenerative disorders. In contrast, only few mutations were identified in patients with autism.

Medical exome thus appears as a good compromise because of its moderate cost and its capacity to diagnose efficiently genetically heterogeneous conditions in a medical purpose. It provides a way to reduce the probability of subsequent sequencing and may thus limit diagnostic odyssey.

V. David: A. Employment (full or part-time); Significant; Employment. **E. Cherot:** A. Employment (full or part-time); Significant; Employment. **C. Mignot:** A. Employment (full or part-time); Significant; Employment. **C. Dubourg:** A. Employment (full or part-time); Significant; Employment. **W. Carré:** A. Employment (full or part-time); Significant; Employment. **A. Lavillaureix:** A. Employment (full or part-time); Significant; Employment. **D. Héron:** A. Employment (full or part-time); Significant; Employment. **F. Demurger:** A. Employment (full or part-time); Significant; Employment. **M. Fradin:** A. Employment (full or part-time); Significant; Employment. **C. Quélin:** A. Employment (full or part-time); Significant; Employment. **C. Depienne:** A. Employment (full or part-time); Significant; Employment. **S. Odent:** A. Employment (full or part-time); Significant; Employment. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Groupama. **C. Nava:** A. Employment (full or part-time); Significant; Employment. **L. Pasquier:** A. Employment (full or part-time); Significant; Employment. **B. Keren:** A. Employment (full or part-time); Significant; Employment.

P14.060D

A novel high-multiplex homogeneous PCR assay format

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Background: Multiplex PCR has become an increasingly popular method to provide more, clinically relevant answers from the same sample. In many clinical settings, achieving multiplex answers from the same sample provide benefits both in terms of cost, speed, added clinical value as well as preservation of limited samples. In cases such as sepsis, RSV-testing, gastrointestinal testing and many others, a broad spectrum of agents are relevant for testing to assess possible infections. However, PCR readout is commonly limited to the current maximum of 4–5 fluorophores on most instruments. We have developed MeltPlex - a homogenous assay method to allow read-out of more than 20 answers from a single PCR reaction.

Material/methods: MeltPlex utilizes a system of labelled probes allowing each to be read out by subsequent melting curve analysis by more than 5 probes per fluorophore. By utilizing meltcurve readout of modified probes - one for each target - rather than the only amplicons, the system adds an extra level of specificity to meltcurve analysis. Reaction and melting analysis is performed without the need to re-open PCR reaction tubes.

Results: We will present proof-of-principle of the method and demonstrate its relevance in routine clinical infectious agent testing.

Conclusions: MeltPlex comprise a robust, high-multiplex, homogeneous system to provide 20+ readouts per PCR reaction.

S.M. Echwald: A. Employment (full or part-time); Significant; Anapa Biotech A/S.

P14.061A

A highly flexible and fast approach for high-throughput genetic testing using next-generation amplicon sequencing

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Introduction: Parallel sequencing of hundreds of patients for a small number of genes has become very cost-efficient using next-generation amplicon sequencing. We have established amplicon-based assays to screen patient samples for disease-associated mutations in a broad variety of treatable rare diseases in parallel. This approach utilizes multiplexing that allows large numbers of libraries to be pooled and sequenced simultaneously during a single sequencing run using the Illumina MiSeq system.

Material and Methods: Our assay design consists of two subsequent PCR amplification reactions. The first

multiplex PCR of ~150bp amplicons is designed to cover all coding exons, part of the promoter region and deep intronic regions previously reported to harbor disease-associated variants. A second PCR amplification is performed with the purified amplicons. This reaction adds Illumina-compatible and patient-specific barcoded index primers to each amplicon. After second purification, samples are pooled and prepared for sequencing. After sequencing on the MiSeq (2×150bp paired-end), de-multiplexing, alignment of sequence reads and variant calling is performed with an in-house bioinformatics pipeline.

Results: This setup allows multiple combinations, eg. complete sequencing of 250 patients for the GLA gene AND 10 BRCA1/BRCA2 gene assays AND CFTR gene sequencing for 20 patients (10.000 amplicons total). To enable high-throughput analysis, the whole workflow is automated including order entry, variant interpretation with CentoMD®, batch report writing and portal-based report-transmission.

Conclusions: This automated amplicon-based NGS approach using the MiSeq System allows high-throughput genetic testing of multiple genes and a high number of patient samples in parallel by a single sequencing run.

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P14.062B

A new methodology for controlling mutagens using cancer cell line SKOV3

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Sister Chromatid Exchanges (SCEs) is a known mutagenicity test and its frequency enhances as a consequence of exposure to various mutagenic agents. It is appeared also to indicate DNA damaging effects and/or subsequent repair by homologous recombination. The purpose of this study was to test the applicability of cancer SKOV3 cells, as a mutagenic and genotoxic screening test for various environmental agents using SCEs alone or in comparison with normal human peripheral lymphocytes (NHPL). SKOV3 cells are human ovarian cancer cells and our results are compared using the mitotic index (MI), the proliferation rate index (PRI) and the frequency of SCEs. By this way, we managed to determine qualitatively and quantitatively the damage caused by camptothecin (CPT), a known anticancer agent, used as a positive control. SCEs methodology resolves problems concerning the structure and function of chromosomes and DNA replication and it detects mutagenicity and/or genotoxicity, even at low concentrations and it can be applied to normal or cancer cells, both in vivo and in vitro experiments. The results of our study showed that cancer SKOV3 cells are more sensitive in CPT, in very low concentrations, in comparison to lymphocytes. Additionally, they showed that cancer SKOV3 cells need much less quantity of BrdU and any possible mutagen to display high levels of SCEs. We also approved, that cancer SKOV3 cells appear concurrency which makes easier the calculation of MI, PRI and SCEs.

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P14.063C

Diagnosing unsolved patients by combining DNA sequencing with bloodtranscriptomics

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Introduction: The University Medical Center Groningen has a rapid whole genome sequencing project for newborns and young children admitted to the intensive care that are suspected to have a genetic disorder. Unfortunately, thus far only about 30% of these patients can be successfully diagnosed. We aim to provide a diagnosis for additional patients by using gene expression data (RNA-seq) obtained from blood.

Material and Methods: Using RNA-seq data, we identified genes with aberrant gene expression levels and

alternative splice junction/exon usage. We compared the patients' RNA-seq data to a reference panel of blood RNA-seq data from 4,000 population based Dutch samples, while correcting for metabolic and physiological variation that has been defined by a re-analysis of 32,000 public RNA-seq samples.

Results: We show that severe aberrant expression/splicing events can indeed be observed in the causal gene 3 out of 9 patients in whom a genetic diagnosis has already been made. Interestingly, although these disorders do not manifest themselves in blood, these effects are detected in this easily accessible tissue. Subsequently, we have identified aberrantly expressed genes in the unresolved patients and compared this with variants of unknown significance, which have been found through whole genome sequencing of these patients. This resulted in the identification of a small number of aberrantly expressed genes with mutations that we are currently following up.

This work is supported by a grant from the European Research Council (ERC Starting Grant agreement number 637640 ImmRisk) to Lude Franke, two VIDI grants (917.14.374 and 917.16.455) from the Netherlands Organization for Scientific Research (NWO) and a grant from the Gratama Stichting (2015–06) to Patrick Deelen.

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P14.064D

Copy number variation detection using small, targeted panels and the NEBNext Direct enrichment method

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Variations in copy number are the most common type of DNA structural variations. These alterations are often associated with disease or indicate disease susceptibility. Clinical next-generation sequencing applications require fast and cost effective methods such as targeted resequencing, but copy number variations (CNVs) can be difficult to detect with these methods.

Here we describe the use of the NEBNext Direct® hybridization-based target enrichment method to identify somatic CNVs with high sensitivity. This approach captures gDNA fragments prior to library preparation and PCR amplification then converts the targeted fragments into an Illumina-compatible library that has defined 3′ ends and contains unique molecular identifiers (UMIs). The UMIs both enhance the identification of duplicate reads to ensure confident allelic frequency calls and allow for duplicate consensus-based error correction to increase sensitivity.

To validate this approach, we used two control systems. First, we titrated DNA from a cell line known to contain a deletion in the CDKN2A gene into a HapMap DNA sample that contains full diploid copies of CDKN2A. Second, we used a primary ductal carcinoma cell line containing several copy number variations sites combined with a matched control. In each case, we were able to detect the gene deletions and duplications as a somatic CNV in a dose dependent manner with high sensitivity and accuracy. Thus, we demonstrate that the NEBNext Direct approach is an efficient technique to detect somatic CNVs of high and low frequencies.

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P14.065A

Implementation of an automated sample quality control tool in a whole exome sequencing workflow

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Sample quality control is of major importance in the Next Generation Sequencing (NGS) workflow as it avoids time- and cost-intensive repetition of experiments. Here the German Cancer Research Center (DKFZ) demonstrates how an automated electrophoresis system can be used to control an exome sequencing workflow to assure the generation of high quality sequencing data.

88 genomic DNA samples from formalin-fixed paraffin-embedded (FFPE) tumor tissues were analyzed. Samples that passed the initial quality assessment, with respect to sample quantity and integrity, were submitted to the library preparation workflow. Depending on the DNA integrity, a suitable DNA fragmentation protocol was used to enable library preparation from degraded DNA. Implementation of quality control steps at various steps throughout the protocol allowed the monitoring of the workflow by checking for

library preparation artifacts, as well as controlling sample concentrations and average fragment sizes to increase read number during sequencing.

The stringent quality control criteria used by the DKFZ allow for an increase in efficiency during library preparation and ensure reliable sequencing results in high throughput sample analysis.

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P14.066B

The evaluation of a new NGS system (GeneReader NGS System) in clinical use of cancer diagnostics: The first report

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Introduction: As a result of technological and practical advances, next-generation-sequencing(NGS) becomes a standard molecular diagnostic tool. However, the interpretation of NGS data is still the critical step by allowing clinicians to act on genomic information for new therapies that target somatically mutated genes. Thus, we evaluated new NGS system(GeneReader-NGS-System) that is the most recent one in the market for the diagnosis of somatic mutations in various cancer types.

Materials and Methods: *KRAS, NRAS, KIT, BRAF, PDGFRA, ALK, EGFR, ERBB2, PIK3CA, ERBB3, ESR1* and *RAF1* genes were sequenced in FFPE tumor samples by a new NGS system (GeneReader-NGS-System) according to the manufacturer's manuals but with modifications.

Results: 127 tumor samples were analyzed which; 15 of 127 were malign-melanoma(11.8%), 89 were lung(70.1%), 19 were colorectal(14.9%) and 4 were ovarian cancer (3.2%). 66.7%(n = 11) malign-melanoma patients had pathogenic variations in *BRAF, NRAS, KIT* and *PIK3CA* genes. 50.6%(n = 45) lung-cancer patients had mutations in *EGFR, ALK, PIK3CA, BRAF, ERBB2, ERBB3, KIT, NRAS* and *KRAS* genes while 4 (4.5%) patients had uncertain significant variants in *PDGFRA* and *KRAS* genes. 36.8%(n = 7) colorectal patients had clinically significant variations in *KRAS, NRAS, BRAF* and *EGFR* genes while 1 patient (5.3%) had an uncertain significant variant in *EGFR* gene. One ovarian-cancer patient(25%) had pathogenic variations in *PIK3CA* and *RAF1* genes.

Conclusion: NGS systems became most successful diagnostic tool, only in hands of experienced medical geneticists for detection of clinically relevant variants. This is the first report of this new NGS system as a useful diagnostics in clinical practice across wide range cancers.

A. Bisgin: None. **O. Sonmezler:** None. **I. Boga:** None.

P14.067C

Validation of OS-Seq panels for clinical diagnostics of inherited disorders

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We have developed a comprehensive set of next-generation sequencing tests for detecting single-nucleotide variants (SNVs), insertions and deletions (INDELs) and deletions and duplications (Del/Dups) in all inherited diseases. The 214 panels contain 2053 clinically actionable genes that span 2243 conditions in 14 medical specialities. We adopted Oligonucleotide-Selective Sequencing (OS-Seq) method for targeted sequencing and implemented the flow-cell capture using the Illumina NextSeq500 sequencing system. We generated six OS-Seq assays that targeted 374 to 585 genes' coding exons, 15 adjacent bases of the splice regions and pathogenic intronic variants. Analytic validity of the NGS assays was demonstrated for SNVs, INDELs and Del/Dups using cohorts of reference samples with high-quality variant calls. Analytic validation results showed that the NGS assays achieve, on average, 0.993 sensitivity, 0.999 specificity, 0.993 positive predictive value for detecting SNVs and 0.961, 0.885 and 0.668 sensitivity for detecting INDELs of 1–10, 11–20, and 21–30 bases, respectively. Longest detected INDEL was 46 bases. Repeatability and reproducibility of the OS-Seq assays were 0.994 and 0.998, respectively. 99.6% of the target regions were covered with over 15x sequencing depth and mean sequencing depth at nucleotide level was 234×. We demonstrated the assays' sensitivity to detect different size (1–4 exons) Del/dups: 0.715, 0.952, 0.990 and 0.999. Del/dup detection was demonstrated to detect >316bp deletions and >544bp duplication and clinical sensitivity of the del/dup detection was 92.42%. Our results demonstrate the analytic validity of the Blueprint Genetics' sequencing panels and show that the technology is well-suited for clinical diagnostics of inherited disorders.

S. Myllykangas: A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **P. Salmenperä:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint

Genetics. **M. Gentile:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics. **S. Bruce:** A. Employment (full or part-time); Significant; Blueprint Genetics. **M. Valori:** A. Employment (full or part-time); Significant; Blueprint Genetics. **M. Muona:** A. Employment (full or part-time); Significant; Blueprint Genetics. **A. Sarin:** A. Employment (full or part-time); Significant; Blueprint Genetics. **J. Koskenvuo:** A. Employment (full or part-time); Significant; Blueprint Genetics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Blueprint Genetics.

P14.068D

Diagnosis of mendelian disorder using a comprehensive 4813 genes next-generation sequencing panel - review of 111 cases

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Introduction: The accurate choice in each patient between a (broad vs focused) gene-targeted panel or whole-exome sequencing (WES) and their respective interpretation constitute crucial challenges for the clinical geneticist. In this study, we sought to review our experience analysing one-hundred-eleven consecutive cases using a broad multi-gene panel. Methods: A predesigned next generation sequencing (NGS) panel for 4813 genes with known associated clinical phenotypes (Illumina Trusight One) was performed in selected patients with unknown genetic or with a diagnosis with genetic heterogeneity. We analysed the laboratory reports which complied with the ACMG 2015 guidelines, clinically re-evaluated each patient and their medical records, performed family studies when appropriate and confirmed or reclassified the variants. These variants were categorized in four groups: pathogenic or likely pathogenic variants in disease genes associated with the reported phenotype (Group 1), variants of uncertain significance in disease genes associated with the reported phenotype (Group 2), secondary findings according to the ACMG 2013 recommendations (Group 3) and other variants not associated with the reported phenotype (Group 4). **Results:** One-hundred-eleven index unrelated patients were tested. The laboratory reported a total of 150 variants. After our classification, we concluded that 30 variants should be included in Group 1, 6 in Group 2, 3 in Group 3 and 111 in Group 4. A diagnosis was achieved in 31 patients which represents an overall diagnosis yield of 28%. Discussion: We will present a comparison with the few other studies in the literature which used a broad multi-gene NGS panel and also WES.

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P14.069A

A novel method for building custom AmpliSeq™ panels using optimized PCR primers

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AmpliSeq™ is a next generation sequencing library preparation method for targeted re-sequencing that utilizes highly multiplexed PCR to amplify regions of interest. A key to successful AmpliSeq libraries is the primer panel used for target amplification. Until now primers have been available as pre-assembled ready-to-use panels, or as custom "made-to-order" panels. We describe a new process for creating customized panels consisting of optimized and verified PCR primers. The primer sets are available as whole genes (i.e., all of the primers needed to create libraries that cover the entire coding regions of genes) and

are selectable on the ampliseq.com website by either uploading gene lists or choosing genes from disease research areas.

We show NGS sequencing data from 10 disease research-oriented panels, including newborn screening research and inherited cancer research, assembled from individual pre-verified gene sets. Panel performance data include coverage uniformity, reproducibility, and sensitivity and specificity of variant calling. To demonstrate flexibility of panel content and performance, the coverage uniformity of the 59 genes recommended by the American College of Medical Genetics and Genomics for reporting of incidental findings (ACMG59) was evaluated in various panels with 50–250 additional genes and shown to be ≥ 97% in all contexts. We also demonstrate the robustness of this method using a variety of sample types (fresh and dried blood, saliva, cheek swabs) with both manual and fully automated library preparation methods. For Research use only. Not for use in diagnostic procedures.

S.J. Roman: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **A. Kothandaraman:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **C. Van Loy:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **A. Broomer:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **T. Biorac:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **X. Duan:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **J. Kilzer:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **M. Allen:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **J. Chang:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **D. Mandelman:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **F. Hyland:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **M. Manivannan:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Fu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Zhu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **E. Williams:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **A. Hatch:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Tian:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Sovan:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **M. Andersen:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific.

P14.070B

Multiplexed next generation sequencing reference materials for testing of inherited disorders

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Introduction: Next Generation Sequencing (NGS) has rapidly advanced genetic testing for inherited disorders. However, these assays present challenges for test development, validation, and quality control. Reference materials are essential for validating and monitoring NGS assays during wet-lab procedures and subsequent software analysis. We developed a reference material technology that can be used to assess the detection of variants, including those that are rare and challenging, such as large indels (>20 bp).

Materials and Methods: GM24385 human gDNA was blended with biosynthetic constructs containing variants introduced into the endogenous sequence. The construct was titrated to 50% allele frequency, confirmed using digital PCR (dPCR). A pilot reference material for hypertrophic cardiomyopathy was developed and tested using multiple capture-based NGS assays. The same concept was then extended to select mutations in seven inherited cancer genes.

Results: NGS testing showed that variants in the construct were detected as heterozygous sites and these data mimic that of the same variant when seen in a patient. SNVs were detected with allele balances between 47–52%. A 25 bp deletion was detected at ~30%, similar to the bias observed in patient samples for this variant even though the frequency was 50% by dPCR.

Conclusions: The Seraseq™ Inherited Disease Reference Materials are designed to challenge NGS assays and fulfill needs for accurate, multiplexed quality controls. These materials can be used to challenge variant detection pipelines, aid in assay optimization, and provide assurance when detecting complex mutations.

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P14.071C

SALSA: a fast, accurate, validated software package for clinical NGS panel data analysis

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The demand and volume of clinical NGS panel testing is increasing rapidly. However, the speed, accuracy and consistency of clinical NGS analytical pipelines would benefit from improvement in many labs. We have developed a Standardized Automated Large-scale Sequencing Analysis pipeline (SALSA), to quickly and accurately analyze NGS panel data to clinical standards. Importantly, SALSA accurately identifies both small variants (base substitutions, small indels) and exon CNVs (including single exon deletions/duplications). SALSA provides comprehensive quality metrics, allowing identification of exons that do not meet user-specified quality and coverage thresholds. Additional, customisable interpretation information can also be integrated. SALSA returns easily readable outputs, to clinical standards. We used SALSA to analyse >8.000 samples tested in batches of 96 with the TruSight Cancer panel, which targets 100 genes. The average SALSA runtime for 96 samples is eight hours. We evaluated SALSA performance with orthogonally generated data of 995 results in 52 genes; 234 negative results, 303 base substitutions, 383 indels and 75 exon CNVs. Sensitivity was 100% for all variant classes. Specificity was 229/233 (98%). All four false positives were flagged as poor quality by SALSA. SALSA also correctly flagged four results as failing predefined quality thresholds, avoiding false negative results. SALSA does not require bioinformatic expertise to run and it easily, accurately and quickly processes clinical NGS panel data. SALSA is now in use in TGLclinical, a clinical genetic testing laboratory accredited to ISO15189, and a freely-available version is being released for other labs to use. Funder, Wellcome:098518/Z/12/Z

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P14.072D

Incidental findings related to sex determinism in a NIPT workflow

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Non-invasive Prenatal Testing (NIPT) has been implemented in many genetic labs for the screening of trisomies of chromosomes 13, 18 and 21. More than 6000 samples have been tested in our facility. Our workflow was not validated to detect sex chromosome aneuploïdies but it can reliably identify fetal sex. This experience lead to the detection of 4 interesting clinical situations.

1. A male 46, XY fetus with an ambiguous female phenotype by ultrasound. Because of this sexual ambiguity and in utero growth retardation sequencing of DHRC7 was performed. Two pathogenic mutations responsible for Smith-Lemli Opitz syndrome were observed : c.452G>A (p. Trp151*) and c.1190C>T (p.Ser397Leu) .

2. A suspicion of monosomy X (Turner) was reported given negative Z-score values for X chromosome. At birth baby's karyotype was 100% 46, XX but mother's karyotype showed a mosaïcism 45×(28%) /46,XX (72%).

3. Another suspicion of monosomy X in a male fetus by ultrasound. FISH analysis demonstrated the presence of mosaicism in amniocytes with a mixture of 45,X cells (20%) and male cells with an iso-chromosome Yp (80%).

4. A male 46, XY fetus with a non ambiguous female phenotype by ultrasound. Further analysis showed that the mother is carrier of a pathogenic mutation in exon 1 of the androgen receptor gene (c.1421_1425dupAGGCG, p. Gly476Argfs*5) transmitted to the fetus as well as to her 2 year old 46,XY daughter.

These results illustrate some of the incidental findings which can be identified in the context of NIPT with an adequate genetic follow-up.

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P14.073A

Optical mapping of enriched, megabase-sized DNA molecules in nanodevices

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Despite the enormous progress in next-generation sequencing (NGS), *de novo* assembly of genome sequence and the detection of genomic structural variations (SVs) remain a challenge. Single DNA molecule optical mapping, considered as a complementary technique to NGS, enables the analysis of megabase-sized DNA molecules and is useful for the detection of large-scale SVs. Sequencing and optical mapping of a whole human genome is time consuming and costly, and frequently only a specific genomic region is of interest. Here, we present a method for selection and enrichment of megabase-sized DNA molecules for single-molecule optical mapping in nanodevices based on *NotI* rare-cutting enzyme digestion and size-selection by pulsed-field gel electrophoresis. More than 600 sub-megabase to megabase sized DNA molecules were recovered from the gel and analysed by optical mapping in a nanodevice. Size-selected DNA from the same gel were sequenced by NGS. Both the molecules analysed by optical mapping and the reads from NGS showed enrichment for long molecules from regions defined by the *NotI* recognition sites. The method provides a low-cost option for investigation of SVs in enriched human genomic regions, including SVs in human diseases. We can identify molecules that flank and reach into the unannotated part of the genome, and thus allow the visualization of these regions that are extremely difficult to analyse with other DNA sequencing methods. Moreover, the method could be adapted to work with any other genome or target other regions by applying multiple restriction enzymes or other genomic editing tools, such as the CRISPR/Cas9 system.

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P14.074B

Off the street phasing (OTSP): Free no hassle haplotype phasing for molecular PGD applications

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Introduction: Parental phasing of mutation-flanking haplotypes is an essential, yet time-consuming, laborious, and costly pre-requisite for preimplantation genetic diagnosis (PGD) of monogenic disorders. Accordingly, the aim of this study was to validate rapid, low cost, population-assisted haplotype derivation in a pre-clinical setting.

Materials and Methods: Targeted sequencing of *CFTR* variants and gene-flanking polymorphic SNPs in 38 Jewish individuals from 9 different PGD families was performed at the SZMC PGD lab. Heterozygous genotype calls were both trio-phased to obtain ground-truth haplotypes, and also population-phased using Shapeit software. Reference panels for population phasing were derived from either '1000 Genomes' or from 128 or 574 sample Ashkenazi Jewish whole genome sequences (kindly provided by The Ashkenazi Genome Consortium). Accuracy of resulting haplotypes was benchmarked against trio-phased haplotypes. The study population consisted of 4 subgroups as shown in Table 1.

Results: Haplotype phase benchmarking results are summarized in Table 1. In general, the 574 sample Ashkenazi genome reference was the most accurate and appropriate for population-based phasing. Importantly, virtually all phase errors in the FA group (and the PAWM group, along a subregion of 3Mb) were traceable to low-coverage sequencing errors in the ground-truth.

Discussion: These striking results indicate that it may soon be possible to replace experimental haplotype phasing with clinical "OTSP," population-based phasing, provided that one has access to an appropriate population-matched reference dataset of sufficient size.

Table 1

Study subgroup (Abbreviation; No. individuals)	1000 Genomes Avg. No. Heterozygous SNPs per individual (+/-SEM)	1000 Genomes Phasing Accuracy (+/-SEM)	128 Ashkenazi Ref. Avg. No. Heterozygous SNPs per individual (+/-SEM)	128 Ashkenazi Ref. Phasing Accuracy (+/-SEM)	574 Ashkenazi Ref. Avg. No. Heterozygous SNPs per individual (+/-SEM)	574 Ashkenazi Ref. Phasing Accuracy (+/-SEM)
Non-Ashkenazi (NA;10)	589.4+/-66.2	71.0% +/-4.2%	519.9+/-57.3	72.7% +/-4.2%	579.4+/-62.3	84.9% +/-3.3%
Partial Ashkenazi no CFTR mutation carriage (PANM; 13)	544.5+/-35.9	73.1% +/-4.3%	484.9+/-32.8	78.9% +/-4.9%	547.8+/-34.7	86.7% +/-5.2%
Partial Ashkenazi W1282X Ashkenazi mutation carriage (PAWM; 5)	741.2+/-48.7	70.9% +/-4.0%	665.6+/-40.3	94.3% +/-2.2%	732.8+/-47.0	**91.4%** +/-5.3%
Full Ashkenazi (FA; 10)	626.5+/-48.5	65.2% +/-6.4%	559.4+/-42.0	88.4% +/-5.5%	621.4+/-47.4	**98.2%** +/-1.1%

G. Altarescu: None. **F. Zahdeh:** None. **Y. Kling:** None. **P. Renbaum:** None. **E. Levy-Lahad:** None. **S. Carmi:** None. **D.A. Zeevi:** None.

P14.075C

Automated patient matching from facial photos - initial feasibility study Automated patient matching from facial photos - initial feasibility study

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Introduction: The importance of matching patients for diagnosis and gene discovery necessitates connecting clinicians and researchers from around the world. This is an initial feasibility study to assess if the automated facial recognition technology is able to provide connections between undiagnosed patients with similar facial phenotype.

Methods: We composed a survey of 53 pairs of possibly matching facial photos of diagnosed, undiagnosed and control patients, asked four geneticists to rate the similarity between each pair and compared their results to the Patient Matching technology of the Facial Dysmorphology Novel Analysis (FDNA) algorithm. We used Pearson Correlation coefficient and the area under the curve (AUC) of the ROC curve to assess correlations.

Results: Of the diagnosed pairs, the phenotype comparison performance of the FDNA technology (3.6 on similar pairs, 3.25 on different pairs) and the geneticists (2.5 on similar pairs, 2.25 on different pairs) was comparable, with a slight advantage to the technology. This trend is also seen by AUC: 0.65 for the technology vs. AUC 0.51 for the mean of the clinician's answers. The overall agreement level between the four geneticists is relatively low (mean correlation 0.52).

Conclusions: The importance of the task and the comparable results is a strong motivator to continue with a second stage of a feasibility study, with more photos, more clinicians and without the intrinsic biases - familiarity of geneticists with the patients. Future applications of this technology could complement next generation sequencing in undiagnosed patients and lead to the discovery of rare novel syndromes

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P14.076D

Proteomic Peptide Screening of Dried Blood Spots: A Potential Clinical Application

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Background: Many proteins of great interest for NBS are often in the low ng/mL range existing only inside the cells. Recently, peptide immunoaffinity enrichment coupled to SRM (immuno-SRM) has emerged as a promising technique for the quantification of low abundance proteins in complex matrices such as dried blood spots (DBS). We investigated the immuno-SRM methodology to determine the concentrations of ATP7B protein in DBS from unaffected and 13 Wilson disease (WD) patients and demonstrated a possible screening for WD in newborns. **Methods:** Several candidate peptides of each targeted protein for 11 primary immunodeficiencies, Wilson disease and Cystinosis were screened by *In Silico*, BLAST search, then selected based on detectability and response in LC-MS/MS analyses followed by polyclonal/monoclonal antibody generation for enrichment. We then analyzed these target peptides in DBS using SRM mode with 6500 QTRAP (ABSCIEX) to develop a high-throughput, multiplex assay. **Results:** Cystinosin and three SHPK target peptides were absent in two Cystinosis patients (57-kb del homozygotes). A multiplexed immuno-SRM assay was configured using polyclonal-based antibodies targeting two to three peptides from the three-targeted proteins (BTK, CD3e, and WASP). Two BTK targeted peptides were absent or markedly reduced in two XLA patients, while the other target peptides designed to detect SCID, WAS in the XLA patients were normal. **Conclusions:** We have demonstrated that extremely low abundant proteins can be detected by immuno-SRM analysis in complex samples such as DBS. When multiplexed, there is a significant potential to increase our capacity to screen various congenital disorders lacking specific protein markers.

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P14.077A

New deep intronic mutation c.1029+384 A to G in *SERPING1* gene creates *de novo* donor splice site and causes aberrant splicing

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Traditionally, the diagnostics of hereditary diseases is primarily focused on detection of mutation in exons or immediately flanking regions of introns. However, the occurrence of the cases with no detected mutations is not unusual. The diagnostic process in these cases is very variable and may include analyses at mRNA level and/or applying next generation sequencing approach. In this work, we have investigated a family diagnosed with hereditary angioedema with no detected exonic or flanking intronic mutation in *SERPING1* gene encoding C1 inhibitor protein. Nevertheless, subsequent capillary analysis of the blood-derived mRNA revealed a low abundant variant prolonged by approximately 89 bp, which had not been detected using standard gel electrophoresis. After intron sequencing, mutation c.1029+384 A to G was found 384 bp downstream of the exon 6, which co-segregated with HAE phenotype in all available family members. According to *in silico* splicing analysis the mutation results in strong donor splice site creation with possible subsequent pseudoexon activation. This hypothesis was confirmed by sequencing of the patient's cDNA using a specific primer hybridizing to the predicted pseudoexon and independently by a minigene analysis. The low amount of the aberrant transcript variant may be explained by its degradation by a natural mechanism called nonsense mediated decay. In conclusion, these findings highlight the importance of mRNA analysis and minigene assay as useful approaches in diagnostics.

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P14.078B

Optimising next generation sequencing data analysis to tackle pseudogene sequences in the PMS2 and PKD1 loci

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Introduction: Pseudogenes are segments of DNA related to real genes that have lost their ability to encode proteins. As sequence homology between the pseudogenes and the parental gene can be very high, such regions complicate molecular diagnostics. Famous examples are the pseudogenes of *PKD1* (involved in autosomal dominant polycystic kidney disease) and *PMS2* (associated with Lynch syndrome). For these genes several pseudogenes are described with a sequence homology up to 98% for certain regions. Materials and Methods: We optimized long range PCRs with primers located in unique regions. All coding exons of *PKD1* and *PMS2* are amplified in respectively 9 (ranging 2–4kb) and 3 (9kb, 9kb and 18kb) reactions. The fragments underwent Nextera XT library preparation and were sequenced on a Miseq. Data for 80 PKD1 and 20 PMS2 samples were used to set up a reliable data analysis workflow in CLCbio v7, detected variants are validated in an independent NGS run. Results and conclusion: Applying standard settings true sequence variants ($n = 108$) could be easily distinguished from false positives ($n = 22$) in PMS2 sequences. The *PKD1* locus showed more variation and it seems that in certain patients some co-amplification of pseudogene sequences occurs despite the use of gene-specific primers. Literature suggests to retain variants with VAFs of 20–30%. However, we found that in mosaic mutations may be missed applying these cutoffs. Careful visual inspection in combination with masking reads with putative mutations may distinguish mosaicism from pseudogene interferences.

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P14.079C

DName barcodes allow absolute quality control of genetic test processes based on NGS

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Genetic tests based on next generation sequencing (NGS) are complex and consist of many handling steps from patient sampling to result, which can lead to errors (e.g. sample switching and/or contamination). Genetic testing labs take extreme measures to overcome errors and will often process patient samples in duplicate to validate the obtained results, amongst many other QC procedures. However, this still does not guarantee 100% quality assurance and comes with a considerable extra cost. We have developed a powerful technology that provides quality assurance throughout the entire genetic testing process. The technology allows to economically produce over millions of unique spiking DNA molecules (DName barcodes), which are used only once. Proof of principle experiments illustrate that these barcodes can be universally applied with respect to the used DNA extraction method, NGS template preparation method and NGS platform, and detect sample switching and allows detection of contaminations. Different unique barcodes can be added at different time points in a genetic test process, such as at the time of blood collection, after DNA extraction before NGS sequencing, so that quality assurance problems can be traced to different sub processes in a genetic test process. NGS testing can thus be outsourced, while retaining full control of the process. The unique quality assurance properties of these barcodes will boost the reliability of genetic tests while maintaining patient confidentiality. Furthermore, it will help to unleash the unprecedented informative power of NGS by improving accuracy, fidelity and automation of genetic test processes at a reduced cost.

H. Cuppens: A. Employment (full or part-time); Significant; DName-iT. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; DName-iT. **S. Dillen:** A. Employment (full or part-time); Significant; DName-iT. **M. Jaspers:** None.

P14.080D

Check your cultures! Identification of genetic instability through karyotyping

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Many useful guides and guidelines exist for those working with cell cultures to be aware of contamination with microorganisms, phenotypic and genetic instability or even cell-line misidentification. A lot of these problems are avoidable with the nescessary foresight. Though, simple rules are frequently ignored. With depressing regularity retraction or modification of these data is seen.

Among the problems that continue to affect cell culture, genetic instability is an important mosaic stone. Depending on the cell type (senescent or immortal cell lines, human or not human, iPSC, cancer cell lines) the chromosomal content can be euploid, aneuploid (abnormal chromosome content) and heteroploid / mosaic (variable chromosome content within the population). Aneuploidy, heteroploidy or even special structural aberrations can be typical for specific cell lines. The latter can be very useful for authentication. Descendents of normal lines or iPSC lines usually are euploid. Anyway, pluripotent stem cell lines (i.e. iPSCs), which are usually clonal in origin, are well known to be susceptible to developing chromosomal changes. Therefore, they need to be periodically karyotyped.

The primary purpose of this poster is to increase awareness for genetic instability to those new in the field and those engaged in teaching and instruction. Results of conventional karyotyping (which has the advantage to detect balanced structural aberrations compared to array technologies) of different cell types and cell lines will be presented.

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P14.081A

The importance of External Quality Assessment for a molecular diagnostic center: the experience of a Brazilian Cancer Reference Center

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Introduction: The Barretos Cancer Hospital (BCH) is a Brazilian reference cancer center, offering genetic testing to patients and relatives at-risk for hereditary cancer predisposition syndromes (HCPS).

Methodology: Genetic tests are performed using laboratory developed and validated methodologies (Sanger/NGS sequencing and MLPA). Since 2011, the Molecular Diagnostics Center (MDC) of BCH has participated annually in the EMQN External Quality Assessment (EQA) schemes (BRCA, FAP, HNPPC, VHL schemes).

Results: Hitherto, over 3,200 individuals from 1,600 families with criteria for HCPS have been referred for genetic testing, including 742 families tested for *BRCA1/2* genes (25% mutated), 125 for APC/*MUTYH* (50% mutated), 478 for *TP53* (15% mutated). 344 HNPCC families have received MSI/IHC screening, and 99 of them went on to have genetic testing (61% mutated). Furthermore, *CDH1/PTEN/CHEK2/RET* tests are also offered. Since participating in the EMQN-EQA schemes, we have observed a five-fold increase in the number of tests performed, not only from BCH patients, but also from other Brazilian hospitals and private laboratories. In addition, we have been able to raise awareness of the importance of verifying/certifying the quality of tests offered nationally, and making participation in EQA schemes a mandatory requirement for all Brazilian genetic laboratories/centers.

Conclusions: Participation in EQA programs has resulted in a significant improvement in the quality of our genetic test results and reporting. In addition, it brought national visibility to the center and enabled other Brazilian centers to see the importance of participating in EQA as a tool for benchmarking performance and improving test quality.

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P14.082B

Quality Sequencing Metric (QSM) a concise, transparent, description of NGS data quality

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Consistent, transparent NGS data analysis is essential for research and clinical applications. Conventional descriptions typically give basic summary information, e.g. the average coverage across the target. This is inadequate for clinical requirements and many research settings. More comprehensive quality information, available per base, is typically required. However, it is also essential that the information can be compared across platforms, which requires common standards and a transparent, easy-to-use, easy-to-understand notation. Here we describe the QSM, a quality sequencing metric that aims to achieve these objectives. The QSM includes information about coverage (C), Base Quality (B) and Mapping Quality (M). This is given per base, but can also be provided per target, or per exon, as appropriate. For example, the QSM for small variant detection in a pipeline will be given per base whereas the QSM for exon CNV detection will be given per exon. Importantly, fulfillment of the QSM can be assessed directly from BAM files, allowing seamless integration in NGS pipelines. As an example, the QSM for TruSight Cancer Panel analysis in TGLclinical is C50B10M20. This means that every base has minimum Coverage of ≥50 reads with Base quality of ≥10 (in at least 85% of reads) and Mapping quality of ≥20 (in at least 95% of reads). A fuller description would be $C50B10_{85}M20_{95}$, to encompass the proportion of reads that are required to meet the base and mapping quality standards. The QSM allows comprehensive, transparent information about quality thresholds of NGS data, and could have broad utility.

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P14.083C

From Face to Gene - Identifying the Genotype of RASopathies with FDNA

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Introduction: RASopathies comprise a group of disorders caused by germline mutations in RAS-MAPK pathway genes. Characteristic features are growth retardation, heart defects and craniofacial dysmorphism. Facial Dysmorphology Novel Analysis technology (FDNA) was used to deeply phenotype and objectively evaluate the craniofacial features of RASopathy patients. We aimed to analyze the ability of the technology to discriminate between the facial phenotype associated with mutations in different genes.

Method: 256 images of 211 patients with mutations in either one of the genes PTPN11, SOS1, BRAF, RAF1 and RIT1 were analyzed by FDNA and grouped according to the pathogenic gene. The mean area under the curve (AUC) was used as a means of comparison between the samples together with ROC curve plotting the true positive rate as function of false positive rates. Binary as well as multiclass analysis was conducted.

Results: Mean AUC results yielded high values with relatively low STD, showing the best result for RIT1 and the lowest for SOS1. The multiclass challenge is able to assign the case to the correct gene out of five with a mean accuracy result of 61% with low STD (5.9), which is roughly three times bigger than the random chance accuracy of 20%.

Discussion: Facial recognition technology that detects dysmorphic features from 2D photographs holds the promise to assist in deep phenotyping of syndromic patients and automatically associating these clinical findings with

disease-causing genes. However, perfect recall is naturally limited by the significant overlap within a clinically and pathogenetically related group of genetic entities.

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P14.085A

Analysis of 477 systematic Sanger sequencing validations of NGS identified variants

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Introduction: The NGS technologies that have revolutionized genetic diagnostic are expecting to be generally implemented in clinical practice. The role of routine Sanger validation of NGS variants is actually under consideration. A proposal, specific for each technology, about which variants should be systematically evaluated, is necessary.

Material and Methods: We have analysed by Sanger sequencing a total of 477 variants (48 *indels* and 429 missense), with 302 unique changes, identified in exome analysis (Ion AmpliSeqTM Exome RDY, Ion ProtonTM/Ion S5TM) of patients with a variety of genetic disorders. Sanger sequencing was used to validate all variants classified as pathogenic or probably pathogenic. Additionally, variants located in homoplymeric, repetitive regions and/or variants located in low coverage regions were also analysed.

Results: Sanger sequencing results confirmed 462 out of 477 variants (97%) identified on the exome analysis. All SNVs not located in homoplymeric or repetitive regions with a coverage >20x and an heterozygous ratio >40%, were validated. Non-validated variants presented: (i) low coverage (<20×) (n = 8), (ii) heterozygous ratio < 20% (n = 3), and/or (iii) they were mainly located in homopolymeric or repetitive regions (n = 4).

Conclusions: Reported results show that Sanger confirmation should be considerer in all variants with low coverage (<20×), heterozygous ratio <20% and/or variants located in homopolymeric or repetitive regions. According to these results and in concordance with previous studies, systematic evaluation of Sanger validation in clinical practice should be restricted to variants with specific features. Validation criteria should be established based on the NGS selected approach.

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P14.086B

Elucidating of novel genetic causes: Exome sequencing at the Center of Rare Diseases Lübeck, Germany

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Background: Rare diseases are defined by a prevalence of <5/10,000 persons. However, a large number of different rare diseases affects millions of people. Notably, ~80% of rare diseases are thought to have a genetic origin. We here present data from an exome-sequencing project conducted in 2015–2016 which included ~100 patients. **Methods:** After careful clinical phenotyping and exclusion of 1–5 candidate genes, patients and, if available, their parents were exome-sequenced. We analyzed 20 parent-offspring trios, 12 quadros (two affected siblings and unaffected parents), 3 duos (affected offspring, one parent), and 54 single samples. **Results:** Most of the patients had a movement disorder with features of dystonia, parkinsonism, ataxia, or spasticity, possibly combined with intellectual disability. We identified novel mutations in 14 established disease genes including *ANK3, ATCAY, CACNA1A, ECEL1, MCOLN1, MT-TK, PANK2, PLA2G6, PNPLA6, PNPT1, POLG, SACS, SPG11, SYNGAP1*, often with an extension of previously described phenotypic features. In addition, we detected novel mutations in recently described disease genes such as *CAPN1, GNB1, KMT2B*, *TMEM230*, and *VAC14*. For some of the mutations, functional investigations were performed and demonstrated altered functionality for many but not all variants. The diagnostic yield in our study was 40–50% for trios and quadros but <10% in exomes of single patients. **Conclusions:** Exome sequencing is a useful tool to dissect the likely genetic cause in rare diseases and should be established in standard diagnostics. Currently, interpretation of the data poses the greatest challenge, particularly for variants of unknown significance requiring functional characterization. **Funding:** DysTRACT, DFG, UKSH foundation

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P14.087C

AUTOMATED APPROACH FOR RAPID AND ACCURATE SNP GENOTYPING

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Improvements to next-generation sequencing technologies have enabled extensive discovery of single nucleotide polymorphisms (SNPs) in numerous organisms. Due to their large numbers and genome-wide distribution, SNPs are the molecular marker of choice in plant, animal, and human genetic research. As the discovery of SNP markers continues to expand, there is a need for a more efficient method for routine genetic analysis. The IntelliQube® real-time qPCR instrument in conjunction with BHQplus® probe-based SNP genotyping assays provides an effective solution to address this need. BHQplus probes incorporate duplex-stabilizers allowing enhanced binding stability, enabling compact probe sequences with excellent mismatch discrimination. Utilizing Array Tape® technology, the IntelliQube integrates liquid handling with qPCR analysis in miniaturized reaction volumes. In this study, we assess the performance of custom BHQplus genotyping assays run in a duplex fashion using fast thermal cycling protocols. Accuracy and reproducibility of this platform was assessed using purified gDNA samples from cell lines obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. Genotype results were compared to previously published literature. The results demonstrate that the IntelliQube, when used in conjunction with BHQplus assays, provides an accurate and streamlined real-time PCR-based method for genetic analysis.

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P14.088D

Impact of probe density in single nucleotide polymorphism arrays (SNP-Array) tests for the detection of genomic alterations

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Background: SNP-Array is a molecular cytogenetics assay that genotypes DNA at thousands of SNPs across the genome simultaneously and detects copy number variation (CNVs), loss of heterozygosity (LOH) and low level mosaicism. Commercial SNP-array platforms vary in probe density, which impacts costs, but alters test sensitivity. We compared the results of two Affymetrix assays, Cytoscan HD (2.6 million probes) and Cytoscan 750k (750,000 probes) in selected patients. Methods: 13 patients presenting developmental delay and dysmorphisms with known previous results from KT/MLPA, FISH or CGH-Array were analyzed. Genomic alterations tested are shown in Table 1. Cytoscan HD was performed for all patients. Raw data from Cytoscan HD was used to simulate Cytoscan 750k data. Results between platforms were compared. **Results:** Cytoscan HD reproduced previous results, regardless the alteration size. Cytoscan 750k reproduced most results, except from patient T_4 (7.6%), with the smallest alteration tested: arr[hg19]9q34.3(140,651,785–140,659,056)x1 (7 kbp). Cytoscan HD contained 17 markers in this region, while 750k contained none. Two RefSeq genes (*EHMT1*, *FLJ40292*) were deleted. Region 9q34.3 encompasses gene *EHMT1*, associated with Kleefstra syndrome (OMIM #610253), a recognizable mental retardation syndrome among other phenotypical features, which matched the patient's clinical indication. **Conclusion:** Lower probe density may hinder identification of small genomic alterations, despite being a rare event. Overall, Cytoscan 750k is able to identify correctly most alterations with comparable results to that of Cytoscan HD.

Table 1 Genomic alterations for each sample used in this study.

Sample	Genomic Alteration	Size (kbp)	N° Markers in HD	N° Markers in 750k
T_1	arr5p15.33p15.1 (113,576–15,525,664) x1	15412	18836	3844
T_2_a	arr18q21.32q23 (56,315,322–78,014,123)x1	21699	18756	5555
T_2_b	arr15q11.2 (22,770,421–23,286,507)x3	516	784	281
T_3	arr4p16.1(9,501,60–9,953,835)x3	452	400	119
T_4	arr9q34.3 (140,651,785–140,659,056)x1	7	17	N/A
T_5	arr14q11.2 (22,855,144–22,940,138)x1	85	100	15
T_6_a	arr21q22.12 (35,944,343–37292,915)x1	1349	2876	860
T_6_b	arr16p11.2p11.1 (34,449,594–34,755,816)x3	306	176	64
T_7_a	arr8p23.3q24.3 (158,048–146,295,771) x3(0.7)	146138125254		35590

T_7_b	arr5p12p11 (45,365,046–46,365,391)x3	1000	712	235
T_8	arr5p14.2p14.1 (23,909,889–24,990,295)x1	1098	712	194
T_9_a	arr7p22.3q36.3 (43,360–159,119,707) x1	159076153640		42417
T_9_b	arr1q21.3q22 (154,567,653–155,322,846)X1	755	664	224
T_9_c	arr2p25.3p16.2 (12,770–54,147,279)x3	54135	50004	14343
T_9_d	arr2q24.1q24.2 (159,644,037–159,947,059)x3	303	258	65
T_10	arrXp22.33q28 (168,546–155,230,271) x1	155062189800		43954
T_11	arr16p11.2 (29,628,661–30,177,807)x1	549	393	393
T_12	arr16p11.2 (29,421,694–30,240,227)x3	819	452	188
T_13	arr21q11.2q21.3 (15,381,880–30,421,277)x1	15039	12605	3542

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Screening for causative structural variants in neurological disorders using long-read sequencing

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Over the past decades neurological disorders have been extensively studied producing a large number of candidate genomic regions and candidate genes. The SNPs identified in these studies rarely represent the true disease-related functional variants. However, more recently a shift in focus from SNPs to larger structural variants has yielded breakthroughs in our understanding of neurological disorders.

Here we have developed candidate gene screening methods that combine enrichment of long DNA fragments with long-read sequencing that is optimized for structural variation discovery. We have also developed a novel, amplification-free enrichment technique using the CRISPR/Cas9 system to target genomic regions.

We sequenced gDNA and full-length cDNA extracted from the temporal lobe for two Alzheimer's patients for 35 GWAS candidate genes. The multi-kilobase long reads allowed for phasing across the genes and detection of a broad range of genomic variants including SNPs to multi-kilobase insertions, deletions and inversions. In the full-length cDNA data we detected differential allelic isoform complexity, novel exons as well as transcript isoforms. By combining the gDNA data with full-length isoform characterization allows to build a more comprehensive view of the underlying biological disease mechanisms in Alzheimer's disease. Using the novel PCR-free CRISPR-Cas9 enrichment method we screened several genes including the hexanucleotide repeat expansion C9ORF72 that is associated with 40% of familiar ALS cases. This method excludes any PCR bias or errors from an otherwise hard to amplify region as well as preserves the basemodication in a single molecule fashion which allows you to capture mosaicism present in the sample.

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P14.090B

When solo diagnostic exomes fail: parent's sequencing added-value

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Background: Advent of next generation sequencing and in particular whole-exome sequencing (WES) has helped resolving many Mendelian diseases.However, clinical WES does not identify the disease cause in approximately 70% of patients. WES pitfalls have emerged namely that candidate variations might not be in exons or that copy number variations and structural variations are poorly detected. Accordingly, whole-genome sequencing might increase diagnostic yield. However, the presence of disease causing variant(s) in exon of genes not yet involved in human diseases remains the most likely hypothesis.

Methods: To evaluate this hypothesis, we secondarily sequenced the parents after negative clinical solo-WES strategy in 8 selected individuals with syndromic development disorders and diagnostic odyssey. Bioinformatics analyses following a trio strategy allowed to efficiently filter *de novo* variants and candidate variations were intensively shared with the Matchmaker Exchange system.

Results: Trio-WES strategy allowed identification of *de novo* likely pathogenic variants in a new candidate gene in at least 2/8 patients. Gene matching permitted to gather enough additional affected individuals with a similar phenotype to conclude towards a new disease-causing gene. In one patient, a *de novo* variant of unknown significance has been identified in a new candidate gene and gene matching is in progress.

Conclusion: Trio-WES combined with intense data sharing seems to be a valuable strategy as a second step after negative diagnostic solo-WES in patients with diagnostic odyssey, before WGS would be routinely available in clinical practice. This strategy allowed identification of new causing genes in at least 25% of this small series.

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P14.091C

Improvement of the molecular diagnostics in tuberous sclerosis complex (TSC) by NGS deep sequencing of the entire TSC genes and inclusion of mTOR pathway genes

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Conventional molecular genetic testing consisting of Sanger sequencing and deletion/duplication analysis of the*TSC1* and *TSC2* coding regions during the past 20 years detected pathogenic variants in about 85% of patients with a definite clinical diagnosis TSC. In the remaining "no mutation identified (NMI)" cases the presence of low level mosaicism and regulatory variants in both TSC genes or variants in other genes leading to TSC-like phenotypes may have escaped mutation detection.

We reanalysed TSC NMI patients by resequencing the entire genomic regions of *TSC1* and *TSC2* applying deep Next Generation Sequencing (NGS). A custom designed target enrichment applying the Agilent SureSelect technology covered 65.000 bp *TSC1* and 46.000 bp *TSC2* genomic sequence and the coding regions of 13 additional genes of the mTOR signalling pathway. Furthermore, routine diagnostics was converted to hybridization based enrichment and Illumina NGS technology for the coding regions of both TSC genes.

In a series of 25 NMI patients, deep resequencing identified pathogenic *TSC2* variants in 11 cases (44%). These included 4 mosaic variants in the coding region/canonical splice sites, 4 heterozygous deep intronic variants, 2 mosaic deep intronic variants and one mosaic genomic deletion. Additionally, revised routine NGS diagnostics of TSC

genes in 29 patients detected 3 additional mosaic coding/splice site variants (10%). Interestingly, two patients with uncovered pathogenic variants in mTOR pathway genes (mTOR and DEPDC5) presented with additional clinical features like complex focal epilepsy, cataract and hemimegalencephaly therefore supporting that mutations in these genes are not a cause of "classic" TSC phenotypes.

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P14.092D

UDPICS, a laboratory-medical informatics system for translational research in the NIH Undiagnosed Diseases Program

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Introduction:

The Undiagnosed Diseases Program (UDP) is a translational medicine initiative based in the NIH Clinical Center which aims to diagnose patients with mysterious conditions and study unique and rare genetic disorders. The program has received over 10,000 inquiries, reviewed 3,500 applications, and seen almost 900 patients. Patient presentation is heterogeneous and our evaluation process initially treats each patient as independent. Therefore, managing patient and research data became increasingly difficult after the first years of the UDP.

Materials and Methods:

To allow clinicians and researchers to seamlessly collaborate on UDP cases, we built a web-based workflow management system called the Undiagnosed Diseases Program Integrated Collaboration System (UDPICS). The system is based on a commercial Ruby on Rails platform, which we expanded and customized for translational research. UDPICS integrates external biobanking, model organism, and genome analysis systems for easy access. Furthermore, we incorporated the open source tool PhenoTips for ontological patient phenotyping and Phenogrid for phenotype-genotype comparisons in humans and model organisms.

Results:

UDPICS has allowed us to track patients and associated research from application to followup. Benefits of the system include rapid transit of records and tasks, reduced error in workflows, and ease of collaboration.

Conclusions:

By implementing complex process management, genomic analysis, biospecimen management, ontological phenotyping, and electronic laboratory notebooks, UDPICS has become a scalable collaborative workspace for research, clinical, and bioinformatics staff. The system is a mechanism for efficient, transparent, and scalable translational research and addresses many of the scientific and logistical problems of the NIH UDP.

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Diagnostic exome sequencing of Danish families with rare genetic diseases

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Introduction: The advancement in DNA sequencing has in recent years highly increased the likelihood of identifying causative variants among patients with various disorders. Whole Exome Sequencing (WES) facilitates an unbiased screening for genetic defects. We have implemented WES for the genetic work-up of patients primarily under suspicion for neurological and mitochondrial disorders, or complex syndromes involving intellectual disability.

Materials and methods: This is a retrospective study of in-house whole exome sequencing conducted at the Department of Clinical Genetics at Copenhagen University and includes more than 100 probands and their families. All the patients/families were prior to analysis thoroughly assessed by medical specialists and counselled by a clinical geneticist, in order to provide detailed and complete clinical information, aiding variant classification.

Results: Causative mutations were identified in approx. 35% of the patients. One fifth of the mutations had arisen de novo or was found in mosaic form in one parent. Approx. 75% of the identified mutations were absent from the literature and common mutation databases, i.e. novel mutations.

Conclusion: In this study, WES facilitated a genetic diagnosis in approx. 35% of patient with complex aetiologies. The tight collaboration and discussions between clinicians and laboratory scientists is instrumental in reaching a conclusive diagnosis. WES will move to the forefront of genetic work-up and become the first tier analysis for disorders of complex aetiology. Most likely, this will result in an even higher diagnostic yield of WES, when it is used in the initial screening in patients with suspected genetic disorders.

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P14.094B

Clinical exome sequencing as a powerful tool for the diagnosis of complex phenotypes

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Introduction: Complex phenotypes, manifested by a collection of clinical findings pertaining to complex multisystem disorders, constitute one of the most challenging cases in the diagnostic field. Clinical whole exome sequencing (WES), which has now been increasingly adopted to diagnose single-gene disorders, is also a powerful tool for complex phenotypes lacking a definitive diagnosis. Materials and Methods: Samples were prepared using the Nextera Capture System (Illumina, San Diego, CA) and the enriched exome libraries were sequenced using paired-end, 300-cycle chemistry on the Illumina NextSeq or HiSeq (Illumina, San Diego, CA). **Results:** Three trio-WES were performed for three probands with their families. One case was diagnosed with Sanflippo syndrome along with ichthyosis vulgaris, with a new mutation in *FLG* gene. In the second case, new likely pathogenic mutations were detected in *PIGO* and *TGFB2* genes, with the diagnoses of hyperphosphatasia with mental retardation and Loeys-Dietz syndromes for the proband. For the final case, two mutations which had already been defined as pathogenic were co-located in the proband, in *SLC5A2* and *MMAB* genes, rendering the diagnoses of renal glucosuria and methylmalonic aciduria, type cbIB possible for her. **Conclusions:** This study demonstrates the utility of diagnostic exome sequencing in the context of complex phenotypes resulting from two underlying pathogenic genetic alterations, which would otherwise elude discovery (i.e. with traditional single-gene testing methods) and offers hope to those patients who do not exhibit a classic disease phenotype. This study is founded by TUBITAK-112S398 (ERA-Net consortium, CRANIRARE2). Acknowledgement: Centogene AG, Germany

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P14.095C

Clinical WES quality assessment scheme shows opportunities for improvement and harmonization: a national collaborative study of the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL)

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Clinical whole exome sequencing (WES) has proven to be very effective for diagnosing heterogeneous genetic diseases, and has therefore been adopted in standard Dutch genetic diagnostic services. Quality standards have been published for gene panel based Next Generation Sequencing, but for WES these have not been determined yet. Goal of this study is to define quality standards for WES and to enable laboratories to improve and harmonize quality. The exome of Genome in a Bottle (GIAB) sample NA12878 was sequenced and processed by all Dutch genetic diagnostic centers according to their standard diagnostic protocols. Anonymized VCF and BAM files were collected and used for comparisons. VCF files were used to calculate variant detection sensitivity using the GIAB high confidence call set. BAM files were used to calculate informative exome coverage statistics based upon a standardized target defined by all coding exons of UCSC and Ensembl +/- 20bp intron flanks. Results showed that single nucleotide variant (SNV) detection sensitivity varied between 93% and 99% (average 97%) and that INDEL detection sensitivity varied between 73% and 96% (average 88%). Exome coverage at ≥15x varied between 80% and 96% (average 92%). Precision of SNV detection was not substantially different, but varied between 75% and 97% (average 91%)

for INDEL detection. These results show a relatively variable variant detection sensitivity and exome coverage between Dutch genetic diagnostic centers. We will present quality standards and guidelines to harmonize WES quality between laboratories. These efforts will enable optimal and standardized patient care within the Netherlands.

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P14.096D

Whole exome sequencing in rare syndromes using family trios - Clinical experience and outcome

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Exome sequencing in trios is becoming a common molecular diagnostic tool for rare genetic disorders.

At Clinical Genetics, Karolinska University Hospital, clinical trio exome sequencing has increased tenfold from 2012 - 2016. In total, trio exome sequencing and analysis has been finalized for 254 patients with undiagnosed, suspected rare genetic conditions. Data was analyzed for sequence variants consistent with Mendelian traits. In general CGH-array and, when indicated, FRAXA analyses had been performed on the patients prior to exome analysis.

Of the 254 cases, the molecular diagnosis rate was 36%. *De novo* heterozygote sequence variants were the most prevalent causes of genetic disorder, 54%. Sequence variants consistent with autosomal recessive inheritance were detected in 30% and X-linked recessive inheritance in 11% of the cases. In five cases, an autosomal dominant inherited sequence variant was identified - this can be explained by a known dominant trait or variable expressivity. Most patients were referred regarding intellectual disability and/or a syndromic phenotype. The spectrum of affected genes varied. The only genes affected in three or more of our patients were *GRIN2B* and *ANKRD11*. The different detected genetic diagnoses are rare, and for most cases the genetic outcome could not be predicted.

In conclusion, trio exome analysis in families with an unknown rare disorder has proven to be a valuable tool to obtain a genetic diagnosis. The identification of the genetic aberration in these patients is crucial for estimation of recurrence risk. It is also a pre-requisite for carrier testing, prenatal- or preimplantation diagnostics.

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P14.097A

Utility of whole-genome sequencing in the clinical diagnostic of rare inherited anaemias

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Whole-genome sequencing (WGS) has clear advantages over other next-generation sequencing and genomic approaches, making it an attractive tool for the genetic diagnostic of rare diseases. It is unbiased compared to whole-exome sequencing and gene panels, allowing to explore non-coding regions and to detect copy number and loss of heterozygosity events with good resolution and sensitivity. We are evaluating the clinical utility of WGS in healthcare, taking rare haematological diseases as case studies, and discuss here the lessons learnt from the first cases analysed.

We performed WGS of 7 trios (30X coverage), where all probands have a rare anaemia of suspected genetic origin and were pre-screened with a targeted panel containing ~50 genes, none of which had yielded causative variants. The analysis involved Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering.

Known causative variants in genes not included in the targeted panel were detected in two patients (28%), whereas candidate variants in novel genes not previously associated with these diseases were identified across the other five cases. Familial segregation and functional studies are underway to provide further evidence of causality for these novel variants.

The results illustrate the phenotypic overlap existing among these conditions and the importance of identifying the genetic defect for the correct diagnostic and clinical management of the patients. We also demonstrate the benefit of using WGS over gene panels given the difficulty of having comprehensive gene panels and keeping them up-to-date as new candidate genes are identified.

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P14.098B

Investigation of simplex patients born to consanguineous parents: Whole exome sequencing has a high yield, with significant contribution of de novo mutations

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Whole exome sequencing (WES) is an effective diagnostic and research tool. It is recommended as a frontline investigation in undiagnosed consanguineous rare disease patients. Here we review the results from two research initiatives, the FORGE Canada and Care4Rare consortia, which leveraged consanguinity for gene discovery. We analyzed the yield in isolated (simplex) subjects with confirmed or suspected (including founder populations) consanguinity and contrasted to projects with multiple affected individuals.

In simplex cases (n = 48), 73% were solved (known or novel genes). Of these, 71% were autosomal recessive (57% homozygous, 14% compound heterozygous) while 29% were *de novo*. When we defined two groups within this cohort based on degree of relatedness, in individuals born to second cousins or closer (n = 28), 75% were solved (90% homozygous and 10 % *de novo*). However, with more distant relatedness or presumed consanguinity (n = 20), 70% were solved, but strikingly, the variant was *de novo* in 57%. In consanguineous families with multiple affected individuals (n = 54) 78% were solved, with 97.5% of solved cases being inherited (90% homozygous) and apparent *de novo* variants accounting for only 2.5%.

Our data underscores the high yield of WES in the context of consanguinity. These results are influenced by the selected and highly studied nature of these research patients, and hence are higher than other unselected or non-consanguineous cohorts. Nevertheless, in simplex cases, particularly in the absence of close consanguinity, trio WES remains essential due to the higher proportion of *de novo* mutations.

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P14.099C

Analytic validation of whole exome sequencing for clinical diagnostics of inherited disorders

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Utility of Whole Exome Sequencing (WES) in clinical diagnostics has been limited by the non-uniform sequencing coverage across exons, leaving typically 5–10% of the regions with shallow coverage that prevents accurate variant detection. We have implemented a WES assay that enables wide breadth of coverage resembling high-coverage gene-panel based assays and provides high sensitivity in variant detection. We have performed analytic validation of the WES assay, which utilizes Agilent SureSelect Human All Exon V6 hybrid capture selection to capture 60 Mb of exonic targets in high coverage. Sequencing was performed using an Illumina HiSeq sequencing system. Analytic validity of the WES assay was demonstrated for single nucleotide variants (SNVs) and insertions and deletions (INDELs) using reference samples from The Genome In a Bottle Consortium with high-quality variant calls obtained. In the analytic validation, the WES assay achieved 99.5% sensitivity, 99.9% specificity and 99.4% positive predictive value for detecting SNVs and 97.2%, 95.7% and 97.0% sensitivity for detecting INDELs of 1–10, 11–20, and 21–30 bases, respectively. Longest detected INDEL was 35 bases. Repeatability of the WES assay was 99.4%. Of the target regions, 98.86% were covered with over 15x sequencing depth and the mean sequencing depth at nucleotide level was 149×. The results demonstrate that comprehensive sequencing coverage allowing high variant calling sensitivity makes this WES assay well-suited for clinical diagnostics of inherited disorders.

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P14.100D

A feasibility study for rapid Whole-Exome Sequencing for paediatric genetic disorders in Hong Kong

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Traditionally genetic tests have long turn-around-time (TAT) and are unhelpful in acute patient care. Our objective is to test the feasibility of rapid WES in Hong Kong.

Methods

We recruit patients suspected to have a genetic disorder and (i) are critically ill; or (ii) a timely diagnosis may affect decision in management. We use a trio-based WES approach. First, we analyse genetic variants within one of the 13 gene panels based on clinical information, then the Mendeliome (4,278 OMIM genes) if negative. We aim to complete this stage of analysis within 14 working days. We will proceed to the exome-wide analysis with no time constraints if the first-tier testing is negative.

Results and Conclusion

This is an on-going study. So far, we have made 2 molecular diagnoses out of 10 cases. The first patient (M/4y) presented with Juvenile Myelomonocytic Leukemia (JMML). A de-novo mutation in *NF1*, p.R1947X, was identified as heterozygote in buccal mucosa and as homozygote in blood. This resulted in a diagnosis of NF1 and the "2nd hit" mutation leading to JMML. The second patient (F/9d) had prenatally detected bilateral echogenic kidneys. We identified 2 disease-causing mutation in *PKHD1* (p.D703N and p.A3057fs), and substantiated the diagnosis of AR polycystic kidney disease. In both, the TAT was 5 days. Our preliminary results support the feasibility of a timely and accurate genetic diagnosis by WES.

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P14.101A

Experiences from whole genome sequencing in diagnostics - broad *in silico* panels and introducing structural variant detection

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We employ whole genome sequencing (WGS) and analysis by *in silico* gene panels for patients (N = 330) with a range of suspected genetic disorders since 2015 (e.g. neuromuscular disorder, ataxia, skeletal dysplasia, connective tissue disorder, neutropenia). Panels achieve a high diagnostic yield by including a large number of genes with established causation, each rare contributors to total disease burden. Indication driven panels enables us to triage variants in a limited number of genes. Even without parent samples, to ascertain inheritance model already at the variant triage stage, gene panels of hundreds of genes can be analysed confidently and in a timely fashion.Our diagnostic rate varies by group of disorder, ranging from 35% to 67%, averaging 43% overall. Coverage increases in panels from a typical 92% bases at minimum 15x using exome capture data to >99% for whole genome data.

We have previously developed bioinformatic tools for detecting chromosomal structural aberrations from WGS data (e.g. Hofmeister 2014; Nilsson 2017). These allow study of not only imbalances, but also balanced translocations and inversions. In the gene panels studied, several genes have structural variation as a common mechanism of variation. When these tools were applied in the clinical setting, a discovery rate of 10% was seen (3 out of N = 30). Using this approach, we detected a SATB2 duplication exons 5–7 (Lieden, 2015), GNAS inversion (Grigelioniene, 2017), and a LAMA2 duplication exon 30.

In summary WGS can succesfully be applied in a clincal setting using *in silico* gene panels in combination with structural aberration detection.

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P14.102B

Clinical benefit of Whole Genome Sequencing: report on 226 families

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Introduction: Clinical practice in medical genetics has dramatically changed in the last decade with the introduction of next generation sequencing. Today, whole genome sequencing (WGS) is holding the potential to stirring human genetics to a higher level.

Patients and Methods: Samples from 226 families underwent WGS analysis in Centogene, using Illumina's technology. Diagnostic yield was calculated for the total group and subgroups with (n = 172) or without (n = 54) prior whole exome sequencing (WES). Most of the families had undergone WES previously (76%).

Results: Overall pathogenic or at least likely pathogenic variants were identified in 41 index cases from the 226 families (**18%**). Additionally, in 15% of the cases (n = 34) a variant of uncertain significance (VUS) was reported, for a total diagnostic yield of 33%. Importantly, in 27 of the 172 cases with prior negative WES analysis, a genetic diagnosis was achieved by WGS (**16%**). In 23 additional cases (13%) a VUS was identified (total 29%). Positive WGS diagnosis following a negative WES was among others, due to deep intronic variants known to be causative, exon deletions or previously uncovered regions. For the group without prior WES (n = 54) the diagnostic yield was lower than expected with **26%**. Additionally, in 20% a VUS was reported.

Conclusion: These results highlight the strength of WGS and should direct the decision for WGS as the first line test in clinically undiagnosed complex cases. WGS is providing genetic diagnosis for many patients for whom all previous genetic testing including WES failed to identify the suspected genetic cause.

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P14.103C

Classification of *LMNA*-variants by means of zebrafish phenotyping

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Implementation of next-generation sequencing (NGS) techniques in human genetics improved the diagnostic yield, but also resulted in an increase of many genetic variants with unknown clinical significance (VUS). Despite the presence of *in silico* prediction programs and databases to predict the functional consequences of a variant, the

effect of the majority of variants still remains unknown. Functional follow up is therefore crucial to enable further classification of a variant as pathogenic or not.

In this proof-of-concept project we are currently characterizing 20 variants in *LMNA* by means of phenotypic evaluation in zebrafish embryos. To this end, we have set up a phenotyping pipeline to evaluate cardiomyopathy in zebrafish. The parameters that we evaluate are: heart morphology, pericardial edema, heart rate and fractional shortening.

The cardiac read-out parameters were first validated using another known genetic cardiomyopathy model (*tnnt2a*-loss-of-function) in zebrafish. In the next step we characterized the phenotype of a morpholino-mediated *lmna* model. We observed a concentration-dependent increase of the *lmna*-knockdown embryos displaying cardiac edema and a significantly reduced heart rate. Additionally, significant cardiac arrhythmia was observed in the knockdown condition. We now are evaluating 20 genetic variants in our model; 5 known (pathogenic and non-pathogenic) controls from the literature and 15 VUS, found in our cardiomyopathy patient cohort. We intend to use this diagnostic phenotyping pipeline as a routine functional evaluation of *LMNA*-variants, detected in cardiomyopathy patients, leading to improved diagnostics. If desired, this pipeline can be extended to include other genes or phenotypes.

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P15 Personalized/Predictive Medicine and Pharmacogenomics

P15.01A

A *GGPS1* mutation found by WES in three sisters with bisphosphonate-associated atypical femoral fractures

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Introduction: Atypical femoral fractures (AFFs) are a rare but devastating type of fracture often associated with long-term bisphosphonate (BP) therapy, the main treatment for osteoporosis and cancer-related bone disease. The mechanisms underlying the pathogenesis of AFFs are unclear. Given its low incidence, there may be underlying genetic causes that might interact with BPs to give rise to AFF.

Materials and methods: We identified three sisters and three additional unrelated patients, all presenting with AFF and treated with BPs for more than 5 years. Their genetic background was explored by whole exome sequencing. Rare non-synonymous mutations shared among the three sisters were selected, considering either a dominant or a recessive inheritance model.

Results: We detected 37 rare heterozygous mutations in 34 genes. Among them, a novel mutation was found in the gene encoding geranylgeranyl diphosphate synthase (GGPPS), an enzyme crucial for osteoclast function, which can be inhibited by BPs. Other identified variants, such as those found in the *CYP1A1* and in the mevalonate pyrophosphate decarboxylase (*MVD)* genes, may also contribute to susceptibility to AFF. Pathway analysis among the mutated genes showed enrichment of the isoprenoid biosynthetic pathway (GO:0008299), containing these three genes (p-value 0.0006).

Conclusions: Our results are compatible with a model in which an accumulation of susceptibility variants (including some in relevant genes, notably *GGPS1*) constitutes a possible genetic component of AFF causality and may lead to novel risk assessment tools to personalize osteoporosis therapy.

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P15.02B

Characterization of two novel non-synonymous genomic variations altering drug response of the DNA topoisomerase II alpha in the Iranian population

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Top2-α is an important target for many anti-cancer drugs. Variations in this enzyme may influence drug responses in different populations. A variety of mutations and non-synonymous genomic variations in the gene coding Top2-α have been found associated with the development of drug resistance. The aim of the study was to characterize two novel non-synonymous genomic variations in TOP2A gene, which could affect its response to Amsacrine and Mitoxantrone as important inhibitors of the enzyme. The docking studies of Amsacrine and Mitoxantrone with structures of Top2-α and two linked variations including rs762022284 (c.1585A>G, p.Lys529Glu) and rs764177670 (c.1589C>T, p.Thr530Met) were performed using Autodock 4.2.6. AutoDock tools, LigPlot 4.5.3 and Chimera 1.5.3 were used for analysis of Top2-α/drug complexes. Furthermore, the genomic variations were genotyped in the Iranian population and the data were analyzed using PLINK and PICcalc programs. Docking and simulation data showed that the presence of rs762022284 and rs764177670 could significantly change the protein-drug interactions in a synergistic manner. Genotyping data indicated the allele frequency of 0.30 (PIC=0.42) and 0.05 (PIC=0.09) for rs762022284 and rs764177670, respectively. Together, the data suggested that the presence of rs762022284 and rs764177670 could affect Top2-α response to Amsacrine and Mitoxantrone, suggesting the necessity of consideration of population dependent genotypes in cancer chemotherapy using these drugs.

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P15.03C

Cross disease pharmacogenetic analysis predicting anti-TNF response identifies SNPs predicting adalimumab response in Crohn's disease patients

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Introduction: Crohn's disease (CD), rheumatoid arthritis, psoriatic arthritis and other inflammatory diseases comprise a group of chronic diseases with immune-mediated pathogenesis which share common pathological pathways as well as treatment strategies including anti-TNF biologic therapy. However, response rate to anti-TNF therapy among those diseases varies and approximately one third of patients do not response. Since pharmacogenetic studies for anti-TNF therapy have been more frequent for other related diseases and are rare in CD, the aim of our study was to further explore markers, associated with anti-TNF response in other inflammatory diseases, in Slovenian CD patients treated with adalimumab. Materials and Methods: We enrolled 102 CD patients on adalimumab for which response has been defined after 4, 12, 20 and 30 weeks of treatment, using IBDQ questionnaire score and blood CRP value. 45 SNPs, significantly associated with response to anti-TNF treatment in other diseases, were genotyped using PCR-RFLP, PCR-HRM or TaqMan technique. **Results:** Out of 45 SNPs, 13 SNPs showed at least one statistically significant association with either IBDQ and/or CRP response. Out of 13 SNPs, two loci (rs755622 in gene *MIF* and rs767455 and rs1800693 in gene *TNFRSF1A*) were associated with anti-TNF response in CD patients for the first time. For SNP rs755622 (*MIF*) consistent association during all 30 weeks of treatment has been observed (p=0,004). In addition, strong and consistent association has been replicated for SNP rs2275913 (*IL17A*) and response to adalimumab (p=0,006). **Conclusions:** These results suggest common mechanism of response/nonresponse to anti-TNF treatment in related diseases.

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P15.04D

Tumour heterogeneity and FGFR3 mutation detected in urinary sediment by digital PCR in bladder cancer patients

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Introduction: Genomic alterations in the fibroblast growth factor receptor 3 (FGFR3) are frequently observed in urinary bladder cancer. The aim of the study was detection of FGFR3 mutation (Y373C) in urinary sediment by droplet digital PCR technique as a highly sensitive and specific method. Materials and methods: Urinary DNA was analysed from 150 primary bladder cancer patients (87 cases G1 and 63 cases G2-G3). There were 33 females in that study group and the average age of patients was 68 (range

41–88). DNA was also isolated from tumor tissue and we sequenced FGFR3 gene. **Results:** We identified FGFR3 mutation (Y373C) in urinary sediment using droplet digital PCR in 27% cases (28/150:23 G1;5 G2). Both methods (sequencing and digital PCR) detect mutation in 40/150 (27%) bladder cancer patients. Only in 8/40 (20%) cases the mutation was observed both in urine and tissue. In 12/40 (30%) cases (5G1 and 7 G2-G3) we did not detect FGFR3 mutation in urine although it was confirmed by sequencing. In 20/40 (50%) cases (15 G1, 5 G2-G3) we found mutation only in urine. We analysed overall survival of patients and we found correlation with presence of FGFR3 mutation in urinary sediment and higher grade of the disease (G2-G3). **Conclusion:** In our opinion two factors plays a crucial role in explanation such results: tumour hetrogeneity and randomly taken urine sample. Further study are needed to prove clinical usefulness of genetic markers in routine practice.

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P15.05A

Improving breast cancer risk prediction by combining 18 SNPs, mammographic density and the Tyrer-Cuzick model

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Introduction: Integration of mammographic density (MD) measurements improves the accuracy of breast cancer risk prediction models necessary to stratify screening and improve disease prevention. Many single nucleotide polymorphisms (SNPs) have been associated with breast cancer susceptibility. However, current breast cancer risk prediction models do not routinely include SNP data. This study investigates the predictive ability of 18 SNPs (SNP18) associated with breast cancer risk adjusting for classical risk factors and MD.

Methods: 462 individuals with breast cancer and 8899 controls prospectively ascertained through the Predicting Risk Of Cancer At Screening study were genotyped. Logistic regression was applied to assess the calibration and inter-quartile range odds ratio (IQR-OR) of SNP18 alone and after adjustment for MD and Tyrer-Cuzick.

Results: SNP18 was predictive of breast cancer risk; IQR-OR=1.56 (95%CI 1.38–1.77), with increasing discriminative power when adjusting for MD and Tyrer-Cuzick IQR-OR=1.70 (95%CI 1.51–1.91). SNP18 substantially improved the ability to identify high-risk women from classical risk factors alone. SNP18, MD adjusted Tyrer-Cuzick 10-year risk of ≥5% identified 17.7% of the population, 31.3% of breast-, 33.3% of interval- and 40% of stage 2+ cancers. In contrast, at $<2\%$ risk, 29.9% of the population developed only 17.2%, 14.8% and 11.7% of the total-, interval- and stage 2+ breast cancers, respectively.

Conclusion: SNP18 is an independent predictor of breast cancer risk. Incorporation of SNP18 and MD into Tyrer-Cuzick gives a better discrimination between risk groups and therefore can be used for risk stratification and personalized treatment.

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P15.06B

Multi institutional evaluation of a new NGS assay for mutation detection from cfDNA

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Introduction: The circulating cell-free tumor DNA (ctDNA) isolated from plasma of cancer patients is an alternative, minimally invasive source of tumor DNA that also allows rapid determination of the mutational status of the tumor. Here we report a multi-institutional validation of the Oncomine cfDNA Lung Cancer assay for the analyses of ctDNA in molecular pathology laboratories. Methods: The Oncomine cfDNA Lung assays is a multiplexed sequencing assay for liquid biopsy that generates reads containing targeted ctDNA regions along with a molecular tag. In order to allow an initial evaluation, the Multiplex I cfDNA Reference Standard (Horizon Dx) was used. The same lot of control sample was distributed to all participating laboratories within OncoNetwork Consortium. Samples were sequenced twice in each laboratory either using the Ion PGM or Ion S5 system. A bioinformatics pipeline within Torrent Server software allowed for automated variant calling. **Results:** Laboratories involved in the study were able to detect the 8 hotspot base changes and indels present in the control samples with an average 94.81% sensitivity and 99.82% specificity. When only considering variants at the 0.1% allelic frequency, the average sensitivity was 83.93% and the average specificity was 99.88%. Notably, at the 0.1% allelic frequency, the participating laboratories were able to detect the challenging EGFR p.T790M variant that is a marker of sensitivity to EGFR tyrosine kinase inhibitors. **Conclusions:** These preliminary data confirm the potential of Oncomine cfDNA lung assay for plasma genotyping to allow noninvasive, multiplexed detection of targetable genomic alterations in lung cancer.

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P15.07C

Strategies for the implementation of Pharmacogenetics in the clinical setting: Application of a custom SNP-array (PharmArray) in a Clinical Pharmacogenetics Unit

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Introduction: In 2013 a Pharmacogenetics (PhGx) Unit was created willing to ease the implementation of PhGx in the clinical practice. This unit was based on a Clinical PhGx consultation and a SNP-microarray which allowed testing of 180 SNPs associated to drug response (PharmArray®). In this study we report our experience over the past 3 years with the aim of evaluating the benefits of implementing a PhGx strategy in the clinical practice of a third care level hospital.

Material and Methods: The PhGx Unit evaluates all inquiries in order to determine if a genetic test is indicated for the selection of a pharmacological treatment, dose adjustment or the identification of therapeutic failures or adverse reactions. Our PhGx test consists on the simultaneous screening of 180 SNPs in genes coding drug metabolizing and transporter enzymes with a custom platform (PharmArray®). HLA-B*57:01 determinations before Abacavir prescription are performed by classic sequencing and INNOLIPA®. Taking into account both the clinical and genetic information the clinical pharmacologist develops a final clinical report.

Results: Over the past 3 years we have performed 1687 HLA-B*57:01 determinations for Abacavir prescription and 600 PharmArray® studies before the administration of different pharmacological treatments: Thioguanines (60%), Methotrexate (20%), Immunosuppressants (6%), Voriconazol (2%), Anticoagulant drugs (3%) and Antineoplasic agents (3%) mainly.

Conclusions: 32% of our patients showed molecular profiles related to alterations in any of the included metabolism and transporter enzymes and therefore treatment revision and adjustment was recommended in the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines and EMA or FDA recommendations.

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P15.08D

Haplotype analyses of CYP2C19*2 and CYP2C19*17 genetic polymorphisms in clopidogrel non-responsiveness after percutaneous coronary intervention with stent implantation

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Dual antiplatelet medication with aspirin and clopidogrel is currently recommended strategy for patients undergoing percutaneous coronary intervention (PCI) with stent implantation. However, emerging non-responsiveness to clopidogrel often results in fatal stent thrombosis. Previous findings suggest that the CYP2C19*2 polymorphism is associated with non-responsiveness to clopidogrel and the CYP2C19*17 polymorphism enhances antiplatelet activity of clopidogrel. We therefore aimed to analyze the haplotypes of these polymorphisms in clopidogrel non-responsiveness. Antiplatelet activity of clopidogrel activity was measured by the VerifyNow $P2Y_{12}$ assay in blood samples collected from patients that took a standard dose of clopidogrel (75 mg/day) for at least 7 days. 243 responder and 104 non-responder patients underwent PCI with stent implantation are included in our study. The CYP2C19*2 (c.681G>A) and CYP2C19*17 (c.-806C>T) polymorphisms are genotyped using the Sequenom MassARRAY system. The genotype frequencies for each polymorphism were in good agreement with the predicted Hardy-Weinberg equilibrium values. Haplotypes with a frequency of <0.03 were excluded. Only frequencies of the A-T haplotype were <0.03 in our study group. The comparison of the G-T haplotype frequencies between the responder and non-responder group did not reveal any significant difference. The A-C haplotype was significantly associated with non-responsiveness to clopidogrel (OR = 2.855, 95%CI: 1.868–4.364, $P<0.001$). Conversely, the G-C haplotype was associated with responsiveness to clopidogrel ($P = 0.002$). Our findings suggest that the A-C haplotype has a 2.8-fold increased risk and the G-C haplotype enhances antiplatelet activity of clopidogrel. Checking the A-C haplotype as a pharmacogenetics test for risk patients will make treatment effective.

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P15.09A

Cross-validation of next-generation sequencing and digital PCR technologies for tumor profiling of colorectal cancer patients

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Introduction: Success of precision oncology relies on accurate tumor profiling. Use of next-generation sequencing (NGS) to detect somatic variants in FFPE tissue DNAs (tDNAs) is challenging for clinical diagnostic laboratories due to low quality of these analytes, frequently impairing the possibility to assign patient to targeted treatments. Here we compare the performance of two different NGS platforms, further validating our results with digital PCR (dPCR) technology. **Materials and Methods:** Performance of MiSeq and Ion PGM sequencers with TruSight® Tumor 15 and Ion AmpliSeq® Colon/Lung Cancer Research Panel v2 were evaluated on 32 tDNAs from non-metastatic colorectal carcinoma (CRC) patients. dPCR assays were customized to screen point mutations detected by both NGS platforms. **Results:** A total of 28 point mutations were identified in 32 CRC patients. Somatic variants were concordantly detected by both MiSeq and Ion PGM in 21/21 (100%) tDNAs, even when samples presented low allele frequencies (<15%). Negligible differences were found between platforms (2.5%±2.4%, range 0.0–9.5%). MiSeq and Ion PGM revealed potentially actionable targets in 14/32 patients (43.7%). dPCR analysis validated all NGS data, confirming the previously identified somatic mutations in 21/21 (100%) of cases. A mean difference in allele frequencies of 1.0%±0.9% (range 0.0–2.4%) was found between NGS and dPCR analysis. **Conclusions:** Our results demonstrate that a combined workflow including different

NGS platforms and dPCR enables to successfully profile tumor aberrations from tDNAs, even when mutational burden is low, thus providing a useful approach for the assignment of personalized therapies to CRC patients.

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P15.10B

Screening for CYP2B6 polymorphisms in the Roma (Gypsy) population

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Introduction: ADME genes exhibit significant variation among the human populations due to the past demographic and evolutionary events. Generally, the knowledge on distribution of ADME genes in isolated populations is limited, particularly in the Roma, transnational minority population of Indian origin. The aim of this study is to determine the variation of CYP2B6 among Croatian Roma. It is one of the most polymorphic ADME genes in humans and plays role in several drug metabolizing pathways (e.g. efavirenz and nevirapine, bupropion).

Methods: Within the broadly designed project, we investigated the variation of a large panel of ADME genes among the Roma minority populations of Croatia. Genotyping was done using KASP method. Here we present CYP2B6 gene variation, which was detected by genotyping seven SNP loci (rs12721655, rs2279343, rs28399499, rs34097093, rs3745274, rs7260329, rs8192709) in 440 DNA samples belonging to the three socio-culturally and geographically distinct Croatian Roma groups.

Results: Three of the investigated loci (rs12721655, rs28399499, rs34097093) were monomorphic in all samples. MAFs of polymorphic loci (rs2279343, rs3745274, rs7260329, rs8192709) ranged 17–30%, 12–26%, 24–45%, 5–17%, respectively, with significant differences among the investigated groups. Moreover, MAF of locus rs8192709 is in two of the three Roma populations four times higher than the average global frequency. Significant LD values between different pairs of loci were detected in all three investigated populations indicating recent founder effect.

Conclusion: Our results suggest that each investigated Roma population has unique CYP2B6 genetic profile which should be considered in the modulation of pharmacotherapy due to its possible clinical importance.

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P15.11C

Impact of pharmacogenetic on voriconazole plasma concentration in patients with invasive fungal disease

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Introduction: Voriconazole is a second-generation triazole antifungal agent with a broad spectrum of activity. It exhibits a nonlinear pharmacokinetic and its interindividual variability has been reported to be influenced by cytochrome (mainly CYP2C19) polymorphisms. Voriconazole therapeutic drug monitoring is suggested in major guidelines to improve treatment outcomes. Our study aimed to investigate the relationship between voriconazole exposure and single nucleotide polymorphisms (SNPs), to guide the adjustment of drug individual dosage regimen. Materials and methods: Adult patients with invasive fungal disease and without HIV-infection, severe malnutrition, liver or kidney failure, treated with voriconazole (oral or intravenous route), were enrolled. *MDR1 3435C>T* (*rs1045642*), *1236C>T* (*rs1128503*), *2677G>T* (*rs2032582*) and *1199G>A* (*rs2229109*), *OATP1B1521 T>C* (*rs4149056*), *PXR 63396C>T* (*rs2472677*), *BSEP T>C (rs228762), MRP2 1249G<A (rs2273697), BCRP1 T>C (rs13120400), MRP2 24G>A (rs717620), CYP2C19*2G>A (rs4244285)* and *SLCO3A1 T>G (rs8027174)* SNPs was performed by real-time PCR. Plasma concentrations at the end of dosing interval (Ctrough) was measured using HPLC-MS validated methods. **Results:** Three hundred fifty-one adult patients (249 males, 338 Caucasians, median age 57 years and median BMI 23.32 Kg/m^2) were included in the study. Median voriconazole Ctrough was 2150.00 ng/mL (interquartile range, 1122–3927 ng/mL). We found significantly higher voriconazole Ctrough in patients with *OATP1B1 521TCC* ($p<0.001$), *MRP2 24GA/AA* ($p = 0.006$), *CYP2C19*2GA/AA* ($p = 0.001$) and *SLCO3A1 GG* ($p=0.004$) genotypes. **Conclusions:** We here reported the influence of single nucleotide polymorphisms in OAT1B1,

MRP2 and SLCO3A1 thus confirming the role of CYP2C19 on voriconazole exposure. This study suggests the usefulness of genetic-based antifungal therapy and highlights the needed of therapy personalization.

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P15.12D

Comprehensive analysis of CYP2D6 variants and copy numbers using reverse-hybridization and real-time PCR based assays

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Introduction: The cytochrome P450 2D6 (CYP2D6) is an important liver enzyme involved in the metabolism of up to 25% of clinically used drugs. The CYP2D6 gene is highly polymorphic, with numerous (sub)variants described in the Human Cytochrome P450 Database (www.cypalleles.ki.se). While the most frequent allelic variations are caused by single nucleotide polymorphisms and small insertions/deletions, highly homologous regions in the CYP2D6 gene locus facilitate unequal cross-over leading to large deletions, duplications and gene conversions.

Material and Methods: We developed a reverse-hybridization assay (PGX-CYP2D6 XL StripAssay) for the simultaneous detection of 19 sequence variations in the CYP2D6 gene, which define the most prevalent alleles impacting enzyme activity in Caucasians. For the detection of copy number variations a real-time PCR based assay (CYP2D6 RealFast CNV Assay) was established. The StripAssay and real-time PCR assay were validated on 118 and 98 samples, respectively.

Results: The PGX-CYP2D6 XL StripAssay correctly identifies allelic variants with normal (*1, *2, *35, *39), reduced (*9, *10, *17, *29, *41) and no (*3 to *8, *11, *12, *14, *15, *40, *58) enzyme activity, and hence allows the classification of individuals into extensive (EM), intermediate (IM) and poor (PM) metabolizers. In addition, ultrarapid (UM) metabolizers and CYP2D6*5 carriers can be identified by quantifiying their abnormal copy number status using the CYP2D6 RealFast CNV Assay. Both assays demonstrate a test accuracy of > 0.99.

Conclusions: The metabolizer phenotype of patients treated with CYP2D6 substrates can be accurately determined by the combined use of both assays.

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P15.13A

Small molecules for the recovery of wild-type CFTR splicing

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Introduction: Cystic fibrosis is a recessive disorder caused by > 1900 mutations in the CFTR gene. About 13% of CFTR mutations are classified as splicing defects. Among them, the polymorphic locus TG(9–13)T(5,7,9) is known to influence exon-10 inclusion and has been associated with monosymptomatic forms of the disease.

Recent advances in therapy demonstrated the efficacy of drugs targeting specific molecular defects (potentiators, correctors). However, no personalized treatments are available for splicing mutations. For this reason, we explored the possibility to improve exon-10 inclusion in CFTR mRNA using small molecules.

Methods: We started with kinetin and its analogue RECTAS, previously found to correct specific aberrant splicing events in other genetic disorders. We then selected other kinetin derivatives, prioritized by in-silico structural-based screening, to identify molecules with a better efficacy. We treated Caco-2 cells with different concentrations of the selected drugs for 24h and we measured the wild-type CFTR mRNA levels by competitive-fluorescent RT-PCR.

Results: These experiments showed a dose-response increase of exon-10 inclusion confirming a remarkable impact on splicing fidelity by all the tested molecules. Among them, RECTAS seems to have the best efficacy at the lowest dose (25% increase in exon-10 inclusion; $P < 0.022$). The effect of kinetin and RECTAS on CFTR splicing have also been confirmed in a more disease-relevant cell system, i.e. primary cultures of epithelial cells isolated from patients' nasal brushings. We are also planning to validate new compounds by cell-based screening using a dual-color fluorescent reporter vector.

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P15.14B

Novel endometrial receptivity markers detected in large-scale RNA-sequencing study combining samples from different populations

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Introduction: Numerous genes regulate the endometrial transition from pre-receptive to receptive state. Additionally, transcriptome dysregulation and problems with endometrial receptivity have been proposed as a potential mechanism behind repeated IVF failure. Due to small between-study overlap, there is only a handful of clinical biomarkers for diagnosing endometrial receptivity problems or selecting the optimal receptivity window for embryo transfer in IVF. **Material and methods:** Paired samples from pre-receptive (n = 35) and receptive (n = 35) endometrium in healthy fertile women (aged 23–36) and receptive phase samples (n = 39) from women with repeated IVF failure (aged 26–49) were collected in Estonia and Spain. Biopsies were analysed using Illumina paired-end RNA-sequencing. For cohort-level differential expression analyses, edgeR was used, while meta-analysis was performed using METAL. Results were selectively validated using qPCR. **Main results:** After multiple testing correction, 3,591 differentially expressed transcripts (DETs) were identified in the pre-receptive vs receptive endometrium analysis of healthy women. Up-regulated DETs were enriched for immune system and cellular adhesion terms, while cell-cycle and DNA repair-related processes were down-regulated. Comparison of receptive phase samples from healthy women and repeated IVF failure patients resulted in 338 DETs. These transcripts were enriched for cellular adhesion and immune response. **Conclusion:** Differentially expressed transcripts identified in our study have consistent effects in different populations and therefore have the potential for being evaluated as robust and efficient markers for endometrial receptivity in the clinical setting. Comparison between healthy women and IVF patients provides novel information on potential causes of repeated IVF failure. Funding: EU41564 (NOTED), EU48695, EU692065 (WIDENLIFE)

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P15.15C

Electrophysiological modeling of *Hnrnpu*-related epileptic encephalopathy in mice for purpose of targeted therapy identification

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Introduction: Despite the availability of over twenty anti-epileptic drugs, nearly one third of epilepsy patients remain refractory to treatment. Fortunately, with recent advancements in genomic technologies, genetic causes of epilepsy are increasingly recognized, thereby offering a window into the underlying disease biology and the opportunity to identify targeted therapies. Here we model *Hnrnpu*-related epileptic encephalopathy using both *in vitro* and *in vivo* electrophysiology screening methods performed on a knockout mouse line. Phenotypes that emerge from these studies will serve as the basis for future drug screening.

Materials & Methods: A heterozygous *Hnrnpu* knockout mouse line was established using CRISPR/Cas9 genome editing technology. Spontaneous seizures and seizure thresholds of 6–8-week-old mutant and wildtype mice were subsequently assessed using electroencephalography (EEG) and electroconvulsive threshold studies (ECT). Neuronal network activity was further evaluated using a multi-electrode array (MEA) performed on primary cortical neuron cultures derived from postnatal day 0 pups.

Results: Although EEGs performed on three mutant mice thus far have not revealed any evidence of spontaneous seizures, preliminary data from ECT studies reveal a 10–20% reduction in seizure threshold compared to wildtype littermates (N = 3 mutants, 5 wildtypes). MEA studies have yet to demonstrate any significant differences in the spontaneous firing patterns of mutant neuronal networks.

Conclusion: While preliminary electrophysiological data thus far do not reveal abnormalities in spontaneous neuronal firing, *Hnrnpu* knockout mice show an overall lower threshold to seizure induction. This ECT phenotype can

thereby serve as a reliable and quantifiable trait for future drug screening efforts.

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P15.16D

The Challenge of Whole Genome Sequencing in a clinical setting

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The creation of a national network of NHS Genomic Medicine Centres (GMCs) is integrating genomics into mainstream medicine in England. The 100,000 Genomes Project is an initiative to sequence 100,000 genomes from patients with rare disease and cancer, has driven the implementation of a framework to enable the acquisition of samples and clinical data of sufficient quality to enable whole genome sequencing (WGS). For patients with rare diseases, pathways have been developed to collect appropriate family samples and extract high quality DNA for WGS to enable a diagnosis previously unobtainable, and the family with reproductive options. The cancer programme pilot phase identified difficulties with genomic testing of tumour samples and led to the embedding of formalin-free tissue handling pathways from theatres to laboratories to ensure samples were WGS conducive. The ability to simultaneously detect small variants, copy number variants and structural variants for cancer patients provides opportunities e.g. new treatment options and clinical trials. The standardisation of processes from sample handling, DNA extraction, quality control, validation and reporting has enabled the delivery of WGS and evidences the provision of genomic services within a national healthcare system is beneficial and deliverable. An external quality assessment has been integrated into all pathways from the outset to monitor and measure the quality of the service and has driven the improvement of patient care.

This approach has adopted innovative approaches to design the GMC genomics services, created genomics multi-disciplinary teams spanning a multitude of specialisms, driven creative workforce training and forward-thinking approaches to informatics.

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P15.17A

Association of *IL28B* and *CCL5* polymorphisms with chronic hepatitis C treatment outcome

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Chronic infection with hepatitis C virus (HCV) affects approximately 3% of people worldwide and can cause serious liver damage. Although the recommended treatment involves pegylated interferon (PegIFN) combined with ribavirin (RBV), it is well-known that for many patients the mentioned therapy is not effective. This makes the search of factors that affect the treatment outcome extremely important. We aimed to analyze the role of rs12979860 (*IL28B*) and rs2107538 (*CCL5*) in the outcome of the HCV infection treatment with PegIFN and ribavirin. 130 Belarusians with HCV after full course of PegIFN/RBV therapy were studied. They were divided into two groups on the basis of treatment outcome. The first group included 49 patients with sustained virological response (SVR), the second - 81 individuals with non-response (NR). Significant differences in the genotype distribution of *IL28B* were revealed between the two groups ($p=2.04*10^{-7}$). Among CC genotype carriers the SVR rate was much higher (81.5%) than among CT and TT groups (31.1% and 13.8%, respectively) ($p=2.29*10^{-7}$). The frequencies of *CCL5* genotypes were alike in two cohorts ($p>0.05$). But when combined with *IL28B*, it turned out that carriers of CT/GA and CT/AA combinations (*IL28B*/*CCL5*, respectively) had significantly higher probability to achieve SVR than patients with CT/GG ($p=6.61*10^{-3}$). Thus, SNP rs12979860 (*IL28B*) can be considered as an independent predictor of the treatment outcome in the Belarusian population. On top of that, SNP rs2107538 (*CCL5*) can definitely enhance the predictor properties of *IL28B*.

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P15.18B

Actions, roles, and responsibilities in implementation of pharmacogenomics in primary care

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Introduction: Barriers and facilitators of pharmacogenomics have been widely studied. Nonetheless, limited implementation of pharmacogenomics is observed in routine health care, especially in primary care. To study how to overcome barriers and to draw on facilitators, we defined actions, roles, and responsibilities in consultation with stakeholder groups.

Materials and methods: Interviews were conducted with general practitioners (n = 8), patients (n = 21), and pharmacists (n = 24) to elicit perceived barriers and facilitators for pharmacogenomics. Main outcomes were grouped into themes, which were used to organize an expert meeting to further define needed actions, roles and responsibilities.

Results: From interviews it appeared that existing pharmacogenomic dosing advices are not included in routine health care guidelines for general practitioners, only for pharmacists. Lack of evidence on clinical utility was mentioned as a general barrier to include pharmacogenomic dosing advices in guidelines for general practitioners. Besides lack of evidence other main structural prerequisites were mentioned, such as reimbursement of the test and subsequent therapy, user-friendly software systems, and data sharing infrastructures. Furthermore, protocols when to test a patient are considered essential to implement pharmacogenomics successfully. However, disagreement exists about the best division of responsibilities between general practitioners and pharmacists, and the patient's role.

Based on the interview data, four themes were defined and discussed in an expert meeting: generating evidence for guideline development, reimbursement, division of responsibilities, and data registration and sharing.

Conclusions: Prioritization of the distilled actions with actionable stakeholder groups is needed as a roadmap to progress implementation of relevant pharmacogenomic applications in primary care.

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P15.19C

Dynamics of changes in the genome of hepatitis B virus for chronic HBsAg-carrier after Lamivudine treatment

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Background: targeted therapy is the main goal of personalized medicine. High-throughput molecular genetic studies are particularly important in antiviral therapy and oncology to overcome drug resistance, to save time, and to predict the effectiveness of medical treatment. The aims of study: analysis of the dynamic effect of Lamivudine to HBV isolate with escape-mutation Gly145Arg under treatment of chronic HBsAg-carrier.

Methods: serum samples in time 2004–2007 were studied for patient 53 y/o with a combination of chronic hepatitis B and chronic Hodgkin's disease. Initial HBV isolate got Gly145Arg mutation, affecting changes in serological properties of the HBV samples. Medical treatment by Lamivudine since 2006 was started. High-throughput sequencing was performed by Ion Torrent PGM (2150×-2700x coverages). Informed consent was obtained from patient according local ethical approval. **Results:** serological markers in the time series of sample were stable the entire period of observation (+/+/+/+/- profile for HBsAg/HBeAg/anti-HBe IgG/anti-HBc IgM+IgG/anti-HBsAg, respectively; HCV and HDV markers were negative). HBV isolate samples from 2004 and 2005 were identical. After Lamivudine therapy was started the HBV genome was mutated. HBV isolate acquired the resistance to Lamivudine by Y(M/V)DD, as well as codon of escape-mutation Gly145Arg got the second mutation resulting heterogeneity Gly145(Arg/Lys). The other important feature was the appearance of heterogeneous Gly10Lys mutation. Sequencing of HBV isolates from 2006 and 2007 proved their identity. **Conclusion:** in chronic HBsAg-carrier with Gly145Arg mutation in HBsAg region Lamivudine treatment resulting to the emergence of a drug-resistance mutation. This case illustrate importance of HBV isolate features for personalized therapy.

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P15.20D

Variants in *CD5* and *IRF4* impact into melanoma survival

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Introduction: The *CD5* gene is a negative T-cell regulator, and p.Pro224Leu (rs2241002) and p.Ala471Val (rs2229177) variants constitute a more immunoreactive haplotype that worsens clinical autoimmune outcome. The *IRF4* gene is transcription factor associated with immune system development and response. The rs12203592 *IRF4* modulates an enhancer element that controls the *IRF4* expression and is associated with high nevi count. The study asses the role of these variants in melanoma survival. **Material and Methods:** The *CD5* variants (p.Pro224Leu and p.Ala471Val) and *IRF4* rs12203592 variant were genotyped in two independent sets of melanoma patients from Barcelona (N = 500 cases) and Essen (*CD5* variants and *IRF4* variant were genotyped in 215 cases and 432 cases, respectively). **Results:** The p.Ala471 *CD5* variant correlated with better melanoma outcome in both datasets. In contrast, increased melanoma-specific mortality was associated with p.Leu224 *CD5* variant and *IRF4* rs12203592 T allele in both datasets (Table1).Survival analyses showed that *CD5* Pro224-Ala471 haplotype in homozygosis improved melanoma survival in the entire set of patients (hazard ratio [HR]=0.27 [0.11–0.67], Adj. p=0.005). While, *IRF4* survival analyses only showed significance for the Barcelona set (HR=4.58 [1.11–18.92], Adj. p=0.03). **Conclusions:** *CD5* immunoreactive haplotype associates with better melanoma outcome and *IRF4* rs12203592 T allele with worse melanoma prognosis. These results highlight the relevance of immune-related genes for clinical outcome in melanoma. **Funding**: This work was funded by Instituto de Salud Carlos III, Spain, grant 12/00840, co-financed by European Development Regional Fund "A way to achieve Europe"

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P15.21A

SNPs in microRNAs associated with vincristine peripheral neurotoxicity in Spanish children with acute lymphoblastic leukemia

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Background

Vincristine (VCR), an important drug in childhood Acute Lymphoblastic Leukemia (ALL) treatment protocols, often causes peripheral neurotoxicity in early phases of the therapy. Association between genetic variants in VCR pharmacokinetic (PK) genes and neurotoxicity has been reported. It is known that these PK genes are regulated by microRNAs (miRNAs). Polymorphisms affecting the levels or function of miRNAS targeting those genes could affect their expression having in turn a role in the VCR-related neurotoxicity. Nowadays, a large amount of new miRNAs have been annotated. Therefore, the aim of this study was to determine if variants in miRNAs are associated with VCR neurotoxicity during induction.

Methods

Blood samples of 175 Spanish pediatric B-ALL patients treated with LAL/SHOP protocol were analyzed. We selected all the SNPs described in pre-miRNAs with a MAF>1% (213 SNPs in 206 miRNAs) that could regulate VCR PK/PD genes. Genotyping was performed with VeraCode GoldenGate platform.

Results

Statistically significant association was found between 8 miRNA SNPs and neurotoxicity during induction phase. The most significant result was found for rs12402181 in mir-3117 seed region. This miRNA was predicted to regulate *ABCC1* and *RALBP1*, genes involved in VCR transport.

Conclusion

In this study we detected a SNP in miR-3117 that might alter *ABCC1* and *RALBP1* VCR transport genes regulation and could affect VCR-induced neurotoxicity in patients with pediatric B-ALL.

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P15.22B

Ensemble Random Forest Classifier for predicting myeloproliferative neoplasms subtype using patient's genomic profile

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Introduction: Myeloproliferative neoplasms (MPN) are a group of related hematological disorders identified by clonal expansion of one or more myeloid cell lineages. Six different types of MPN are distinguished; the most prominent of these are polycythemia vera (PV) and essential thrombocythemia (ET), respectively characterized by erythrocytosis and thrombocytosis. Acquired somatic mutation of $JAK2^{V617F}$ underlies more than 96% of PV and 50–60% ET. We applied machine learning models to germline genetic variants and constructed a robust prediction model for alternative classification of ET and PV patients.

Materials and methods: A cohort of 499 $JAK2^{+ve}$ ET-cases and 505 $JAK2^{+ve}$ PV-cases were genotyped using the Illumina Human OmniExpressExome-v1.2. Quality control involved removal of SNPs with MAF < 0.01, SNPs and samples with > 10% missing genotypes and SNPs deviation from HWE proportions. In order to account for multicolinearity and avoid overfitting SNPs with r^2>0.2 within 50kb window were excluded. PCA was used as an unsupervised approach for data visualization and classification. 7144 SNPs with significant alternative allele association (p-value> 0.05) between ET and PV were used to construct classifier model.

Result: Our MPN ensemble classifier model was highly predictive of MPN subtypes. Using 5 fold cross-validation; Random Forest model achieved superior accuracy (AUC: 0.9) compared to Decision Tree model (AUC: 0.87).

Discussion: The results demonstrate that the germline variant information is useful for prediction of MPN type with machine learning modelling. To our knowledge, this study is the first investigation of the MPN classification using machine learning and germline mutation data.

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P15.23C

Study of correlation between the NAT2 phenotype and genotype status among Greenlandic Inuit

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Introduction: Greenland has one of the highest tuberculosis incidences in the world and the standard treatment includes isoniazid. *N*-acetyltransferase 2 (NAT2) is the main enzyme metabolizing isoniazid and genotype-based treatment has been studied for years without becoming common practice yet.

Material and Methods: We sequenced the coding sequence of *NAT2* and determined the NAT2 enzyme-activity by caffeine test in 260 Greenlanders.

Results: No additional genetic variants were identified in the coding sequence of *NAT2*, so that genotype status could be assessed by the well-established 7-SNP panel. Studying the enzyme activity by the ratio of the two caffeine metabolites AFMU and 1X showed that all 42 individuals with two haplotypes associated with slow metabolism had slow metabolism. The remaining individuals with intermediate or rapid genotype status displayed some phenotypical variation, with 79 out of 218 (36%) misclassified with slow metabolism. Further investigation revealed that drinking coffee and not tea or cola was important for high levels of both metabolites, resulting in fewer misclassifications. Furthermore, taking a second urine sample of 15 participants on another day indicated individual variation between two measurements in our study group.

Conclusion: The concordance between phenotype and genotype status with regard to slow metabolism supported the recommendation of lower isoniazid doses in individuals with slow genotype status in order to avoid liver-injury, a frequent side effect.

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P15.24D

Association between genetic variants of the nicotinic receptor genes and smoking cessation: a pharmacogenetic study

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Introduction: Many smoking cessation methods have been developed that may help quit smoking, however, no single method is effective for all smokers. Previous studies identified genetic variants on nicotinic acetylcholine receptors (nAChRs) for nicotine dependence. However, their relationship with pharmacotherapy remains inconclusive. This study aims to examine whether nicotinic receptors gene variants could predict short/long-term effect of smoking cessation and their interaction with medication on smoking cessation. Methods: A total of 697 ever-smokers receiving nicotine replacement therapy or Varenicline treatment were recruited from medical centers. Fifty-five SNPs on ten nAChRs subunit genes were selected for genotyping. The short/long-term effect of smoking abstinence were evaluated at 3-month and 12-month. Multiple logistic regression was used to assess the effects of genetic variants and cessation methods on smoking cessation using gPLINK and SAS 9.3v. software. **Results:** Our results showed that five variants on *CHRNA5-A3-B4*, *CHRNA2*, and *CHRNA7* are associated with short/long-term smoking cessation. Subjects carrying one additional minor allele of *CHRNA7* rs35114543 and rs11637923 would significantly increase the odds of short/long-term abstinence if receiving Varenicline treatment (OR=5.04, 95% C.I.: 1.32–19.3; OR=3.22, 95% C.I.: 1.36–7.66, *respectively*). However, subjects carrying one additional minor allele of *CHRNA5* rs680244 and *CHRNA3* rs6495308 would significantly decrease short/long-term abstinence odds (OR=0.38, 95% C.I.: 0.15–0.98; OR=0.36, 95% C.I.: 0.15–0.90, *respectively) despite* receiving Varenicline. **Conclusion:** Our study demonstrated that variants on *CHRNA5-A3-B4*, *CHRNA2*, and *CHRNA7* genes are significantly associated with short/long-term smoking cessation among subjects receiving Varenicline treatment. Further studies are warranted to replicate our findings. Grant No: NHRI-EX105-10304PI & NHRI-EX106-10304PI

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P15.25A

Clinical cancer diagnostics and treatment using next generation technologies

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Introduction: Cancer is a very variable disease and molecular links between cancer susceptibility and progression alteration levels remain mostly unknown. That's why our cancer patients were screened towards germline and somatic mutations, looking for the potential of integrating genomic data for a comprehensive and personalized cancer medicine.

Materials and Methods: Using NGS technology, our cancer patients were screened towards 170 genes, suspected to play a role in predisposing to cancer. We also screened those patients towards a wide range of "hot spot" mutations in 56 tumor suppressor genes and oncogenes annotated in the COSMIC database. In order to confirm the occurrence of somatic mutation we additionally did a comparison of 3 techniques: Sanger sequencing, qPCR and ddPCR.

Results: NGS technology speeds up the identification of causative alterations, proper diagnostics and treatment of patients. Comparison of 3 techniques highlighted the sensitivity of ddPCR, which can precisely quantify mutant allele frequency of a rare tumorigenic mutations in a high background of "normal" cells, routinely down to 0.01%, much deeper than the standard real-time PCR (1–5% limit detection) and Sanger sequencing (often over 30%).

Conclusions: There is a great potential for integration of new generation technologies looking for germline-somatic links in terms of development, usage of individualized biomarkers and monitoring response to chemotherapy. Targeted cancer therapies may give medical oncologists a better way to customize cancer prevention and treatment.

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P15.26B

Personalized medicine via Pharmacogenetics: Investigating critical success factors for clinical implementation

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Background and aim: Pharmacogenetics (PGx) has the potential to improve drug efficacy and safety by identifying patients likely to respond well to a certain drug or who could be at risk. Although substantial evidence has been published to support the clinical use of PGx, clinical implementation is limited. The University Medical Center Groningen (UMCG) is developing a PGx implementation pilot. As a first step, we assessed impeding and facilitating factors as well as needs of pharmacists, prescribers and patients in applying PGx. **Materials and Methods:** Qualitative interview and focus group studies were conducted with 7 pharmacists, 13 prescribers and 19 patients. **Results:** Both professionals and patients were positive towards PGx. A lack in PGx knowledge among pharmacists and prescribers was, however, considered a barrier. For PGx implementation, clinical decision support was considered a prerequisite for prescribers. The distribution of responsibilities also needs addressing. Prescribers and pharmacists differed in views, both holding the other responsible. For patients the offer of an animation in addition to an information brochure aided in understanding PGx. Since the term PGx was found to be difficult, it was suggested to speak of personalized medication in the implementation process. **Conclusions:** A positive attitude towards clinical implementation of PGx was found. The availability of clinical decision support is regarded as a critical success factor. In the UMCG implementation pilot, PGx will be implemented within three departments of the UMCG (psychiatry, internal medicine, geriatrics) and clinical decision support software based on the GASTON® framework will be used and evaluated.

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P15.27C

Genome-wide association study of angioedema induced by ACE inhibitors or ARBs in Sweden

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Introduction: Angioedema is a rare and potentially serious adverse reaction to angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) treatment. It is characterized by a sudden swelling that can be fatal if the upper airways are involved. Associations between angioedema and candidate genes in the bradykinin pathway have been inconsistent. A previous genome-wide association study (GWAS) found no genome-wide significant association with a single-nucleotide polymorphism (SNP).

Materials and Methods: We performed a GWAS on 173 patients with angioedema induced by ACE inhibitor or ARB treatment from Swedegene (http://www.swedegene.se) and 4890 population controls from TwinGene (http://ki.se/en/meb/twingene-and-genomeeutwin). Cases and controls were genotyped using the Illumina arrays HumanOmni2.5 and HumanOmniExpress 700K. After quality control, the merged genotyped set contained 600K SNPs. The genotyped data was adjusted for gender and principal components 1–4. After phasing and imputation, the dataset contained 7.6 million SNPs. The genome-wide significance p-value threshold was set to $p<5\times10^{-8}$ to correct for multiple testing.

Results: Angioedema was significantly associated with SNPs on chromosome 10 with minor allele frequencies of 0.14 in cases and 0.06 in controls. The strongest associations were with rs2253201 and rs2253202 located in an intron of the *KCNMA1* gene (odds ratio 2.47 [95% confidence interval 1.79, 3.42], $p = 4.31\times10^{-8}$).

Conclusions: Our GWAS detected a novel association between angioedema induced by ACE inhibitor or ARB treatment and the calcium-activated potassium channel gene *KCNMA1*.

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P15.28D

Influence of CYPA4*1B, CYP3A5*3, GSTT1 and GSTM1 polymorphism with treatment-related Chronic Myeloid Leukemia

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Imatinib Mesylate (IM) (Gleevec™ [STI571]; is used in Brazil as a first- line drug inhibitor of bcr-abl for Chronic Myeloid Leukemia treatment. However, cases of treatment failure or of suboptimal response are sometimes seen. This study aimed to investigate if variations in polymorphic genes *CYP3A4*, *CYP3A5* (involved in IM metabolism) and *GST*s (xenobiotics metabolism in general) influence the IM treatment response. A total of 107 CML patients in use of IM from Hospital das Clínicas-UFG, Goiânia, Goiás, Brazil, were recruited. Response criteria were based on European Leukemia Net recommendations. Genotyping was carried out for *CYP3A4*1B* (-392A>G (rs2740574)), *CYP3A5*3* (6986A>G (rs776746)), *GSTT1*0* (null allele) and *GSTM1*0* (null allele) polymorphisms by q-PCR. Statistical analysis was carried out using GraphPad Prism5® software. The Kaplan-Meier survival analyses method was used to estimate if there are difference at the time for achieve and lost response for each genotype. No statistical significance was observed for none genotypes and time for achieve and lost response. Despite this, a tendency to achieve faster cytogenetic response in patients with *GST1* presence, *GSTM1* deletion and *CYP3A5*3* polymorphism together with functional *CYPA3A4* gene was observed. Unlike CYP3A4 and CYP3A5, GSTT1 and GSTM1 are not specific imatinib metabolizers. This study highlights the importance of population studies to investigate metabolizing genes influence in imatinib treatment response.

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P15.29A

Identification of a novel locus associated with asthma treatment response with inhaled corticosteroids in African-admixed children

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Introduction: Inhaled corticosteroids (ICS) are the most widely prescribed asthma controller medication. High variability in response to ICS has been described among individuals and populations, with African-Americans having a lower response. In fact, ICS treatment can be ineffective and patients can suffer severe exacerbations despite

the use of them. This can be partially attributed to genetic factors, since a large heritability has been estimated for ICS response (40–60%). Here, we aimed to identify loci associated with asthma exacerbations in children taking ICS.

Methods: We performed a meta-analysis of two genome-wide association studies (GWAS) of asthma exacerbations including 1,401 African-admixed individuals from the GALA II and SAGE II studies. Imputation of genetic variants was carried out using the Haplotype Reference Consortium as reference panel and association testing was performed with logistic regression models. A total of 8.6 million variants with minor allele frequency ≥1% were meta-analyzed. Variants with $p \leq 5 \times 10^{-6}$ were followed up in 1,204 participants from three European studies (PACMAN, followMAGICS, and PASS).

Results: Suggestive association was found for 27 polymorphisms ($p \leq 5 \times 10^{-6}$) in African-admixed individuals, 3 of them showing evidence of replication in the European populations ($p \leq 5.4 \times 10^{-3}$). The most significant variant was located in the intergenic region of the *APOBEC3B* and *APOBEC3C* genes.

Conclusions: We identified the association of a novel locus with asthma exacerbations in children despite the use of ICS.

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P15.30B

Management of secondary findings from whole exome/genome sequencing: a first step into genomic medicine

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Introduction: Genomic medicine is defined by the NHGRI as "an emerging medical discipline that involves using genomic information about an individual as part of their clinical care and the health outcomes and policy implications of that clinical use". With next generation sequencing (NGS), beyond identifying the cause of manifestations that justified prescription of the test, other information with potential interest for patients and their families, defined as secondary findings, can be provided to patients once they have given informed consent, in particular when therapeutic and preventive options are available. The disclosure of such findings has caused much debate amongst ethicists and healthcare professionals.Materials and Methods: A review of the literature was performed, focusing on all PubMed articles reporting qualitative, quantitative or mixed studies that interviewed healthcare providers, patients, or society regarding this subject. The methodology was carefully analysed, in particular whether or not studies distinguished between actionable and non-actionable secondary findings.

Results: From 2010 to 2016, 35 articles were compiled including 29 from North America, three from Australia and three from Europe. A total of 11,827 people were interviewed in these studies (1,098 patients, 3,224 healthcare providers, 7,505 representatives of society at large). When actionable and non-actionable secondary findings were analysed separately (76%), about 93% of respondents were keen to have results regarding actionable secondary findings, against 71% for non-actionable secondary findings. **Conclusions:** This review shows the need to consider this option among diagnostic labs. An effort should be made to develop educative tools to facilitate decision-making.

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P15.31C

Using genome editing to identify and characterize functional variants that determine response to metformin

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Introduction: Several pharmacogenetics studies have identified that the minor allele at rs2289669 in intron 10 of the *SLC47A1*, which codes for the metformin transporter multidrug and toxin extrusion 1 (MATE1), is associated with a greater reduction in HbA1c levels. However, it is currently not known if rs2289669 is itself causal or how this functional variant operates at the molecular level. To address these questions, we combined in silico analysis with in vitro genome engineering using the CRISPR-Cas9 system in human Huh-7 hepatocytes. **Materials/Methods:** Functional annotation using ANNOVAR for all SNPs in tight LD with rs2289669 revealed rs8065082 as the top ranking functional candidate. Two gRNAs per SNP target were designed, cloned into a Cas9 plasmid vector and optimized for high transfection efficiency in Huh-7 cells. Further, to generate *SLC47A1*/MATE1 KO cells, predesigned gRNAs were used to excise a ~2kb region for functional downstream analysis using metformin treatment. Finally, the same gRNAs will be used to perform CRISPR-Cas9 individual SNP editing experiments. **Results:** *SLC47A1*/MATE1 KO cells show ~60% reduction in *SLC47A1*/MATE1 mRNA expression as well as ~88% lowered MATE1 protein level in a heterogeneous cell population. Functional downstream experiments, comparing clonal cell populations of SLC47A1/MATE1 KO and WT cells, and testing metformin-dependent inhibition of gluconeogenesis as well as the effect of lifestyle mimetics (e.g. AICAR) on potential gene x environment interactions, are ongoing. **Conclusion:** This study will contribute to illuminate the underlying functional effect of metformin in hepatocytes as well as the identification of the functional polymorphism.

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P15.32D

New susceptibility loci for cold medicine-related Stevens-Johnson syndrome with severe ocular complications identified by GWAS using an ethnicity-specific SNP array

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A genome-wide association study (GWAS) for cold medicine-related Stevens-Johnson syndrome (CM-SJS) with severe ocular complications (SOC) was performed in a Japanese population with 117 patient and 691 control samples. A recently developed ethnicity-specific array (Japonica Array) with genome-wide imputation that was based on the whole-genome sequences of 1,070 unrelated Japanese individuals was used. Validation analysis with additional samples from Japanese individuals and replication analysis using samples from Korean individuals identified two new susceptibility loci on chromosomes 15 and 16. Moreover, combination of variants' effects between the previously identified risk allele, HLA-A*02:06, and the newly detected risk variants were observed. This study confirmed the usefulness of GWAS using the ethnicity-specific array and genome-wide imputation based on large-scale whole-genome sequences. These findings contribute to the understanding of genetic predisposition to CM-SJS with SOC.

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P15.33A

Tacrolimus troughs in Caucasians with two CYP3A5 loss-of-function alleles: Evidence for additional genetic influences using a genome wide association study

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Background. The commonly used immunosuppressant tacrolimus (TAC) is metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5 enzymes. The presence of the *CYP3A5*1* allele is well-known to increase TAC metabolism. However, it is common for Caucasian recipients to carry two *CYP3A5* loss-of-function (LoF) variants which profoundly reduces TAC metabolism. We observed that individuals who carry two LoF alleles have considerable variability in TAC trough blood concentrations, even in the absence of CYP3A5 enzyme activity. We hypothesize that variants in genes outside of *CYP3A5* will provide insight into this variability.

Methods. We analyzed TAC trough levels in 1,446 adult Caucasian kidney allograft recipients having two *CYP3A5* LoF alleles (*3*, rs776746; *6*, rs10264272; or *7*, rs41303343) resulting in reduced or absent CYP3A5 function. We used a genome wide association study (GWAS) to identify additional genetic variants associated with dose normalized TAC trough variation.

Results. The GWAS of Caucasian recipients identified several variants within *CYP3A4*, including *CYP3A4*22* (rs35599367, p=2.21e^{-17}), previously associated with TAC trough variation. After adjusting for this variant, no other common variants were genome wide significant. However, we identified several less frequent variants showing promising evidence of association including rs34104306 in *EPHB2* (p=1.98e^{-9}, MAF=0.015) rs147025569 in *POLQ* (p=4.22e^{-7}, MAF=0.011) and an Alu ins/del in *NEBL* (p=3.97e^{-7}, MAF=0.06).

Conclusion. Additional high allele frequency genetic variants associated with TAC trough variability are unlikely to be present in Caucasian recipients. These data suggest that low allele frequency variants identified by DNA sequencing should be evaluated and may contribute to pharmacokinetic variability of TAC metabolism.

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P15.34B

Utilization of genetic data can improve the prediction of type 2 diabetes incidence in a Swedish cohort

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The aim of this study was to measure the impact of genetic data in improving the prediction of type 2 diabetes (T2D) in a Swedish cohort. The current study was performed in 3,835 Swedish individuals and utilizes a set of genetic and environmental risk data. We first verified that the genetic data has a statistically significant positive correlation with incidence of T2D in the studied population. We also verified that adding genetic data slightly but statistically increased the Area Under Curve (AUC) of a model based only on environmental risk factors (AUC shift + 1.0%, p-value = 0.0194). We verified that the improved AUC will result in 2% improvement in sensitivity or specificity of T2D predictions. To study the dependence of the results on the environmental risk factors, we divided the population into two equally sized risk groups based only on their environmental risk and repeated the same analysis within each subpopulation. While there is a statistically significant positive correlation between the genetic data and incidence of T2D in both environmental risk categories, the positive shift in the AUC remains statistically significant only in the category with the lower environmental risk (p-value = 0.0189). These results demonstrate that genetic data can be used to increase the accuracy of T2D prediction. Also, the data suggests that genetic data is more valuable in improving T2D prediction in populations with lower environmental risk. Finally, it suggests that genetic association studies should be performed in light of the underlying environmental risk of the population.

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P15.35C

The pharmacogenetics of metformin and its effect on plasma metformin concentration and glycated hemoglobin

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Introduction: Metformin is the most often used first line pharmacotherapy for Type 2 Diabetes (T2D) treatment; however considerable interindividual variability in clinical efficacy of metformin is recognized. The goal of this study was to investigate variability in genes coding for metformin

transporters (OCT1, OCT2, OCT3, MATE1, MATE2, and PMAT) and metformin molecular targets (LKB1, ATM) to identify the specific genetic variants associated with intolerance and efficacy of metformin therapy.

Materials and Methods: A total of 206 treatment naïve newly diagnosed T2D patients were included in this study. To identify the genetic alterations, Ion PGM next-generation sequencing method was used. Patients received 500 mg dose of metformin followed by collection of blood and urine samples for pharmacokinetics study of metformin.

Results: In this study, 121 variants were identified in metformin responders and non-responders. Three functional variants: R287G, G401S and 420del identified in OCT1 gene and correlate with metformin non-responsiveness. The rs2457574, rs4724524, rs587781368, rs7225817, rs139394521 and rs7223097 are significantly associated with metformin non-responder, whereas rs3218681 is associated with metformin response. Pharmacokinetic properties of metformin, including AUC, V/F, and Cmax were significantly different between the individuals who carried one of the reduced function alleles, R287G, G401S or 420del and those who carried only OCT1- reference alleles.

Conclusions: Genetic alteration in OCT1, is the most important aspect for the metformin glycemic response in West Bengal, India. OCT1 activity affects metformin steady-state pharmacokinetics, and OCT1 genotype has a bearing on HbA1C during metformin treatment.

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P16 Omics/Bioinformatics

P16.01A

GeneHancer: Genome-wide integration and scoring of enhancers and target genes in GeneCards

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A major challenge in the field of transcription regulation is identifying the connections between enhancers and genes, key to the understanding of developmental and disease mechanisms. We present GeneHancer, a novel database of human enhancers and their inferred target genes, in the framework of GeneCards (www.genecards.org). First, we integrated data from four databases that reported enhancers in the human genome: a) ENCODE, with ~176,000 predicted enhancer-like regions; b) Ensembl, with ~213,000 regulatory build-predicted enhancers; c) FANTOM, with ~43,000 enhancer RNA (eRNA) enhancers; d) VISTA, with ~1,700 enhancers validated by transgenic mouse assays. Subsequently, we generated gene-enhancer maps and combinatorial scores using information that helped link enhancers to genes: 1) GTEx expression quantitative trait loci (eQTLs), whereby a significant genetic association occurs between variants in enhancers and the expression of candidate target genes; 2) Capture Hi-C promoter-enhancer long range interactions; 3) FANTOM expression correlations between eRNAs and candidate target genes; 4) Cross-tissue expression correlations between transcription factors interacting with enhancers and candidate target genes; 5) Enhancer-gene physical distance. GeneHancer presents ~285,000 integrated enhancers connected to ~101,000 genes. Defining "elite" as supported by ≥2 data sources, we highlight ~40,000 double-elite gene-enhancer pairs as most relevant to whole genome sequence analyses. To this end we are also upgrading our VarElect/TGex WGS analysis and interpretation tools so as to map variants to unified enhancers, identify their high-scoring target genes, and interpret the results in terms of gene-phenotype/disease keywords. Supported by LifeMap Sciences Inc.

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P16.02B

Comorbidity landscape of the Danish patient population affected by chromosomal abnormalities

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Introduction: In this work we present a systematic and comparative analysis of comorbidities in the Danish population for patients affected by the chromosomal abnormalities such as Klinefelter (47,XXY), Turner (45,X) and Down syndromes (47,XX/XY + 21).

Material and Methods: We analysed data from electronic patient records in the Danish National Patient Registry. The registry includes 6.9 million patients hospitalized between 1994 and 2015 and comprises more than 108 million hospital encounters. We quantified the association between chromosomal abnormalities and diseases by calculating the age and sex corrected relative risk, using a matched background population.

Results: We identified 9,803 patients with a chromosomal abnormality in this dataset (ICD-10 codes Q90-Q99). This epidemiological and data driven approach pointed out a specific signature of comorbidities for each chromosomal instability considered. However, there was also interesting overlaps between co-occurring diseases including diabetes, hearing loss and infectious diseases.

Conclusions: Patients with chromosomal abnormalities are predisposed for a wide range of diseases. By studying comorbid diseases we can shed light on the genetic mechanisms that drive these diseases. In this study we presented a population wide comparative analysis of comorbidities in patients affected by chromosomal abnormalities. We found that even though each chromosomal disease had a unique comorbidity signature, there are still overlaps pointing towards shared genetic mechanisms or pleiotropic effects. Taken together, these results highlighted a deeper understanding of chromosomal disorders that can be exploited in prospective studies. Future directions will consider the integration of more data types including genomic, proteomic or even socioeconomic data.

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P16.03C

panelcn.MOPS: CNV detection in targeted NGS panel data for clinical diagnostics

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Targeted next-generation-sequencing (NGS) panels have largely replaced Sanger sequencing in clinical diagnostics. Enrichment-based targeted NGS panels allow for the detection of copy-number-variations (CNVs) in addition to single-nucleotide-variants and small insertions/deletions. However, existing computational CNV detection methods have shortcomings regarding accuracy, quality control, incidental findings, and user-friendliness.

To solve these problems we developed panelcn.MOPS, a novel pipeline for detecting CNVs in targeted NGS panel data. Using NGS panel data from 170 samples, we compared panelcn.MOPS with 5 state-of-the-art methods.

We present the first thorough comparison of CNV detection methods for targeted NGS panel data. Most methods achieved comparably high sensitivity and/or specificity, but panelcn.MOPS led the field with a sensitivity and specificity of 100%. panelcn.MOPS reliably detected CNVs ranging in size from 20 nucleotides (only part of a region-of-interest - ROI), to whole genes, which may comprise all ROIs investigated in a given sample. The latter is enabled by analyzing reads from all ROIs of the panel, but presenting results exclusively for user-selected genes, thus, avoiding incidental findings. Additionally, panelcn.MOPS offers quality control criteria not only for samples but also for individual ROIs within a sample which increases the confidence in called CNVs.

panelcn.MOPS is freely available both as an R package and as standalone software with an intuitive graphical user interface. It can therefore readily be used by clinical geneticists without any programming experience or integrated into existing analysis pipelines. Taken together, panelcn.MOPS combines high sensitivity and specificity with user-friendliness rendering it highly suitable for routine clinical diagnostics.

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P16.04D

High-throughput clonal analysis of tumors with droplet microfluidics

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Single cell analysis tools are crucial to understand the role that rare or heterogeneous cancer cells play in the evolution of tumor progression. To enable the characterization of

genetic diversity within cancer cell populations, we developed a novel approach that barcodes amplified genomic DNA of individual cells confined to microfluidic droplets. The barcodes are used to reassemble the genetic profiles of individual cells from next-generation sequencing data. A key feature of our approach is the "two-step" microfluidic workflow. The microfluidic workflow first encapsulates individual cells in droplets, lyses the cells and prepares the genomic DNA for amplification with proteases. Following this lysate preparation step, the proteases are inactivated and droplets containing the genomes of individual cells are then paired with molecular barcodes and PCR reagents. We demonstrate that the two-step microfluidic approach is superior to workflows without the two-step process for efficient DNA amplification on tens of thousands of individual cells per run with high coverage uniformity and low allelic dropout of targeted genomic loci. To apply our single-cell sequencing technology to human tumor samples, we developed a targeted panel to sequence genes frequently mutated in acute myeloid leukemia (AML) including TP53, DNMT3A, FLT3, NPM1, NRAS, IDH1 and IDH2. Using this panel, we were able to sensitively identify clonal populations from AML research samples; moreover, the single-cell nature of our approach enabled the correlation of multiple mutations within subclones. Collectively, our results show a greater degree of heterogeneity in AML tumor samples than is commonly appreciated with bulk sequencing methods.

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P16.05A

Myeloid gene expression in acute lymphoblastic leukemia with very early relapse

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Introduction: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer in Mexico, where a high mortality rate from this disease has been reported. ALL very early relapse (VER: within 18 months from diagnosis) in one of the main causes of death in this country. To identify genes involved in VER as well as potential prognostic biomarkers we performed a whole gene expression analysis in Mexican pediatric patients.

Materials and methods: We obtained 57 total bone marrow samples (49 at diagnosis and 8 at VER) from patients under 18 years old with pre-B ALL. Gene expression analysis was carried out using oligonucleotide microarrays HTA 2.0 (Affymetrix). To identify differentially expressed genes in VER samples we used TAC software (Affymetrix). Genes with a FC of 1.5 and FDR <0.05 were considered statistically significant. Pathway enrichment analysis and biological processes were performed with IPA (QIAGEN) and Gene Ontology.

Results: We found 288 differentially expressed genes in the VER group, of which, genes (*EBF1, BLNK, PAX5* and *DNTT)* involved in B-cell differentiation were down regulated. Meanwhile, myeloid lineage genes such as *CEBPA*, *S100A9*, *RAB31* and *MPO (*$p < 0.02, 0.004, 0.0007, 0.001$, respectively) were up regulated. Other differential expressed genes were associated with infectious and respiratory diseases.

Conclusion: Our analysis support previous evidences suggesting that low expression of *PAX5* and *EBF1* switch from B-cell to myeloid lineage gene expression during ALL relapse and exhibited those genes as potential biomarkers in VER in ALL. More studies are needed to validate these findings.

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P16.06B

Allele balance based variant callability score for whole exome sequencing

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Introduction: Next generation sequencing (NGS) significantly changed biomedical research in the last years. Nonetheless, the use of high-throughput DNA sequencing for identification of causal/associated variants is highly sensitive to technical –random and systematic- errors, which can lead to false associations. Therefore, understanding and identifying systematic errors is needed to avoid misleading results in NGS analysis.

Methods: We present a callability score based on allele balance (AB) (proportion of reads supporting alternative alleles in a specific genomic position) to detect systematic errors. Specifically, the relation between the recurrent AB bias (ABB) and false positive (FP) calls. In a cohort of 987 exome samples, we obtained AB distribution models for all possible DNA genotypes and measured the recurrence of ABB across all positions. Using these measures, we developed a logistic regression model able to predict recurrent ABB in independent samples (benchmarked in 200 independent samples).

Results: Our callability score (ABB-score) showed high precision and recall for detecting ABB (positively correlated with FP rates) in germline variant calling pipelines (~4% of the GATK variants calls), ~50% of which were found to be FP with Sanger validation. Additionally, it labeled ~8% of the confident somatic calls reported by MuTect for 450 cancer exomes (Chronic lymphocytic leukemia) as likely FP calls.

Conclusion: We have developed a variant callability score (ABB-score) able to identify FP calls caused by systematic sequencing or alignment errors in the human exome. This model can be integrated in disease variant prioritization-association pipelines to remove spurious results and refine true positive variants.

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P16.07C

Improving gene annotation to identify missing variants of clinical significance

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High quality gene models provide a foundation for the annotation of sequence variation in clinical samples. Complete and accurate annotation of gene structure and function is essential to reduce both false negative (from missing annotation) and false positive (from incorrect annotation) errors in identification of disease-associated variants. As part of the GENCODE project, we produce detailed reference annotation of all human and mouse protein-coding genes. We will describe the re-annotation of 70 genes on reference diagnostic panels for Early Infantile Epileptic Encephalopathies (EIEE), a group of disorders characterized by early onset seizures and developmental delay.The re-annotation utilised public transcriptional evidence, focussing on next-generation sequence data from human brain. We used RNAseq, SLRseq and PacBio reads to find new alternative splicing (AS) events; novel exon inclusion and skipping, and shifted splice sites. Although these genes had been annotated previously by GENCODE, with 685 AS transcripts compared to 193 in the RefSeq geneset, our re-annotation added a further 1092 AS transcripts, 706 novel exons, 224 shifted splice sites and more than 141kb additional genomic coverage of which approximately 15.2kb represented novel CDS. More than 80% of these novel splice features were expressed in foetal brain and ~30% of those in the CDS show high cross-species sequence conservation, suggesting functionality. Variants in the SCN1A gene are responsible for the majority of cases of the EIEE Dravet syndrome. We will describe a study using updated SCN1A annotation to identify variants missed using pre-existing annotation and discuss the novel pathogenic variants found.

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P16.08D

Efficient de novo structural variation analysis and annotation using Bionano's Next-Generation Mapping (NGM) technique

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De novo mutations are known to cause genetic diseases. With the advent of family-based whole genome techniques, *de novo* mutations can now be detected. *De novo* structural variation (SV), beyond large deletions and duplications, remains elusive by sequencing. Short reads can miss complex SVs, and makes false calls due to high error rates and misalignments.

The Bionano Saphyr™ system captures long DNA to delineate SV structures. DNA molecules measuring greater than 100 kbp are extracted from 2 mL of whole blood, labelled at specific motifs, and linearized through Nano-Channels arrays for subsequent visualization. Digitized images of those molecules are then assembled *de novo*, creating megabases long assembled optical maps. While our calling algorithm can sensitively detect homozygous and heterozygous SVs in these long maps, we also use a variant-annotation workflow to specifically uncover *de novo* SVs, a key feature for family studies.

We ran a quintet family with three autistic children. For each sample, we collected 100x coverage depth across the genome, generated *de novo* assemblies, detected and annotated heredity for SVs. This process, from sample collection to SV-discovery took only one week on one Saphyr system. In each sample, we detected >3,500 insertions and deletions >500 bp. We found 3–4 *de novo* SVs in each child, one of which was shared, suggesting that this was a mosaic in a parent. This workflow enables efficient selection of candidates for curation. With one comprehensive platform, Saphyr may replace conventional approaches for discovery of functionally-relevant variants, and improves our understanding of genomes.

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P16.09A

Cross-Species Genomics Explorer™ for disease mutation identification through cross-species analysis

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Recent developments in ngs technologies have made them widely used in the diagnosis of inherited diseases and developmental conditions. However, in a sizable fraction of cases, no causative mutation can be pinpointed with high certainty. This is likely to be the case especially when only a single member of a family is affected, or the presentation of a condition is atypical. In these cases, the use of phenotypic data on animals with targeted genetic alterations is an intriguing possibility to narrow down on a specific mutation as likely causative. However, effective cross-species use of genotype-phenotype data is hampered by the difficulty in effectively accessing this data and using it together with the sequencing data itself.

To solve this problem, we have developed Cross-Species Genomics Explorer™, a browser based system that enables efficient and flexible analysis of (human) genomics data in a cross-species context. It incorporates a variant storage and analytic system for big variant data together with efficient exploration of human genetic variants. This exploration is done in the context of the variants' orthologous positions in the genomes of model organisms, through multi-species genome tracks and on the fly cross-species genome build conversion. Together with an extensive database of the known phenotypic consequences of genetic alterations in model organisms, this allows researchers to quickly test human hypotheses against data on organisms such as mouse, zebrafish or fruitfly and, conversely, starting from model organism data and specific genotype-phenotype observations, generate hypotheses about human gene function in health and disease.

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P16.10B

CAW - Cancer Analysis Workflow to process normal/tumor WGS data

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As whole genome sequencing is getting cheaper, it is viable to compare NGS data from normal and tumor samples of numerous patients. There are still many challenges, mostly regarding bioinformatics: datasets are huge, workflows are complex, and there are multiple tools to choose from for somatic and structural variants and quality control.

We are presenting CAW (Cancer Analysis Workflow) a complete open source pipeline to resolve somatic variants from WGS data: it is written in Nextflow, a domain specific language for workflow building. We are utilizing GATK best practices to align, realign and recalibrate short-read data in parallel for both tumor and normal sample. After these preprocessing steps several somatic variant callers scan the resulting BAM files; MuTect1, MuTect2 and Strelka are used to find somatic SNVs and small indels.For structural variants we use Manta. Furthermore, we are applying ASCAT to estimate sample heterogeneity, ploidy and CNVs.

The software can start the analysis from raw FASTQ files, from the realignment step, or directly with any subset of variant callers. At the end of the analysis the resulting VCF files are merged to facilitate further downstream processing, though the individual results are also retained. The flow is capable of accommodating further variant calling software or CNV callers. It is also prepared to process normal - tumor - and several relapse samples.

Besides variant calls, the workflow provides quality controls presented by MultiQC. A docker image is also available, the open source software can be downloaded from https://github.com/SciLifeLab/CAW .

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P16.11C

MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations

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Published genetic associations can be used to infer causal relationships between phenotypes, bypassing the need for individual-level genotype or phenotype data. We have curated complete summary data from 1094 genome-wide association studies (GWAS) on diseases and other complex traits into a centralised database, and developed an analytical platform that uses these data to perform Mendelian randomization (MR) tests and sensitivity analyses (MR-Base, http://www.mrbase.org). Combined with curated data of published GWAS hits for phenomic measures, the MR-Base platform enables millions of potential causal relationships to be evaluated. We use the platform to predict the impact of lipid lowering on human health. While our analysis provides evidence that reducing LDL-cholesterol, lipoprotein(a) or triglyceride levels reduce coronary disease risk, it also suggests causal effects on a number of other non-vascular outcomes, indicating potential for adverse-effects or drug repositioning of lipid-lowering therapies.

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P16.12D

Patient Archive: A platform for clinical deep phenotyping

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Phenotype has long been used to substantially reduce the search-space for genomic variation and to determine underlying genetic etiologies. The incomplete linking of detailed phenotypic terms to genomic variants is now the major factor limiting rapid diagnostics. The Monarch Initiative develops data models, algorithms and platforms to enable translational deep phenotyping. Patient Archive (PA), a platform part of the Monarch suite, focuses on accurate clinical phenotyping to facilitate disease stratification and exploration of the candidate gene space. PA uses the widely adopted Human Phenotype Ontology to create structured patient phenotype profiles and supports both bottom-up, as well as top-down phenotyping. The former emulates the standard clinical workflow and is driven by the automatic extraction of HPO concepts from free text. The latter, starts from a provided disease model and enables clinicians to create a structured profile by externalizing the alignment between this model and the phenotypes exhibited by the patient. Additional analytical capabilities are provided via HPO-driven semantic similarity matching algorithms. These can be used for a variety of tasks, ranging from patient matchmaking or disorder exploration to personalized gene list generation to power the variant filtering process. A second aim of the platform is to create a seamless data exchange channel for the clinicians. Consequently, it supports fine-grained access control in both individual and group contexts, and full integration into the Global Alliance for Genetic Health MatchMaker Exchange Program. PA provides a seamless solution for the harmonization of phenomic information to enable the acceleration of translational and clinical applications.

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P16.13A

Interactome mapping of copy number variations for candidate process prioritization in intellectual disability and autism

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Introduction The interpretation of CNVs represents a major challenge. Various methodologies are used to establish consequences of genetic variants. In this light, bioinformatic methods play a crucial role. Here, we describe an original approach towards revealing the effects of CNVs through walking the interactome (interactome mapping) and candidate process prioritization. **Materials and methods** Eleven children with resembling phenotypic manifestations of intellectual disability, congenital malformations and autism were analyzed using molecular karyotyping (SNP array) and an original bioinformatic technology for prioritizing genetic changes (Iourov et al., 2014) including interactome analysis. **Results** In all the patients, likely pathogenic CNVs involving 43 genes were revealed. Using STRING Database, BioGRID and NCBI Gene, we walked their complete interactome encompassing 813 genes. Employing bioinformatic algorithms, we found an interconnected net of 544 elements (genes). According to KEGG, these genes are involved in 240 pathways. Among the pathways which scored the highest according to our algorithm, we selected following candidate processes: viral carcinogenesis pathway (44 genes), cell cycle pathway (38

genes), PI3K-Akt signaling pathway (33 genes) and MAPK signaling pathway (33 genes). **Conclusions** Although dysregulations of these pathways are not unique for intellectual disability and autism, data mining and original bioinformatic methodologies have confirmed the associations. Consequently, we concluded that interactome mapping can be an effective bioinformatics tool for uncovering molecular and cellular mechanisms of genomic pathology. Supported by the Russian Science Foundation (project #14-35-00060).

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P16.14B

WISEexome: Detection of copy number variations in clinical exome sequencing data based on a within-sample comparison scheme

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Clinical exome sequencing (CES) can provide a molecular diagnosis in families with an unexplained genetic disease. Both SNPs and CNVs can be pathogenic and are searched for in diagnostic settings. At the moment most clinical labs perform both CES and array to be able to detect SNPs and CNVs (resolution ~20 kb). To be able to replace array with CES we developed a method, based on a combination of our previous work (WISECONDOR; https://github.com/rstraver/)) and a segmentation algorithm. Our method fully depends on an internal comparison of DNA fragments per probe, rather than comparisons with a reference set of samples. All 18 known CNVs in our dataset of 24 samples were correctly identified. Size ranges from 4 kb to 5,2 Mb. The smallest CNV contained a deletion of 3 exons. Results can be sorted by a score that indicates the reliability of a call, usually providing the true positives in the top 5. We are now in the process of retrospectively analyzing our diagnostic exome cohort (~500 trio analysis) and expect to detect small pathogenic CNVs that may have been missed by the routinely used array analysis (resolution of 20 kb). Our work provides an alternative method to find CNVs in exome data. We do not require that reference samples are resequenced in the same run, and we are able to detect variable-sized CNVs. Consequently, WISEexome has the potential to replace array analysis and maybe even MLPA tests in a diagnostic setting in a cost-effective way.

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P16.15C

Systematical identification of essential genes in Acute Lymphoblastic Leukemia (ALL)

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Deletion of chromosomal material is a hallmark of cancer genomes. Genomic deletion of tumor suppressor genes frequently encompasses essential neighboring genes, rendering cancer cells with hemizygous deletions vulnerable to further suppression of such genes. Identifying these vulnerabilities is one of the current challenges in cancer genomics.

We developed a computational method to identify genomic regions harboring frequently deleted, yet essential genes, by applying pattern recognition techniques to large sets of DNA copy-number profiles. In total, we identified four regions, located on chromosomes 9, 13 and 17, that fulfilled the criteria of our pattern recognition framework and harbored multiple candidate essential genes. The strongest signals were located in the hemizygously deleted regions flanking the commonly deleted region containing *CDKN2A*, a well-known tumor suppressor gene that is frequently homozygously deleted in several tumor types, including Acute Lymphoblastic Leukemia (ALL). To validate the essentiality of the genes in this region, we used pooled CRISPR/Cas9 editing in ALL cells with and without a *CDKN2A* deletion. This provided further evidence for the essentiality of several genes in the identified regions, including one gene that was essential only in *CDKN2A*-deleted cells.

Haploinsufficiency for the identified genes could be further exploited as a therapeutic advantage for ALL treatment.

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P16.16D

CnvHunter: a new tool for highly accurate detection of single exon copy number variants in next generation sequencing data, validated in 994 samples from a targeted hereditary breast cancer panel

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Introduction: Copy number variants (CNVs) represent an important proportion of all detectable pathogenic variants in certain genes. Due to an ever increasing amount of genes in next generation sequencing (NGS) panels, the use of additional methods like multiplex ligation-dependent probe amplification (MLPA) for CNV detection becomes impractical. Using NGS data for the detection of CNVs would greatly reduce costs and yield important data of diagnostic value. However, high resolution single exon CNV detection is challenging, especially in data sets with variable sample sizes or different sequencing platforms used.

Methods: CnvHunter was validated with a data set of 994 samples from a targeted panel (TruSight Cancer panel; 12 to 24 samples per run on MiSeq or NextSeq platforms, Illumina,). CNVs were independently validated with MLPA in up to five genes (*BRCA1, BRCA2, CHEK2, RAD51C, RAD51D*). CnvHunter takes depth of coverage (DOC) profiles from several targeted NGS samples as input that were processed with the same enrichment kit. It detects CNVs for each sample individually, based on reference data from the most similar samples in the input dataset.

Results: In this highly heterogeneous validation data set, using MLPA 27 CNVs were detected in 119 exons. CnvHunter detected CNVs in 116 exons, including 5 single exon CNVs. CnvHunter achieved 98% sensitivity and 99% specificity and a positive predictive value of 78%.

Conclusions: CnvHunter is a robust high resolution tool for CNV detection in heterogeneous NGS data with a very high sensitivity and specificity rate.

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P16.17A

Whole genome copy number variation analysis using a SNP-focused targeted sequencing panel for tumor analysis

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Introduction: Accurate detection of somatic copy number variation (CNV) is crucial but presents challenges due to tumor purity, heterogeneity, and ploidy. While there's been a shift towards new Next Generation Sequencing (NGS) for other mutation types, somatic copy number detection often still relies on fluorescence *in situ* hybridization, array comparative genomic hybridization, or SNP arrays. There are significant throughput, coverage, cost, and sample input advantages to broadening NGS-based targeted sequencing to include accurate somatic CNV detection.

Material and Methods: An xGen® Lockdown® CNV panel targeting evenly spaced SNPs across the genome was developed. For additional resolution, probe density was increased 3-to-10-fold for chromosome 7 and ten additional genes known to commonly exhibit somatic. Coverage and allele fraction at the SNP sites were used to determine tumor purity and ploidy, thus enabling higher accuracy somatic CNV detection.

Results: We demonstrate use of the panel as an addition to the xGen® Exome Research Panel. SNPs present in NA12878 were used to validate the panel's ability to detect heterozygous germline SNPs with >95% sensitivity and specificity. In addition, mixtures of cell lines from the Cancer Cell Line Encyclopedia were tested with varying levels of background copy-neutral genomic DNA. Sensitivity and specificity of the panel to detect CNV and loss of heterozygosity events were compared to expectations given pure cell line sequencing and SNP array data.

Conclusion: The xGen® Lockdown® CNV panel enables the detection of somatic copy number alterations with high resolution and accuracy, which will be a valuable resource for cancer research.

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P16.18B

RBV: Allele-specific copy-number validation of whole genome sequence and whole exome sequence data

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The increased use of genome resequencing has led to the development over 80 software tools created to identify copy number variants (CNVs) from sequence data. These generally utilise characteristics of the sequence data including: read depth, split reads, read pairs, and assembly-based techniques.

The assessment of the relative number and read coverage of heterozygous loci has not been extensively used for NGS CNV investigations. We sought to develop a bioinformatic tool to validate CNVs using the relative number of heterozygous alleles and the distribution of reads for heterozygous alleles within putative CNVs.

The number of heterozygous single nucleotide polymorphisms (SNPs) in a given length of the genome follows a Poisson distribution. Therefore, the probability of a specific region being deleted can be calculated using a Poisson distribution centred on the average number of heterozygous SNPs in a sample of genomic regions of the same length. The relative proportion of reads at each heterozygous position of the aligned genome, reflects the relative copy number at that position. Hence, the distribution of the ratio of reads for the allele with higher coverage at heterozygous loci in the putative CNV region can be compared to the distribution of those in a sample of diploid regions.

RBV is currently applied to validate CNVs in a cohort of New Zealand cases with undiagnosed neurodevelopmental disorders (Minds for Minds cohort) using the relevant aligned genome and a list of coordinates for the predicted CNVs. Potential further uses include ploidy estimation, and calculating degree of mosaicism.

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P16.19C

An integrated analysis tool for analyzing hybridization intensities and genotypes using new-generation population-optimized human arrays

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Background: Affymetrix Axiom single nucleotide polymorphism (SNP) arrays provide a cost-effective, high-density, and high-throughput genotyping solution for population-optimized analyses. However, no public software is available for the integrated genomic analysis of hybridization intensities and genotypes for this new-generation population-optimized genotyping platform.

Results: A set of statistical methods was developed for an integrated analysis of allele frequency (AF), allelic imbalance (AI), loss of heterozygosity (LOH), long contiguous stretch of homozygosity (LCSH), and copy number variation or alteration (CNV/CNA) on the basis of SNP probe hybridization intensities and genotypes. This study analyzed 3,236 samples that were genotyped using different SNP platforms. The proposed AF adjustment method considerably increased the accuracy of AF estimation. Th e proposed quick circular binary segmentation algorithm for segmenting copy number reduced the computation time of the original segmentation method by 30%–67%. The proposed CNV/CNA detection, which integrates AI and LOH/LCSH detection, had a promising true positive rate and well-controlled false positive rate in simulation studies. Moreover, our real-time quantitative polymerase chain reaction experiments successfully validated the CNVs/CNAs that were identified in the Axiom data analyses using the proposed methods; some of the validated CNVs/CNAs were not detected in the Affymetrix Array 6.0 data analysis using the Affymetrix Genotyping Console. All the analysis functions are packaged into the ALICE (AF/LOH/LCSH/AI/CNV/CNA Enterprise) software.

Conclusions: ALICE and the used genomic reference databases, which can be downloaded from http://hcyang.stat.sinica.edu.tw/software/ALICE.html, are useful resources for analyzing genomic data from the Axiom and other SNP arrays.

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P16.20D

Turning *big data* into *small data* through crowdsourced curation: integrating all types of medical and scientific knowledge

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Big data in the form of scientific media comes in many languages and formats: journal articles, books, images, videos, etc. Text media often includes additional embedded or associated information such as tables, figures and supplementary data. While there are many resources for browsing, searching and annotating some of this media, there is no single place to search them all at once, and generalized search engines do not allow for the comprehensive and precise searches researchers require. One could

argue that any scientific media that is on the web is therefore connected, but much of it is inaccessible and is neither discoverable nor connected. To this end, we created **iCLiKVAL**, a web-based tool that uses the power of crowdsourcing to accumulate annotation information for all scientific media found online. Annotations in the form of key-relationship-value tuples (**any language**) added by users through various means can make vast amounts of unstructured data easier to comprehend and visualize by turning it into *small structured data*. This allows for richer data searches and discovery of novel connections by integrating all forms of scientific knowledge through common terminology. It currently supports PubMed, YouTube, Flickr, SoundCloud and anything with a DOI name, allowing for the inclusion of **hundreds of millions of media objects**. We also created a Chrome Browser extension that allows any non-password protected online media to be annotated, **even while the user is offline**. The iCLiKVAL database is completely searchable, and all of the data is freely available to registered users via our API.

T.D. Taylor: None. **M. Hebrard:** None. **N. Kumar:** None.

P16.21A

Optimizing data compression technique determined by decoding HIV-1 HXB2 DNA genome assists in deciphering the means the human genome is transcribed and translated

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An optimizing data compression technique involving multiple genes is revealed in analyzing HIV-1 HXB2 genome K03455.1. Understanding HIV's data compression assists in an expanded understanding of the human genome. The data compression technique ingeniously utilizes features of the codon code and nucleotide code to generate overlapping genetic information, which facilitates 8 segments of HIV's pre-mRNA code to be read twice producing differing mRNA sequences each time the pre-mRNA code is deciphered.

The HIV-1 HXB2 genome k03455.1, 9719 nucleotides, which produce 9 genes/master proteins designated as gag PRO_0000261216, gag-pol PRO_0000223620, vif PRO_0000042759, vpr PRO_0000085451, tat PRO_0000085364, rev PRO_0000085279, vpu PRO_0000085433, env PRO_0000239240, and nef PRO_0000038365.

Demonstrated in Table 1, is evidence of a frameshift involving each gene. There are 8 segments where the codon code overlaps, which facilitate the HIV genome to be 825 nucleotides shorter than if such a data compression technique were not utilized. Env, tat-2, rev-2 all share a 45 nucleotide sequence. This data compression technique represents a higher order complexity in the construct of the genome, beyond a simple linear read, facilitating the HIV genome to fit inside HIV virion.

HIV genome utilizes the same genetic cellular machinery as human genes; recognizing the microcosm of data compression present in HIV genome broadens the analysis regarding how the spliceosome and ribosome complexes interact with human DNA, which will inspire innovative human genetic therapies.

Table 1: Data compression frameshift present in 8 genes

Gene 1 Protein 1	Start-Stop Positions	Point of Frameshift	Nucleotide Code at Point of Frameshift	Codon Code at Point of Frameshift
Gene 2 Protein 2		# Nucleotides Which Overlap		
gag	790-2292	2091	…ttt tta ggg aag	……NFLGK…
261216			…….agg	……RED…
gag-pol	2091-5096	202	gaa gat	^
223620			I	2091
			2091	
gag-pol	2091-5096	5041	…tat gga aaa	…DYGKQ…
223620			cag…	……MENR…
vif	5041-5619	56	…….atg gaa	^
042759			aac…	5041
			I	
			5041	
vif	5041-5619	5559	…aga aga tgg	…DRWNK…
042759			aac…	…….
vpr	5559-5850	61	…….atg	MEQA…
085451			gaa..	^
			I	5559
			5559	
vpr	5559-5850	5831	…aga aat gga gcc	….ARNGA…
085451			…….atg	……
tat-1	5831-6049	20	gag…	MEPV…
085364			I	^
			5831	5831
tat-1	5831-6049	5970	…tcc tat ggc	…GISYGR…
085364			agg…	…….MAG…
rev-1	5970-6045	80	……atg gca	^
085279			gga	5970
			I	
			5970	
vpu	6062-6310	6225	…ggc aat gag agt	….SGNES…
085433			……..atg aga	……
env	6225-8795	86	gtg..	MRV…
239240			I	^
*33-856			6225	6225
env	6225-8795	8379	..cag acc cac..	…SFQTH…
239240			……((a)ac)	…..(N)PP*…
rev-2	8379-8653	275	cca*	^
085279			I	8379
			8379	*combined 'aac'
			*combine 'a' from codon for 'N'	

			rev-1, 'ac' from rev-2	
env	6225-	8380	…cag acc cac ctc..…..FQTHL…	
239240	8795	------	……ccc	……PTS…
---------	----------45		acc tcc	------^
tat-2	8380-		--------I	------8380
085364	8424		--------8380	
env	6225-	8797	…cta taa…	ILL(stop)…
239240	8795	------	……..……	
---------	---------- 0		atg ggt ggc…	MGG
nef	8797-		----------I	----------^
038365	9417		----------8797	----------8797

L.B. Scheiber: None. **L.B. Scheiber:** None.

P16.22B

Boosting genotype-phenotype and translational research on rare diseases by establishing Findable, Accessible, Interoperable and Reusable (FAIR) data resources through data linking technologies

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Genotype-phenotype and translational research on rare diseases (RDs) depends on combining data from biobanks, knowledge-bases, patient registries, and 'omics' sources. RD data are sparse, often sensitive, and distributed across countries and institutes. Infrastructure that boosts RD research allows researchers to avoid spending too much time reconciling data ambiguities for every analysis, but with 5000–7000 RDs, the scale and ethical and legal bottlenecks make a central data warehouse impossible to maintain. We present a 'rare disease data linkage service plan' that applies the principles of Findable, Accessible, Interoperable, and Reusable data for humans and computers (FAIR) at the source to create a decentralised infrastructure. It follows 'Bring Your Own Data' workshops and an ELIXIR-supported pilot that demonstrated the concept for answering cross-resource questions. RD stakeholders, infrastructure experts, programs such as ELIXIR and BBMRI, and patient organisations support the plan. The plan brings together an international collaboration of FAIR data engineers and RD software engineers. They support RD data managers in incorporating FAIR data elements and services that reduce the effort to combine data for computational analysis (using globally unique identifiers, ontologies, Linked Data, and an application programming interface to provide controlled access to data). Specific ontology choices include the Human Phenotype Ontology and the Orphanet Rare Disease Ontology. Software and ontological models that are used for each resource are shared with the community to be reused for similar cases, thus stimulating convergence within the RD domain. We acknowledge the generous support from RD-stakeholders, RD-Connect, ELIXIR, ELIXIR-EXCELERATE, BBMRI-NL, ODEX4All and FAIR-dICT.

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P16.23C

Trusted Friend Computing: data mining federated OMICS knowledge source

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Introduction: Interpretation of OMICs data requires comparison and thus access to a large number of previously analysed samples, but sharing of human OMICs data has many complex legal and ethical constraints. Because of this, many laboratories restrict their comparison to internal OMICs knowledge bases. To solve this problem, we propose a new approach called TFC (Trusted Friend Computing), which lets laboratories transparently and securely share their internal knowledge bases, as well as other resources, especially computing resources. Methods: We are extending POP-Java, a Java language extension for distributed computing, with the features to implement the sharing of both data and computing power through a network of friends. A network of friends is created through a process similar to social networks, allowing for detailed

control with whom to share resources. The open-source implementation of TFC is used to implement those features inside GensearchNGS, a graphical NGS data analysis pipeline developed by Phenosystems SA. **Results:** We implemented a prototype that demonstrates the functionality of TFC. We also show the feasibility to integrate this functionality into an existing application like GensearchNGS. We also present the larger concept of Trusted Friend Computing and how it can be used to securely share and datamine OMICs knowledge across laboratories. **Conclusion:** We propose a new method to share securely OMICs knowledge sources as well as computing resource. We demonstrate this through a prototype of the technology and show how the integration into an existing software, like GensearchNGS, is possible. Grants: CTI no. 18781.1 PFES-ES

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P16.24D

Towards automated sharing of genetic variants between genome diagnostics laboratories and beyond: an initiative of the Dutch diagnostic data sharing consortium

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The use of genomic information in both research and clinic has expanded enormously in the last decade. Exome analyses have become part of routine diagnostics. Yet, the interpretation of the obtained data is a huge challenge, because information on individual DNA variants is often absent in public databases. In order to improve patient care the Dutch genome diagnostic laboratories decided to share their data, most importantly the individual variant pathogenicity classifications and observed variant frequencies. When a variant has been previously carefully classified by one or more expert centers, there is less need to (re-) evaluate this variant when encountered for the first time by another center. Towards this goal we created a central national database with connections to each of the laboratories for automated bi-directional sharing. Data are stored separately for variant classifications and variant frequencies, using open source MOLGENIS and VARDA systems so that this procedure can be reused by other countries. Through this national node data will be shared with international databases. Currently the database contains 46.500 classified DNA variants in 4.500 genes, of which 40.000 variants have been classified by only one laboratory and 6.500 were classified by multiple laboratories. For 650 variants (10%) classifications differ, but only 2 variants have an opposite classification. First data has already been shared with the LOVD3 and CafeVariome; variant sharing is the most effective way forward to annotate WES and WGS data.

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P16.25A

LOVD: variant annotation software and a public database

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As genotyping becomes cheap and widespread in both research and diagnostics, new genetic variants are discovered at an ever increasing rate. Recording, storing and sharing this information, including any consequences of the variants (their classification), is critical to allow efficient evaluation of the sequencing data. We have developed the LOVD software to facilitate these processes.

LOVD is a freely available open source database system for recording, storing, curating and annotating genetic variants, running on any platform. LOVD processes information on individuals, their phenotype, screenings performed and variants detected. LOVD offers great flexibility to store information via custom defined columns, aids automatic validation and translation of HGVS variant descriptions and directly links to external resources like PubMed, OMIM, UCSC, Ensembl, etc. Recent improvements include an API to submit new data automatically and the possibility to share access to data submissions with other users.

Besides providing software we host public LOVD databases where >2000 users (submitters) share data and >250 gene experts curate the incoming information. Data is stored per allele at the DNA, RNA and protein level and variant classifications are shown separately based on the opinion of the submitter and the expert curator. Locally installed databases have an option to share information with a central LOVD server where the data are shared via a web service. Currently 71 databases are sharing nearly 1 million variants. LOVD and its services are maintained by the Leiden University Medical Center and is available through http://www.lovd.nl.

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P16.26B

Locus Reference Genomic (LRG): reference resource for the accurate reporting of clinically relevant variants

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Accurate and unambiguous annotation is essential for the correct interpretation of clinically relevant sequence variants. Central to this is the reference sequence against which a variant is reported. Ongoing changes to the reference assembly, the existence of numerous versioned reference sequences and the use of legacy reporting systems present challenges in the clinic. The Locus Reference Genomic (LRG), a manually curated resource designed specifically for the reporting of clinically relevant variants, addresses these challenges. Each LRG record provides a stable and non-versioned genomic DNA sequence for a human locus, establishing a coordinate system that is independent from upgrades to the reference assembly. The record also contains a minimal set of transcripts to be used as reference standards and defines the relationship between these and existing RefSeq and GENCODE models. Only transcripts required for reporting of disease-causing variants, and for which there is currently good biological understanding, are included. LRGs are based on RefSeqGenes, a sibling project at the NCBI, and are created in consultation with locus-specific experts. Over 1100 LRGs have been requested, of which almost 700 are public. LRGs have been recommended by HGVS and other key organisations. Their widespread use will ensure consistent variant reporting over time. LRGs are created jointly by the NCBI and EMBL-EBI (http://www.ebi.ac.uk), are available on the LRG website (http://www.lrg-sequence.org/) and can be visualised in all genome browsers. This work is supported by the Wellcome Trust [WT200990/Z/16/Z], the European Molecular Biology Laboratory, and the Intramural Research Program of the NIH, National Library of Medicine.

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P16.27C

Tissue-specific expression and co-expression analyses of gene families in human brain regions to prioritize genes potentially implicated into brain diseases

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A gene family is a set of genes formed by duplication of a common ancestor originated from Whole Genome or Small-Scale (SSD) duplications. The retention of duplicates in the genome may favor gain of functions and tissue-specific expression contributing to organism evolution. Moreover, duplicates are enriched in brain disease genes compared to singletons. Our objective is to better understand contributions of gene families to tissue differentiation and brain diseases.

We carried out expression and tissue differentiation analyses of 13 brain sub-regions using RNA-Seq data from GTEx consortium. Tissue-specificity was assessed by a score computed per gene and co-expression of gene families across brain regions was explored by weighted gene co-expression network analysis.

We determined that duplicates better differentiate brain sub-regions than singletons based on their expression profiles. Therefore, we developed a comprehensive resource on gene family expression in brain regions by integrating evolutionary, co-expression network, tissue-specificity and disease information. First, from our integrated data, we found that brain disease genes are enriched in tissue-specific genes and in SSD duplicates. Then, we pointed out that co-expressed families are enriched in SSDs. This result is consistent with previous studies, indeed it has been shown that tissue-specific SSD families tend to be expressed in the same tissue.

We generated a unique resource on gene families to facilitate associations between evolutionary, tissue-specificity, co-expression and brain disease characteristics. Interrogation of these integrated data may highlight new genes potentially implicated in brain pathologies.

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P16.28D

GARFIELD: Genomic vARiants FIltering by dEep Learning moDels

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Exome sequencing approach is extensively used in research and diagnostic laboratories to discover pathological variants and study genetic architecture of human diseases. Even if present platforms produce high quality sequencing data, false positives variants remain an issue, particularly for indel detection, and can confound subsequent analysis and result interpretation. Advanced filtering methods have been developed for large datasets, while strategies for single samples or trio analysis are usually limited to hard filtering of variants based on quality parameters. Here, we propose a new tool named GARFIELD (Genomic vARiants FIltering by dEep Learning moDels), which uses deep learning algorithm to predict false and true variants assigning a prediction value from 0 to 1 (P true). GARFIELD consists of 4 distinct models specifically trained for INDELs or SNPs variants and applies to both Illumina and ION data. In our tests using the gold-standard exome variants dataset NA12878 (NIST v.3.3.2), GARFIELD outperformed previously proposed hard filters. AUC values: (i) ION SNPs 0.96; (ii) ION INDELs 0.98; (iii) Illumina SNPs 0.71; (iv) Illumina INDELs 0.90. The method is robust also at low coverages down to 30X and performs well also for the recently introduced Illumina two-colour data. GARFIELD is implemented as Perl and R scripts and it can be easily applied to regular VCF files to add P true annotation for variants. Different thresholds on P-true value can be applied based on the desired level of sensitivity and specificity.

E. Giacopuzzi: None. **V. Ravasio:** None.

P16.29A

eDiVA: Exome sequencing pipeline to identify clinically relevant mutations in disease studies

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Introduction: Next generation sequencing boosted causal variant identification in people affected by Mendelian diseases, helping to link genetic variation to phenotype. Multiple tools exist to call, annotate, and prioritize genetic variants, but with currently 25–30% success rate, identifying the causal mutations is still an issue.

Materials: We developed eDiVA to identify coding mutations causing rare diseases in small families using exome-seq data, focusing on optimizing functional annotation and prioritization.

eDiVA calls variants using best practices to reduce false positives. Thereafter, variants are annotated with functional, OMICs and clinical information from many sources like CADD, SFIT, PhyloP, dbSNP, 1000genomes, EVS, ExAC, Clinvar, or OMIM to provide contextual information.

eDiVA integrates annotation into a machine-learning based variant pathogenicity classifier (eDiVA-score). eDiVA-score is integrated with domain-knowledge filtering to correctly prioritize variants following specific disease models, (autosomal dominant, recessive, de novo, X-linked, and compound heterozygous) in small families and parent-child trios.

Results: We compared eDiVA-score with M-Cap, CADD, Eigen-score and Revel in thousands of semi-synthetic cases, as well as the full prioritization pipeline against PhenoDB and Phen-Gen. eDiVA consistently outperformed existing methods in terms of ROC, precision and recall of pathogenic variants.

We also validated eDiVA studying 35 families affected by rare diseases like ataxia, myasthenia, and immunodeficiency. On average eDiVA reported less than 30 candidate variants per family, which included the (subsequently validated) causal variant in about 50% of the cases.

Conclusion: eDiVA, available at www.ediva.crg.eu, provides a clinically tested prioritization method to identify causal variants for genetic diseases with remarkable success rate.

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P16.30B

Automating clinical interpretation of NGS data in oncopathology using by an expert system xGenCloud

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Introduction: The interpretation of the results of NGS sequencing associated with the problems of processing large quantities of genetic and biomedical information, especially in cases of genetically heterogeneity pathologies presented in oncology. This requires the development and use of specialized computer software.**Materials and methods:** Publicly available online sources of Internet used for filling the knowledge base «xGenCloud» expert system. Information from Database OMIM used to form the catalog of monogenic diseases. Information about the pathogenicity of mutations was taken from ClinVar database. TruSight Cancer by Illumina used to generate test cases. Sequencing was carried out on MiniSeq system. Genome assembly and alignment was carried out using Illumina software, with GRCh37 as the standard reference assembly sequence used. **Results:** Expert biomedical xGenCloud computer program was created. Report on the diagnostics results includes the following sections: an introduction to the methodology of interpretation, the description of the genetic panel, the ranking criteria based on pathogenicity, the list of the identified pathogenic mutations and associated monogenic and multifactorial diseases, the total risk assessment, recommendations for additional laboratory tests and instrumental investigations, the literature, list of detected variants. Automation of the process of data analysis and report generation can reduce expended time from 3–5 hours, required previously, up to 5–10 minutes, depending on the number of pathogenic variants detected.**Conclusion:** The using of specialized software service for interpreting of the NGS data can significantly optimize work of the clinician and thus enhance the effectiveness of the genetic counselling in the oncopathology.

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P16.31C

eQTLs affecting annotated and un-annotated alternative splicing events in human basal ganglia are enriched for risk variants for adult neurological disorders

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Alternative splicing (AS) is a form of post-transcriptional RNA processing whereby a single gene encodes for multiple distinct transcripts. With the widespread use of RNA sequencing, it has become clear that alternative splicing is more prevalent than previously recognised particularly in human brain. Given that AS can modify the properties of encoded proteins, it has the potential to contribute to adult neurological disease. As part of the UKBEC project, we performed eQTL analyses on whole transcriptome RNA sequencing (RNA-seq) data originating from 134 neuropathologically normal post-mortem human brains and including 180 samples dissected from the putamen and substantia nigra. We performed conventional gene-level quantification together with quantification of AS events. This was done using: i) the software Altrans, which detects both annotated and novel AS events present within gene boundaries, ii) inferring AS using exon-level quantifications tested for beta heterogeneity across a given gene, and iii) an in-house pipeline to identify and quantify novel exons and the associated AS events outside gene boundaries (additional 5′ and 3′ novel exons). The results of cis-eQTL mapping performed using MatrixEQTL were integrated with information on risk loci identified through genome-wide association studies (GWAS). A significant enrichment of risk SNPs for adult neurological diseases was seen amongst eQTLs affecting AS events. This was particularly evident amongst diseases, characterised by dysfunction of the basal ganglia, such as Parkinson's disease. Thus, we demonstrate that the identification of eQTLs regulating AS events, both annotated and novel, provides insights that would be hidden by conventional analyses.

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P16.32D

Integrated high-throughput biomolecular analyses of *FRA10AC1* altered expression in a human cell model

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Introduction: The *FRA10A* fragile site causative gene, *FRA10AC1*, encodes a well-conserved nuclear protein with yet, uncharacterized function (Sarafidou et al., *Genomics*, 84:69,2004). The FRA10AC1 position in the human protein interactome (Klapa et al., *BMC Syst. Biol*. 7:96,2013; www.pickle.gr) suggests its involvement in pre-mRNA processes (Galliopoulou et al, in prep.). To elucidate the biological role of FRA10AC1, we developed *HeLa* cells with impaired *FRA10AC1* expression and performed integrated transcriptomic, proteomic and metabolomic analyses.

Materials and Methods: The respective profiles were acquired using GeneChip® U133 Plus2.0 arrays, LC-MS with iTRAQ-labeling and GC-MS, capturing information for ~24000 transcripts, ~6400 proteins, and ~130 metabolite derivatives, respectively. After normalization and filtering, 5153 proteins and 69 metabolites were further considered.

Results: Multivariate statistical analysis indicated that *FRA10AC1* downregulation strongly affected the abundance of ~2000 transcripts with unique UniProt_IDs, 560 proteins and 32 metabolites. Its overexpression had a strong impact on ~1540 transcripts with unique UniProt_IDs, 215 proteins and 16 metabolites. Ontological analysis of the differentially expressed transcripts and proteins indicated that most are implicated in mRNA splicing and metabolism, alternative splicing, transcription and translation, strongly supporting a role for FRA10AC1 in pre-mRNA processing.

Discussion: The educated integration of all three omic profiles indicated that the aberrant *FRA10AC1* expression affects the cytoskeleton and cell membrane organization, the endoplasmic reticulum and Golgi apparatus function, the cell signaling through protein phosphorylation and response to glucocorticoids, the glucose and cholesterol homeostasis, the oxidative phosphorylation and the anti-inflammatory response activity. Interestingly, the observed alterations can be associated with certain neurological disorders.

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P16.33A

Gene prioritization tools facilitate identification of novel variants in neuromuscular disorders

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Neuromuscular disorders (NMDs) are a phenotypically and genetically heterogeneous group of diseases whose accurate diagnosis continues to be a challenge for clinicians. Next Generation Sequencing (NGS) technology is gaining wide acceptance in routine diagnostics with targeted sequencing of the exome being increasingly adopted for clinical diagnosis of NMD patients. However, a current challenge in variant analysis is the efficient ranking of potential disease associated variants based on genotype-phenotype correlations. In this study, we reviewed 42 web-based gene prioritization tools and developed an in-house variant analysis pipeline incorporating three platforms - VarElect, Phenolyzer and Manteia, for candidate gene ranking. Our variant analysis pipeline was used to resolve four undiagnosed cases of NMD. Genomic DNA isolated from peripheral blood of patients was sequenced on Agilent SureSelect Targeted capture platform for a custom panel of 110 genes which are known to be associated with NMD. We identified and validated four single nucleotide variants likely to be causative for given patient phenotypes, viz., a novel truncating *DMD* mutation (p.Leu268X); a novel protein coding *BICD2* variant (p.Arg141Cys); and lastly, compound heterozygous mutations in *TRAPPC11* gene comprising a rare nonsense (p.Arg48X) and a known pathogenic mutation (p.Gly980Arg). Our study highlights the significance of variant analysis pipeline based on gene prioritization tools for more efficient and faster clinical dignosis of rare genetic disorders like NMDs. In addition, this pipeline can be applied to data from any sequencing platform besides that described in this study.

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P16.34B

Sharing dog exome/genome variant and phenotype data in Varda and LOVD3

The BPSDA Dog Project team, W. Suring, **P. Taschner**; Generade Center of Expertise Genomics, Leiden, Netherlands.Finding sufficient evidence to prove causality is the main hurdle in current whole exome (WES) and whole genome sequencing (WGS) studies aimed at cracking rare disease. Roughly, studies give a yield of 1/3 proven causality, 1/3 likely causality and 1/3 unresolved cases. Other resources providing additional positive or negative evidence

for candidate variants might improve overall yield. Important obstacles: other resources often not easy to find and access; lack of standards and metadata may interfere with reuse of data. We hypothesize that efficient sharing of genetic variants and phenotype data from other species may help overcoming these problems

Reference protein sequences from other organisms are already used to predict amino acid conservation scores [1, 2]. These might be improved if protein variants from individual breeds could be included in the calculations. Variants causing disease in animals are currently stored in Online Mendelian Inheritance in Animals (OMIA, http://omia.angis.org.au/), which partially follows standards resembling HGNC and HGVS nomenclature. We demonstrate that variants from VCF files can be stored and annotated with their frequency in the variant frequency database Varda (Dog Varda, http://varda.generade.nl). Information about functional effects of variants and the resulting phenotypes in individual animals can be stored in the LOVD3 gene variant database platform [3] (Dog LOVD, http://databases.generade.nl/dog/). We hope that this example will be followed by research and diagnostic centers world-wide for other species.

[1] PolyPhen-2, PMID:20354512, http://genetics.bwh.harvard.edu/pph2/

[2] SIFT, PMID:11337480, http://sift.bii.a-star.edu.sg/

[3] LOVD, Leiden Open-source Variation Database, PMID: 21520333, http://www.LOVD.nl

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P16.35C

Simplifying access to human genomic data: community platform Repositive.io

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Introduction: Common practice suggests that human genomic data should be deposited in public repositories for further reuse. Finding and accessing deposited genomic datasets, however, is often cumbersome, with data and metadata being scattered throughout the Internet, annotated inconsistently and often machine unreadable. This makes it difficult for researchers to find existing genomic datasets of interest.

Materials and Methods: Genomic data sources were identified from our interactions with the genomics community, by monitoring announcements and by targeted searching of the Internet. State-of-the-art interfaces have been deployed on top of our database to facilitate the discovery of existing human genomic datasets through a variety of filters and querying options such as assay type or keyword.

Results: More than 300 data sources are identified, more than 1,1 million datasets from 38 sources are currently indexed. We created the Repositive platform (https://repositive.io/?ESHG17) as a free online portal and community of users that facilitate finding, accessing, and sharing of published genomic data. Indexed datasets cover population studies, microbiomes, methylomes and other types of NGS data. Many of these datasets are further classified into curated collections.

Conclusions: Repositive.io is a free community platform that allows users to find, annotate, curate and share datasets, and form data collaborations. It facilitates data access and re-use in the genomics community and is a "one-stop shop" to discover and explore the most relevant human genomic datasets published to date.

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P16.36D

Increasing the utility of the NHGRI-EBI genome-wide association study (GWAS) Catalog for users

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The GWAS Catalog (www.ebi.ac.uk/gwas) is a manually curated summary resource of human genotype-phenotype associations from GWAS publications. The Catalog contains a vast amount of data, encompassing over 35,000 SNP-trait associations from 2,700 publications as of February 2017. This data is used by a growing community of biologists and bioinformaticians to identify causal variants, understand disease mechanisms and establish targets for treatment. We have made several recent improvements, with the aim of improving the utility of GWAS Catalog data

in these analyses. A prototype GWAS Catalog variant-specific page is now available (http://wwwdev.ebi.ac.uk/gwas/beta/variants) that integrates additional variant information from Ensembl, along with links to co-located genes and regulatory elements, population genetics and phenotype information. It includes an interactive visualisation, allowing users to identify and explore variants in linkage disequilibrium with the Catalog variant. Updates have also been made to support hosting of full p-value data sets for GWAS Catalog studies, vastly increasing the potential of downstream analyses. Published summary statistics are now available for download via the search interface and will be added for additional studies when curated. We encourage authors of GWAS Catalog publications to contact us if summary statistics are available so we can add these. We welcome feedback from the user community on these improvements and any other changes they would like to see. Funding: NHGRI, NHLBI, the NIH Common Fund (U41-HG006104, U41-HG007823) and the European Molecular Biology Laboratory.

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P16.37A

Amplification-free targeted SMRT sequencing using CRISPR/Cas9 for studying repeat expansions in Huntington's disease

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Targeted enrichment of specific loci is a widely used approach in next generation sequencing. Most available methods for targeted enrichment require amplification during library preparation, which can be challenging for example when targeting low complexity regions or long repeat expansions. Here, we present a novel amplification-free method for targeted enrichment utilizing the CRISPR/Cas9 system for long-read Single Molecule Real-Time (SMRT) sequencing. Guide RNA (gRNA) was designed in proximity to the region of interest and was used to target and digest whole genome libraries. Capture adapters were subsequently ligated to the digestion site and magnetic beads used to enrich for libraries containing the target sequence. We used this approach to target the Huntingtin (HTT) gene, the causative gene for Huntington's disease by a CAG repeat expansion. Twelve individuals with Huntington's disease were sequenced and analyzed. In all cases the CAG repeat count from our data agreed with previous results from fragment analysis. The alleles for all samples were resolved. Furthermore, novel trinucleotide repeats in the flanking regions of the CAG repeats were found. With this amplification-free technique and the long reads of SMRT sequencing, we demonstrate that we accurately can sequence through repeat expansions that are difficult or impossible to investigate using PCR based methods. We are currently optimizing this method to allow for multiplexing of several samples/targets in the same run. Also, since this method captures native DNA it is also possible to detect epigenetic modifications in the data.

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P16.39C

Machine learning-based gene prioritization identifies novel candidate risk genes for inflammatory bowel disease

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Background: The inflammatory bowel diseases (IBD) are chronic inflammatory disorders, associated with genetic, immunologic and environmental factors. While hundreds of genes are implicated in IBD etiology, it is likely that additional genes play a role in the disease process. We developed a machine learning-based gene prioritization method to identify novel IBD risk genes. **Methods**: Known IBD genes were collected from genome wide association studies and annotated with expression and pathway information. Using these genes, a model was trained to identify IBD risk genes. A comprehensive list of 16,390 genes was then scored and classified. **Results:** Immune and inflammatory responses, as well as pathways such as cell adhesion, cytokine-cytokine receptor interaction, and sulfur metabolism were identified to be related to IBD. Scores

predicted for IBD genes were significantly higher than for non-IBD genes ($P<10^{-20}$). There was a significant association between the score and having an IBD publication ($P<10^{-20}$). Overall 347 genes had a high prediction score (>0.8). A literature review of the genes, excluding those used to train the model, identified 67 genes without any publication concerning IBD. Those genes represent novel candidate IBD risk genes which can be targeted in future studies. **Conclusions:** Our method successfully differentiated IBD risk genes from non-IBD genes by utilizing information from expression data and a multitude of gene annotations. Crucial features were defined and we were able to detect novel potential risk genes for IBD. These findings may help detect new IBD risk genes and improve the understanding of IBD pathogenesis.

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P16.40D

DOMINO: a bioinformatic tool for the identification of dominant disease genes

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Introduction: In a typical exome or genome sequencing analysis, ~400 heterozygous, rare and nonsynonymous or splicing DNA variants are found per individual. In patients with dominant conditions, these variants include a single pathogenic mutation. To help ascertaining the causative mutation among these many variants, we developed DOMINO, a bioinformatic tool able to identify genes susceptible to be associated with dominant conditions. No such tool seems to be currently available.Methods: DOMINO utilizes linear discriminant analysis to compute the probability of a gene to harbor mutations that are pathogenic at the heterozygous state. It is based on a broad array of features including genomic, expression, interaction, structural, and functional data. We instructed this analytical framework with a set of >500 genes extracted from OMIM and manually curated for well-established inheritance patterns (the "training set"). We used 10-fold cross-validation to determine the essential features.**Results:** In the training set, a small number of features was sufficient to provide an AUC higher than 0.85. We validated the model on different sets of genes associated with Mendelian conditions, including skeletal nosology, retinal degeneration, and genes published in AJHG in 2016. In these validation sets, AUCs were even higher than in the training set.**Conclusion:** DOMINO displays high sensitivity and specificity for genes associated with dominant disorders. It may fill the need for a method to prioritize the numerous heterozygous variants identified in highly parallel sequencing. Its precision may increase as novel annotations and features become available.PhD Fellowship of FBM, UNIL; Grant #156260, SNSF

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P16.42B

Primary and metastatic melanoma reveal specific lncRNAs expression signatures

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Introduction: Melanoma is the deadliest form of skin cancer with known aggressiveness. Cutaneous melanoma accounts for only 2% of skin cancers, but melanoma death rate is approximately 80%. Previous studies demonstrated an association of many noncoding RNAs with melanoma development. Nevertheless, the long noncoding RNAs (lncRNAs) and their role on melanomagenesis just have emerged and remained poorly understood. The recent identification of melanoma-associated lncRNAs BANCR, SPRY4-IT1 and others opened a research field with many challenges and questions to elucidate. Until now, others studies has not entirely focused on lncRNAs involvement in every step of melanoma progression. Materials and Methods: We performed RNA-Seq in melanocyte, primary melanoma, and metastatic melanoma cell lines to detect

lncRNAs associated with melanoma progression. To identify lncRNA differentially expressed (adjusted P-value < 0.01 and -1>fold change>1) we used a public dataset of RNA-Seq from melanocyte and melanoma. **Results:** melanocyte, primary and metastatic melanoma showed lncRNA specific expression. Comparison between melanocyte versus all melanoma samples revealed 110 upregulated and 52 downregulated lncRNAs. Principal Component Analysis (PCA) presented primary melanoma with some discrepancy from the melanocyte and metastatic melanoma. Some differentially expressed lncRNAs were predicted to interact with proteins, suggesting their participation in a range of biological processes associated with cancer progression. **Conclusions:** We identified potential lncRNAs candidates with involvement in melanoma progression, as the recently described lncRNA SAMMSON, that have a fundamental role in melanoma survival, and which we also identified in our study. Grants and fellowships: FAPESP, CNPq, CAPES, FUNDERP and FAEPA.

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P16.43C

Salivary microbiome changes associated with asthma in African American children

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Introduction: Several studies have shown that the airways of asthma patients contain higher diversity of bacteria and are enriched in certain pathogenic species. However, sampling the airways in children is challenging. Here aimed to identify differences in the salivary bacterial composition between children with and without asthma.

Methods: Saliva samples from 147 Latinos from the GALA II study (33 asthma cases and 114 controls) and 113 African Americans from SAGE II (56 asthma cases and 57 controls) were analysed. The V4 16S rRNA gene was sequenced in a MiSeq sequencer (Illumina) with 250 bp paired-end reads. Data processing was performed using a custom QIIME-based pipeline. Differences in bacterial abundance between cases and controls were assessed with Explicet, using the non-parametric Wilcoxon test and adjusting for multiple comparisons.

Results: A total of 5 Phylum and 19 genera were identified as core microbiome (>1% abundance), being Prevotella, Streptococcus and Veillonella the most abundant genus. In African Americans, differences were observed for the genera Veillonella (14.0% in cases vs 9.7% in controls, p=3.1×10–4) and Streptococcus (16.9% in cases vs 23.9% in controls, p=2×10-3). Conversely, no differences were found at the taxonomic levels assessed among cases and controls in Latinos.

Conclusions: We identified changes in the salivary microbiome composition associated with asthma susceptibility in African American children.

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P16.44D

The methylome of duodenal cell populations reveals epigenetic effects of the HLA region in celiac disease

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Introduction: The Human Leucocyte Antigen (HLA) *locus* and other DNA variants identified in GWA (Genome-Wide Association) studies explain around 50% of the heritability of celiac disease (CD). However, the pathogenesis of CD could be driven by other layers of genomic information independent from inherited sequence variation, like DNA methylation. It could also be that allele-specific DNA methylation explains a fraction of SNP associations.

Methods: To answer these questions, knowing that the DNA methylation landscape may vary across cell types, we analyzed the methylome of cellular fractions enriched for epithelial and immune populations from duodenal biopsies in CD patients and controls. We also obtained Immunochip SNP genotypes and cell-type specific transcriptomes from a subset of the samples.

Results: We find a cell type-specific methylation signature that includes the HLA region and is characterized by a loss of CpG island boundaries, often resulting in altered gene expression, and by increased variability in the epithelial fraction. The overlap between differentially methylated positions and those SNPs that are associated with the disease or correlated with methylation levels is minimal. There is a remarkable enrichment for immune-related genes (including several previously unrelated to CD) around differentially methylated regions.

Conclusions: These results support the idea of methylation as a genotype-independent, disease-related event and confirm its role in the HLA region (apart from the well-known DQ allele-specific effect). Additionally, differential methylation does not explain a significant fraction of the GWAS-SNP associations. Interestingly, the methylation profile of the celiac epithelial fraction resembles early stages of solid tumors.

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P16.45A

The Human-Mouse: Disease Connection: facilitating the quest for translational mouse models

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The mouse is a pivotal model system for investigating etiopathogenesis and therapeutic targets for human hereditary disease. The Human-Mouse: Disease Connection (HMDC, www.diseasemodel.org), developed by MGI (www.informatics.jax.org), is a translational tool providing integrated access to human-mouse genomic, phenotypic, and disease information. Researchers can dynamically explore phenotypes and disease correlations, identify candidate genes, and assess experimentally defined mouse genotypes modeling clinically relevant profiles. The latest HMDC release features a redesigned search form, and parallel integration of human phenotype terms and known disease-to-phenotype associations from the Human Phenotype Ontology (HPO) along with human disease terms from the Disease Ontology (DO). Users can initiate searches from a human or mouse viewpoint using gene names, symbols or IDs, human or mouse phenotype terms or IDs, disease (OMIM or DO) terms or IDs, or genomic locations. Multiple search criteria may be combined or used individually via Booleans (e.g., "Charcot-Marie-Tooth" **AND** PMP22); or users can select specific disease/phenotype terms of interest (e.g., "Charcot-Marie-Tooth Disease, Type 4B2") using the optional autocomplete feature. Initial results appear in a visual, color-coded grid juxtaposing phenotypes and diseases associated with human and mouse orthologs, and may be further refined by filters. Importantly, grid cells link to underlying MGI and HPO data, including phenotypic detail, disease information, available mouse model resources, and supporting references. Alternate views present gene- and disease-centric data in tabular format. We highlight HMDC content and enhanced flexibility to visually compare human-mouse phenotypes, prioritize candidate disease genes, and identify mouse models of translational value. Supported by NIH grant HG000330.

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P16.46B

DNA methylation differences in CD4 + and CD8 + T cells of multiple sclerosis patients and healthy controls

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory, demyelinating disorder of the central nervous system and one of the most common neurological conditions among young adults. The cause of MS is largely unknown, however the best-supported hypothesis is that MS onset is triggered by common environmental and genetic factors and interactions between these. Epigenetics has emerged as a critical factor of gene regulation that may link genetic makeup and environmental exposures in complex diseases

such as MS. Here we investigate the role of DNA methylation in immune cells relevant for MS.

Materials and Methods: CD4 + and CD8 + T cells were collected from a highly homogeneous group of 30 newly diagnosed, treatment-naive Norwegian female MS patients and 29 age-matched healthy controls. We measured DNA methylation at ~450,000 sites using a chip-based assay. Since DNA methylation often occurs on groups of CpG sites, we looked for differently methylated regions (DMR) using the *Bumphunter* R package.

Results: We identified five DMRs between MS patients and controls in our sample. Interestingly, some of these DMRs were identified in both CD4+ and CD8+ T cells, suggesting that these regions are more global DNA methylation patterns associated to MS.

Conclusions: DNA methylation may play a role in the risk of getting MS. We replicate the previously identified DNA hypermethylation in CD8+ T cells from MS patients compared to healthy controls. In addition, we identified five DMRs that may mark processes involved in MS risk.

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P16.47C

Using neural networks to predict effects of mutations on protein binding

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Introduction: Modern sequencing technologies has made sequencing affordable in the clinical setting at a scale previously unheard of. With the wealth of data comes the problem of understanding variations and assessing their effect on the phenotype. In particular, the potential pathogenic effect of intronic and silent sequence variations are challenging, since they can have strong effects by disturbing the splicing process and cause insufficient protein production. Moreover, it is often overlooked that also missense mutations can disrupt splicing. Mutations that affect splicing often do so by disrupting binding of regulatory splicing proteins (SRPs), such as SR and hnRNP proteins. SRPs bind short degenerate motifs within splicing enhancers (SE) or splicing silencers (SS), but their exact binding preferences *in vivo* are often insufficiently characterized and the effect of a mutation on SRP protein binding to an SE or SS does not always correlate with in *silico* predictions.

Results: To address this problem we have developed DeepCLIP, a neural network than can be trained on protein bound sequences, such as those from *in vivo* cross-linking studies and can predict binding of a protein to a given sequence. We show here that it can efficiently discriminate between bound and un-bound sequences and that the binding of protein to mutants correlates with the computed score and splicing pattern.

Conclusions: We suggest that DeepCLIP can be used to predict the effect of mutations on protein binding and in larger contexts be used to correlate genotype with phenotype.

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P16.48D

Methods for low and ultra-low variant detection using molecular tagging, targeted sequencing and error correction algorithms

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Introduction: Low frequency variant detection is crucial for clinical sequencing, particularly with the advancement of testing "liquid biopsy" samples with very low tumor fraction. Accurate calling of these rare mutations is further hindered by artifacts introduced during sample preparation, target enrichment, and sequencing. Here, we report error rates for canonical base substitutions and show how consensus building from molecular tags enables *in silico* correction of false positives, greatly improving accuracy for sub-1% variants.

Materials and Methods: Low frequency variants were modeled using mixtures of well-characterized normal cell line DNA, resulting in 1% and 0.5% alternative allele frequencies. Libraries were constructed using adaptors containing Unique Molecular Identifiers (UMIs) and captured with custom IDT xGen® Lockdown® probes targeting a 35kb region of highly polymorphic SNPs. Positive and negative variant calls were assessed for accuracy using high confidence regions annotated in the Genome in a Bottle database.

Results: The most prevalent artifactual mutations were G > A/C > T substitutions likely introduced via oxidative damage during sample preparation. Consensus building using a minimum family size of 20 improved positive predictive value (PPV) from 45% to 85%. PPV was further increased to 98% by increasing the minimum alternate-allele consensus read count for variant calls symptomatic of oxidation damage.

Conclusions: Building consensus sequences enables *in silico* error correction, dramatically increasing variant calling specificity. Due to the prevalence of artifacts arising from oxidative damage, mutation-specific thresholding is necessary to accurately detect variants present below 1%.

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P16.49A

Optimal workflow for next generation sequencing data processing using existing technology

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Introduction Next Generation Sequence (NGS) instruments are becoming the standard tool in genetics, however, this process has not been accompanied by the development of a "gold standard" pipeline for germline NGS data processing. Our goal in the present study is to determine the optimal workflow in the specific case of whole exome sequencing (WES) to maximize the cost-efficiency of the process.

Material and Methods For our analysis, we selected a cohort of 200 individuals sequenced for WES. All the samples considered were processed using sixteen different workflows composed of the different algorithms and software available. We selected up to ten quality parameters and indices to discern the best possible workflow.

Results Using bwa mem for the alignment compared to using bwa sampe improved the number of aligned reads by roughly a 10%. When performing and not performing BQSR and indel realignment, there were no significant differences in the number of variants called or the quality of those, but there was an increase of computing time (4:32 versus 2:24). Regarding the variant calling, there were no significant differences in the number of variants called or their quality between algorithms, while the depth of read was significantly higher in unified genotyper (73.63 versus 53.83). Also, the computational time was significantly lower for unified genotyper (2:24 versus 11:13).

Conclusions Our results indicate an optimal workflow for the processing of NGS WES germline data which can cut the time per sample down to a tenth while maintaining the quality of the results produced.

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P16.50B

Innovative technologies for high performance Next Generation Sequencing data processing delivering fastest turn arounds

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Next Generation Sequencing (NGS) is rapidly being adopted and implemented in clinical settings to improve patient diagnostics, prognosis and therapy. Simultaneously new platforms produce more sequencing data at a higher pace. Consequently, NGS data analysis is becoming the bottleneck. It takes more resources to process sequence data into clinically interpretable variants. Both time and costs are crucially important for clinical diagnostics. As a solution to this growing pain we present GENALICE MAP, a high performance NGS data analysis software solution. It maps sequence reads, calls variants, detects somatic mutations and discovers copy number variations (CNV), all at significantly reduced processing times. GENALICE MAP can do read mapping and variant calling of a human 30x whole genome sequencing data set in approximately 30 minutes. Daunting tasks, such as migration of large data sets to reference build 38 of the human genome can be performed in a few days. Because of its unexceeded processing speed, trio- and large cohort size analysis becomes easy and affordable. Its unique methods ensure high quality results. For example, *Consensus Based Call Enhancement* enables high accuracy and low Mendelian error rates in trio analysis, facilitating *de novo* variant discovery. Likewise, *profiles* give higher sensitivity for detecting pathogenic variants. We

conclude that GENALICE MAP is an accurate and high performance software solution to accelerate NGS data processing, consuming little resources. As such, it is an excellent solution to process an ever-increasing NGS data stream supporting relevant diagnostic improvements such as faster clinical outcome enabling more cost-effective decisions.

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P16.51C

Methodical comparison of variant calling pipelines for next generation sequencing data

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Several variant calling pipelines have been developed to identify the genome variants from next generation sequencing (NGS) data. Still, the concordance rate between different pipelines is poor and the reliability of identified variants is often questionable. We aim to address this problem by systematically evaluating the performance of preprocessing to variant caller algorithms, using combination of different tools. In the pre-processing steps – mark duplicates (tools: Picard and samblaster), indel realignment (tools: Genome Analysis Tool Kit (GATK) indel realigner and ABRA), adapter trimming by SeqPurge and quality score recalibration by GATK base quality score recalibration along with three different freely available variant callers - GATK HaplotypeCaller, Freebayes, Platypus will be compared. Furthermore, we compare the performance of previously mentioned variant callers with a commercial platform known as Dynamic Read Analysis for Genomics (DRAGEN) from Edico genome. As a gold standard variant list we use a set of high-confident variant calls for one individual (NA12878) from the Genome in a Bottle (GIAB) consortium to bench mark different variant calling pipelines. After bench marking the pipelines, we will validate the efficacy of our pipeline by using publically available exome data from the Parkinson's Progression Markers Initiative (PPMI) study, which is an effort to identify the biomarkers of PD progression, and our in-house data comprising of whole-exome and whole-genome sequencing data. The results of this study will not only help to streamline the variant calling pipeline strategies, but also provide appropriate ground rules for dependable variant detection for future NGS based studies.

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P16.52D

Need for speed in high-throughput sequencing data analysis

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Introduction: Rapidly evolving high-throughput sequencing (HTS) technologies produce huge amounts of data, requiring high-throughput data analyses with appropriate sensitivity/recall. However, widely-used alignment and variant calling tools are either too slow (e.g., BWA +GATK) or have insufficient sensitivity/recall (e.g., Isaac). In this study, we compared speed, disk footprint, and sensitivity/recall of the current gold standards BWA+GATK and Isaac with GENALICE MAP (genalice.com), a recently introduced ultra-fast HTS data analysis software solution. Materials and Methods: We performed alignment and variant calling on short-read (2×150bp) 60x PCR-free WGS data of NA12878 using BWA+GATK, Isaac, and GENALICE MAP. While measuring analysis time and disk footprint, we assessed the sensitivity/recall of the three pipelines according to GIAB v3.3 using RTG Tools. **Results:** Our data demonstrate more than 95 and 15 times higher speed as well as more than 45 and 20 times decreased disk footprint of GENALICE MAP compared to BWA+GATK and Isaac, respectively. The sensitivity/recall of GENALICE MAP was considerably higher than that of Isaac and close to BWA+GATK. **Conclusion:** The GENALICE MAP software offers hitherto unprecedented high speed and low disk footprint in alignment and variant calling with reasonable sensitivity/recall. Thus, it is a promising new tool in HTS data analysis either as a stand-alone/primary or a secondary solution, enabling realignments and reanalyses in ever-growing patient cohorts (e.g., due to updates of the reference genome such as hg19 to hg38).

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P16.53A

Genomics England PanelApp: A Key Open Source Genetic Resource for the Rare Disease Community

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The Genomics England 100,000 Genomes Project is sequencing, analysing and interpreting genomes from NHS rare disease patients, their relatives, and cancer patients in order to provide diagnoses and consequently deliver better clinical care for NHS patients. Curation and community engagement are fundamental to the interpretation of genomes within the project. Gene panels are used in the interpretation of sequencing data and aid identification of candidate pathogenic variants for rare disease diagnoses. Historically, gene panels for the same disease differed across diagnostic labs, thus, Genomics England curators aim to establish consensus lists. Within the open-source PanelApp database (https://panelapp.extge.co.uk/crowdsourcing/PanelApp), virtual gene panels are constructed, expert reviews are crowd-sourced, and panels are revised to establish diagnostic-grade lists for each disease category. A range of biological databases are used in this process and knowledge is sourced from experts in clinical and scientific communities. Using a traffic-light system genes are classified: "green" (diagnostic level) these genes are used for genome interpretation and "red" candidates for further investigation if additional data becomes available, thereby, providing a useful resource for both clinical and research communities. PanelApp has >500 registered reviewers from >20 countries. To date, it contains 3696 genes and 127 revised gene panels (publicly available to view and download via the user interface, or query via web services) and currently ≈160 rare disease categories can be analysed. PanelApp is a publicly available, dynamic resource for the rare disease community and demonstrates how a coordinated curation effort combined with community involvement can be key to genome interpretation.

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P16.54B

Population variation improves genetic variant interpretation within protein domains

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Large datasets of population variation resulting from exome sequencing studies have made it feasible to identify differences in variation patterns among genes. Many protein domains have homologues in multiple human genes, and we hypothesize that genetic variation in each protein domain can be extrapolated to its homologues, which subsequently can improve variant interpretation.

We built a novel framework that enables mapping of the longest translations for all 20,345 human protein-coding genes to protein sequences in Swiss-Prot. This resulted in 16,684 genes with 100% sequence identity and similarity to a Swiss-Prot canonical or isoform sequence. Of these genes 90% has at least one Pfam domain of which for 2,699 unique Pfam domains there are multiple homologues among these human genes. We used the high quality (PASS) filtered variants from the Exome Aggregation Consortium (ExAC), to compute genetic intolerance scores for all Pfam domains based on Dn/Ds.

First, we investigated whether tolerance to normal genetic variation is preserved for each unique domain with respect to its homologues and this is indeed the case (p-value < e-30). Next, we tested if unique ExAC variants in one domain predicts the same variation in its homologues and this is significant for 83% of the homologous Pfam domains (Bonferroni p-value < 0.05). These results show that aggregation of variant information across homologues can potentially be used to improve variant effect predictions within protein domains.

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P16.55C

Predicting disease genes from cancer somatic mutations

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Genomic sequence mutations in both the germline and somatic cells can be pathogenic. Several authors have observed that often the same genes are involved in cancer when mutated in somatic cells and in genetic diseases when mutated in the germline. Recent advances in high-throughput sequencing techniques have provided us with large databases of both types of mutations, allowing us to investigate this issue in a systematic way. Here we show that high-throughput data about the frequency of somatic mutations in the most common cancers can be used to predict the genes involved in inheritable phenotypes and diseases. The predictive power of somatic mutation frequencies is largely independent of that of prioritization methods based on germline mutation frequency, so that the two can be successfully integrated into a powerful disease gene predictor. These results confirm the deep relationship between pathogenic mutations in somatic and germline cells, provide new insight into the common origin of cancer and genetic diseases and can be used to improve the identification of new disease genes.

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P16.56D

Database of protein features mapped to the human genome

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Introduction: Nowadays there are many tools for NGS variants evaluation, none of them provides information if a specific variant is translated into an annotated domain or feature of protein. NCBI database provides information about domains and features but without connection to genomic coordinates. The availability of such mapped information will be advantageous in characterizing and annotating DNA variants detected in exome and genome studies.

Materials and methods: For gathering all of the necessary data were used Entrez Programming Utilities. This tool helps to access all desired information about proteins. After processing protein features, they were reverse translated and mapped to the reference genome hg19 and stored in a MySQL database.

Results: The resulting database provides information about 760,487 features, from 42,371 proteins. There are 522,660 protein Regions (19,375 unique types) with an average length 647.37 bp and 237,827 protein Sites (19 unique types) with average length 14.78 bp.

After entering genomic coordinates (e.g. chr2:15229777) into our website a user will retrieve a list of protein features, which covers the entered coordinates. Each record is structured into Gene name, Refseq IDs (NM_xxxx and NP_xxxx), type (Region/Site), all NCBI content and chromosomal location of start and end of feature (divided to cover only exons).

Conclusion: We have created the most comprehensive mapping of protein features of chromosomal coordinates to enhance DNA variant annotation.

The information in this database will help identify novel functional variation and further assists in sifting through the large number of coding variants produced by NGS.

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P16.57A

PLATELET PROTEOMICS PROFILING IN TRANSFUSION ADVERSE EVENTS

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Introduction: Blood platelets destined for transfusion release panoply of molecules during preparation and storage. The leukoreduction process made the transfusion safer but did not completely abolish the adverse events. The rationale of this study is to identify potential proteins in Platelet Components (PCs) involved in serious adverse events (SAEs). **Methods:** Pellets from leukodepleted PCs were sampled from 5 PCs implicated in adverse events and 5 PC matched controls. We performed a Label-Free quantitative analysis using an LC-MS/MS method: LC system coupled to an Electrospray Q-Exactive quadrupole Orbitrap benchtop mass spectrometer. Subsequently, data were searched by SEQUEST through Proteome Discoverer 1.4. Raw LC-MS/MS data were imported in Progenesis QI 2.0 for peptide quantification and statistical comparison.Functionnal analysis was performed using Ingenuity Pathway Analysis software.**Results:** 1000 proteins were identified in our samples of which 423 were differentially expressed ($p<0.05$, Fold Change>2) between the two studied groups. These 423 proteins revealed increased activation of platelets with degranulation and an intense modification of the structure of their cytoskeleton as well as an increase in their inflammatory functions in the event of an SAE. The most enriched signaling pathways are: actin cytoskeleton signaling, the intrinsic pathway of mitochondrial apoptosis, integrin signaling, remodeling of epithelial adherens junctions, RhoA signaling, oxidative phosphorylation, signaling of the acute phase inflammatory response, and protein ubiquitination pathways.

Conclusion: The proteomic study of PC pellets induced by SAE may help to better understand the physiopathological aspect of SAE and thus may help to better prevent EIR in platelet-transfused patients.

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P16.58B

Share and solve rare diseases with RD-Connect's analysis platform

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RD-Connect is a platform for facilitating rare disease research through the integration of sequencing and phenotypic data while incorporating associated biosample and patient registry information. It is open to any rare disease researchers willing to share their sequencing and phenotypic data. RD-Connect provides both a centralised data repository and a user-friendly online analysis system to registered users. Whole-genome, exome and gene panel data are freely shared within the platform, and transferred to the EGA for archival. Raw genomic data is fully reprocessed through a standardised analysis pipeline, while associated clinical information is recorded in PhenoTips using HPO, OMIM and ORDO, allowing machine-readable querying. Results are made available through the genomics analysis interface, which enables filtering and prioritisation of variants by location/gene(s) of interest, impact, pathogenicity, control population frequencies, and phenotype-gene associations using Exomiser/OMIM. This allows users to perform entire primary genomic analyses of their own patients online, and compare findings with other submitted cohorts. Future developments include tools to study non-coding regions, obtain pharmacogenomic profiles, and incorporate other -omics data types (e.g. transcriptomics, metabolomics). The platform facilitates external data sharing at various levels, including through the GA4GH beacon network (www.beacon-network.org), and Matchmaker Exchange (MME, www.matchmakerexchange.org). MME allows users of different systems to exchange non-reidentifiable information to find similar cases, which is particularly useful for complex phenotypes and/or cases proving refractory to resolution. The platform already includes several thousand cases from partner projects such as NeurOmics (www.rd-neuromics.eu) and BBMRI-LPC (www.bbmri-lpc.org). RD-

Connect is free and open for contributions: https://platform.rd-connect.eu/.

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P16.59C

mRNA Half-Life in Single T Cells

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Introduction: Gene expression profile is dictated by the delicate equilibrium of RNA synthesis and turnover. Accurate regulation of mRNA turnover - whether by microRNAs or other post-transcriptional mechanisms - is crucial for immune system development and disease. However, there is still lack of methods to accurately measure this dynamic aspect of gene regulation in a high throughput manner to understand post-transcriptional mechanisms that affect the immune system.

Materials and Methods: We sorted single T cells into 96 well plates at different time points after transcription inhibition to profile the RNA decay rates of over 300 T cell marker genes in hundreds of cells with molecular barcoding. Unlike other traditional assays, molecular barcoding allows accurate counting of originally tagged mRNA transcripts by eliminating PCR bias.

Results and Conclusions: From our single cell sequencing data, we calculated the half-lives of T cells marker mRNAs based on molecular barcode counts. First, we identified a class of steady transcripts that are likely important for T cell maintenance. On the other hand, we identified a class of transcripts with extremely rapid RNA turnover, suggesting that they are important in dynamic cellular responses such as cell cycle or T cell activation. Unlike traditional RNA-seq assays that measure the steady state expression of genes, RNA half life profiling in single cells uncovers the underappreciated dynamics of gene regulation in development and disease.

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P16.60D

Developing custom next-generation sequencing panels using pre-optimized assays: an integrated approach from disease research area to functionally annotated variants

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Targeted next-generation sequencing panels enable interrogation of multiple genes across many samples to more deeply understand human genetic disease. However, finding all relevant genes, developing robust, high performing, multiplex panels, and implementing scalable, reproducible and accurate analysis pipelines is challenging. We present a coordinated suite of tools to facilitate genetic disease research. First we developed the Disease Research Database which organizes human diseases hierarchically, and links all diseases to a set of associated genes; and the Gene Scoring Algorithm which ranks genes by clinical relevance. We developed optimized assays for the most studied 1000 disease research genes. An interactive web interface allows scientists to select any disease of interest, display all associated genes, select any genes and add additional genes, for any number of diseases. Empirical coverage for each gene can be visualized in IGV. A custom Ion Ampliseq gene panel can be built using the optimized assays from all the selected genes. Optimized gene panels can be developed narrowly targeted to specific diseases, or larger gene panels can be developed for broader phenotypes. Disease categories include early onset neonatal phenotypes such as metabolic disorders, Severe Combined Immunodeficiency, heme disorders; cancer predisposition; and late onset phenotypes such as cardiovascular disorders. The panel information is integrated into a seamless end to end analysis. Single sample, Trio, and Tumor-Normal pipelines are available. Pipelines include mapping and variant calling, followed by functional annotation, protein prediction, and detailed annotation of variants and genes based on their presence in public databases.

F. Hyland: A. Employment (full or part-time); Significant; Thermo Fisher Scientific. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Thermo Fisher Scientific. **M. Manivannan:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **B. Krishnaswami:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **C. Van Loy:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **A. Broomer:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **T. Biorac:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **D. DeWolf:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Fu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **E. Williams:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Zhu:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **Y. Tian:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **F. Hernandez Guzman:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **A. Kothandaraman:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific. **S. Roman:** A. Employment (full or part-time); Significant; Thermo Fisher Scientific.

P16.61A

Comparing exome performance and Sanger sequencing in the clinical setting

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Background: Clinical utilities of Next Generation Sequencing (NGS) are continuously expanding. Here, we aimed to examine the performance, accuracy, sensitivity and specificity of NGS vs Sanger-sequencing, and to estimate the possibility of replacing targeted gene sequencing, or gene-panel sequencing with standard, commonly available, whole exome sequencing (WES) kits.

Methods: Genomic DNA was extracted from peripheral leukocytes and buccal swabs. Sanger-sequencing was performed for 258 genes. NGS data was obtained in duplicates from the Illumina HiSeq2500 platform using the Nextera (FC-140-1006) and Agilent (SureSelect^{QXT}) capture kits for both DNA sources. Standard NGS analytic pipelines with predefined quality thresholds followed.

Results: The Nextera and the SureSelect assays yielded >20x coverage for >91% and >98% of the expected genomic regions, respectively. The average coverages were 103x for Nextera and 253x for SureSelect. A total of 449 variants were identified by at least one of the experiments, of which 407 (90.6%) were detected by all experiments. We determined that 23 of the 42 discordant variants were true calls, summing-up to a truth set of 430 variants. The calculated sensitivities ranged between 97%-100%.

Conclusions: The overall concordance between Sanger-sequencing and NGS assays was satisfactory. Despite clear differences in the obtained sequencing coverage for the two capture kits, the detection rates of the true calls were not significantly different. We concluded that neither Sanger nor NGS can be regarded as a "gold-standard" method, as both had false calls. Accordingly, high suspicion of a given diagnosis should override negative molecular results of either Sanger-sequencing or NGS.

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P16.62B

Cost effective DNA copy number profiling of singlecells without upfront whole-genome amplification

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Single-cell sequencing is a powerful tool to investigate the biology of cellular heterogeneity and is revolutionizing different fields of life sciences. To sequence a single cell, whole-genome amplification (WGA) and subsequent library preparation is currently still the standard. While there are many approaches to achieve this, the methods for single-cell library preparation are time consuming and very expensive, often far exceeding the actual sequencing cost per cell. Indeed, we and others have previously shown that the detection of DNA copy number aberrations (CNAs) can be performed by sparse sequencing of single-cell sequencing libraries. To enable studies that require sequencing of hundreds to thousands of single cells, we developed an accessible method that is cost and time effective, called SC-NxtSeq. In SC-NxtSeq we omit the upfront whole-genome amplification step and immediately prepare sequencing libraries from single-cell lysates. We evaluated the robustness of our method by comparing SC-NxtSeq to conventional single-cell sequencing approaches using 7 different WGA technologies. The accuracy of single-cell DNA copy number profiles following SC-NxtSeq was at least as good as the other methods and often even better, while the cost is more than an order of magnitude lower. Finally, to demonstrate that SC-NxtSeq can be used for primary tissue, we applied it on single cells biopsied from a human breast tumor. In conclusion, SC-NxtSeq is a readily available, low-cost and highly accurate method for CNA profiling of single-cell genomes.

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P16.63C

Faster Workflow, Lower Inputs and Longer Read Lengths for SMRT Sequencing of the Human Genome

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Single Molecule Real-Time (SMRT) sequencing platforms offered by Pacific Biosciences (PacBio) provide continuous, low bias DNA reads. This long-read technology facilitates sequencing of regions beset by low complexity, repetitive elements, or large structural variants. These capabilities enable enhanced coverage and assembly metrics for applications like de novo assembly, whole-genome sequencing, and full-length RNA isoform detection. Despite these benefits of long-read SMRT sequencing, the current limits of read length and input requirements could be improved to complete genome assemblies with less difficulty and lower inputs.

Here, we push the boundaries of long-read sequencing with a novel library preparation optimized for long-read SMRT sequencing. The Accel-NGS Long Insert Library Kit combines Swift's enhanced repair and ligation chemistries to produce high library conversion rates while simultaneously eliminating adapter dimers and chimeric inserts. In addition to enhanced performance, this method offers a single-tube library prep workflow that can be completed in as little as 4 hours.

To validate performance, large insert libraries were prepared from human gDNA, enriched for large inserts using the Sage BluePippen, and run on the PacBio platform. Swift libraries resulted in mean and N50 subread lengths that were 2 kb larger than SMRTBell libraries. Additionally, the Swift Long Insert library yields generally exceeded that of traditional SMRTBell libraries, allowing for reduced large shear DNA inputs as low as 2µg, opening up SMRT sequencing to difficult-to-acquire DNA samples. Overall, the Accel-NGS Long Insert Library kit provides both a convenient workflow and enhanced performance optimized for the needs of long-read SMRT sequencing.

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P16.64D

Breaking the curse of dimensionality for machine learning on genomic data

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Introduction: Genomic research is performed on ever larger cohorts with insights jointly drawn over the full dataset. However, traditional approaches are unable to cater for this task, with especially the finite resource of computer memory becoming the bottleneck. Systems like Apache Hadoop and Spark aim to overcome these obstacles.

Materials and Methods: Leveraging the power of Spark and its machine learning libraries (Spark ML), we built VariantSpark: A flexible framework for analysing genomic data. We previously demonstrated VariantSpark's ability to cluster 2,500 individuals with 80 million genomic variants each (1000 Genomes Project, phase 3) into their super-population groups achieving an ARI=0.82 (1 perfect and -1 random clustering). However, when aiming to improve this performance and distinguish between the American and European populations, Spark ML's supervised machine learning methods suffer from "the curse of dimensionality". That is, they scale well with samples (n) but not with features (p, variants), quickly exceeding memory limits.

Results: We hence extended VariantSpark to include CursedForest, a bespoke random forests implementation able to scale to whole genome approaches. We successfully trained a random forest on the same data achieving a cross-validated accuracy of ARI=0.96. Our tailored random forest implementation is 80% faster than comparable implementations on subsets of the data and the only method coping with the full dataset; classifying all individuals in just 30 minutes.

Conclusions: VariantSpark allows real-time classification of population-scale patient cohorts enabling advanced machine learning applications like finding "patients like mine" from whole genome sequencing profiles.

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P16.65A

SNP2TFBS - a database of regulatory SNPs affecting predicted transcription factor binding site affinity

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Introduction: Transcription factor (TF)-target site interactions are the elementary molecular events of gene regulation. Knowing the potential effects of regulatory genetic variants on TF-target site interactions is thus essential to understand their molecular mode of action.

Methodology: We present SNP2TFBS, an automatically generated resource aimed at annotating genetic variants with predicted effects on transcription factor binding affinities. The database consists of a collection of text files providing specific annotations for human SNPs, namely whether they are predicted to abolish, create or change the affinity to one or several TFs. A SNP's effect on TF binding is estimated based on a position weight matrix (PWM) model. SNP2TFBS is regenerated at regular intervals by an automatic procedure that takes as input a reference genome, a comprehensive SNP catalogue, and a collection of PWMs. SNP2TFBS is also accessible over a web interface, enabling users to view the information provided for an individual SNP, to extract SNPs based on various search criteria, to annotate uploaded sets of SNPs, or to display statistics about the frequencies of binding events affected by selected SNPs. All scripts and binary programs that were used to generate SNP2TFBS are available from our FTP site and can potentially be used to generate similar resources for other organisms from analogous inputs.

Conclusions: SNP2TFBS is a useful resource for any type of research that aims at understanding the phenotypes of regulatory genetic variants at the level of TF-target site interactions. SNP2TFBS Home page: http://ccg.vital-it.ch/snp2tfbs/.

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P16.66B

Next-Generation Mapping: Application to Clinically Relevant Structural Variation Analysis

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Next-generation mapping (NGM) from Bionano Genomics allows researchers to interrogate genomic structural variations (SVs) in the range of one kilobase pairs and above. It uses extremely long range information to span interspersed and long tandem repeats making it suitable for elucidating the structure and copy number of complex regions of the human genome, such as loci with complex pseudogene and paralogous gene families. Because NGM is a *de novo* process and because molecules analyzed are longer than almost all genomic repeats, NGM is able to detect a wide range of SVs including insertions of novel sequence, tandem duplications, interspersed duplications, deletions, inversions and translocations, a range of SV types detectable by NGM alone. Because of the high speed and comprehensiveness of the SV types detected, NGM is increasingly being applied to the analysis of clinical genomes for the detection of SVs potentially involved in disease pathogenesis. We present several *in silico* and biological validation experiments to demonstrate the

sensitivity and specificity of NGM for insertion, deletion and translocation SVs and compare it to benchmark studies using short read and long read sequencing. We also show the application of NGM to studying somatic variation in a breast cancer cell line, finding hundreds of somatic structural variations. Finally, we applied NGM to several leukemia patient samples to find more than 50 cancer related SVs in each patient. NGM is a fast and cost effective method for detection of a broad range of traditionally refractory SVs across the genome.

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P16.67C

Detecting Pathogenic Structural Variants with Low-Coverage PacBio Sequencing

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Though a role for structural variants in human disease has long been recognized, it has remained difficult to identify intermediate-sized variants (50 bp to 5 kb), which are too small to detect with array comparative genomic hybridization, but too large to reliably discover with short-read DNA sequencing. Recent studies have demonstrated that PacBio Single Molecule, Real-Time (SMRT) sequencing fills this technology gap. SMRT sequencing detects tens of thousands of structural variants in the human genome, approximately five times the sensitivity of short-read DNA sequencing.

As the application of PacBio SMRT sequencing to detect structural variants moves from initial studies to broad adoption, it is important to produce consistent results at reasonable costs. Towards this end, we have developed a workflow and implemented supporting software for low-coverage (about 10-fold), whole genome sequencing on the PacBio Sequel System and a reference-based, re-sequencing approach to variant calling.

To benchmark performance, we applied the workflow to NA12878, a genome that has been deeply characterized by NIST Genome in a Bottle. With 10-fold coverage sequencing, we identify over 85% of known structural variants in NA12878. We also applied the workflow to an individual with Carney complex for whom short-read whole genome sequencing was non-diagnostic. Filtering for rare, genic structural variants left six candidates, one of which was determined to be likely causative. These applications demonstrate the ability of low-coverage PacBio sequencing to detect structural variants and suggest the potential to increase diagnostic yield for individuals with undiagnosed genetic disorders.

L. Hickey: A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **A.M. Wenger:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **P. Baybayan:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **P. Peluso:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **J. Korlach:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences.

P16.68D

Seeing the whole picture: an integrated view of CNVs, AOH, and sequence variants leads to improved results

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Introduction: Given the separate technologies used to detect CNVs, AOH, and Sequence Variants (SNP Microarrays for CNVs and AOH, and NGS for Sequence Variants), this data commonly has been reviewed in isolated silos. This segregation has also been exaggerated by the separate expertise in Cytogenomics and Molecular Genetics. Materials and Methods: As the fields are now coming together, the importance of an integrated view of the data has become even more apparent. Here we present a new system that can integrate data from any array as well as NGS platforms to create a single genomic view of structural changes in a sample.

Results: The data is presented in a view familiar to cytogenomicists with aberrations displayed across an ideogram with supporting evidence for each call (e.g. probes from arrays and reads from NGS). Historical data and outside database knowledge is integrated into this view allowing the analyst access to all information needed to make assessments of the results. We will demonstrate the utility of this system using a few samples with compound heterozygous aberrations that are detected using different technologies. This includes a case with a microdeletion detected by custom exon array and SNV detected with a custom targeted NGS panel. Another case includes a variant in a large region of homozygosity.

Conclusion: An integrated view of CNVs, AOH and sequence variants leads to improved results.

S. Shams: None. **A. Venier:** None. **A. O'Hara:** None. **Z. Che:** None.

P16.69A

Improving the interpretation of exonic changes by combining *in silico* predictions, RNA splicing assays and protein functional data

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Introduction: Even if next-generation sequencing has improved the detection of nucleotide changes, the biological interpretation of most exonic variants remains challenging. Recently, we found that exonic splicing mutations are more prevalent than currently estimated and can be predicted by using new algorithms. Here again, we used the exon 10 of *MLH1*, a gene implicated in hereditary cancer, as a model system for evaluating variant interpretation methods.

Materials and Methods: First, newly described *MLH1*-exon 10 variants (n = 6) were analyzed for their impact on exonic splicing regulatory elements (ESR) both by using the new algorithms and by performing minigene assays. Then, all currently known, but not-yet-fully-characterized, *MLH1*-exon 10 missense variants (n = 11) were analyzed at the protein level by resorting to bioinformatics predictions and experimental analyses focused on protein expression and mismatch repair activity levels. Bioinformatics predictions were compared with experimental results both from this study and from published reports for the 9 remaining *MLH1*-exon 10 missense variants.

Results: We found that the ESR-dedicated algorithms could predict the impact on splicing of the new variants: either increased exon inclusion or skipping. In contrast, protein-dedicated algorithms showed poor performance in discriminating non-damaging (n = 15) from damaging missense variants (n = 5) as assessed in protein functional assays. We identified 2 new mutations with a negative impact on splicing and 4 new loss-of-function missense variants.

Conclusions: This study confirms the pertinence of using ESR-dedicated algorithms for prioritizing variants for functional RNA splicing analyses, and pinpoints the importance of combining RNA and protein experimental data to interpret missense variants.

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P16.70B

Comparison of multiple hybrid capture technologies: high quality oligonucleotide capture baits improve performance

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Introduction: NGS target enrichment is cost effective and efficient but is historically less uniform than whole genome sequencing. Some studies show that coverage of coding exons is better in whole genome data than in targeted sequencing, particularly for GC-rich regions like first exons.

Materials and Methods: We compared the performance of 4 different exome capture sequencing technologies of similar size (~40–50 Mb). Prepared libraries were pooled

into a 12-plex and split into each capture. Each 12-plex capture was sequenced on a single lane of an Illumina HiSeq 2500 in high output mode. We compared the uniformity of coverage, coverage of RefSeq and sequencing requirements for each technology.

Results: Per base coverage histograms indicate that the xGen® exome had the tightest coverage distribution and the lowest fraction of uncovered bases. For RefSeq genes, 93% of bases had ≥20x coverage for the IDT exome, whereas the other three technologies ranged from 57–71%. When considering only first exons, the disparity increased significantly. 94% of first exon bases were covered at ≥20x with IDT's exome, whereas poor coverage of GC-rich targets in the other exome captures resulted in only 49–60% of first base exons being covered at ≥20×. This led to better end-to-end gene coverage with every position in a gene covered at ≥20x for 92% of genes with the IDT panel verses 58–73% for other methods.

Conclusions: Here we present how individually synthesized and QC'ed capture baits dramatically improve the performance of target enrichment, changing the targeted vs. whole genome calculation.

K. Giorda: A. Employment (full or part-time); Significant; Integrated DNA Technologies. **S. Groenewold:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **K. Lai:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **C. Walworth:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **D. Kupec:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **L. Lewis:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **Y. Bao:** A. Employment (full or part-time); Significant; Integrated DNA Technologies. **M. Jarosz:** A. Employment (full or part-time); Significant; Integrated DNA Technologies.

P17 Epigenetics and Gene Regulation

P17.01A

Characterization of the expression of the imprinted *Kcnk9*-gene in specific brain regions and the phenotypic analysis of *Kcnk9*-knockout mice

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Kcnk9/KCNK9 is a maternally expressed imprinted gene whose mutations are responsible for the maternally inherited Birk-Barel mental retardation syndrome. It encodes a member of the superfamily of K + -channel and is involved in the modulation of the resting membrane potential and excitability of neurons.

In this study, we set out to characterize the allele-specific expression of *Kcnk9* in various regions of the mouse brain and to analyze several parameters of brain function in homozygous (Kcnk9KO^{hom}) and heterozygous *Kcnk9* KO mice with inactivation of the maternal *Kcnk9* allele (Kcnk9KO^{mat}).

Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) method was performed for different brain areas of (C57BL/6xCast/Ei) F1-hybrid mice. Exclusive expression from the maternal *Kcnk9* allele was detected in the hippocampus, mesencephalon, brainstem and thalamus. Biallelic expression was observed in the olfactory bulbs, cortex, cerebellum and striatum.

The Phenotype of WT, Kcnk9KO^{mat} and Kcnk9KO^{hom} mice were examined in a behavioral test battery. Results show a clearly impaired working memory of the Kcnk9KO^{mat} and Kcnk9KO^{hom} mice in comparison to WT mice. The spontaneous alternation in the Y-Maze was significantly reduced by approx. 10–20%. In acoustic startle response and prepulse inhibition tests Kcnk9KO^{hom} mice showed significantly reduced acoustic startle response (ASR), Kcnk9KO^{mat} and Kcnk9KO^{hom} mice also showed reduced pre-pulse inhibition compared to B6-WT. Investigations of the circadian rhythm reveal an increased locomotor activity during the dark phase in Kcnk9KO^{hom} and Kcnk9KO^{mat} mice. Our findings shall further elucidate the role of *Kcnk9/KCNK9* in pathophysiology and open new avenues for treatment of cognitive dysfunctions in Birk-Barel syndrome.

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P17.02B

Functional dissection of the enhancer network in human embryonic stem cells by ChIP-STARR-seq

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Enhancers are genetic elements that direct correct spatio-temporal regulation of gene expression and are often predicted by co-localization of transcription factors (TFs) and enhancer-associated histone-modifications (e.g. H3K27ac, H3K4me1/3). Only a limited number of putative enhancers

identified can be experimentally validated, indicating that current-state-of-the-art methods to identify functional enhancers have limitations. To identify functional enhancers quantitatively in a genome-wide manner, we have developed a new high-throughput screen, which combines chromatin-immunoprecipitation for TFs or histone modifications, with a massively-parallel-reporter-assay. Non-coding DNA sequences bound by TFs or marked by histone-modifications are cloned *en masse* in reporter plasmid libraries, which can be used in cell-transfection experiments, where enhancer-activity of cloned sequences can be quantified by RNA-seq. Using this ChIP-STARR-seq approach, we can directly functionally assess enhancer-activity of non-coding sequences at an unprecedented scale. We have applied this approach to stem cell biology, focusing on the TFs NANOG, OCT4 and the histone modifications H3K4me1 and H3K27ac in human primed and naive embryonic stem cells. We found that from 270K regions marked by these factors, spanning >320Mb of sequence, only approximately 20K regions display enhancer-activity, allowing distinction of functional from non-functional sites, and dissection of super enhancers into smaller active elements. Active ChIP-STARR-seq enhancers are associated with highly expressed genes displaying cell-type specific gene ontology, and a group of housekeeping genes. Commonly, they are marked by a unique activator signature of related TFs and chromatin-modifiers, thereby providing new biological insights in enhancer biology and further clarifying the functionality of the non-coding genome

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P17.03C

Unveiling the regulatory landscapes of genes involved in pancreatic cancer using a zebrafish model

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Pancreatic carcinoma (PC) is a highly aggressive disease with very poor prognosis. Mutations in non-coding regions, particularly cis-regulatory elements (CREs), have been associated with cancer, but their role is yet to be uncovered. Enhancers are CREs controling the transcriptional output of target genes in specific tissues and developmental times, being crucial for gene expression and when mutated may cause loss-of-expression. The aim of this work is to unveil the effect of mutations in CREs of tumor-suppressor genes (TSGs) involved in PC. By using a zebrafish we can identify and validate the impact of those non-coding mutations in an *in vivo* model. Zebrafish has been used as a model of PC and genetic regulators of zebrafish pancreatic development can be translated into potential clinical biomarkers and therapeutic targets in human PC. Currently we are exploring the transcriptional regulatory genome (regulome) of zebrafish pancreas, using genome-wide techniques combined with next-generation sequencing, such as Assay for Transposase-Accessible Chromatin (ATAC-seq), Chromatin-Immunoprecipitation (ChIP-seq) and Circularized Chromosome Conformation Capture (4C-seq). Analyzing these data, we have identified putative enhancers of pancreas TSGs and we are now validating their activity by transgenic assays. The demonstration of regulatory regions determining the expression of TSGs in pancreatic tissue will have a major impact on understanding pancreatic cancer genetics. These results may further be translated to human genetics and the same strategy be applied to identify human CREs involved in PC, which will broaden the perspectives of new therapeutic approaches and clinical management of PC patients.

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P17.04D

DNMT inhibitor 5-Azacitidine modulates immune responses in CD4^{+}T cells from patients with Cystic Fibrosis

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Introduction: Cystic fibrosis (CF) characterized by the overproduction of the pro-inflammatory cytokines and reduced expression of anti-inflammatory cytokines. Epigenetic factors such as altered DNA methylation in T cells might play a role in cytokine dysregulation during CF. In the present study we examined expression of IFN-γ and IL-4 by T cells prior to and following the DNA methyltranspherase inhibitor 5-Azacytidine (5-azaC) treatment. Materials and Methods: Eight CF patients (age: 5–12 years) were included in the study and compared to six age-matched healthy subjects. The Ethical Committee of the TSMU (Tbilisi, Georgia) had approved the protocol and the parents of each CF patient and healthy subject provided informed

consent. $CD4^+$T cells were isolated from PBMC using CD4 MicroBead kit (Miltenyi Biotec GmbH) and were cultured in RPMI 1640 medium at 37°C with 5% CO_2, in presence or absence of 5-Azacytidine (Sigma Aldrich, Germany) at a dose of 2μM. Cells were activated with PMA (20ng/ml)/ Ionomycin (250 ng/ml). Concentrations of IL-4 and INF-γ in $CD4^+$ T Cell were measured by ELISA (eBoiscience, san Diego, CA, USA). **Results:** In our study we showed that 5-Azacytidine modulates cytokine exspression in $CD4^+$T cells derived from CF patients. After 5-azaC treatment secretion of IFN-γ was significantly decreased in CF T cells, while amount of IL-4 was elevated by ~2.8 times compared to controls. **Conclusions:** Our data demonstrates that epigenetic mechanisms such as DNA methylation may be considered as a one of the potential therapeutic target in a treatment of Cystic Fibrosis.

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P17.05A

5-Azacitidine and NSAIDs alter levels of DNA methyltransferases in nucleus raphe magnus in formalin induced inflammatory pain

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Introduction: It has been established that nucleus raphe magnus (NRM) is one of the important parts of antinociceptive system of CNS. Epigenetic mechanisms, mainly DNA methylation, may play a major role in regulating nociception.The aim of the present study was to evaluate the changes of a subset of DNA methyltransferases level in NRM neurons in inflammatory pain and prior intraperiotonal injection of 5-AzaC and NSAID. **Materials and Methods:** All animal studies conformed to the Guidelines of International Association for the study of Pain regarding investigations. Adult rats were divided into seven groups. In first group 50 ml of 10% v/v formalin solution (Sigma-Aldrich) unilateral intraplantar injection was made. Second group was injected with saline as vehicle. 3^{rd}-7^{th} groups were administrated with different doses and combinations of various drugs (Xefocam, Diclofenac, ketorolac, 5-AzaC, Naloxone - Sigma-Aldrich) 30- 45 min. prior to pain induction with formalin. Mechanical pressure paw withdrawal thresholds were used to assess pain. The levels of DNMT1, DNMT3a and DNMT3b were measured in nuclear extracts of NRM neurons using DNMTs assay kits (Abcam). **Results:** Received data demonstrated increased levels of DNMT3a/b in NRM neurons of rats after formalin induced pain compared with controls. NSAIDs diminished levels of DNMT3a/b in NRM, as well as 5-AzaC dose-dependantly. Prior preinjection of naloxone, 5-AzaC and Xefocam didn't cause decrease of DNMT3a/b. **Conclusions:** Our results suggest that reduction of DNMTs with 5-AzaC and NSAIDs may be mediated via DNA methylation-dependent antinociceptive mechanisms in the NRM and also these alterations may be opioid dependent.

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P17.06B

Long-term prenatal exposure to paracetamol is associated with DNA methylation changes and ADHD: identification of dysregulated pathways involved in oxidative stress and the olfactory system

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Epidemiological studies have shown that long-term exposure to paracetamol during pregnancy is associated with attention-deficit/hyperactivity disorder (ADHD). The mechanism by which paracetamol modulates the increased risk of developing ADHD is currently unknown. We have conducted an epigenome-wide association study to investigate whether prenatal exposure to paracetamol is associated with DNA methylation changes and ADHD. Participants ($n = 384$) were selected from a large prospective birth cohort (Norwegian Mother and Child Cohort Study), which contains information about medication use during pregnancy. The ADHD diagnoses were obtained from the Norwegian Patient Registry. Case-control analysis of paracetamol or ADHD alone did not reveal any differential DNA methylation. However, comparison of samples with ADHD exposed to paracetamol for more than 20 days to healthy controls identified differentially methylated CpGs ($n = 6\ 211$). In addition, these samples were differentially methylated compared to samples with ADHD exposed to paracetamol for less than 20 days ($n = 2\ 089$ CpGs) and not exposed to paracetamol ($n = 193$ CpGs). Interestingly, several of the top genes ranked according to significance and effect size have been linked to ADHD, neural

development and neurotransmission. Gene ontology analysis revealed enrichment of pathways involved in oxidative stress, neurological processes and the olfactory sensory system, which have previously been implicated in the etiology of ADHD. These results lend novel insights into the epigenetic aspect of ADHD and provide a possible pharmaco-epigenetic link between the prenatal exposure to paracetamol and ADHD development.

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P17.07C

A mediating role for *F2RL3* methylation in the relationship between smoking and cardiovascular disease: evidence from population and laboratory studies

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Introduction: Smoking is robustly associated with hypomethylation of *F2RL3* (coagulation factor II receptor-like 3) which encodes protease-activated receptor 4 (PAR4), a thrombin receptor involved in platelet activation. Epigenetic control of PAR4 could be important in mediating cardiovascular risk from smoking.

Materials and Methods: We performed epidemiological analyses of the association of *F2RL3* methylation at four CpG sites (CpG_1–4) with smoking and acute myocardial infarction (AMI) in 853/2,352 nested cases/controls from the Copenhagen City Heart Study (CCHS). We investigated platelet activity in 41 never smokers from the Avon Longitudinal Study of Parents and Children (ALSPAC) recruited on methylation at *F2RL3*. In *in vitro* studies we: 1) exposed primary human coronary artery endothelial cells (HCAEC) to cigarette smoke extract (CSE) and measured methylation at *F2RL3* and PAR4 expression; and 2) transfected HEK-293 cells with reporter constructs containing different fragments of *F2RL3* and assessed gene expression.

Results: In CCHS, hypomethylation of *F2RL3* was associated with smoking and higher odds of AMI (smoking-adjusted OR:1.12 per 10% decrease in methylation, 95% CI:1.05,1.20). In ALSPAC participants, lower methylation was associated with increased PAR4 specific platelet activity. CSE exposure reduced *F2RL3* methylation and increased *F2RL3* expression in HCAEC. Transfection of HEK-293 cells confirmed that exon 2 (containing CpG_1–4) has enhancer activity that is partially enabled by a CCAAT recognition site located 2 bp 3′ of CpG_1.

Conclusions: These findings strengthen support for methylation-dependent platelet activation as a mediator between smoking and AMI, highlighting this pathway for clinical intervention to reduce harmful consequences of smoking.

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P17.08D

Pathogenic SNPs with allelic imbalance show higher expression in the major allele

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In recent years eQTL studies have been conducted on a large scale. However, due to limited sample-sizes most studies have been statistically powered to identify effects of common genetic variants. To overcome this, we investigated RNA-seq data from 3,685 unrelated population-based blood samples from BIOS, a large Dutch biobank consortium, and performed allele specific expression (ASE) analysis to identify effects of proven pathogenic variants on gene expression.

To identify these pathogenic variants, we called genotypes from the RNA-seq directly. We observed that the quality of this genotype calling and subsequent imputation was very high, as determined by comparing genotypes of 446,096 called SNPs with DNA array and whole genome sequencing (WGS) genotype data of 155 samples (average concordance with WGS data was 99%). We subsequently calculated allelic ratios, and observed that 48,207 SNPs showed at least one sample with an allelic imbalance. Out of 2,661 pathogenic SNPs from Clinvar, we identified 15 SNPs where at least one individual was heterozygous and also showed an allelic imbalance. This was significantly more as compared to a null-distribution of coding SNPs, matched for minor allele frequency. This suggests that it is possible to use whole blood RNA-seq reference datasets to make inferences on the molecular consequences of rare pathogenic variants. As such this provides avenues for prioritizing likely pathogenic variants that cause disease by ascertaining their effects on gene expression levels.

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P17.09A

Decreased mitochondrial DNA methylation in peripheral blood cells of Alzheimer's disease patients

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Several evidences suggest that mitochondrial dysfunction occurs early in Alzheimer's disease (AD), both in affected brain regions and in peripheral blood cells, likely promoting neurodegeneration through increased oxidative stress. Oxidative stress induces cell death through several cellular pathways, including epigenetic mechanisms. In recent years several investigations highlighted an impairment of nuclear DNA methylation in AD pathogenesis but little attention was given to the mitochondrial epigenome itself. In the current study we developed a protocol for the study of mtDNA methylation by means of the cost effective technique Methylation Sensitive-High Resolution Melting. We investigated the presence of DNA methylation modifications in the mitochondrial displacement loop (*D-loop*) region, which regulates mitochondrial DNA replication and transcription, in peripheral blood cells of 133 AD patients and 130 healthy matched controls. *D-loop* methylation analyses showed the existence of an interindividual variability with methylation levels ranging from 0 to 9 %. *D-loop* methylation levels were lower in AD patients respect to control subjects in a statistically significant manner ($P = 0.04$). No significant correlation between *D-loop* methylation levels and age was observed ($r = -0.09$, $P = 0.14$), and no differences in methylation levels between males and females were detected ($P = 0.55$). Present data indicate that the *D-loop* region is hypomethylated in peripheral blood DNA of AD patients, suggesting that mtDNA epimutations deserve further investigations in AD pathogenesis.

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P17.10B

DNA methylation levels in blood of amyotrophic lateral sclerosis patients carrying not fully penetrant *SOD1* mutations

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting from the degeneration of motor neurons in the motor cortex, brainstem and spinal cord. Superoxide dismutase 1 (*SOD1*) has been the first identified causative gene for ALS and more than 180 different mutations have been described so far in ALS patients, including not completely penetrant ones, leading to a phenotypic heterogeneity ranging from asymptomatic or paucisymptomatic individuals to slowly progressive or even very aggressive ALS forms, suggesting that the severity of the phenotype

may be modulated by other genetic variants and/or environmental factors that could act by means of epigenetic mechanisms. In the present study we collected DNA samples from five ALS families with not fully penetrant *SOD1* mutations (p.Asn65Ser, p.Gly72Ser, p.Gly93Asp, and p.Gly130_Glu133del) and searched for the presence of additional mutations in the major genes of ALS, namely *FUS, TARDBP* and *C9orf72* as well as for epigenetic differences between ALS patients, asymptomatic/paucisymptomatic carriers and non-carriers of *SOD1* mutations. No additional mutations were found in the analyzed subjects. Global DNA methylation levels were significantly higher in blood DNA of ALS patients than in asymptomatic/paucisymptomatic carriers or in familial non-carriers of *SOD1* mutations. However the promoters of *SOD1*, *FUS*, *TARDBP* and *C9orf72* were completely demethylated in all the analysed samples. The present study suggests that global changes in DNA methylation might contribute to the ALS phenotype in carriers of not fully penetrant *SOD1* mutations, but those changes do not occur at the promoter of major ALS genes.

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P17.12D

Role of natural antisense transcripts in human gene expression regulation

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Introduction Antisense transcription is a frequent but insufficiently studied phenomenon. It was shown that antisense RNAs play role in the development of some genetic diseases. Natural antisense transcripts (NATs) transcribe from opposite DNA strands of the same genome locus (*cis*-NATs) or different genome loci (*trans*-NATs). One of the mechanisms of antisense regulation is realized by formation of complementary RNA-RNA duplex.**Materials and methods** We used qPCR to measure expression of *cis*-NATs in different samples. Knockdown was performed using siRNAs. Overexpression was performed using expression vector pEYFP-C1. Besides, we made RNA-RNA pull-down control experiments to identify the most appropriate conditions for detection of RNA-RNA interactions. **Results** Based on bioinformatic analysis, we chose four pairs of *cis*-NATs to study their antisense regulation (AFAP1 - AFAP1-AS1, SIRT3 - RIC8A, HSPE1 - HSPD1, S100A13 - CHTOP). For each pair we selected suitable cell line for further experiments using two criteria: expression of both antisense transcripts and their overlapping regions. Next, knockdown of one gene from each pair was performed in the selected cell line. We showed that knockdown of HSPE1 and S100A13 do not affect expression level of their *cis*-NATs HSPD1 and CHTOP, respectively. Whereas AFAP1-AS1 and RIC8A knockdown and overexpression showed the possible positive antisense regulation of their corresponding *cis*-partner. **Conclusions** We have shown possible positive antisense regulation for *cis*-NATs pairs AFAP1-AFAP-AS1 and SIRT3-RIC8A. We hypothesize that this regulation could be realized through formation of RNA-RNA duplexes. Our further study will be aimed at performing RNA-RNA pull-down to test this hypothesis.

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P17.13A

Overgrowth and Undergrowth/ID: Two faces of a unique *CDKN1C*-mutation

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LoF mutations in the maternal *CDKN1C* gene may cause Beckwith-Wiedemann syndrome (BWS) and overgrowth, while missensemutations in the gene's PCNA-binding motif are associated with IMAGe syndrome (Intrauterine Growth retardation, Metaphyseal Dysplasia, Adrenal Insufficiency, Genital abnormalities) and Silver-Russel-syndrome (SRS).

We present a male patient with elements of both BWS (large omphalocele, macroglossia, facial nevus flammeus and ear creases) and IMAGe (IUGR, genital abnormalities, prolonged neonatal hypoglycemia, small kidneys, feeding difficulties, no adrenal insufficiency). Examination of *CDKN1C* revealed a frameshift change (c.822_826delinsGAGCTG p.Asp274GlufsTer12) in the same region where the IMAGe mutations have been found. *CDKN1C* RNA-analysis suggested that nonsense mediated RNA-decay (NMD) occurred in blood leukocytes but not skin fibroblasts. This was supported by Western blots, showing a stronger band of right size in patient fibroblasts than in control fibroblasts. In theory, the mutated allele will give rise to a shortened protein with substitution of IMAGe-related amino acids (in pos. 276–281) and loss of a likely nuclear localization signal.

Interestingly, the *CDKN1C*-mutation was inherited from an asymptomatic mother and a mildly affected mosaic grandfather. Haplotype analysis showed that the mutation was *de novo* on the grandfather's maternal allele.

To the best of our knowledge, this is the first time the same mutation leads to both LoF (in blood) and GoF (in fibroblasts) and therefore the presence of elements of two mirror-image syndromes in the same patient. The patient is also microcephalic with delayed development, without any other findings on WES and methylation analysis. Further functional analysis is planned.

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P17.14B

A novel 11p15.5 microduplication upstream *IGF2* associated with Beckwith-Wiedmann syndrome

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Beckwith-Wiedemann syndrome (BWS, MIM 130650) is an overgrowth disorder characterized by variable clinical manifestations and may include omphalocele, macroglossia, hemihyperplasia, ear pits, umbilical hernia, hypoglycemia and high risk of tumor development. Several molecular mechanisms have been involved in the dysregulation of imprinted growth regulatory genes on chromosome 11p15. This genomic region contains two imprinted domains regulated by two independent imprinting control regions (ICR), ICR1 and ICR2. We report on a 1 year-old patient with high clinical suspicion of BWS. Evaluation of ICR1 and ICR2 methylation and CDKN1C sequencing were normal. SNP array analysis revealed a 102 kb microduplication at 11p15.5 which encompassed the non-imprinted genes *LSP1, TNN3, MRPL23* but not *H19* and *IGF2* nor the ICR2 region. Duplications or deletions of the chromosome 11p15.5 region are rares and can be inherited or *de novo*. All microdeletions reported were maternally inherited while duplications were maternally or paternally inherited, sometimes spanning both domains in the chromosome 11p15.5 region. In the present case, the microduplication has occurred *de novo* on the maternal chromosome 11 and only covered the regulatory elements located upstream *IGF2*. We hypothesized that this duplication could interfere with the structure of topologically associating domains (TAD), a structural unit shared between the different cell types and conserved between species. The structural modification of the TAD generated by 11p15.5 duplication could lead to an overexpression of IGF2. Since genomic structural variants that disrupt TAD boundaries have been reported to cause developmental disorders, this observation suggests a new mechanism behind the BWS.

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P17.15C

C9orf72 epigenetic modifications in patients with amyotrophic lateral sclerosis and fronto-temporal dementia

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A non-coding GGGGCC repeat expansion (RE) in *C9orf72* represents the main genetic cause of amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD). Previous findings strongly suggest that methylation of mutant *C9orf72*, resulting into decreased pathological RNA foci/dipeptide repeat protein aggregates in brain tissues, may have a neuroprotective role and act as a modifying factor in disease presentation. To consolidate these findings, we investigated *C9orf72* promoter and RE methylation in a cohort of 60 Italian ALS and FTD mutation carriers. By using bisulfite conversion of blood genomic DNA and methylation-specific repeat-primed PCR, we confirmed that methylation of RE is present in all mutation carriers. By bisulfite Sanger sequencing, we assessed methylation status of 33 CpG sites within the promoter CpG island and observed a significant hypermethylation (methylated CpG

sites >3) in about 60% of carriers, but not in *C9orf72*-negative patients. NGS approach was used, for the first time, to accurately estimate methylation at individual CpG sites, revealing higher levels of methylation at the 5′ of the island. We found that promoter methylation levels were inversely correlated with *C9orf72* expression of both total mRNA and the three different isoforms in blood. In our cohort, *C9orf72* promoter methylation was associated with disease duration in ALS, but not with age at onset, and was inversely associated with RE size. This study confirms that assessment of *C9orf72* RE and promoter methylation, frequent in both ALS and FTD mutation carriers, is important when considering genotype-phenotype correlations.

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P17.16D

Integrating Capture Hi-C with RNAseq timecourse data to uncover the regulatory interactions modulated by genetic variation in disease

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Introduction: There is compelling and growing genetic evidence as to the importance of T-cells in rheumatoid arthritis (RA). Overlaying RA GWAS hits with cell specific expression data and epigenetic marks of gene activity (histone marks, DNaseHS), suggests T-cells, and specifically CD4 T-cells, play the driving role in RA genetic susceptibility. A large number of likely RA genes are differentially expressed in primary CD4 T-cells under stimulatory conditions suggesting it will be most informative to initiate studies under stimulatory conditions, to define the key genetic pathways for RA. Here, we incorporate Capture Hi-C and nuclear RNAseq data from a CD4 T-cell stimulation timecourse to link chromatin modelling and gene expression over time. Materials and Methods: Primary human CD4 T-cells were isolated from PBMCs using an EasySep T-cell isolation kit then stimulated with CD3/CD28 Dynabeads over a period of 24-hours. Pooled cells from multiple samples were used to make libraries for Capture Hi-C and RNAseq. Capture Hi-C enriched for promoters and disease-associated enhancers was used to identify physical interactions and how these change through time. Nuclear RNAseq was used to quantify nascent transcription. **Results:** Here we show that interactions between GWAS SNPs and genes associated with RA, can be correlated with gene expression. *IRF8* gene expression occurs only after four hours of stimulation and at this point interactions with GWAS SNPs can also be detected. **Conclusions:** These data suggest that dynamic chromatin remodelling takes place post-stimulation allowing interactions with the target gene to take place. Funded by MRC project no MR/N00017X/1

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P17.18B

Epigenetic changes mediated by UHRF1 (ubiquitin-like with PHD and ring finger domains 1) are associated with silencing of *MIR137HG*, a tumor suppressor gene frequently downregulated in multiple solid tumors

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Introduction: The miR-137 encoding gene (*MIR137HG*) is epigenetically silenced in colorectal adenomatous tissues to the same extent as in colorectal cancer and in diverse solid tumors. Restoration of miR-137 expression decreases proliferation and promotes cell cycle arrest at the G0/G1 stage in different human cancer cell lines. These observations suggest that miR-137 down-regulation is an early event in carcinogenesis and that it may act as a tumor suppressor. *MIR137HG* is embedded in a CpG island, but the mechanism through which it is repressed is unknown. **Results:** Chromatin immunoprecipitation of HCT116 human colon carcinoma cells nuclear extracts showed the presence of UHRF1, DNMT3b and the transcriptional repressors HDAC1 and G9a on the *MIR137HG* promoter. Identical epigenetic marks were found at the *PPARG* locus, a well-known tumor suppressor gene. SiRNA-mediated UHRF1 silencing relieved repression of endogenous miR-137. Using bisulfite-treated DNA pyrosequencing and western blotting in a panel of colorectal tumors, we observed a correlation between UHRF1 expression and *MIR137HG* hypermethylation. An opposite result was obtained from paired adjacent normal colon mucosa specimens. We further showed that miR-137 negatively regulated Cdk6 (cyclin dependent kinase 6) and Cdc42 (cell division cycle 42), two enzymes that play a critical roles in regulating cell cycle progression. Consistently, ectopic miR-137 expression inhibited proliferation and promoted senescence of HCT116 cells. **Conclusion:** We provide

evidence that UHRF1 coordinates MIR137HG epigenetic silencing. We propose that UHRF1 overexpression is a mechanism underlying DNA hypermethylation at diverse microRNA-related tumor suppressor gene loci, a mechanism that could constitute an early event in carcinogenesis.

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P17.19C

Epigenetic and gene expression changes in patients with Crohn's disease, and responsiveness to adalimumab therapy

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Introduction: Inflammatory bowel disease (IBD) is a chronic autoimmune intestinal inflammation with two subtypes, ulcerative colitis and Crohn's disease. Treatment with TNF inhibitors presents an important therapy advancement. However, it is only successful in a subset of patients, with about 20–40% failing to respond. Due to anti-TNF therapy's side effects and high cost, ability to determine which patients are likely to respond would be beneficial. Although some biomarkers predictive of anti-TNF treatment response have already been identified in IBD, epigenetic alterations correlated with anti-TNF responsiveness (e.g. DNA methylation changes) have not yet been explored. Additionally, epigenetic modifications that indicate or contribute to the pathophysiology of IBD have not been well characterized.

Materials and methods: Pre-treatment whole blood samples from Crohn's patients receiving anti-TNF drug adalimumab (responders and nonresponders), together with blood from healthy subjects, were used to correlate Crohn-specific DNA methylation/expression profiles. These patterns were compared in responsive and non-responsive patients. Methylation profiling was carried out by HRM analyses and bisulfite sequencing, and integrated with gene expression levels (RT-qPCR).

Results: Several genes previously implicated in IBD through genome-wide studies were analyzed, as well as genes that had previously been implicated in anti-TNF responsiveness in other autoimmune diseases, including inflammatory cytokines from the CXC chemokine family, S100 family of calcium-binding proteins, WNT-signaling pathway and Low Density Lipoprotein Receptor-Related Protein (LRPAP1). Discussion: Our preliminary expression results identify two Crohn's-associated candidates (LRPAP1, Axin1), and studies aimed at identification of biomarkers that will facilitate prediction of Crohn patients' responsiveness to adalimumab are under way.

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P17.20D

Dies1/VISTA expression loss is a recurrent event in gastric cancer due to epigenetic regulation

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Introduction: *Dies1/VISTA* induces embryonic stem-cell differentiation, via BMP-pathway, but also acts as inflammation regulator and immune-response modulator. *Dies1* inhibition in a melanoma-mouse model led to increased tumour-infiltrating T-cells and decreased tumour growth, emphasizing *Dies1* relevance in tumour-microenvironment. *Dies1* is involved in cell de/differentiation, inflammation and cancer processes, which mimic those associated with Epithelial-to-Mesenchymal-Transition (EMT). Despite this axis linking *Dies1* with EMT and cancer, its expression, modulation and relevance in these contexts is unknown.

Materials and Methods: We analysed *Dies1* expression, its regulation by promoter-methylation and *miR-125a-5p* overexpression, and its association with BMP-pathway downstream-effectors, in a TGFβ1-induced EMT-model, cancer cell-lines and primary samples.

Results: We detected promoter-methylation as a mechanism controlling *Dies1* expression in our EMT-model and in several cancer cell-lines. We showed that the relationship between *Dies1* expression and BMP-pathway effectors observed in the EMT-model, was not present in all cell-lines, suggesting that *Dies1* has other cell-specific effectors, beyond the BMP-pathway. We further demonstrated that: *Dies1* expression loss is a recurrent event in GC, caused by promoter methylation and/or *miR-125a-5p* overexpression and; GC-microenvironment myofibroblasts overexpress *Dies1*.

Conclusions: Our findings highlight *Dies1* as a novel player in GC, with distinct roles within tumour cells and in the tumour-microenvironment. Grants and Fellowships: POCI-01-0145-FEDER-007274; SFRH/BPD/89764/2012; SFRH/BPD/86543/2012; SFRH/BD/63300/2009; SFRH/BPD/104208/2014.

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P17.21A

Integrated analysis of DNA methylation and gene expression fingerprints of human atherosclerotic plaques

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Aberrant DNA methylation and gene expression have been reported in human atherosclerotic plaques of patients with carotid atherosclerosis. In the present study, the DNA methylation and gene expression fingerprints of arterial tissue were investigated together. The Illumina HumanMethylation27 BeadChip and Illumina HumanHT-12 version 4 Expression BeadChip microarrays were used for analysis of advanced carotid atherosclerotic plaques and healthy internal mammary arteries of five statin-treated patients with atherosclerosis. The advanced atherosclerotic plaques in comparison with the healthy arteries were characterized by the predominant DNA hypermethylation changes. There were no biological processes in Gene Ontology terms associated with the hypermethylated genes. In contrast, hypomethylated genes encode molecules belonging to different biological processes such as development, lipid storage, programmed cell death, immune and inflammatory responses. The majority of differentially expressed genes were down-regulated in advanced atherosclerotic plaques. “Cellular response to metal ion” and “extracellular matrix organization” were the most significant Gene Ontology terms among the down- and up-regulated genes, respectively. Unexpectedly, genes involved in immune and inflammatory responses were down-regulated in advanced atherosclerotic plaques compared to the healthy arteries. Statins are likely to attenuate the expression of inflammatory genes in blood vessels. After integration of the DNA methylation and gene expression data, we identified four hypermethylated-downregulated genes (*LIPE, CIDEC, TMEM88, GATA2*) and one hypomethylated-upregulated gene (*S100A4*). In conclusion, we identified the atherosclerosis-specific DNA methylation and gene expression fingerprints that provide new insights into the pathogenesis of atherosclerosis. The

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P17.22B

Genome-wide miRNA profiling in plasma of pregnant women with Down syndrome fetuses

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Introduction: Down syndrome (DS) is the most common aneuploidy. Impact of epigenetic mechanisms is currently often discussed in the context of DS. We follow up on a previous study comparing miRNA expressions in normal and trisomic placentas, where seven miRNAs were found as being significantly upregulated in DS placentas. To further investigate biological functions of miRNA, in the present study we focused on plasma samples. Methods: Genome-wide miRNA expressions were profiled using Affymetrix miRNA 4.1 array strips. A total of 26 plasma samples from pregnant women - 13 bearing DS fetuses and 13 normal karyotype fetuses, were included. Results were evaluated using Partek Genomics Suite. The one-way ANOVA with a cut-off p-value < 0.05 and Benjamini-Hochberg correction for multiple testing was used for detection of differentially expressed miRNAs. **Results:** We identified 61 human miRNAs as being significantly dysregulated between compared groups of samples. Unlike the placenta, most of these miRNAs (44) were down-regulated in plasma of women bearing the DS fetus. Many biological pathways were identified as being potentially influenced by altered miRNA levels. Cell communication and signalling pathways were between the most enriched ones. Seven miRNAs, previously determined as upregulated in DS placentas, did not differ on the level of plasma. **Conclusion:** Our study shows that differences of miRNA profiles between normal and DS fetuses are detectable even on the level of maternal plasma. Such differences could mirror alterations in placental development and they deserve further study. Supported by the Ministry of Health of the Czech Republic RVO VFN64165.

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P17.23C

Effects of genetic variation on promoter usage (pmQTL) and enhancer activity (enQTL)

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Identification of genetic variants affecting gene expression, splicing, and chromatin states have increased our understanding of mechanisms underlying human traits. We hypothesized that some eQTLs (expression_quantitative_-trait_loci) act on differential promoter-usage and enhancer-activity. Transcriptomes of 154 unrelated individuals were profiled using CAGE on total-nuclear-RNAs extracted from LCLs. Sequences were mapped to promoters of the FANTOM atlas, yielding to the quantification of 38,759 promoters/transcripts that we tested for association against 7,508,202 genetic variants; we discovered 5,491 *cis*-promoter-QTL (pmQTL,*FDR*<0.05). As eQTLs, pmQTLs localize preferentially near to TSSs and are marked with active transcriptional marks. Approximately 90% of pmQTLs are found to also affect mRNA-levels ($\pi 1$=0.904) as quantified with RNA-seq, half of them are not associated with main but with alternative promoters and 26.3% of the associated genes have more than one promoter linked with a pmQTL. Opposite regulatory effects of pmQTLs were detected on ≥2 promoters for 139 genes. The integration of pmQTL with eQTL allows the evaluation of the relative participation of alternative promoters to mRNA abundance and the detection of variants associated with promoter-usage and not with mRNA-levels. Together this gives insights into eQTL mechanisms involving differential promoter-usage. Furthermore, using the FANTOM enhancers and the quantification of enhancer-RNAs (eRNAs) as a proxy for enhancer-activity, we mapped 108 *cis*-enhancer-QTL (enQTL,*FDR*<0.05). For each enQTL-enhancer pair, we tested causal inference of eRNAs as molecular-mediators for the expression of enhancer-target-genes, using causal inference testing. Causality was detected for 48 triplets (*p-value*<0.05). This approach provides insights into eQTL mechanisms delineating effects on both distant-enhancers and proximal-promoters.

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P17.24D

Is the FADS1-FADS2-FADS3 gene cluster enriched with variants that interact with dietary fats in cardiometabolic traits?

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Introduction: Recent analyses in in Greenlandic Inuit identified novel genetic variants in the fatty acid desaturase gene cluster (*FADS1-FADS2-FADS3*) that are associated with with multiple metabolic and anthropometric traits.

Objective: We extended this work by testing whether these associations are driven by high polyunsaturated fat intake using a Swedish cohort.

Material and Methods: Adults from the GLACIER cohort (max N = 5,160) were genotyped with the Metabochip array. Height, body weight, fasting and 2hr-glucoses, triglycerides, and HDL- and LDL-cholesterol, total cholesterol were assessed. Dietary intakes of polyunsaturated fat acids (PUFA), n-3 fatty acids, and n-6 fatty acids were calculated from food-frequency questionnaires. We tested: 1) interactions between FADS genetic variants and dietary fat intake using linear regression models; 2)

centric joint analyses to detect overall interaction signals in FADS region; 3) haplotype blocks joint analyses to pinpoint the causing region.

Results: 1) The strongest interactions were observed between rs174570 and n-6 fatty acid intake on fasting glucose ($P_{interaction} = 0.007$); 2) Borderline significant joint and random interaction effect was emerged for *FADS* and n-3 PUFA on triglycerides ($P_{JOINT} = 0.014$, $P_{INT\_RAN} = 0.005$); 3) With n-3 PUFA intake, haplotype block analyses showed that haplotype 12 ($P_{INT\_RAN} = 0.011$), haplotype 16

($P_{INT\_RAN} = 0.010$), and haplotype 21 ($P_{INT\_RAN} = 0.011$) are the driving force of random effect interaction on triglycerides.

Conclusion: These findings support that the association between fat intake and triglycerides can be modified by genetic variants in the FADS cluster.Funding: Swedish Research Council, Swedish Heart-Lung Foundation, and the Novo Nordisk Foundation

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P17.25A

Epigenetic silencing of *FBXW7* through promotor hypermethylation in ovarian cancer

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FBXW7, is a tumor suppressor gene with a critical role in cell cycle progression regulated by E_2F_5. While *FBXW7* is silenced in several cancers; its role in ovarian cancer pathogenesis is still unclear. This study explored *FBXW7* methylation status in ovarian cancer and its functional significance. *FBXW7* mRNA and protein expression were assessed in OV2008, OVSAHO and MCAS cells by qRT-PCR and western blotting. Expression of *FBXW7* was high in MCAS and low in OV2008 and OVSAHO cells. *FBXW7* gene methylation status was analyzed in OVSAHO and in 8 EOC patients by methylation-specific PCR. Five out of eight (62.5%) patients analyzed as well as OVSAHO showed methylation. To evaluate its functional importance, *FBXW7* expression was restored in OVSAHO cells by treatment with the demethylating agent, 5′-aza-2′deoxycitidine. Western blotting in *FBXW7*-restored OVSAHO cells and *FBXW7*-positive cell line (MCAS) showed an upregulation in caspases-2, 3, 7, BAX, P53, BAD, Cyclin D1, pNFkB and pGSK3-beta, whereas Bcl-2, Bcl-x, pBad, STAT3, GSK3-beta and NFkB were downregulated; indicating cells to undergo apoptosis *via* the STAT3 pathway. Response of OV2008 and OVSAHO cells to platinum and 5-azacitidine drugs were investigated. While OVSAHO was sensitive to platinum-drugs, OV2008 was highly resistant. Interestingly, resistant OV2008 cells treated with 5-azacitidine, showed an enhanced sensitivity to platinum. This study described an alternative mechanism for *FBXW7* inactivation, namely promoter specific methylation in ovarian cancer and identified *FBXW7* as a potential target for guiding the development of therapeutic strategies against ovarian cancer.

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P17.26B

Chromosome 18p deletion is a risk factor for FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscle disorder with a prevalence of 1:8500. FSHD is associated with somatic expression of DUX4, a germline transcription factor normally repressed in somatic tissue. A copy of DUX4 is located within each unit of the D4Z4 repeat array, which adopts a repressive chromatin structure in somatic tissue. Repeat array contraction in FSHD1, or mutations in D4Z4 chromatin repressors such as SMCHD1 (FSHD2) lead to partial D4Z4 chromatin relaxation and derepression of DUX4 in skeletal muscle when occurring on FSHD-permissive chromosomes that contain a DUX4 polyadenylation signal. The SMCHD1 gene maps to chromosome 18p and is often deleted in 18p deletion (18p-) individuals together with other genes. We hypothesized that 18p- individuals harbouring a FSHD permissive 4q35 allele are at risk of FSHD because of SMCHD1 haploinsufficiency.

We studied a cohort of 18p- and control primary fibroblast cell cultures and quantified SMCHD1 mRNA and protein levels. We found that 18p- samples have significantly lower levels of SMCHD1, which is associated with decreased amounts of SMCHD1 associates and increased levels of H3K27me3 at the D4Z4 repeat, similar to FSHD2. Transdifferentiation of 18p- fibroblasts with a

permissive and moderately sized D4Z4 repeat into myotubes resulted in DUX4 and DUX4 target expression in 6 out of 8 cell cultures.

Our results show that SMCHD1 haploinsufficiency can lead to DUX4 expression through a similar mechanism as in FSHD2 and that some 18p- individuals are at risk of developing FSHD.

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P17.27C

The methylome of the inner ear: implications for regulation and pathogenesis of the auditory system

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Mechanisms of epigenetic regulation have been studied vigorously, gaining a major boost by coupling traditional molecular biology methods with high-throughput Next Generation Sequencing (NGS). The mammalian inner ear is a complex morphological structure responsible for hearing and balance, and its pathology is associated with deafness and balance disorders. To evaluate the role of epigenomic dynamics for mouse inner ear sensory epithelium development and maturation and its implications in deafness, we performed whole-genome bisulfite sequencing (WGBS) on inner ear tissue for three key time points. We identified both unmethylated (UMR) and low methylated (LMR) regions across all datasets. UMRs and LMRs have been shown to predict promoters and enhancers, respectively, allowing us to generate the first regulatory maps in the mouse inner ear sensory epithelium. We also determined differentially methylated regions (DMRs) among these three time points. Approximately 75% of the identified DMR sequences were conserved between the mouse and the human genome. These regions may include regulatory mutations associated with deafness in the human genome. Finally, we also assessed nonCG context and observed that between 5% and 12% of CHH/CHG were methylated. Our DNA methylation study is part of an integrated analysis to build a hierarchical regulatory scheme of auditory pathways including non-coding RNAs, nucleosome occupancy and histone post-translational modifications. The breakthroughs enabled by epigenomic analysis may help guide the development of therapeutics for hearing loss, by providing multiple intervention points for manipulation of the auditory system.

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P17.28D

Induction of fetal hemoglobin by modulation of epigenetic and genetic factors in beta thalassemia major patients

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Objective: Our aim is to reveal relationship between genetic variations of *KLF1* and *BCL11A* genes which are implied on HbF induction pathways, and modifying properties of epigenetic mechanisms of resveratrol and sodium butyrate on both K562 cells and primary erythroid cells in patiens with beta thalassemia major.

Method: RNA profiles of globin genes, p38, ERK1/2 and NRF2 signalling pathways are evaluated in both K562 cells and primary erythroid cells in 30 well-characterized BTM patients in which were induced with resveratrol and/or sodium butyrate. Among the factors which regulates the amount of HbF, *KLF1*whole gene, *BCL11A* rs11886868 variation and *XmnI* polymorphisms were investigated using both RFLP and Sanger Sequencing.

Results: Resveratrol and sodium butyrate caused dose-dependent hemoglobinization pattern in K562 cells. No synergistic effect was found between these two agents. Apoptotic effect was observedat high dose resveratrol in K562 cells. The potential of gamma globin induction of both resveratrol and sodium butyrate were elucidated. NRF2, ERK1/2 and p38 activation were screened in K562 cells while there was activation of p38 and inactivation of ERK1/2 pathways in primary erythroid cells of patients.

BCL11A rs11886868, *XmnI* polimorphisms and *KLF1*variations were found to be compatible with the literature.

Conclusion: Guidance on selection of pharmacological molecules in HbF induction studies, individual genetic and epigenetic factors should be taken account in patients with beta thalassemia major

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P17.29A

Targets of DNMT3b identified in Enteric Precursor Cells as candidate genes for Hirschsprung disease

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Hirschsprung disease (HSCR) is attributed to a failure of neural crest cells (NCCs) to migrate, proliferate, differentiate and/or survive in the bowel wall during embryonic Enteric Nervous System (ENS) development. ENS formation is the result from a specific gene expression pattern regulated by epigenetic events, such DNA methylation by the DNA methyltransferases (DNMTs), among other mechanisms. Specifically, DNMT3b *de novo* methyltransferase is associated with NCCs development and has been shown to be implicated in ENS formation and in HSCR. Aiming to deep into the specific gene expression pattern established by epigenetic mechanisms during these processes, we have performed a study based on the identification of DNMT3B target genes in enteric precursor cells (EPCs) from mice, through a chromatin immunoprecipitation coupled with massively parallel sequencing analysis (ChIP-seq). In addition we have applied several bioinformatic tools to determine the potential target genes. This approach led us to find 20 genes whose expression could be maintained at basal levels by DNMT3b in EPCs. These target genes may be part of the signaling pathways required for the proper ENS formation. Therefore this finding leads us to propose that a failure in their expression might contribute to the onset of HSCR.

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P17.30B

DNA methyltransferases and MBD genes expression levels in HPV-induced cervical lesions

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Human papilloma virus (HPV) is considered the aetiologic agent of cervical neoplasia and infection with high risk types HPVs was associated with cervical dysplasia and carcinogenesis. DNA methylation and histone modifications are the main mechanisms of epigenetic regulation. MBD proteins exhibit methyl-CpG-binding domains that mediate the interaction with methylated DNA. The aim of this study was to establish the influence of hrHPVs infection on MBDs, DNMTs expression pattern and 5-mC percentage in cervical oncogenesis. In this purpose DNA and RNA were isolated (TriZol) from cervical samples (159 patients and 40 control samples). Gene expression levels (MBD1,3,4 and DNMT1,3a) were investigated using qRT-PCR. $2^{-\Delta\Delta Ct}$ method was used to establish n-fold gene expression. 5-mC percentage was estimated with 5-mC DNA ELISA (Zymo Research). Significant differences were found in DNMT1 gene expression especially between CIN2+ and SCC samples ($p=0.0013$), whereas for DNMT3B gene the expression level was increased in tumor samples (mean = 2.406 ± 1.088) as compared to the precursor lesions ($p=0.0027$). A significant increase was observed for MBD1 gene between CIN2+ and SCC ($p=0.046$) samples, while MBD3 gene found a significant difference in expression levels between CIN1 and CIN2+ ($p=0.0229$). MBD4 gene showed a significantly high level of expression in tumors (mean = 2.824 ± 0.9320) ($p=0.004$). The correlation between the MBDs level of expression, and the percentage of 5-mC, showed a good connection with MBD1 levels ($r^2=0.3551$, $p=0.0056$). These results illustrate the involvement of epigenetic alterations in cervical oncogenesis and could serve as a starting point for diagnosis and prognosis.

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P17.31C

Widespread recovery of methylation at gametic imprints in hypomethylated mouse stem cells following rescue with DNMT3A2

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Introduction: Imprinted loci are paradigms of epigenetic regulation and are associated with many genetic disorders in human. A key characteristic of the imprints is the presence of a gametic differentially methylated region (gDMR) which must be passaged through the germline to be reprogrammed.

Materials and Methods: We examined the most complete current set of imprinted gDMRs that could be assessed using quantitative pyrosequencing assays in ESCs that lacked DNMT1 (1KO) and those cells lacking a combination of DNMT3A and DNMT3B (3abKO). We further verified results using clonal analysis and combined bisulfite and restriction analysis.

Results: Our results showed that loss of methylation was approximately equivalent in both cell types. 1KO cells rescued with a cDNA-expressing DNMT1 could not restore methylation at the imprinted gDMRs, confirming some previous observations. However, nearly all gDMRs were remethylated in 3abKO cells rescued with a DNMT3A2 expression construct (3abKO + 3a2). Transcriptional activity at the *H19/Igf2* locus also tracked with the methylation pattern, confirming functional reprogramming in the latter.

Conclusions: These results suggested (1) a vital role for DNMT3A/B in methylation maintenance at imprints, (2) that loss of DNMT1 and DNMT3A/B had equivalent effects, (3) that rescue with DNMT3A2 can restore imprints in these cells. This may provide a useful system in which to explore factors influencing imprint reprogramming.

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P17.32D

Detection of imprinted genes by single-cell allele specific gene expression

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Genomic imprinting results in parental-specific gene expression. Imprinted genes are involved in the etiology of rare syndromes and have been associated with common diseases such as diabetes and cancer. Standard RNA bulk cell sequencing applied to whole tissue samples has been used to detect imprinted genes in human and mouse models. However, lowly expressed genes cannot be detected by using RNA bulk approaches. Here, we report an original and robust method that combines single-cell RNAseq and whole genome sequencing into an optimized statistical framework to analyze genomic imprinting in specific cell-types and in different individuals. Using samples from the probands of 2 family trios and 3 unrelated individuals, 1084 individual primary fibroblasts were RNA sequenced, and more than 700000 informative heterozygous single nucleotide variations (SNVs) were genotyped. The allele specific coverage per gene of each SNV in each single cell was used to fit a beta-binomial distribution to model the likelihood of a gene being expressed from one and the same allele. Genes presenting a significant aggregate allelic ratio (between 0.9–1) were retained to identify of the allelic parent of origin. Our approach allowed us to validate the imprinting status of all of the known imprinted genes expressed in fibroblasts and the discovery of 9 putative imprinted genes, thus demonstrating the advantages of single-cell over bulk RNAseq to identify imprinted genes. The proposed single-cell methodology is a powerful tool for establishing a cell type-specific map of genomic imprinting.

C.B. and F.S. contributed equally to this work.

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P17.33A

IRS1 DNA promoter methylation in human adipose tissue mediates the relationship of rs2943650 near *IRS1* to fat distribution and metabolic traits

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Aims/Hypothesis: Body Fat composition measures fat distribution more precise than BMI. A SNP near the *IRS1* locus was reported that associates with decreased body fat, *IRS1* gene expression and an adverse metabolic profile in humans. We hypothesized that these effects may be mediated by epigenetics.

Methods: We measured DNA methylation within the *IRS1* promoter and mRNA expression in paired human adipose tissue samples (SAT and OVAT) from 146 and 43 individuals, respectively. Genotyping of the body fat associated variant rs2943650 near *IRS1* was performed ($N=$ 146).

Results: The total cohort shows significantly higher *IRS1* promoter DNA methylation in OVAT compared to SAT ($P=8.0\times10^{-6}$), while expression levels show the opposite effect direction ($P=0.011$). OVAT and SAT methylation is correlated to gene expression in obese subjects ($P=0.007$ and $P=0.010$). The major T-allele is related to increased DNA methylation in OVAT ($P=0.019$). Finally, DNA methylation and gene expression in OVAT correlates with clinical variables such as waist-circumference, waist-to-hip ratio (WHR) and glucose metabolism in obese individuals.

Conclusions/Interpretation: Anthropometric and metabolic variables may be influenced, in addition to and in concert with genetic factors, by changes in DNA methylation that translates into altered gene expression. Larger studies are warranted to further dissect the interplay between rs2943650, DNA methylation and gene expression of *IRS1* and its influence on metabolism.

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P17.34B

Identifying distal interactions between RUNX1 and a Juvenile Idiopathic Arthritis associated SNP by Chromosome Conformation Capture

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Background: 17 genetic loci have been identified which confer susceptibility to juvenile idiopathic arthritis (JIA). Several of these loci harbour genes involved in the Interleukin-2 (IL2*)* pathway: an important signalling cascade involved in many autoimmune diseases including JIA. Capture Hi-C data has shown several interactions occur between the non-coding, JIA susceptibility SNP rs9979383 and the haematopoiesis master regulator gene Runt-Related Transcription Factor (*RUNX1*), a crucial transcription factor involved in the regulation of IL2. **Methods:** To prioritise the most likely functional candidate SNPs and select appropriate cell types, several bioinformatics databases were interrogated. Chromosome conformation capture (3C) experiments were designed to try to validate these interactions using T-cell lines and genotype specific B-cell lines. **Results:** The highest prioritised SNP in the *RUNX1* gene region was identified as rs9979383 Stebased on transcription factors, Hi-C data and histone marks. 3C data showed significantly increased interactions between the anchor fragment containing rs9979383 and several fragments near the *RUNX1* promoter in B-cells when compared to controls, with some evidence of genotype specific interaction frequencies. In contrast no interactions could be detected at this locus in the T-cell line. **Conclusion:** The bioinformatics approach proved to be highly informative and aided the design of 3C experiments. rs9979383 may be regulating *RUNX1* expression in B-cells, with one interaction in particular revealing a long-range, 400kb interaction between the SNP fragment and the *RUNX1* promoter region fragment. Expression analysis, chromatin immunoprecipitation and CRISPR-Cas9 are now being employed to further investigate the regulatory mechanisms driving this interaction.

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P17.35C

Effects of Kaiso (binding) to the differentially methylated ICR1

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Introduction: The correct dosage of imprinted genes is important for normal development and anomalies of genomic imprinting often manifest as developmental disorders or are causative for cancer. The expression of imprinted genes is generally regulated by differentially methylated imprinting control regions (ICRs). The methylation of many ICRs is maintained by the ZFP57/KAP1/SETDB1-complex, with the exception of the ICR1 that controls the uniparental expression of IGF2 and H19.

Methods: Using EMSA and ChIP we identified ZBTB33 (Kaiso) as a factor binding to the ICR1. Lentiviral Kaiso knock-down experiments and CRISPR/Cas9 mediated deletion of the ICR1-Kaiso-bindingsite were used in combination with bisulfite-conversion and pyrosequencing to address the relevant function of Kaiso for the ICR1 methylation.

Results: We show that Kaiso binds to methylated CpGs and to an unmethylated consensus sequence in the ICR1. Reduction of the Kaiso transcript as well as the deletion of the unmethylated binding-site both show reduced methylation within the ICR1 and also effect transcription of IGF2 and H19. Other ICRs, e.g. the KvDMR on the same chromosome, showed no difference in the methylation after manipulation of Kaiso.

Conclusion: We show the binding of Kaiso to the ICR1 and proof its essential role for the methylation maintenance in the ICR1. Kaiso binding to the ICR1 also effects the expression levels of IGF2 and H19. Our work thus establishes Kaiso as a previously unrecognized indispensable factor for a specific ICR1 function in imprinted gene regulation.

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P17.36D

Mutations in the DNA-binding Domain of KLF1 lead to Autosomal Dominant Anemia via altered sequence specificity and ectopic gene expression

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The average human genome contains nearly 100 missense mutations within the DNA-binding domain (DBD) of various sequence-specific transcription factors (TFs). Despite their potential medical importance, the consequence of these mutations remains largely unknown. Missense mutations in the DBD of TFs can alter both the affinity and the specificity of DNA-binding leading to disruption of gene regulation. Here, we demonstrate how a missense mutation in the second zinc finger of Krüppel-like factor-1 (KLF1) leads to an altered DNA-binding specificity in vivo, resulting in ectopic transcription in a murine model of Congenital Dyserythropoietic Anaemia (CDA). To investigate how this mutation might cause disease, we introduced tamoxifen-inducible versions of wildtype and mutant KLF1, an essential TF required for nearly every aspect of red blood cell formation, into a $Klf1^{-/-}$ erythroid cell line. We employed ChIP-seq and 4sU-RNA-seq to identify aberrant DNA-binding events genome wide and the ectopic transcriptional consequences of this binding. We confirmed novel sequence specificity of the mutant recombinant zinc finger domain by performing biophysical measurements of in vitro DNA-binding affinity. Together, these results shed new light on the mechanisms by which missense mutations in DNA-binding domains of transcription factors can lead to autosomal dominant diseases.

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P17.37A

Long noncoding RNAs abnormally expressed in relapsed acute lymphoblastic leukemia

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Introduction: Long noncoding RNAs (lncRNAs) are emerging as important players in normal biological processes as well as pathological conditions, including cancer. In fact, several of them have been proposed as potential prognostic, poor survival, and cancer metastasis biomarkers. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide, which displays a differential incidence rate between ethnic groups. Mexican population exhibits one of the highest incidence and rate mortality due to early relapse (within the first 18 months after diagnosis). We performed a comparative transcriptome analysis to identify lncRNAs differentially expressed in ALL with relapse.

Materials and Methods: We included 60 bone marrow samples from pediatric ALL patients, of which 11 were relapsed samples. Expression analysis was performed using the Affymetrix Human Transcriptome Array 2.0 platform following the manufacturer's protocol. Annotation was carried out using Transcriptome Analysis Console software (Affymetrix). P-values <0.05 were considered as statistically significant.

Results: Regarding gender, age at diagnosis, leukocyte count, immunophenotype, risk classification and frequency of gene rearrangements (*ETV6-RUNX1, TCF3-PBX1, BCR-ABL1* and *MLL* rearrangements), no statistically significant differences between no-relapsed and relapsed groups were observed. On overage, relapse occurred 10.6 months after diagnosis and survival of this group was three months (range 0–8 months). Expression analysis showed five differentially expressed lncRNAs (fold-change >1.2 and p value <0.01) between both studied groups, being Linc00341 one of the highest abnormally expressed (p= 0.004). **Conclusions**: Our data provide evidence that lncRNAs could play a relevant role in the relapse of this hematological malignancy.

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P17.38B

Computational mapping of regulatory domains of human genes

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As a result of recent IHEC efforts, a large number of epigenome profiles became publicly-available. However, a systematic, integrative and accessible approach for discovery and documentation of genome-wide cis-regulatory relationships is still missing. In this research, we performed the most detailed data-integration analysis to associate enhancers to the genes they regulate across the human genome.

Cross-cell-types profiles of H3K27ac, H3K4me1, DNA methylation, DNase I hypersensitivity and RNA-Seq were selected from four different IHEC data-sets to quantify enhancer activity and gene expression. Long-range interactions were identified by successfully running linear regressions of gene expression and enhancer activity. Individual gene-enhancer models were meta-analyzed across consortia datasets. To confirm associations, a benchmark dataset was built based on: 1) GTEx database, 2) published chromatin interactions, and 3) massively parallel reporter assays results.

We quantified cross-cell-types enhancer activities and gene expressions based on up to 179 epigenome profiles. More than 32,000 genes we modelled successfully; whereas we meta-analyzed 21,000 genes across all consortia. We tested more than 3.7 million gene-enhancer pairs, and approximately 17,000 genes and 80,000 enhancer regions passed filtering procedure (q-value 10^{-8}). Our results confirm previously reported examples of long-range interactions, including the famous FTO-IRX3-IRX5 long-range interactions. This indicates that we should extract many novel long-range interactions from this database in the future.

This database represents the most detailed regulatory catalog in existence so far. It should empower the future functional interpretations of disease-associated variants by facilitating the precise identification of genes which are affected by variants of interest.

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P17.39C

Genetic variants associated with inflammatory disease can alter m6A RNA methylation and influence immune response

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m6A modifications are a novel RNA-based regulatory layer known to regulate the gene expression programme at a post-transcriptional level. It has been speculated that the m6A methylome can be related to immune tolerance. However, the influence of disease associated regions in the variability of the so called epitranscriptome has not been studied to date. In order to find m6A candidate regions that could be altered by the presence of genetic association of immune-mediated diseases, Immunochip regions were scrutinized for described m6A marks. One of these regions, which is associated to multiple immune diseases (i.e celiac disease, Crohn's disease or psoriasis) harbors a functionally uncharacterized lncRNA. m6A immunoprecipitacion confirmed the presence of mRNA methylation in this lncRNA in different intestinal cell lines. Moreover, when using intestinal biopsies of heterozygous individuals the methylation was preferentially found in the allele that confers risk to intestinal inflammatory diseases. Further characterization of the risk-conferring lncRNA form showed that the m6A mark containing transcript is present at lower levels in intestinal biopsies, correlating with higher expression of a closeby gene, which regulates Hsp90 function, and increased levels of the inflammatory cytokine TNFa. In the same way, induced overexpression of the risk lncRNA in intestinal cells, leads to higher expression of Hsp90 regulator, together with increased NFKB1 levels. In conclusion, we found an uncharacterized lncRNA whose levels are regulated by a genotype/tissue dependent RNA methylation and which seems to induce inflammatory gene expression by an Hsp90 linked pathway.

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P17.40D

miR-125b, miR-182 and miR-200c as prognostic biomarkers in primary cutaneous malignant melanoma and their association with clinicopathological parameters and clinical outcome

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Introduction: Metastatic dissemination causes 15–25% of melanoma deaths. It has been observed that miRNAs mediate melanoma invasion and metastasis. Here, we inquired into the association of miR-125b, miR-182 and miR-200c with clinicopathological parameters and their ability to determine patient clinical outcome at the time of primary tumor as a way to differentiate patients with higher risk to develop metastasis.

Materials and Methods: 65 fresh frozen primary human melanoma samples were analyzed. We determined miR-125b, miR-182 and miR-200c expression of these primary tumors by miRNA RT-qPCR and the results were correlated with clinicopathological parameters and the clinical outcome of patients. Correlation and contrast statistic tests were performed.

Results: In all samples, satisfactory RT-qPCR amplification curves were obtained for miR-125b, miR-182 and miR-200c. Clinicopathological parameters such as Breslow thickness and number of mitoses per mm^2 were negatively correlated with the three miRNAs expression, and there was also a relation between these miRNAs and ulceration and growth phase (radial or vertical). Clinical follow up was prolonged to 134 months. Only 16 cases (24,6%) developed distant metastasis. All three miRNAs were down-regulated in metastatic versus non-metastatic primary melanomas ($p \leq 0{,}001$) and were capable to distinguish patients with different metastatic risk.

Conclusions: miR-125b, miR-182 and miR-200c could be used as determinants of prognosis at the time of primary diagnosis due to their ability to classify patients regarding their invasive potential.

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P17.41A

Development of a robust method to study epigenetic changes in highly degraded FFPE DNA

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Biomarkers for age-related disorders such as Alzheimer´s, dementia and Parkinson's disease can be discovered by assessment of epigenetic markers in the brain. This research is hampered by the massive DNA shredding and partial nucleotide breakdown. For the first time, the methylation status of brain coupes from the Swedish Twin Registry (STR) could successfully be assessed using a novel approach.

Three cutting-edge procedures for DNA restoration prior to methylation assessment were benchmarked. The superior technique was then validated using highly deteriorated DNA from Formalin-Fixed Paraffin-Embedded (FFPE) tissue dating from 1988[(3)]. In all samples ≥98% of the epigenetic loci were detected (R^2≥0.98 for duplicates). The concordance of FFPE DNA with their 30-year old fresh frozen counterparts was excellent (R^2≥0.9), showing that the biological relevance remains intact after restoration. The most degraded samples showed the most significant improvements (R^2 increased from 0.4 to 0.9). The observed call rates could be predicted by qPCR before proceeding to array hybridization.

Epigenetic measurements were performed on frontal cortex and cerebellum of the STR cohort (49 individuals; 99 samples) using Illumina's MethylationEPIC-array. Despite the apparent deterioration of the samples (average fragment length: ~300 bp), 79 samples showed sufficient quality. Overall, sample quality was consistent within individuals. Principal component analysis showed tissue type as the main inducer of variation. The analysis now focuses on detecting epigenetic differences in the progression of dementia and Alzheimer's disease.

We here show a robust and powerful method to study epigenetic changes in previously unattainable cohorts.

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P17.42B

genome-wide DNA methylation variation in maternal and cord blood of gestational diabetes population

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Introduction: Gestational diabetes mellitus (GDM) has been a concerning issue for pregnant women. It will cause various complications such as shoulder dystocia and neonatal hypoglycemia. In recent studies, gestational diabetes was found to be related to epigenetics modification, leading to high probability of developing metabolic syndrome and diabetes later in life. This study was aimed to analyze the genome-wide DNA methylation status of patients and offspring.

Materials and methods: Maternal and cord blood were collected from 16 pregnant women and their newborns, including 8 exposed to GDM. DNA methylation was measured at > 841,573 CpG sites (Infinium HumanMethylationEPIC BeadChip). Ingenuity Pathway Analysis was conducted to identify genes and pathways epigenetically affected by GDM.

Results: We identified the top 200 loci and their corresponding genes in maternal blood group (n = 151) and cord blood group (n = 167), which were differently methylated in GDM and the unexposed group. Metabolic diseases related genes and pathways were also identified in both groups. Suggesting that GDM has epigenetic effects on both mother and their offspring, which may cause future metabolic syndrome or diabetes.

Conclusions: This high throughput platform enabled us to analyze through methylation status and identify and the most promising genes and pathways associated to gestational diabetes, which are worthy for future studies in epigenetics and its effect on GDM offspring.

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P17.43C

Investigation of methylation patterns of SFRP1, SFRP2, SHP1, SOCS3, EBF2 genes before and after treatment in acute myeloid leukemia and chronic myeloid leukemia

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Introduction: Dysregulated epigenetic mechanisms have important roles in the pathogenesis of cancer. This study designed to investigate methylation levels of SFRP1, SFRP2, SHP1, SOCS3 and EBF2 genes possible contributions to the pathogenesis of myeloid leukemia.

Methods: Methylation levels of SFRP1, SFRP2, SHP1, SOCS3 and EBF2 genes of 25 AML and 25 CML patients at initial diagnosis compared with methylation levels of the same genes after treatment and 25 healthy volunteers. Pyrosequencing method was preferred due to its advantages as giving accurate, rapid and quantitative results.

Results: In our study, mean methylation values of SFRP1, SFRP2, SHP1 and EBF2 genes in AML group at initial diagnosis have been identified significantly higher compared to healthy controls. SFRP1, SFRP2 genes methylation values were found significantly low after treatment. Mean methylation values of SHP1 and EBF2 genes in CML group at initial diagnosis was observed significantly different compared to healthy controls and only EBF2 gene methylation was changed with treatment. No statistically significantly difference was observed between AML, CML and healthy control group for mean methylation values of SOCS3 gene and no change in methylation value was determined with treatment.

Conclusion: These results may suggest that SHP1 and EBF2 genes play a role in the pathogenesis of myeloid leukemia and methylation of SFRP1, SFRP2 genes contribute the pathogenesis of AML. SHP1 gene methylation do not seen to be affected by treatment in both AML and CML, this brought to mind the idea that using demethylation agent could be useful.

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P17.44D

Trastuzumab treatment response prediction by micro-RNAs profile in HER2 positive breast cancer tumors

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Introduction: Breast cancer is the most common cancer in women worldwide, showing Her2 oncogene overexpression in 25–30% of them. Current therapies, as the humanized monoclonal antibody trastuzumab, lead to the silence of its activity. However, most of women with metastatic Her2+ breast cancer get resistance to trastuzumab within a year (acquired resistance), and even 40% of Her2+ tumors are resistant or low sensitive since the beginning of the treatment (primary resistance). Thus, the identification of trastuzumab resistance mechanisms would be of huge importance to identify those patients really sensible to trastuzumab.

Taking in account the role of miRNAs regulating several gene expressions and its implication in several cancer pathways, the objective of this work is the identification of a miRNA predictive model which determines the response to trastuzumab therapy in women with Her2+ breast cancer.

Material and Methods: The expresion of 768 miRNA by TLDA cards (Applied Biosystems) was analyzed in 37 Her2+ breast cancer core biopsies, 21 with a complete pathologic response after anthracyclines + taxanes + trastuzumab neoadyuvant treatment.

Results: Nine miRNAs with a good predictive value (AUC 0.8–0.75) were obtained. Samples without complete pathologic response behaved more homogeneously than with a complete response, showing in general overexpression of these miRNAs.

Conclusions: The great number of miRNAs analyzed and our sample size, validation studies are needed to confirm the clinical utility of this predictive model for trastuzumab treatment response.

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Rezola: None. **M.A. Viguri:** None. **I. Guerra:** None. **M. Martinez de Pancorbo:** None. **N.G. Bediaga:** None.

P17.45A

Single Cell Gene Profiling of Cell Migration

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Introduction: RNA-Seq has been a powerful method to understand sample-to-sample differences in gene expression from basic biology studies to translational biomedical research. Recently, single cell RNA-seq (scRNA-seq) is also getting increasingly adopted as a standard procedure to tease out cell-to-cell heterogeneity within biological samples, which is crucial for understanding health and disease. In this study, we first explore why scRNA-seq is important in even in simple biological samples that conventional 'bulk' RNA-seq cannot decipher.

Materials and Methods: Migration assay is initiated after multiple scratch wound is applied in adherent cells. After 12 hours, ~10,000 cells were collected from the same treatment dish to perform either bulk RNA-seq or single cell RNA-seq with molecular barcoding. Gene expression profiles obtained using different gene expression assays were compared.

Results and Conclusions: We explored the gene expression pattern as seen by bulk RNA-seq compared to scRNA-seq studies from 10,000 cell resolution. Bulk RNA-seq revealed average gene expression changes during cell migration; however, using scRNA-seq, we were able to map the dynamic gene expression patterns exhibited during cell migration. For example, we have identified genes that have similar transcripts per million (TPM) value as assessed by bulk RNA-seq, but their unique expression pattern across single cells are different. This heterogeneity in gene expression across single may reveal differences in cellular regulatory mechanisms for these mRNAs in during cell migration or other dynamic cellular phenomenon.

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P17.46B

Micro RNA 196a-5p serum levels show reverse association with severity of coronary artey disease

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Introduction: MicroRNAs (miRNAs) are relatively recently discovered cardiovascular diseases(CVD) biomarkers which have important implications for CVD early diagnosis, treatment and estimation of prognosis. It shown that miRNAs have role in macrovascular/microvascular (dys)function. We aimed to test whether miR-196a-5p has different expression levels according to severity of coronary arter disease(CAD) patients. **Methods:** In this study, 135 patients with angina or acute myocardial infarction(MI) who underwent coronary angiography were recruited and divided into 3 groups:45 normal coronary arteries(coronary lesion<50% stenosis), 45 critical stenotic(≥50%) and 45 MI (complete stenosis or thrombosis). Serum obtained from blood samples which drawn before coronary angiography. After RNA isolation and cDNA synthesis, expression of miR-196a-5p were analyzed by using Qiagen's miScript SYBR Green PCR kit and quantitative real-time PCR method performed. Statistical analysis of real-time PCR results was achieved using the $2^{-\Delta Ct}$ formula. **Results:** $2^{-\Delta Ct}$ results indicate that miR-196a-5p was significantly down-regulated in patients with MI compared to non-CAD group (fold change 0.3,p <0.05). Critical CAD group fold change was0.6 but not statistically significant compare to non-CAD group. Using miRDB target prediction tool, we identifed *HOXA5 i*s a strong candidate (target score=92)for miR-196a-5p. **Conclusions:** It is known that *HOXA5* gene and *HoxA5* transcription factor is effective in the endothelial cell shear stress response and atherosclerosis by epigenetic mechanisms and also play crucial role in antiangiogenesis. These results suggest Hox-related miR-196a-5p have reverse association with severity of CAD and further evaluation by functional gene expression study recommended. This study was funded by the Adiyaman University Scientific Research Projects Unit

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P17.47C

Involvement of miR424-5p and miR-221-3p in tumour metastasis in laryngeal squamous cell carcinoma

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Introduction: miRNAs are one of the most studied biological regulators of gene expression. The aim of our study is to evaluate miR-424-5p and miR-221-3p expressions, and to analyze both miRNAs as potential markers for metastasis in laryngeal squamous cell carcinoma (LSCC). Materials and Methods: The expression of miR-424-5p and miR-221-3p was evaluated in 26 freshly-frozen LSCC tumour and 12 metastatic samples by RT-qPCR technique. Statistical analysis was performed using SPSS17.0 software program. A p-value less than 0.05 was considered statistically significant. **Results:** We observed upregulation of miR-424-5p and miR-221-3p in 69.23% and 26.92% in tumour tissue. MannWhithey Utest showed significant higher expression levels of miR-424-5p (RQ=6.35±6.65; p=0.018) in tumour samples from patients with metastasis in comparison with those without metastasis (RQ=2.52±1.77). Wilcoxon Test showed that elevated expression levels of miR-424-5p are statistically higher (RQ=6.35±6.65; p=0.028) in tumour tissue than metastatic tissue (RQ=3.22±4.00). The ROC curve analysis of miR-424-5p showed discriminative accuracy with AUC=0.736 (p=0.05) (95%CI: 0.530–0.942) with 83.3% sensitivity and 58.3% specificity. In combination both miRNAs reach stronger discriminative accuracy with AUC=0.750 (p=0.03) (95%CI: 0.548–0.952) with 91.7% sensitivity and 58.3% specificity. miR-221-3p did not show significant data for all statistical analysis. **Conclusion:** These findings suggest that miR-424-5p may plays distinct roles in tumor progression and metastasis. The combination of miR-424-5p and miR-221-3p could be potential discriminative biomarker in biomedicine. These results may contribute to future studies on elucidating the metastatic mechanisms of contribution miRNAs to LSCC. Grant: The study was supported by the Science Fund, Medical University - Sofia (12-D/2016).

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P17.48D

Bovine miRNA Mir154-c can survive human digestion and affect human genes in epithelial cells

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Colorectal cancer (CRC) is the second most frequent human cancer with over 1.3 million new cases globally and over half a million deaths per year. The global burden of CRC is expected to increase by 60% by 2030 despite the increased preventing screening efforts. CRC is a complex disease caused by the interaction of both genetic and environmental factors with diet and in particular the high consumption of red meat (especially beef) being a risk factor for CRC initiation. The current research focused on a novel hypothesis regarding the molecular mechanisms behind the initiation and progression of CRC while also addressing a new, emerging and highly controversial scientific field entitled: "The Dietary XenomiR hypothesis" which, if proven, could have imperative biomedical impact, challenging our current knowledge on the nutrition-disease interconnection. In this proof-of-principle study we tested whether a selected ingested beef miRNA (namely bovine Mir154-c) has the potential to regulate human genes initiating or adding to the progression of colorectal cancer. Specifically, Mir154-c was selected after bioinformatic filtering and further studied for its "survival" through cooking as well as through human digestion. Mir154-c was also tested for its effect on human intestinal epithelial cells after transfection. Identification of beef miRNAs as the link between diet and CRC will have implications for prevention, risk-assessment and therapy of an increasingly frequent human cancer while add to the growing and controversial field of "cross-species regulation by dietary miRNA"; a phenomenon that could revolutionize our understanding of the effect of diet on human disease.

K.N. Felekkis: None.

P17.49A

Characterization of genetic variation in transcription binding sites nearby human genes associated with Brugada syndrome

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The genomic sequence of each human individual contains thousands of genetic variations. When underlying noncoding regions, genetic variations may disrupt transcription factor (TF) binding and alter gene expression of nearby genes, contributing to phenotypic disparity within the human population. Here, we aimed to characterize genetic variation at noncoding regions that may host TF binding events nearby genes associated with Brugada syndrome (BrS). BrS is a human cardiac disease often associated with genetic mutations in coding regions of cardiac ion channels (25–30%), but the contribution of non-coding variation remains largely unknown. We have integrated data of topological organization, chromatin accessibility, histone marks, and TF maps in human cardiac cells, and identified 1,300 genomic regions that may host binding events of cardiac TFs. We have selectively captured and extensively sequenced (x100) these regions in a cohort of n = 89 BrS patients, and using a very stringent combination of sequence mappers and variant callers, we have identified 5,388 single nucleotide variants (SNVs) and 1,196 insertions and deletions (INDELs). To investigate their potential effects on TF binding, we have generated TF binding profiles in iPSC-derived cardiomyocytes to identify the exact location of cardiac TF binding. Using a machine-learning algorithm, we have predicted the potential effects of personal combinations of variants (haplotypes) on cardiac TF binding, further supported by experimental validation in luciferase reporter assays. Together, we have identified a rich variation in noncoding variants within BrS-associated TF binding sites that may explain some cases of this cardiac disease. Funding: Agaur-fellowship (MP-A), SAF2015-70823-R (MINECO/FEDER-UE), IRG07-GA-2010-268395 (FP7-PEOPLE)

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P17.50B

Epigenetic studies of ovarian cells: understanding the aetiology and management of Polycystic Ovary Syndrome

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Polycystic Ovary syndrome (PCOS) is a common heterogeneous endocrine disorder of unknown aetiology, with more than 5% prevalence amongst women of reproductive age. Its main characteristic is the hypersecretion of androgens, predominantly of ovarian origin that in turn can cause infertility, hirsutism, psychological distress and metabolic defects, with PCOS women having an increased risk of developing type 2 diabetes and cardiovascular disease later in life. Our hypothesis is that PCOS pathogenesis may be explained by an integrated epigenetic model that in relation with environmental factors, modifies the effect of susceptibility genes and results in disease during adult life. We have conducted a genome wide epigenetic (EWAS) case-control microarray study, using the EPIC BeadChip array from Illumina, with DNA from granulosa cells (GCs) that were obtained during IVF treatment. In total, > 861K CpG sites were interrogated. Data from 32 samples (16 PCOS cases and 16 controls), were analysed with linear regression using as variables age, BMI, ovarian morphology (PCOS vs non-PCOS), cumulative dose of FSH during ovarian stimulation, number of eggs collected and type of egg maturation trigger. Ovarian morphology as linear predictor indicated 106 hits with p values less than the genome wide threshold set to $p<5\times10^{-8}$. Amongst those hits were several genes related to the PCOS phenotype, or had ovarian function or localisation. A number of these targets have been selected for further validation using Pyrosequencing. Preliminary data indicate a trend in differential methylated patterns following the EWAS analysis predictions.Funder by the Genesis Research Trust

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P17.51C

Rapid Identification of Small Molecules Affecting Stem Cell Pluripotency

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96 Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"-Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-

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Introduction: The advancement of stem cell research has lead to a promising future for regenerative medicine. High throughput screening (HTS) of small compounds allows identification of drug treatments that affect cellular maintenance and function. However, conventional HTS methods for identifying compounds of could be a laborious procedure. In this study, we have applied a high throughput 96 well RNA-seq assay as a rapid yet thorough phenotyping screen for a small compound library to identify molecules that may affect stem cell maintenance and differentiation.

Materials and Methods: The small molecule library is applied on a 96-well plate of human mesenchymal stem cells (hMSCs) grown on media that promotes pluripotency. Drug-induced hMSCs are harvested over different time points after treatment and immediately stamped onto the BD Precise Targeted 96-plates for rapid RNA-seq screening studies. Libraries from 96 treatments are completed in one day without the aid of automation.

Results and Conclusions: We were able to perform accurate gene expression profiling using Molecular Indexing on 96 drug treatments with minimal technical errors and PCR bias. We have identified compounds that affects hMSC pluripotency, which can be useful for potential protocols to drive stem cell differentiation. Moreover, this scalable screening procedure can be used to facilitate other HTS studies by reducing screening time and cost. <!--EndFragment-->

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P17.52D

Human sphingomyelin synthase genes: from structure to functions

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Introduction: Sphingomyelin synthase activity is provided by two enzymes in cell – sphingomyelin synthase 1 (SMS1) and sphingomyelin synthase 2 (SMS2). Sphingomyelin synthases are essential enzymes that are involved in the functioning of lipid metabolism in norma and pathology. They are highly conserved in mammals and encoded by paralogous genes (*SGMS1* and *SGMS2*). Previously, we have shown that the *SGMS1* human gene encodes several isoforms of mRNA as well as transcripts, which don't provide the synthesis of SMS1. Particularly, it has been found the circular RNAs (circRNAs) with brain-specific expression.

Materials and Methods: High-throughput RNA sequencing "RNA-CaptureSeq", Sanger sequencing, real-time RT-PCR, bioinformatics.

Results: Detailed analysis of transcripts of *SGMS1* gene in the human frontal cortex and blood was carried out using RNA-CaptureSeq. The recursive exons which are located in the 5'-untranslated region (5'-UTR) of *SGMS1* gene were identified. Some of them are part of the circRNAs that have predominant cytoplasmic localization and can be considered as potential cis-regulatory molecules. In contrast to *SGMS1, SGMS2* gene doesn't contain the multi-exons 5'-UTR, it encodes circRNAs composed of the coding exons of the gene. *SGMS2* gene has a low level of expression, and recursive exons are not found in their structure.

Conclusions: Sphingomyelin synthase genes have different structure and expression pattern that is important for understanding the mechanisms of physiological processes regulation in cells in norma and pathologies associated with lipid disorders.

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P17.53A

The osteogenic differentiation via conditioned medium of human dental pulp mesenchymal stem cell: an experimental study

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Aim: The aim of study was to investigate the effect of conditioned media of osteogenic differentiated cells on undifferentiated mesenchymal stem cells (MSC). Materials and Methods: hDPSCs were characterized with flow cytometry, immunostaining, differentiation assays. In study, hDPSCs were differentiated with osteogenic differentiation medium. At the end of 14 days, differentiation was assessed

by alizarin red staining, mineralization assay, and RUNX2, Osteopontin, Osteonectin gene expression level. Conditional media were collected from differentiatied cells. Collected conditional media were applied on undifferentiated hDPSCs. On days 7 and 14, β-Gal staining and mitocondrial activity were performed, The differentiation in the conditioned medium group was assessed alizarin red staining, mineralization assay and gene expression.**Results:** hDPSCs were CD90 +, CD44 +, CD105 + and CD45-, CD34- by flow cytometry and immunofluorescence staining. The CFU-F assay showed that the cells were clonogenic. In addition, these cells were identified by adipogenic, osteogenic and chondrogenically differentiated with adipo red, alizarin red, safranin O, toluidine blue-positive stains. After 14 days, in group to treatment of conditional media, minerals deposited on cells were positively stained with alizarin red and mineralization assay. Real-time PCR analysis showed that expression levels of RUNX2, Osteopontin and Osteocalcin genes were increased in cells collected at the end of 14 days after induction but not in the control group. There was statistically significant difference between the groups ($p < 0,05$). **Conclusion:** The conditioned medium obtained from osteogenic differentiated hDPSCs can create an alternative medium for inducing osteogenic differentiation of cells.

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P17.54B

Telomerase inhibiton in glioma cells: Epigenetic reflections to therapy

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The telomerase enzyme is low expressed or undetectable in normal cells, but is overexpressed in human cancer cells. Upregulation or reactivation of telomerase enzyme regulates several cellular mechanisms such as gene expression, proliferation, apoptosis, cellular metabolism and therapy resistance. It's emphasized that TERT regulates expression of several gene but interaction mechanism between target genes and TERT is still unknown. Telomerase can be inhibited by several mechanisms. BIBR1532 is the most studied telomerase inhibitor. It targets telomerase components hTR and hTERT, and inhibits cancer cell growth by shortening and decreasing functions of telomeres. We observed that BIBR1532 treatment increased apoptosis by 2.41 fold in U87MG cells compared to control. Histone deacetylases (HDACs) regulate chromosome structure and are linked to the loss of genomic integrity in cancer cells. Recent studies have demonstrated a negative correlation between HDAC gene expression and glioma grade, suggesting that HDAC4, HDAC11 might play important roles in glioma malignancy. We found that BIBR1532 treatment increased HDAC4 and HDAC11 gene expression levels 2,4975 and 3,0008, respectively. HDAC4 and HDAC11 gene expressions are downregulated in gliomas and might play pivotal roles in glioma malignancy. Lysine acetyltransferases (KATs) family member KAT6B has tumor-suppressor properties with histone H3 Lys23 acetyltransferase activity. We observed that BIBR1532 inhibitor has increased KAT6B gene expression levels by 3,3108 fold. Consequently, we thought that inhibition of telomerase enzyme by BIBR1532 regulates epigenetic pathways and it is crucial to target it for glioma treatment.

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P17.55C

Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients

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Purpose: Temple syndrome (TS14) is a rare imprinting disorder caused by aberrations at the 14q32.2 imprinted region. Here, we report comprehensive molecular and clinical findings in 32 Japanese patients with TS14.

Methods: We performed molecular studies for TS14 in 356 Japanese patients with variable phenotypes, and clinical studies in all the TS14 patients including previously reported 13 Japanese patients.

Results: We identified 19 new patients with TS14, and the total of 32 patients consisted of 23 patients with UPD (14)mat, six patients with epimutations, and three patients

with microdeletions. Clinical studies in infancy revealed both Prader-Willi syndrome (PWS)-like marked hypotonia and Silver-Russell syndrome (SRS)-compatible phenotype in 50% of patients, PWS-like hypotonia alone in 20% of patients, SRS-compatible phenotype alone in 20% of patients, and non-syndromic growth failure in the remaining 10% of patients, and those in puberty showed gonadotropin-dependent precocious puberty in 76% of patients.

Conclusion: These results suggest that TS14 is not only a genetically diagnosed entity but also a clinically recognizable disorder. Genetic testing for TS14 should be considered in patients with growth failure (and placental hypoplasia) plus both PWS-like hypotonia and SRS-compatible phenotypes in infancy, and/or precocious puberty, as well as familial history of deletion-type Kagami-Ogata syndrome.

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P17.56D

miRNA expressions of HEK-293 cell lines after Galpha (s) protein related *RIC8B* transfection

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Introduction: MicroRNAs (miRNAs) are small non-coding RNAs that regulate various cellular processes such as proliferation, differentiation, apoptosis and development. RIC8B (RIC8 Guanine Nucleotide Exchange Factor B) is a protein coding gene that plays specific role in G alpha(s) protein levels by positively regulates G(s) signalling. The aim of this study is to determine the expressions of miRNAs after RIC8B transfection.

Material and Method: We examined 16 miRNAs in cell cultures of untransfected, transfected with fluorescent protein (GFP) and GFP + RIC8B in duplicate using 1×10E6 HEK293 cells per well in 2ml of DMEM +5% FBS + 1% Pen/Strep. miRNAs expressions were analysed by real time PCR (RotorGene Q, Qiagen) using miScript miRNA primer assays and SYBR Green PCR Kits (Qiagen) according to manufacturer's instructions.

Results: mir375 and mir146a were selectively expressed after Galpha(s) protein related RIC8B transfection. mir125b and mir218 were expressed by any transfected cells with any vector (Table).

miRNA expression of HEK293 cells before and after transfection

Control	GFP	GFP + RIC8B
mir-106b-3p	mir-106b-3p	mir-106b-3p
mir-155-5p	mir-125b-3p	mir-125b-3p
mir-331-3p	mir-218-3p	mir-218-3p
	mir-331-3p	mir-155-5p
		mir-146a-3p*
		mir-375-5p*

Discussion/Conclusions: **mir375, mir146a and RIC8B has solely been related to increased inflammatory responses. Hence, mir375 and mir146a might be novel potential targets for inflammation through RIC8B related cAMP signalling.**

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P17.57A

gonadal mosaicism in grandfather leading to the transmission of severe hemophilia a in the grandson

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Gonadal mosaicism in grandfather leading to the transmission of severe hemophilia A in the grandson

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This study reports a patient affected with severe hemophilia A in which the germline mosaicism in the maternal grandfather complicate the X-linked inheritance of Hemophilia A. Pedigree analysis suggested that the proband would be a new and sporadic case. Genetic testing of the proband showed the common mutation of inversionI. There for, mother would be the obligate carrier of the mutation

which confirmed by linkage analysis and also direct investigation of the gene. Linkage analysis was performed using STR markers linked to the VIII gene. Testing four uncles revealed normal results with direct methods and also linkage analysis confirmed the obtained results. On the other hand, one of the aunts were carrier by haplotype analysis but were negative for the familial mutation. Maternal grandmother also was investigated. The analysis showed no mutation and also haplotype analyses were consistent with a normal haplotype pattern. According to the results, the only mechanism which could justify the scenario would be differential somatic or germline mosaicism of the grandfather. DNA testing may help carrier detection but negative results will not rule out the possibility of mosaicism. Gonadal mosaicism of the male individual is a rare event which usually occurs in female. This observation suggests the importance of confirming the carrier status of the family members with different approaches. The results have important implication in pedigree analysis and genetic counseling.

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P17.58B

Skewed X-inactivation is common in the general female population

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X-inactivation is a well-established dosage compensation mechanism ensuring similar X-chromosomal gene expression in both sexes. In female carriers of alleles causing X-linked recessive disorders, inactivation of the X-chromosomal copy carrying the healthy allele may cause symptoms. Predominant inactivation of either the maternal or the paternal copy of the X-chromosome in a given individual is usually considered to be driven either by chance or by the presence of a selective allele. We used blood-derived RNA-sequencing data from female offspring of trios in the Genome-of-the-Netherlands (GoNL) project to establish: 1) the parental origin for all X-linked heterozygous SNP alleles and 2) the median ratios of paternal over total read counts in an individual, at all heterozygous SNPs covered by more than ten reads. We observed that median ratios away from 0.5, indicative of skewing, are common in the population. Random X-inactivation in the 8-cell germinal stage is sufficient to explain the observed range. Our observations are consistent with those obtained with assays measuring the methylation status of the *AR* locus, but now extend these to the entire X-chromosome. Strikingly, many of the genes known to escape X-inactivation demonstrate a similar skewing as non-escapee genes, indicating that their escape from X-inactivation is not common in the blood. Collectively, our data suggest that skewed X-inactivation is common in the female population, and affects the far majority of genes. This may contribute to manifestation of symptoms in carriers of alleles causing recessive X-linked disorders. This research was financially supported by BBMRI-NL, NWO grant 184021007, 184033111.

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P17.59C

De novo truncating mutations in ZNF148, a transcriptional regulator with possible involvement in the epigenetic machinery cause global developmental delay, absent or hypoplastic corpus callosum, and dysmorphic facies (GDACCF) syndrome (OMIM # 617260)

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Introduction Mutations in components of epigenetic regulatory networks cause diverse disorders and syndromes of which some are linked to the emerging group of Mendelian disorders of the epigenetic machinery (MDEM). Krüppel-type zinc finger genes (ZNF) constitute a large gene family encoding proteins that recognize specific DNA motifs in

gene promoters. They act as transcriptional co-activators or -repressors via interaction with chromatin remodeling proteins and other transcription factors.

Methods Routine diagnostic exome sequencing data were obtained from 2,172 patients with intellectual disability and/or multiple congenital anomalies.

Results Truncating *de novo* mutations in the last exon of ZNF148 were identified in four individuals with a newly recognized syndrome characterized by global developmental delay, absent or hypoplastic corpus callosum (ACC), and dysmorphic facies (GDACCF, OMIM #617260). Other features include short stature, micro-/macrocephaly, cardiac and renal malformations. Among the target genes hypothetically linked to the phenotype are GHR, PKD1, PKD2, and CTNNB1, the latter associated with ACC. ZNF184 also interacts with histone deacetylases (HDAC) and histone acetyltransferase EP300 suggesting a possible link to the epigenetic machinery. One patient presented with features reminiscent of Floating Harbor syndrome and Rubinstein-Taybi syndrome. Pathways regulated by EP300-ZNF148 interactions may be involved in chromatin remodeling by histone deacetylation via complexing with HDAC.

Conclusion Some phenotypic features observed in individuals with ZNF184 mutations might be related to epigenetic effects. Characteristics shared with MDEM include neurodevelopmental dysfunction, abnormal growth, and variable organ involvement. Identification of a distinct phenotype in individuals with mutations in ZNF184 will help elucidating its role in neurodevelopment.

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P18 Genetic epidemiology/Population genetics/Statistical methodology and evolutionary genetics

P18.01A

A genetic overview of Atlantic coastal populations from Europe and North-West Africa based on a 17 ×-STR panel

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Introduction: The forensic use of X-STRs requires allele and haplotype frequency databases for the populations where they are going to be used. Recently, a new 17 ×-STR panel has been developed and an updated Spanish allele and haplotype frequency database has been performed. Being the only database available up to now for this new multiplex, the objective of this work was to broaden the forensic applicability of this panel to other Atlantic coastal populations since they have experienced genetic exchanges throughout history.

Materials and Methods: 513 individuals from four different populations located on the Atlantic Coast of Europe and North-West Africa, i.e. Brittany (France), Ireland, northern Portugal, and Casablanca (Morocco) were studied, and pairwise F_{ST} genetic distances between the analyzed populations and others from the Atlantic Coast were calculated.

Results: Allele and haplotype frequency databases, as well as parameters of forensic interest are presented. Our results suggest that certain nearby populations located on the European Atlantic coast could have undergone episodes of genetic interchange as they have not shown statistically significant differentiation between them. However, the autochthonous Basque Country and Brittany populations have shown distinctive allele frequency distributions between them. In contrast, the population of Casablanca showed significant differentiation with the majority of the European populations.

Conclusions: These findings seem to support that the use of independent allele and haplotype frequency databases for each population instead of a global database would be more appropriate for forensic purposes.

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P18.02B

How migration and sociopolitical institutions have shaped the genetic structure of present-day Democratic Republic of Congo

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The Democratic Republic of Congo (DRC) has been largely uncharacterised from a genetics perspective. This is despite its pivotal location in West-Central Africa; a region important for understanding the migrations of Bantu-speaking peoples and sub-Saharan African genetic diversity in general. Here we provide the first genome-wide assessment of population structure in the Kasai region of the DRC by analysing novel genotype data from 693 individuals from 27 ethnic groups. We demonstrate that population structure in the region is extremely subtle and haplotype based approaches such as chromosome painting offer greater resolution when inferring fine-scale differences between ethnic groups. Using GLOBETROTTER we detected admixture in the ancestry of multiple DRC groups at the time of a late Bantu expansion associated with metallurgy. In addition, we explored the genetic legacy of the pre-colonial political dynasty of the Kuba Kingdom which existed in Kasai from the early 17th century until the DRC became a Belgian colony in the early 1900s. By comparing patterns of haplotype sharing we demonstrate that the existence of this Kingdom seems to have acted to promote genetic diversity relative to surrounding regions outside the Kingdom. In this way, we provide a characterisation of the genetic structure of the DRC as well as a compelling example of where a socio-political system appears to have directly impacted patterns of human genetic diversity.

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P18.03C

The genetic basis of lactase persistence in ethnically diverse african populations

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In most humans, levels of lactase, an enzyme encoded by *LCT*, decrease after weaning leading to a reduced ability to digest lactose, the primary sugar found in milk, referred to as "lactase non-persistence" (LNP). However, some individuals maintain high expression of lactase and are able to digest lactose into adulthood, "lactase persistence" (LP). LP is a recent adaptive trait and has evolved in populations that practice milk production and consumption. We sequenced 555 bp in intron 13 of *MCM6* gene, a candidate enhancer region for *LCT* where LP-associated variants have been identified in European and African populations. Our sample consists of 641 individuals from 4 Tanzanian populations, 641 individuals from 18 Ethiopian populations and 650 individuals from 8 Botswana populations that practice diverse subsistence methods. In addition to genetic data, phenotypic data from a lactose tolerance test were collected in a subset of 209 Tanzanian individuals, 151 Ethiopian individuals and 50 Botswana individuals. We conducted a genotype-phenotype association analysis, tests of neutrality and we reconstructed the haplotypes network. We confirmed an association between LP and the C-14010 variant in Tanzania and for the first time we identified the C-14010 and G-13915 variants in the Hadza of Tanzania, traditionally hunter-gatherers. The G-13915 variant had not been identified in Tanzanian populations previously studied. The G-13907 variant was not found in the Tanzanians but was identified in the Ethiopians. Tests of neutrality based on EHH and iHS statistics indicated evidence for strong recent selection of these genetic variants.

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P18.04D

Immunochip meta analysis and follow up study in alopecia areata

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Background: Alopecia areata (AA) is a common hair loss disorder that affects both sexes and all age groups. AA is thought to be an autoimmune disease directed against the hair follicle. Genome-wide association studies have identified more than 10 susceptibility loci for AA; however, a large percentage of the heritable risk still awaits identification. To provide further insight into immune nature of AA, we performed an immunochip meta-analysis and are now following up the best results using a Sequenom assay to identify novel susceptibility loci.

Method: We conducted the meta-analysis combining data from our US calibrators and own sample studies on Illumina BeadChip arrays including 1,096 cases and 3,176 controls of CEU origin. Synthesis of regression slopes (MSRS) was used for the analyses which are implemented in METAINER package. We chose the most 25-promising candidate SNPs for the follow-up step. These are examined with the Sequenom MassARRAY iPLEX Platform in an independent AA sample (CEU) comprising 1,459 cases and 970 controls.

Results: The Meta-analysis identified 49 novel loci with a suggestive p-value of $P_{Becker\text{-}Wu} \leq 10^{-3}$ ($P_{Het} \geq 0.01$). Among them, *NFKB* is the most significant locus ($P_{Becker\text{-}Wu} = 1.5 \times 10^{-07}$). We considered the 19 most significant loci (lower $P\text{-}_{Becke\text{-}Wu}$ value) for the follow-up step. The experiments are ongoing and results will be presented at the meeting.

Conclusion: Despite the recent identification of susceptibility loci for AA, our understanding of the genetics of AA is incomplete. Identification of new loci may lead to a better elucidation of disease pathophysiology.

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P18.06B

GWAS meta-analysis yields novel insights into the biology of male-pattern baldness

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Male-pattern baldness (MPB) is a highly heritable trait and the most common form of hair loss among men. It is characterized by androgen-dependent, progressive loss of hair from the scalp. So far, genetic MPB research has identified 12 risk loci and has already implicated several plausible candidate genes. However, a significant fraction of the overall heritable risk still awaits identification and no conclusive data exist on how these genetic risk factors contribute to the key pathophysiological signs of MPB. Here, we carried out a large GWAS meta-analysis of MPB, comprising 10,846 early-onset cases and 11,672 controls from eight independent European cohorts. We identify 63 MPB-associated loci ($P<5\times10^{-8}$) of which 23 have not been reported previously. The 63 loci explain ~39% of the phenotypic variance in MPB and highlight novel candidate genes and pathways that are likely to be implicated in the key-pathophysiological features of MPB such as deregulation of the hair growth cycle (*FGF5*,*EBF1*,*DKK2*,adipogenesis); increased androgen sensitivity (*SRD5A2*, melatonin signalling); and transformation of pigmented terminal hair into unpigmented vellus hair (*IRF4*). In addition to confirming the involvement of well-established pathways that control hormonal status (androgen metabolism, oestrogen signalling) and hair follicle cycling (WNT-signalling, EGF-signalling), our data support the importance of less-well studied biological contexts, such as the involvement of perifollicular macrophages and adipocytes in MPB development. Moreover, our data provide molecular evidence that rather than being an isolated trait, MPB shares a biological basis with numerous other human phenotypes such as body height, skin-/hair-pigmentation, prostate cancer and cardiovascular traits.

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P18.07C

Genetic structure of the Armenian population based on genome-wide data

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Located at the crossroads of Europe and the Middle East, the Armenian Highland played a crucial role in the ancient migrations of Anatomically Modern Human (AMH). The region was a migratory pathway for the Neolithic farmers from the Near East and, relatively recently, for different tribes and ethnic groups. This may have had an impact on the genetic structure of the Armenians, who have inhabited the region over several millennia.

We applied the genome-wide SNP profiles for 31 Armenian samples and compared these with previously published data sets from the populations of Europe, the Near East, Central Asia and the North Caucasus. Furthermore, we analyzed genome-wide data of 202 ancient individuals from across Eurasia, including 17 Chalcolithic and Bronze Age samples from the eastern regions of the Armenian Highland.

The genetic makeup of Armenians mainly consists of the Near Eastern and Caucasus components, of which the former has relatively higher contribution in respect to that of the populations of the North Caucasus. On the genetic landscape, Armenians occupy an intermediate position between the Near Eastern populations, on one side, and European and Caucasian clusters, on the another side. Moreover, we detected the presence of genetic continuity between modern Armenians and ancient inhabitants of the Armenian Highland.

On the whole, the modern Armenian gene pool preserves main features of the ancestral population of the Armenian Highland, the origin of which should be attributed to at least the Chalcolithic.

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P18.08D

GENEVARASSOC and SCOREASSOC - programs to facilitate gene-based analyses of next generation sequence data

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Introduction: Whole exome or genome sequence (WES, WGS) data provides information on very large numbers of variants. We have previously described a weighted burden method of gene-based analysis which is implemented in the SCOREASSOC program. Variants which are rare and/or predicted to have a functional effect are weighted more highly than others.

Method: A program called GENEVARASSOC has been written in C++ to conveniently produce input files for SCOREASSOC from standard VCF files such as would be produced in a case-control WES or WGS study. The program automatically extracts all variants for a particular gene. It annotates them according to either inbuilt functions or using Variant Effect Predictor (VEP). User-defined expressions allow for arbitrarily complex weighting schemes and exclusion criteria to be specified according to a variety of variant features: inbuilt or VEP annotations; annotations stored externally such as SIFT and POLYPHEN results; allele frequencies in ExAC or 1KG; genotype quality scores; other characteristics of the variant. The programs were applied to a WES sample of over 11,000 schizophrenia cases and controls.

Results: All genes can be analysed within a few hours. The weighting schemes and exclusion criteria can be altered and analyses repeated so that the gene-wise p values obtained using different weighting schemes and exclusion criteria can be compared. Discussion: GENEVARASSOC provides a convenient means of setting up analyses for SCOREASSOC and provides the user with a great deal of power and flexibility. The source code, documentation and example files can be downloaded at: https://github.com/davenomiddlenamecurtis/geneVarAssoc

D. Curtis: None.

P18.09A

The causal effect of BMI on the metabolome profile in childhood

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Introduction: Numerous studies have observed strong associations between adiposity and the metabolome, however few of these studies investigated the direction of the causal pathway in these relationships. We used Mendelian randomization (MR) to investigate whether BMI has a causal effect on the metabolome in childhood.

Methods: We investigated the relationship between BMI and 160 metabolite measures in children age 7 years in the Avon Longitudinal Study of Parents and Children (n = ~5400 children). Having observed associations between BMI and several of the metabolite measures, we used a BMI allele score to perform MR analysis to assess whether our observed associations between BMI and metabolites represent a causal effect of BMI on the metabolome.

Results: We observed associations between BMI and 106 metabolite measures (using Bonferroni correction $p < 0.05/160 \approx 0.0003$). We observed associations for almost all of the VLDL concentration measures, the majority of the HDL concentration measures, and several other measures including diacylglycerol, cholines, apolipoproteins, fatty acids and amino acids. The causal effect estimates from MR analyses were mostly directionally consistent with the observational estimates, however the confidence intervals of the causal effect estimates were wide and the majority of them spanned zero.

Conclusions: We observed strong evidence of associations between BMI and several metabolite measures in 7-year-old children. The results of our MR analyses suggest that BMI may have a causal effect on some components of the metabolome, however the evidence is not conclusive.

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P18.10B

XPAT: A toolkit to conduct cross-platform association studies with heterogeneous sequencing datasets

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High-throughput sequencing data is increasingly being made available to the research community for secondary analyses, providing new opportunities for large-scale association studies. However, heterogeneity in target captures and sequencing technologies often introduce strong technological stratification biases that overwhelm subtle signals of association in studies of complex traits. Here, we

introduce the Cross-Platform Association Toolkit, XPAT, which provides a suite of tools designed to support large-scale association studies with heterogeneous sequencing datasets. XPAT includes tools to support cross-platform aware variant calling, quality control filtering, gene-based association testing, and rare variant effect size estimation. To evaluate the performance of XPAT, we conducted whole-exome case-control association studies with 783 breast cancer cases, 272 ovarian cancer cases, and 1,722 shared controls using sequencing data from multiple sources. XPAT greatly reduced Type I error inflation in the case-control analyses, while replicating many previously identified cancer-gene associations. We also show that association tests conducted with XPAT using cross-platform data have comparable performance to tests using matched platform data. XPAT enables new association studies which combine existing sequencing datasets to identify novel genetic loci associated with common diseases and other complex traits.

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P18.11C

Association of *VEGF* Polymorphisms with Breast Cancer Risk in North Indians

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Objectives: To investigate the potential association of ten promoter (-2578C/A, -2549I/D, -460T/C, -417T/C, -172C/A, -165C/T, -160C/T, -152G/A, -141A/C and -116G/A), two 5′-UTR (+405C/G, -7C/T) and one 3′-UTR (+936C/T) polymorphisms of *VEGF* with breast cancer risk in patients from Punjab, North-West India. **Methods:** The DNA samples of 250 breast cancer patients and 250 age and gender matched controls were screened for thirteen selected *VEGF* using direct PCR, PCR-RFLP and Sanger sequencing. **Results**: Of 250 breast cancer patients, 7 were males and 243 were females. About 73.6% of patients developed breast cancer after 40 years of age. There was a significant association of *VEGF* -2578AA, -2549II, -460CC, +405GG, -152AAand -116AA genotypes with increased breast cancer risk. *VEGF* -165CT and -141AC genotypes were associated with decreased risk for breast cancer. Linkage disequilibrium analysis revealed that *VEGF* -2578C/A and -2549I/D, -2578C/A and -460T/C, -2549I/D and -460T/C and -165C/T and -141A/C polymorphisms were in strong linkage disequilibrium. Haplotype analyses revealed that *VEGF* -2578A/-2549I/-460C/+405G/-7C/+936C and -417T/-172C/-165C/-160C/-152A/-141A/-116A combination was associated with increased risk while combination -2578C/-2549D/-460T/+405G/-7C/+936C was associated with decreased risk for breast cancer. Analysis using TFSEARCH software revealed that *VEGF* -2578C/A, -417T/C, -172C/A and +405C/G polymorphisms altered the binding site of specific transcription factors. **Conclusion:** In this case-control study, *VEGF* -2578C/A, -2549I/D, -460T/C, +405C/G, -165C/T, -152G/A, -141A/C and -116G/A polymorphisms were associated with susceptibility to breast cancer in patients from Punjab, North-West India.

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P18.12D

The heritable basis of gene-environment interactions in cardiometabolic traits

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Introduction: Little is known about the heritable basis of gene–environment interactions in humans. Therefore, multiple cardiometabolic traits were screened to assess the probability that they are influenced by genotype–environment interactions.

Materials and Methods: 14 established environmental risk exposures and 11 cardiometabolic traits were analysed in the VIKING study, a cohort of 16,430 Swedish adults

from 1682 extended pedigrees, using a maximum likelihood variance decomposition method in SOLAR software.

Results: All cardiometabolic traits had statistically significant heritability estimates (range: 24%-47%). Genotype–environment interactions were detected for age and sex (for the majority of traits), physical activity (for triacylglycerols, 2h glucose and DBP), smoking (for weight), alcohol intake (for weight, BMI and 2h glucose) and diet pattern (for weight, BMI, glycaemic traits and SBP). Genotype–age interactions for weight and SBP, genotype–sex interactions for BMI and triacylglycerols and genotype–alcohol intake interactions for weight remained significant after multiple test correction.

Conclusions: Statistically significant evidence for genotype-environment interaction was found for several cardiometabolic traits. Age, sex and alcohol intake are likely to be major modifiers of genetic effects for a range of cardiometabolic traits. This information may prove valuable for studies that seek to identify specific loci that modify the effects of lifestyle in cardiometabolic disease.

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P18.13A

Repeated expansion of human amylase genes create multiple independent CNV series

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Introduction: The human amylase gene family is highly copy number variable, and the salivary (*AMY1*) and pancreatic (*AMY2A* and *AMY2B*) amylase genes encode the starch-digesting enzyme expressed in the salivary gland and pancreas, respectively. High *AMY1* copy number (CN) has been shown to be correlated with adaptation to human dietary starch intake, and low *AMY1* CN reported to be a predisposition factor to obesity. Reliable measurement methods and accurate structural characterisation of the region are important to address such findings.

Methods: We used high-resolution measurement methods and segregation analysis in trios to show independent allelic series of amylase CN variants (CNV) in sub-Saharan Africans. To confirm the haplotype compositions we used fibre-FISH (Fluorescence *in situ* hybridization), which revealed some structural complexity difficult to observe using conventional methods.

Results: Our work showed rearrangements including multiple expansion of a unit containing one copy each of *AMY1*, *AMY2A* and *AMY2B*. Overall, our data demonstrated at least five independent rearrangements of pancreatic amylase genes, in which the region has undergone homologous and non-homologous rearrangements to create new haplotypes, some of which contain five copies each of the *AMY2A* and *AMY2B* genes.

Conclusions: Structural features shared by fundamentally distinct lineages strongly suggest that the common ancestral state for the human amylase cluster contained more than one, and probably three, copies of *AMY1*. The additional rearrangements we have discovered in the pancreatic amylase genes suggest that the pancreatic amylase genes should be taken into account when evaluating the adaptive significance of variation in this gene cluster.

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P18.14B

EUROlinkCAT - a HORIZON2020 study 2017–2021

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Introduction: Congenital anomalies (CA) are the leading cause of stillbirth and infant mortality and an important contributor to childhood morbidity. Over 130,000 children with CA are born in Europe each year. Their physical, mental and social handicaps are a significant burden on the health care, social and educational services. Still, comprehensive epidemiological and clinical data on morbidity, treatment, and education of these patients are lacking.

Methods: The Horizon2020 funded EUROlinkCAT project will support 21 EUROCAT registries from 13 European countries to link their data on children with CA to mortality, hospital discharge, prescription and educational databases. Each registry will send standard aggregate tables and analysis results to a Central Results Repository (CRR). Registries will use a novel sustainable e-forum, "ConnectEpeople", to link families with local/national and international registries and information resources allowing a meaningful dissemination of project outcomes.

Results: The CRR will contain standardised data and analyses on an estimated 200,000 children with a CA born 1995–2014 up to age 10, enabling hypotheses on their treatment, health and education to be investigated at the EU level. This will provide evidence to inform national treatment guidelines and screening programs, and to optimise management. The geographical differences in morbidity and mortality and an economic evaluation of the hospitalisation costs associated with CA will be presented.

Conclusion: This enhanced information on prognosis, outcome, geographical differences in morbidity and mortality at the European level will allow optimisation of personalised care and treatment decisions for children with CAs, hopefully reducing health inequalities in Europe.

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P18.15C

Common and rare copy number variants at chromosomes 3q13, 12p12 and 14q11 are associated with sporadic colorectal cancer risk

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Introduction: Multiple single nucleotide polymorphisms have been associated with colorectal cancer (CRC) risk. The role of structural or copy number variants (CNV) in CRC, however, remained unclear. Methods: We performed a genome-wide association study on 1,000 Singapore Chinese patients aged 50 or more with no family history of CRC and 1,000 ethnicity-, age-, and gender-matched healthy controls using the Affymetrix SNP 6 platform. After 16 principal component corrections, univariate and multivariate segmentations followed by association testing were performed on 1830 samples that passed quality assurance tests. **Results:** A rare copy number variant region (CNVR) at chromosome 14q11 (Odds Ratio (OR) =1.92 {95% CI 1.59–2.32}, p=2.7e-12) encompassing *CHD8*, and common CNVR at chromosomes 3q13.12 (OR=1.54 {95% CI 1.33–1.77}, p=2.9e-9) and 12p12.3 (OR=1.69 {95% CI 1.41- 2.01}, p=2.8e-9) encompassing *CD47* and *RERG*/*ARHGDIB* respectively were significantly associated with CRC risk. CNV loci were validated in an independent replication panel using an optimized copy number assay. Genome-wide expression data in matched tumors of a subset of cases demonstrated that copy number loss at *CHD8* was significantly associated with dysregulation of several genes in the *Wnt*, *TP53* and inflammatory pathways. Copy number loss at *RERG* and *ARHGDIB* were associated with up-regulation of *MUC15* and *ST8SIA1*. **Conclusions:** Rare CNV has greater effect size than common CNVs and copy number loss rather than copy number gain significantly altered expressions of genes implicated in colorectal tumorigenesis. This study was funded by National Medical Research Council Singapore.

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P18.16D

CYP2C19 is associated with platelet reactivity, but not with 1-Year major adverse cardiac and cerebrovascular events in Chinese people undergoing percutaneous coronary intervention with stenting

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Introduction: Dual antiplatelet therapy, clopidogrel combined with aspirin, is recommended for patients undergoing percutaneous coronary intervention (PCI). But, therapeutic effects affected by CYP2C19*2 and *3 remain controversial. We evaluated the association of CYP2C19 with platelet reactivity (PR) and 1-Year major adverse cardiac and cerebrovascular events (MACCE) in Chinese people.

Materials and Methods: 3410 unrelated patients undergoing PCI with stenting were recruited from March 25,

2010 to June 29, 2015, who were followed up to 1 year. Association studies were analysis by a logistic regression model, and the Cox proportional hazards model is used to analyze the survival data. PR is grouped by an ADP-maximum amplitude, a platelet aggregation rate and a combination criteria. CYP2C19 *2 and *3 are genotyped by sequencing.

Results: The PR is associated significantly with CYP2C19 *2, *3 and metabolic type (P<0.01). Patients with risk allele A in CYP2C19*3 is susceptibility to high PR (OR=1.29, 95% CI=1.14–1.47, P=0.0001). As the number of risk allele A increase in both CYP2C19 *2 and *3, PR also gradually increase which maybe cause clots (OR=1.29, 95% CI =1.14–1.46, P=0.0001). The results are replicated by the low PR grouping. No significant associations are observed between MACCE and CYP2C19*2 and *3.

Conclusions: Our researches suggest that allele A in CYP2C19*2 and *3 is a risk factor for HPR. Patients with more allele A have a higher risk of clot. But, the two SNPs are not related to the MACCE.

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P18.17A

Analysis of *CFTR* by next-generation sequencing in patients with cystic fibrosis from West Siberia

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Cystic fibrosis (CF) is a common autosomal recessive disorder caused by *CFTR* mutations. *CFTR* encodes cystic fibrosis transmembrane conductance regulator protein. We carried out the NGS analysis by "MiSeq" ("Illumina", USA) in 27 patients from West Siberia. Bioinformatic filtering of the DNA sequencing results was performed using "GeneTalk" (https://www.gene-talk.de/), "UGENE" (http://ugene.unipro.ru/), "Reporter Ion" (https://ionreporter.lifetechnologies.com/ir/), "SIFT" (http://sift.jcvi.org/) and "PolyPhen2" (genetics.bwh.harvard.edu/pph2/). Final results were verified by direct Sanger sequencing using ABI 3130 genetic analyzer ("Applied Biosystems", USA).

The most frequent mutation, *delF508*, was identified in 26% of CF chromosomes. In two patients, we detected three mutations (instead of commonly presented two mutations): *delF508/S466X/R1070Q*. According to the literature, this variant *S466X/R1070Q* is a complex allele carrying a severe disease. Five novel mutations were found: *c.T3983A (p.I1328K), c.43delC (p.L15fs), c.1580dupA (p.E527fs), c.T698G (p.L233R), c.T252A (p.Y84X)*. However, the efficiency of the CF allele detection after NGS was 76%. One of the most important NGS disadvantages is an inability to identify large deletions and insertions. For example, large mutation *CFTR21kbdel* was detected in one patient only by PCR analysis. The low efficiency of the CF allele detection may suggest the presence of the large *CFTR* deletions or insertions in patients with unknown genotype. Also, we identified polymorphic variant *V470M* with quite high frequency 55% in this group.

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P18.18B

A simulation study on power of epigenome-wide association analysis using disease-discordant twin design

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Background: Identical twin pairs discordant for a disease have been widely used in epigenome-wide association studies (EWAS) of complex diseases because the underlying genetic background is cancelled out. Although the twin design is deemed to have enriched power as compared with ordinary case-control design, its statistical power has not been well investigated.

Methods: We perform a computer simulation study on the power of the disease discordant twin design assuming that both genetic and environmental factors contribute to the liability of a disease phenotype. Power is assessed under different levels of genetic contribution or heritability for various effect sizes of environmental exposure that induce change in the level and variance of the normal-distributed M value of DNA methylation.

Results: In general, the discordant twin design outperforms ordinary case-control design especially for CpG sites with moderate effect and for diseases with heritability>0.3. The power advantage in discordant twin design becomes more obvious with increasing heritability. For diseases with no genetic background, the twin design is slightly underpowered, while for high heritability (0.5), high intrapair methylation correlation (0.5) and small effect CpG sites ($R^2 = 0.1$), sample size required for power over 0.8 is 215 for ordinary case-control design, and only 59 for discordant twin design, which is an over three folds reduction.

Conclusion: The twin design is powerful in detecting epigenetic alterations induced by environmental exposure especially for complex diseases with a strong genetic background. The use of twin design should be encouraged in EWAS using microarray and high-cost bisulfite sequencing techniques.

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P18.19C

Predicting the impact of expanded carrier screening on carrier detection rates and healthcare costs

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Introduction: Expanded carrier screening for an ever-increasing number of disorders with pan-ethnic applicability has become available. However, clinical utility, cost effectiveness, and ethical standards for this practice have yet to be fully established. We describe a simple model for predicting carrier detection rates, and some of its implications.

Materials and Methods: We developed a simple mathematical model based on binomial distribution for predicting carrier detection rates. We validated this model using the five most prevalent recessive disorders in the Ashkenazi Jewish population, then incrementally extrapolated the model to include up to ~1300 recessive diseases in a pan-ethnic population.

Results: An increase in the number of tested disorders results in a logarithmic increase in predicted carrier rates. In the Ashkenazi Jewish population, testing for only five disorders results in a carrier detection rate of 13%, but exceeds 50% when a dozen conditions are included. In an exogamous population, the rate of rise in carrier detection is slower, but continues to climb and may exceed 60% when testing for larger numbers of disorders.

Conclusions: This model is concordant with empirical carrier rates observed in both Ashkenazi Jewish and pan-ethnic populations. The carrier detection rate continues rising with increasing size of the panel. The implications for downstream demand on clinical genetics services warrants novel approaches, such as considering the couple as the testing unit, rather than the individual.

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P18.20D

Familial amyloid polyneuropathy (FAP) ATTRV30M in Portugal: *APOE* gene as a genetic modifier of age-at-onset?

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TTR Val30Met related familial amyloid polyneuropathy (FAP) is an autosomal dominant systemic amyloidosis, with a wide variation in age-at-onset (AO). An increasing number of late-onset cases has been identified; anticipation has been found to be a true biological phenomenon, as has the difference in AO according to sex. Hence, AO must be studied in the view of genetic modifiers. Several studies described *APOE* ε4 to be associated with early- and late-onset forms of Alzheimer disease, whereas ε2 allele seems to have a protective effect.

We analysed 10 coding-sequence polymorphisms in *APOE* in 299 patients (113 families), to evaluate whether functionally-significant variants have a modifier effect in AO in Portuguese families with TTR-related FAP. In statistical analysis, generalized estimating equations were used to account for non-independency of AO among relatives.

Mean AO was ≈39y, but higher in females (40.71y vs. 37.35y in men), as previously described. The TT genotype of rs7412 and CG genotype of rs267606661 were

significantly associated with earlier onset ($p<0.001$), corresponding to a decrease of ≈9 and 12 years in mean AO, respectively. No significant results were found regarding the CT genotype of rs7412 and rs429358.

The rs7412 may act as a potential genetic modifier of AO variation in TTR-related FAP, as seen in other neurological diseases. The G allele of rs267606661 is rare in European populations, in accordance to our results. Further investigation of AO variation in FAP is crucial to provide further insight into the mechanisms underlying anticipation, as the potential loss of a protective factor.

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P18.21A

What Constitute Tastes? : A Korean Twin-Family Study

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Introduction: What ones eat matters for health and diseases, still little is known about the food preferences, and most findings had been based on young children. Adults have more autonomy in food selection together with more sociocultural influences than children. We attempted to understand components of food preference and its genetic/environmental architecture.

Materials and Methods: Open questionnaires about fondness/distaste for food were taken for 1029 adults (154 identical twin pairs, MZ), with genome-wide SNP information; asking them to name particular food (up to 3 items each). All items were categorized by a committee. For finalized food categories, food frequency information (~3,500 individuals) was combined where appropriate, to estimate heritability and identify associated genetic variants (family-based GWAS GEMMA-GMMAT).

Results: Food preferences were categorized into following: 1) olfaction/flavor, 2) consistency/texture, 3) psycho-emotional/revolting, 4) particular food groups (vegetable, seafood and offal). For above categories, both preferences (liking/disliking) were found.

Food distastes that showed genetic influences were olfaction/flavors ($h2=0.27$), consistency/texture (0.45), vegetable (0.46), and seafood (0.38). Interestingly, antipathy for psycho-emotional/revolting categories showed environmental influences (no MZ correlation versus strong spouse similarity), whereas fondness was heritable (0.36). GWAS study showed that CFTR on 7q31.2 was association with fondness of offal (OR: 1.985, $P= 2.89E-08$).

Conclusion: Our findings suggest food preferences consist of composite sensory traits as well as perceptional and emotional components. Some components are heritable and probably oligogenic, while some are formulated by cultural/environmental influences, and subject to change gradually.

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P18.22B

Weighted functional linear regression models for gene-based association analysis

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The essence of all gene-based methods is to merge the information about association between the trait and genotypes of every genetic variant in a single value of statistical test for whole region. This can be achieved by either merging genotypes in collapsing-based methods, or summation of score-tests in the kernel-based methods, or construction of continuous smoothing functions in the methods based on functional data analysis (FDA). Differential weighting of genetic variants is a common practice for both collapsing- and kernel-based methods. However, until now, the weights were not incorporated into FDA models, one of the most powerful tools for gene-based association analysis.

Here we present an FDA mixed model with assigned differential weights of genetic variants. We estimate the statistical properties of the new method using independent and family data sets released by Genetic Analysis Workshop 17 in a wide range of simulation scenarios. The weight of each variant was defined in a standard way using beta distribution density function on its minor allele frequency. We show that all type I errors correspond to declared levels, while increasing the weights of causal variants improves power of weighted models. The model is included in FREGAT R-package (https://cran.r-project.org/web/packages/FREGAT/index.html) and can be used for testing the association on both independent and structured samples. Weights can be prespecified using minor allele frequencies, biological functional annotations and any other a priori information available.

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P18.23C

Gene-environment interaction test in generalized linear mixed models with family data

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Introduction: Detection of gene-environment (GE) interactions has important implications for explaining the etiology of complex diseases that are caused by a combination of genetic factors and environment. Several methods have been proposed using linear mixed models (LMM) and generalized linear mixed models (GLMM) to detect the interaction effects assuming independent observations. However, in family data, the correlation between relatives cannot be ignored. Thus, we propose a test for GE interaction in a GLMM with family data.

Materials and Methods: We used semiparametric additive mixed models to develop the GE test. The link function is expressed as the linear combination of fixed (a set of covariates, genetic markers and an interest environmental variable) and random (family and shared household) effects assuming the GE variable as a fixed and as a random effect in the GLMM. We also model this interaction by means of a non-parametrical functional relationship with the link function and by rewriting the generalized additive mixed model (GAMM) model as a GLMM to test if this functional relationship is significant. We compare this latter method with the two former ones, using simulated data assuming the environmental variable is either correlated or uncorrelated with the genetic variants (SNPs).

Results & Conclusions: Our preliminary results using simulated data demonstrate that the random variance components are very similar to the simulated values. We are in the process of analysing complete clinical and genetic information from the Baependi Heart Study which consists of 80 families with 1207 subjects.

M.D. Andrade: None. **M.L. Mazo:** None.

P18.24D

Estimating the correlation between the accuracy of simulation-based genetic risk modelling and its principal prediction parameters interrelationship

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Introduction: Genetic risk modelling using simulated datasets demonstrated to be a useful method having predictive ability comparable with empirical data. The outcome of such modelling is measured as area under the receiver-operating characteristic curve (AUC) and is ultimately defined by simulation and prediction parameters. We aimed to check whether there is correlation between modelling outcome and its principal parameters interrelationship to estimate the workability of the method.

Materials and Methods: Genotype modelling and risk prediction was based on Bayes' theorem and depended on prevalence of disorders, allele frequencies, and odds ratios (OR). These empirical parameters were retrieved from GWAS Catalog. Subsequent risk prediction was performed using c-statistics. Calculations were performed in R version 3.2.3 (the code is available at https://github.com/Olegbor/Statgen).

Results: A total of 3492 entries (associations) related to 32 disorders from GWAS Catalog were selected to be analyzed. Simulated sample consisted of 100.000 individuals. Obtained AUC values demonstrated high discriminative accuracy of genetic risk prediction: median AUC was 0.94 [interquartile range: 0.91, 0.98]. We found no correlation between prediction accuracy and principal prediction parameters interrelationship (allele frequencies and OR): Pearson's product-moment correlation coefficient was -0.06 (p-value 0.74).

Conclusions: Being based on various extrinsic parameters, genotype datasets simulation followed by genetic risk modelling present an autonomous and self-sufficient method that shows high accuracy in genetic risk prediction. This work was supported by the Russian Science Foundation (grant No. 17-15-01436)

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P18.26B

Use of a Bayesian Mixture Model (BayesR) to investigate the genetic control of complex traits

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In this study, we implemented a Bayesian mixture model to perform a Genome-wide Association analysis for nine urine phenotypes. These traits were measured on 2934 individuals from the Generation Scotland: Scottish Family Health Study genotyped at genome-wide ~700K SNPs. The Bayesian model assumes SNP effects are from a mixture of four normal distributions with different variances and was used to investigate the underlying genetic architectures and genome-wide SNP heritabilities for the urinary traits. We also assessed the performance of the BayesR model on simulated phenotypes. We report that more than 99% of the SNPs were sampled to have no effect in the analysis of the urine traits. The number of SNPs sampled to have effect varied from 620 to more than 4000 in some traits. All the traits were estimated to have a polygenic architecture with the small and moderate effects components of the mixture distribution explaining between 70% and 83% of the additive genetic variance. Estimated heritabilities were generally very low, ranging from 3.5% to 12.5%, with only urine glucose having a moderate heritability (44%). These heritability estimates were similar to estimates previously obtained from these data by a variance component analysis which ranged from 0 to 13.07%.Results from the simulation study showed that the Bayesian mixture model provided good estimates of the model parameters (genetic and error variance, overall mean and mixing proportions of SNPs) for traits with moderate to high heritability whilst it underestimated parameters of low heritability traits.

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P18.27C

The position of Bulgarian uniparental diversity in the Slavic genetic landscape

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Introduction: To date, the Bulgarian gene pool has been analyzed from many aspects, but the impact of certain ancestral populations in its formation is unknown. In order to contribute to the understanding of the Slavic component in the Bulgarian genetic make-up, we have performed an analysis of the Bulgarian uniparental genetic diversity in the Slavic context. Materials and methods: The mtDNA phylogenetic analysis was performed on 855 Bulgarian males by sequencing of the control region followed by RFLP analysis of coding markers, whereas the Y-chromosome haplogroup classification was performed on a sample comprising 808 Bulgarian males using DHPLC and RFLP analysis. The obtained frequencies of Y-chromosome and mtDNA haplogroups were analyzed in the context of Slavic speaking populations in order to determine their genetic proximity with Bulgarians. The determination of the matrilineal and patrilineal relationships was performed by Principal Component Analysis (PCA) on 23 and 33 (sub-) populations, respectively. **Results:** In the PCA of Y-chromosome haplogroup profiles, the distribution of the populations is mainly determined by the south-north cline of the first principal component. It positions Bulgarians at the one extreme close to other South Slavic speaking populations; East Slavic speaking populations on the other extreme and West Slavic speaking populations in-between. The Y-chromosome haplogroup PCA profile follows similar pattern separating South and East by West Slavic speaking populations. **Conclusion:** Similarly to previous uniparental and genomic studies, our analysis shows that Bulgarians are genetically most close with Serbs, Croats and Herzegovians and are particularly distant from East Slavic speaking populations.

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P18.28D

The migrations and barriers that shaped the Central AsianY-chromosomal pool

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Central Asia is a contact region in between all other parts of Eurasia, but its Y-chromosomal landscape is very understudied. To compensate for this bias, we first genotyped 5,295 Y-chromosomes from 64 populations representing Mongolia, Russia, Kazakhstan, Uzbekistan, Tajikistan, and Kirgizia. Analysis at the common level of phylogenetic resolution (50 Y-SNPs and 17 Y-STRs) revealed Mongol-Kazakh, Altaian-Uralic, and Persian clusters of populations. Cartographic analysis revealed that a core Central Asian Y-chromosomal pool (centered in Mongolia) spread westward as a narrow stream between the Tian-Shan and Altay mountains, and became much more pronounced in nomads from the lowlands than from the mountains. Thus mountain chains served as one important factor structuring the paternal lineage pool in Central Asia. In phase two of our study, we focused on the major Central Asian haplogroup C2 (formerly C3)-M217. We sequenced 62 Y-chromosomes (~11 Mb each) and constructed a detailed phylogenetic tree. Remarkably, six independent branches expanded simultaneously around 1,000 years ago, indicatingrapid male demographic growth on the eve of the Mongol expansion. We genotyped 1,490 M217-positive samples using 23 branch-defining SNPs discovered in our study, and reconstructed the complex picture of haplogroup C2 branch spread across Central Asia and Siberia. Finally, we observed that barriers between clans were more important for structuring the Central Asian Y-chromosomal pool than geographic barriers. This study was supported by the RSF grant 14-14-00827 (Y-chromosomal sequencing) and the Vavilov Institute for General Genetics theme 0112-2016-0006 (gene pool structure analysis).

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P18.29A

Genetic determinants of healthy ageing

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A long and healthy lifespan has a significant genetic component. It is yet unclear, whether this is due to lack of predisposing genetic variants for various diseases in healthy elderly people or whether there would be specific genetic variants that make the effect of ageing less detrimental for the carriers. We performed a genome-wide association study and a meta-analysis in participants of Finrisk (Finland), the Cohort of Swedish Men and the Estonian Genome Center of the University of Tartu (total N of 11913). We compared individuals with a long (>75 years) healthspan (N = 1323) to persons deceased under 75 years of age or to those being over 75 years old unhealthy survivors diagnosed with (at least one of following condition) CVD, diabetes, dementia, cancer, COPD, asthma, rheuma, Crohn's disease, malabsorption, kidney failure.

We found that a locus including the TRRAP gene, having histone acetyltransferase activity, in chromosome 7 was genome-wide significantly associated with healthy ageing (p=3.8×10-08). TRRAP may affect in the development of several types of cancer. Another locus close to a genome-wide significant level was found to be in close vicinity of SREK1 in chromosome 5 (p=1.1×10-07). SREK1 modulates alternative splicing by modulating the activity of other splicing factors.

Our goal is to further confirm these findings in other available cohorts with European descent. These results offer a good starting point to model and understand longevity and healthy life.

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P18.30B

Inuit in Greenland have significantly higher heritability for anthropometric traits

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Introduction: Heritability is the amount of variation that can be explained by genetic variation. Different models for estimating heritability are applied to different types of datasets, ranging from populations samples to families and twin cohorts. The resulting estimates previously published differ considerably between models, but not between ethnic populations.

Aims: In this study we firstly aim to investigate the heritability of metabolic traits in the Greenlandic population, which is an admixed, highly interrelated and small population. Secondly, we aim to compare these estimates to those of cohorts from the outbred European population.

Results: Using the GCTA-REML unadjusted model, we find systematically higher estimates of heritability in Greenlanders (N = 4724) for anthropometric traits, such as BMI (h^2=0.25, SE=0.016), but not glycemic traits, such as fasting glucose levels, when comparing with the Danish Inter99 cohort (N = 6127) ($BMI_{Inter99}$: h^2=0.11, SE=0.021). The difference does not seem to be explained by admixture, since the heritability estimate for Greenlanders with little or no European admixture (<5%) are similar to estimates for Greenlanders with a range of admixture proportions ($BMI_{Greenlanders\ with\ <5\%\ European\ admixture}$: h^2=0.33, SE=0.070). The shared environment in families does not explain the higher heritability either, since we observe comparable estimates when only including one member for each family ($BMI_{Greenlanders\ with\ <5\%\ European\ admixture\ and\ no\ families}$: h^2=0.46, SE=0.16)

Conclusion: These results suggest that the heritability of some anthropometric traits is markedly higher in Greenlanders than that found in Europeans, and that the difference cannot be explained by admixture or shared family environment.

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P18.31C

A molecular genetic anatomy of Hirschsprung disease and its risk profile

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Introduction: Hirschsprung disease (HSCR), a frequent cause of functional intestinal obstruction in neonates, is a multifactorial disorder with > 80% heritability, harboring associations with rare and common sequence variants in enteric nervous system (ENS) genes, together with monogenic and chromosomal syndromes. We quantified the total genetic burden in individual patients and their population risk profiles.

Methods: We genotyped and exome sequenced 190 European ancestry HSCR probands to identify genetic variants contributing to HSCR. We defined pathogenic alleles (PA) as common functional enhancer variants; rare nonsense, conserved missense (PhyloP>4) and canonical splice-altering single nucleotide (SNV) and INDEL coding variants; or large (>500kilobase) copy number variants (CNV). PAs, genes and loci were identified through a statistically significant excess in cases versus appropriate controls.

Results: 72% of all patients have at least one identifiable genetic risk factor with an estimated population attributable risk of ~69%; of these, 48.4% (92 cases) have a structural or regulatory deficiency in the *RET* gene that regulates an early step in ENS differentiation.

Conclusions: HSCR has major identifiable genetic components with a diversity of molecular lesions, coding and non-coding, SNV and CNV, rare and common, affecting ENS development. Individual patients have combinations of risk variants such that ~2%, 22%, 50% and 26% of probands have all 3, any 2, any 1 or none of these variant types with total risk varying ~130-fold across these classes. We demonstrate that individual patients can arise from multiple diverse common and rare risk factors, challenging assumptions about the etiology of complex disorders.

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P18.32D

Whole genome sequencing of two indigenous high altitude populations identifies adaptive genetic variants for survival in a hypobaric hypoxic environment

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Introduction: There are approximately 140 million people living at altitudes ≥ 2500 m above sea level. These individuals are exposed to a reduced atmospheric pressure. They have between 11% - 14% effective oxygen availability, instead of the 21% available at sea level. We set out to identify signals of selection in the genome that facilitate the adaptation of these populations to their hypobaric hypoxic environment. We also present an insight to the molecular mechanisms involved in the response to hypoxia.

Methods: We performed whole genome sequencing on two populations; the Quechua from the Andes and the Sherpa from the Himalaya. We modelled the demographic history of these populations and performed five tests of selection, iHS, XPEHH, Fst, diHH and DAFF. These five tests will be combined to give a composite of multiple signals score, which will identify regions of the genome that are under the selective force of hypoxia.

Discussion and Conclusion: We identify a list of candidate genes under selection in each of our cohorts. Given the molecular pathways these genes are involved in, they are likely to play a direct biological role in adaptation to high altitude. On-going work will involve the functional characterisation of these genetic adaptive variants in-vitro.

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P18.33A

Towards development of an Irish Traveller Genetic database

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Irish Travellers are an endogamous, ethnically Irish population of ~40,000. Consanguinity is common. Whilst, there is knowledge of Traveller disorders in specialised centres, knowledge gaps exist elsewhere. Most Traveller disorders are published but ethnicity is often not explicit, hampering diagnoses.

Aims: To catalogue inherited Irish Traveller disorders, publications and mutations and develop a database to facilitate diagnoses.

Methods

A literature review was undertaken. Key national and international Clinician/scientists were contacted to identify relevant disorders and publications. Laboratory and clinical databases were searched to retrieve disorders & mutations. Annotations were updated. An Excel database was established listing each disorder, its associated mutation and linked to a relevant publication.

Results

76 distinct rare genetic disorders resulting in 65 phenotypes were identified; 69/76 were autosomal recessive; 4 of these were dominant disorders but presented only in the recessive state. Six dominant disorders with no recessive phenotype were included as > one affected individual existed. One common 17q12 duplication was included, presenting in two unrelated families. Homozygous mutations were found in all recessive disorders bar one. The genetic basis of 72/76 was established. A further 2/76 have common haplotypes; the genetic basis of two disorders remains unclear.

Genetic heterogeneity was observed for eight disorders. We observed disorders in linkage disequilibrium; 4 families with McArdles disease had co-existing microcephaly, 9 individuals have co-existing Friedreich's ataxia & galactosemia.

Conclusion Our work is the first step towards cataloguing inherited Irish Traveller disorders. Future challenges

include development of an NGS panel and moving towards a population screening service.

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P18.34B

Genomic characterization of three Italian isolated populations through low-coverage whole genome sequencing

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The contribution of rare and low frequency genetic variants, as opposite to the contribution of common variants, is still largely incomplete and it has become fundamental in order to search causative variants for mendelian and complex traits.

Here we present a genomic characterization of three South European populations from the Italian Network of Genetic Isolates (INGI). Through the use of low-coverage Whole Genome Sequencing of 926 samples, we were able to identify more than 26 million variants. A fraction of them (between the 11% and the 29%) could be considered rare variants (MAF <1% and AC>1) both shared with outbred populations and private of our cohorts.

Our genomic characterization ranges from the analyses of ROH to the distribution of putatively deleterious variants, in the latter focusing on variants with CADD score over than 20 and significantly enriched in our population respect to European reference (Pvalue Fisher<0.05). As an example, we discovered that in one isolated South Italian population the TYR gene rs1126809-A allele reached the frequency of 0.37, this allele is associated with increased risk of cutaneous malignant melanoma.

Considering the geographical localization of our three isolates we argue that our data could be representative of the genetic variation of a good portion of the Italian population. We also generated a reference panel for imputation in order to improve the coverage of Southern European populations. We add ~6.9M of INGI private sites in comparison to the already available resources, providing a new resource for future GWAS studies on Southern Europeans.

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P18.35C

Investigating the Italian exome: population-specific differences in the coding genome

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Just a small portion of the human genomic variability has been explored so far, especially for rare and population-specific variants. Even within the relatively homogeneous European population, there are variants whose frequencies vary widely across different areas. This is of special interest concerning exome variants, since they allow for better interpretation of phenotypic differences between populations, especially in a clinical context. Although many variant databases exist, population-specific frequencies of exome variants are needed to distinguish real disease associations from population-specific polymorphisms.

In this study, whole-exome sequencing data of around 1700 healthy Italian individuals from almost all Italian regions were analyzed. This sample size provides enough power to detect 90% of variants with an allelic frequency of 0.01% and virtually all the variants with a frequency greater than 0.02%.

Significant differences in allele frequencies of exome variants were found between Italy and Europe and between Northern and Southern Italy as well, focusing on variants with a functional role in diseases and drug response. Several thousands of identified variants were not listed in dbSNP. As expected, most of these novel variants were rare.

However, a few high-quality variants had a frequency greater than 1%, mostly indels in non-coding regions, and were thus polymorphisms private to the Italian population. Comparing allele frequency within Italy, significant differences were found: for instance, in the HERC2 (hair and eye color), LCT (lactose intolerance) and ADH1B (ethanol metabolism) genes and in variants associated with hypertension and with methotrexate-induced toxicity.

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P18.36D

Genetic signature of ancient humans in Lakshadweep Island: A complex scenario as mainland India

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Introduction: The heritage of contemporary Indian population is well studied for the aspects of diverse ethnic, linguistic, genetic and geographical architecture. Although, many questions remain contested and unresolved for Indian ancestral population such as proximal ancestral sources, possible ways of influx and degree of admixture of the ancestral component. To address these aspects many attempts have been made by reconstructing Indian mainland and Island population history, but Lakshadweep Island (LD) population are least studied for their ancestral sources. However, with its distinct geography in the Arabian Sea (western Indian Ocean), obscure history and rigid population structure of LD drawn our interest. The knowledge of ancient ancestry of this population would be desirable to re-evaluate Indian ancestral component.

Material and Method: We determine Mitochondrial DNA (mtDNA) haplogroup (Hg) pattern in 1359 individuals from LD by employing Sanger sequencing and compared with other South Asian (SA) data set.

Results: We observed major mtDNA Hgs (R30, M30 and M35), with significantly less haplotype diversity. These clades are nested within previously reported Indian-specific mtDNA macro Hgs. Our Hg frequency-based analyses reveal that LD shared greater ancestry with Indian mainland population irrespective of geography and language.

Conclusion: Thus, inconsistent mainland affinity in LD population resemblance that LD was a central hub for ancient human admixture. Our result also indicates that Lakshadweep Islands were an important destination during ancient trading practices and it could have been served as a junction between India and other SA countries.

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P18.37A

Genetic Architecture of the longevous Amish population

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Numerous research efforts have focused on searching for 'longevity genes'. However, attempting to decipher the genetic component of the longevous phenotype have resulted in limited success. We conducted a genome-wide homozygosity analysis of the founder population of the Amish community in central Ohio. The Amish provide a large cohort of extended kinships allowing for in depth analysis via family-based approach. The present analysis integrated both phenotypic and genotypic data and led to discovery of a series of variants, distinct for stratified populations across ages and distinct for paternal and maternal cohorts. Specifically 5437 subjects were analyzed and a subset of 893 successfully genotyped individuals was used to assess CHIP heritability. We examined if homozygosity is associated with increased risk of living beyond 90. We delineated 10 significant regions of homozygosity (ROH) specific for the age group of interest (>90). Of particular interest was ROH on chromosome 13, $P < 0.0001$. Furthermore we built a classifier using the obtained SNPs from the significant ROH region with 0.945 AUC giving ability to discriminate between those living beyond to 90 years of age and beyond. The identified regions of interest via ROH analysis could be of profound importance for the understanding of genetic underpinnings of longevity. We will validate all the candidate variants in independent cohorts of centenarians, to test whether they are robustly associated with human longevity. In conclusion our results suggest that a history of longevity does indeed contribute to increasing the odds of individual longevity.

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P18.38B

Identification of likely non-pathogenic loss-of-function-variants in consanguineous families

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Consanguineous marriages could expose autosomal recessive disorders. Consanguinity not only helps to discover causative homozygous variants in families affected from autosomal recessive genetic disorders but also to identify homozygous nonsense and stop-loss variants that are likely benign. Here, we have ascertained 118 consanguineous families with several phenotypes mainly intellectual disability/developmental delay or visual impairment, originating from Arab countries and Pakistan. In the current study, we looked for (likely) non-pathogenic homozygous loss-of-function variants in order to contribute to the improvement of the diagnostic algorithms and reduce the false positive calls for pathogenicity. To identify the non-disease causing homozygous nonsense and stop-loss variants, we used a combination of homozygosity mapping and exome sequencing. At least five individuals per nuclear family were genotyped and one individual per family was sequenced (exome sequencing). We have identified 48 homozygous non-sense that did not segregate correctly with the recessive phenotype and therefore are likely tolerated. We conclude that the discovery of novel non-pathogenic homozygous loss-of-function mutations can be accelerated by analyzing large cohorts of consanguineous families, which in-turn will be beneficial for correct molecular diagnosis, and counselling of the affected families. An international, open-access database of these variants would accelerate the functional analysis of protein-coding genes, and the diagnostic capabilities of (near) Mendelian disorders.

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P18.40D

Genomic and epigenetic signatures of mosaic Y chromosome loss highlight cell cycle genes and overlap with cancer susceptibility

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The Y chromosome is frequently lost in hematopoietic cells, representing the most common somatic mutation in men. However, the mechanisms that regulate mosaic loss of chromosome Y (mLOY), and its clinical relevance, are unknown. Using both genotype array intensity data and sequence reads in up to 85,542 men, we identify 19 genomic regions ($P<5\times10\text{-}8$) and 36 differentially-methylated regions associated with mLOY. Cumulatively, these loci also predict X chromosome loss in women ($N = 96{,}123$, $P=4\times10\text{-}6$). Identified genes functionally converge on aspects of cell proliferation and cell-cycle regulation,

including DNA synthesis (NPAT), DNA damage response (ATM), mitosis (PMF1, CENPN, MAD1L1) and apoptosis (TP53). We highlight a shared genetic architecture between mLOY and cancer

susceptibility, in addition to inferring a causal effect of smoking on mLOY. Collectively, our results demonstrate that genotype array intensity data enable a measure of cell cycle efficiency at population scale, identifying genes implicated in aneuploidy, genome instability and cancer susceptibility.

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P18.41A

Molecular characterization and haplotype analysis in a large group of Mucopolysaccharidosis type IIIC (MPS IIIC) patients reveal the evolutionary history of the disease

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MPS IIIC is an autosomal recessive disorder caused by mutations in the *HGSNAT* gene. So far 64 mutations have been described altering production and/or activity of the HGSNAT enzyme and leading to accumulation of heparan sulfate in lysosomes. In order to identify the origin and evolution of MPS IIIC in human populations we analyzed all exons of *HGSNAT* as well its flanking sequences, a 3′ UTR and two extragenic sequences in 69 MPS IIIC index cases from 20 countries, the largest group studied so far. The haplotypes, defined by polymorphic SNPs, were inferred by PHASE. Amongst all patients, 20 were new cases without previous molecular characterization. Six novel mutations (one deletion, one insertion, one nonsense and three missense) were detected in these patients. Functional studies confirmed that HGSNAT-p.G173D, p.N258I and p.G423W mutant proteins had no enzymatic activity. HGSNAT-p.N258I also lacked lysosomal proteolytic processing suggesting that the mutation interferes with its folding and targeting. Of a total of 44 mutations identified, 19 were shared by more than two populations. Haplotype analysis allowed to suggest the same origin for mutations observed in different populations, and those with potential founder effect such as the novel mutations c.773A>T (p. N258I) and c.164T>T (p.L55X) that represent 58.3% and 25%, respectively, of the mutant alleles in the Paraiba state of Brazil. In conclusion, haplotype results combined with geographic and historical data allowed to get insight into the migration roots and founder effects for a number of *HGSNAT* mutations and helped to reveal the history of MPS IIIC.

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P18.42B

Association between mitochondrial DNA copy-number in leukocytes with plasma lactate levels after glucose loads in non-diabetic women

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Introduction: Increased lactatemia occurs after glucose loads, in a magnitude that is possibly linked to reduced mitochondrial respiratory capacity. Additionally, both plasma lactate and mitochondrial DNA (mtDNA) copy-number have been proposed as biomarkers of aerobic oxidative capacity and insulin resistance. The aim of this study

was to evaluate the association between mtDNA copy-number, measured in DNA from leukocytes, with circulating plasma lactate levels after oral or intravenous glucose loads in non-diabetic women.

Subjects and Methods: 69 Chilean non-diabetic women (age: 26.8 ± 6.3 years; BMI: 23.9 ± 3.5 kg/m^2) were recruited. mtDNA copy-number was determined by quantitative PCR (qPCR) and digital-droplet PCR (ddPCR) techniques in DNA from leukocytes, using the same set of primers. Serial measurements of plasma lactate, glucose and insulin were obtained during a 2-hour Oral Glucose Tolerance Test (OGTT) (75 g. glucose) and from 50-minute Abbreviated Minimal-Model Intravenous Glucose Tolerance Test (MM-IVGTT) (0.3 g/kg). We calculated the Area Under the Curve for lactatemia during OGTT (AUCL) and the difference in plasma lactate levels at 50 versus 10 minutes during the MM-IVGTT (IVL50-10).

Results: mtDNA copy-number measured with qPCR and ddPCR showed strong association (rho=0.52; p<0.0001) with moderate concordance. We found a significant direct association between the mtDNA copy number and plasmatic lactate levels both for AUCL (rho=0.33; p=0.02) and IVL50-10 (rho=0.32; p=0.03).

Conclusion: This research support the hypothesis that increased mtDNA copy-number in leukocytes may represent a biomarker of reduced systemic aerobic oxidative capacity given its direct association with plasma lactate levels after glucose loads. FONDECYT 1150416.

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P18.43C

Ancient Out-of-Africa mitochondrial DNA variants associate with distinct mitochondrial gene expression patterns

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Mitochondrial DNA (mtDNA) variants have been traditionally used as markers to trace ancient population migrations. Although experiments relying on model organisms and cytoplasmic hybrids, as well as disease association studies, have served to underline the functionality of certain mtDNA SNPs, only little is known of the regulatory impact of ancient mtDNA variants, especially in terms of gene expression. By analyzing RNA-seq data of 454 lymphoblast cell lines from the 1000 Genomes Project, we found that mtDNA variants defining the most common African genetic background, the L haplogroup, exhibit a distinct overall mtDNA gene expression pattern, which was independent of mtDNA copy numbers. Secondly, intra-population analysis revealed subtle, yet significant, expression differences in four tRNA genes. Strikingly, the more prominent African mtDNA gene expression pattern best correlated with the expression of nuclear DNA-encoded RNA-binding proteins, and with SNPs within the mitochondrial RNA-binding proteins PTCD1 and MRPS7. Our results thus support the concept of an ancient regulatory transition of mtDNA-encoded genes as humans left Africa to populate the rest of the world.

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P18.45A

Prediction of pleiotropic genes using a multilocus approach

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Introduction: Genome wide association studies (GWASs) used to search for genetic variants associated with quantitative traits. Pleiotropic effect of genes may cause the observed correlations among different phenotypes. The main aims were 1) investigate if the multilocus model was useful for detecting pleiotropic genes using a simulated data set and to investigate pleiotropic genes for testis weight and testis gene expression levels in house mouse. **Material and Methods:** The pedigree included 4100 individuals for 3 simulated quantitative traits (Usai et al., 2014). The genome consisted of 10000 Single Nucleotide Polymorphisms (SNPs) distributed over 5 chromosomes. The mice data consists of 179 males from house mouse (Turner and Harr, 2014). **Results:** We assumed different genetic architectures for the QTLMAS phenotypes. The posterior mean number of major SNPs were 51, 73 and 200 for 3 traits respectively. To identify fertility related pleiotropic genomic regions in 179 male mice: we analyzed relative testis weight and genome wide testis gene expression patterns using multilocus model. The first principal component (PC) account for highest explanatory proportion (14 %) of the data. And each succeeding PCs predicted very small sizes of SNPs effects. Multilocus model detected 50 and 53 major SNP effects using first and the second PCs. **Conclusion:**

We presented a multilocus model to detect pleiotropic SNPs. These findings show that assumption for different pleiotropic SNPs effects sizes lead to better genomic predictions compared with the single SNP approach. The study was supported by Akdeniz University Scientific Research Projects Unit, project number: FBA-2015-1117.

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P18.47C

Reconstructing the origin of Csango people on a genome-wide basis

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Csangos are a closed ethnic group living mostly in Moldavia, Romania, counting approximately 230,000 individuals. Their traditional language is Csango, an old Hungarian dialect, which recently became severely endangered. Their culture has remarkable connections to Hungarian culture, yet their origins are still heavily disputed by Hungarian and Romanian sources. Theories regarding their origin were established since the 19th century. This study concentrates on relationship of Csangos, Romanians and Hungarians to investigate the origin of Csango people.

Here we analyzed genome-wide SNP array data from Csango individuals (n = 30), genotyped on Affymetrix 1M chip. We used datasets at our disposal to represent distinct Eurasian populations. Ancestry estimating and clustering software based on statistical (ADMIXTURE1.22, FineSTRUCTURE2) and algorithmic (EIGENSTRAT6.01) methods were applied. IBD analyses were carried out using BEAGLE4.1 for supporting purposes and to attempt specifying the sources of Csango ancestry.

Results show that investigated populations have strong relationship with East European populations. Csangos are closer to Hungarians, since average pairwise IBD sharings were 3.36Mb with Hungarians and 2.63Mb with Romanians. Latter is close to sharing between Hungarians and Romanians (2.59Mb). East-Central European populations have strong relationship with Turkic people, which is more significant in case of Hungarians. However, Csangos show even higher relationship with Turkic people from the Russian area, which distinguishes them also from the major populations of the region.

Our ancestry analyses of Csangos indicates their highest relationship to Hungarians. They show also a significant common history with nomadic Turkic people who are nowadays settled in the territory of Russia.

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P18.48D

Sequence variants associating with serum protein levels in icelanders

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Introduction: Genome-wide association studies have focused largely on the effect of sequence variants on clinical phenotypes. It remains necessary to bridge the gap between diversity of genotypes and normal phenotypes in order to shed light on pathogenesis of diseases. We attempt this by studying the effects of sequence variants on serum protein levels.

Materials and Methods: We performed a genome-wide association study searching for sequence variants associating with serum protein levels (pQTL). A total of 1,310 proteins were assayed using the SOMAmer proteomics platform (SomaLogic Inc.) in 294 Icelanders. We performed conditional analysis on sequence variants in a systematic manner in order to separate distinct signals from each other.

Results: The study yielded 228 independent associations with a total of 204 proteins. Of these signals, 138 are located within 1Mb of the gene encoding the protein in question (cis signals) and 90 are located more than 1Mb away from the encoding gene, or on another chromosome (trans). Of note, the missense Thr400Met in *VTN* (MAF = 49.3%) associates significantly with levels of 24 proteins (including vitronectin itself). The allele associating with decreased levels for 20 of these proteins is also reported to associate with a decreased risk of age-related macular degeneration. In addition, using our own and public data we assessed the relationship of pQTL with expression (eQTL).

Conclusion: We detect a large number of highly significant distinct signals, shedding light on the genetics of the human proteome. These data have the potential to provide insight into human pathophysiology.

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P18.49A

Polygenic analysis of genome-wide SNP data identifies common variants on allergic rhinitis

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Background: Allergic Rhinitis (AR) is a complex disorder that affects many people around the world. There is a high genetic contribution to the development of the AR, as twins and family studies have estimated heritability of more than 33%. Due to the complex nature of the disease, single SNP analysis has limited power in identifying the genetic variations for AR. We combined genome-wide association analysis (GWAS) with polygenic risk score (PRS) in exploring the genetic basis underlying the disease.

Methods: We collected clinical data on 631 Danish subjects with AR cases consisting of 434 sibling pairs and unrelated individuals and control subjects of 197 unrelated individuals. SNP genotyping was done by Affymetrix Genome-Wide Human SNP Array 5.0. SNP imputation was performed using "IMPUTE2". Using additive effect model, GWAS was conducted in discovery sample, the genotypes and their effect sizes were used to calculate PRS in the testing sample. Gene-based test and biological pathway analysis were performed using "VEGAS2" software.

Results: We found 56 suggestive significant SNPs from GWAS ($p < 10^{-5}$). A significant PRS ($p=0.0016$) was defined by 745 SNPs (P-value cut-off <0.0005) which explains 3% of the variation in AR. Furthermore, we found 1195 significant genes ($p<0.05$) enriched by their relevant SNPs. Biological pathway analysis identified multiple significant gene-sets with the top-most of them implicated in signaling pathways.

Conclusion: Our polygenic analysis identified significant common SNPs underlying AR. Gene-based and biological pathway analysis revealed significant and meaningful pathways implicated in AR.

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P18.50B

Estimating the number of patients with a paediatric onset Rare Disease seen by a single National Genetics centre born in a single year

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Objective: To derive a proxy estimate the number of childhood onset rare diseases through referrals to the country's only Genetics centre, as the Republic of Ireland does not have a centralized rare disease registry.

Methods: A retrospective review of referrals to cytogenetics and clinical genetics for the years 2000–2016, for patients born in the year 2000, was undertaken. Anonymized data was catalogued into rare, common, normal, likely rare & unclassifiable diagnoses by review of records, and assigned Orphacodes based on diagnosis. Census livebirth data was used as the denominator.

Results: 54,789 livebirths were recorded by the census in 2000. 1872 referrals to Genetics (representing 1749 individuals) were retrieved for review. 1006 had cytogenetics testing only, of which 51 had rare chromosomal anomalies. Review of 743 referrals to clinical genetics identified 541 with a rare disease (73%), and 9 with a likely rare disease. Of the 53/1749 who had died (3%), 51 had a rare disease, with congenital malformations (24) the most common cause. A further 89 had trisomy 21, which is not rare in Ireland.

Conclusion: A total of at least 592 rare disease patients presented by age 16 in this cohort, giving a minimum incidence of 1.1% for paediatric rare diseases. Including the Trisomy 21 and likely rare cases, the incidence of 1.3% is still less than international estimates, but represents a first attempt to estimate numbers of Irish rare disease patients. Clearly, extending the study to include other rare disease sources (metabolics, molecular genetics) would improve ascertainment.

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P18.52D

Rare variant association tests for pleiotropy in longitudinal family studies

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Abundant pleiotropy has been observed in many complex traits or disease. When pleiotropy exists, testing rare variants for multiple phenotypes simultaneously is often more powerful than for single phenotype through borrowing additional information from cross-phenotype correlation. Likewise, identifying rare variants associated with repeated phenotypic measurements in longitudinal studies can also have greater statistical power than that in a cross-sectional study. On the other hand, functional rare variants are often enriched in family-based designs. Longitudinal family-based designs therefore provide valuable opportunities to increase statistical power on identifying pleiotropic rare variants associated with multiple phenotypes. In addition, identification of pleiotropic variants is helpful for elucidating shared pathogenesis of multiple phenotypes. However, statistical tests for pleiotropic rare variants detection in longitudinal family studies remain fairly limited. We extended pedigree-based burden and kernel association tests to longitudinal studies with multiple phenotypes. Generalized estimating equation (GEE) approaches were used to account for the correlations from multiple phenotypes at individual time points as well as the complex correlations between repeated measures of the same phenotype (serial correlations) and between individuals within the same family (familial correlations). Extensive simulation studies were conducted to evaluate performance of the proposed tests under various configurations. The proposed tests were illustrated by a real data example. Both simulation study and data example suggested that incorporating multiple phenotypes can increase statistical power of the proposed tests on rare variant detection. (This study has been supported by grants from Ministry of Science and Technology (MOST105-2314-B-400-017) and National Health Research Institutes in Taiwan (PH-104~106-PP-04).)

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P18.53A

A correction for sample overlap in GWAS in a polygenic pleiotropy-informed framework

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Backgroud: There is considerable evidence that many complex traits have a partially shared genetic basis, termed pleiotropy. It is therefore useful to consider integrating genome-wide association study (GWAS) data across several traits, usually at the summary statistic level. A major practical challenge arises when these GWAS have overlapping subjects. This is particularly an issue when estimating pleiotropy using methods that condition the significance of one trait on the

signficance of a second, such as the covariate-modulated false discovery rate (cmfdr).

Methods and Principle Findings: We propose a method for correcting for sample overlap at the summary statistic level. We quantify the expected amount of spurious correlation between the summary statistics from two GWAS due to sample overlap, and use this estimated correlation in a simple linear correction that adjusts the joint distribution of test statistics from the two GWAS. The correction is appropriate for GWAS with case-control or quantitative outcomes. Our simulations and data example show that without correcting for sample overlap, the cmfdr is not properly controlled, leading to an excessive number of false discoveries and an excessive false discovery proportion. Our correction for sample overlap is effective in that it restores proper control of the false discovery rate, at very little loss in power.

Conclusion: With our proposed correction, it is possible to integrate GWAS summary statistics with overlapping samples in a statistical framework that is dependent on the joint distribution of the two GWAS.

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P18.54B

Significance of secondary genetic findings in a large prospective population sample

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Introduction: Genetic studies are encouraged to return secondary findings from clinically important genes to the study participants. The rare but highly penetrant mutations in these genes typically lead to serious consequences, such as cancer or serious heart dysrhythmia, but there are known medical interventions with potential life-saving value. Although classified as pathogenic in web-based databases, the impact of most risk variants at a population level remains unclear.

Material and Methods: We studied variation in the 59 genes of the American College of Medical Genetics and Genomics (ACMG) secondary findings recommendation. Impact of the risk variants on clinical outcomes was evaluated in 25–74 year old FINRISK study participants (N~23 000) using up to 23 years of follow-up. The variants, genotyped with genome-wide microarrays and imputed using population specific reference panel, were analyzed in time-to-event and logistic regression models.

Results: The expected number of risk allele carriers for 75 ACMG risk variants varied between 4 and 300 based on publicly known allele frequencies and similar numbers were seen in FINRISK. Although some variants associated with increased risk of an event during follow up (e.g. carriers of a risk variant in a cardiomyopathy associated gene had a 3.7 times higher risk of heart failure compared to non-carriers in Cox survival analysis ($p=0.0014$)), for a large majority we observed no clinical significance.

Conclusions: Before secondary findings from clinically important genes are returned to study participants, the significance of the variants needs to be carefully evaluated at a population level.

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P18.55C

Genetic signals of selection in Native and admixed populations of Latin America

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The peopling of the Americas represents the last major expansion of human populations worldwide. As the first humans moved into the continent they were exposed to multiple new environments requiring them to adapt. The subsequent colonization of the continent by Europeans, along with the African slave trade, involved a major admixture process that was accompanied by new selective pressures, most notably exposure to new pathogens and disease.

In this study we examine how these major events have shaped the genetic landscape of present day inhabitants of the Americas. Using genome-wide data from over 200 Native Americans genotyped at over 700,000 autosomal SNPs, we test for signatures of positive selection using a suite of haplotype and population differentiation-based methods. Our top candidate regions of selection include genes associated with metabolic traits, highlighting a possible adaptation to dietary changes.

Taking advantage of the complex admixture history of Latin America, we also explore the distribution of Native American, European and African ancestry across the genome of 5 different admixed populations in Latin America. We test for regions of the genome with inferred ancestry

significantly different from the genome-wide average, to identify regions under post-colonial selection. Using this method, we demonstrate that the HLA region, largely involved in the immune response, shows the highest deviation towards African ancestry across all populations tested. This result suggests a major widespread selective pressure throughout the continent likely in response to infectious diseases introduced by the arrival of Europeans.

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P18.56D

The Human Variome Project Global Globin 2020 Challenge and the ITHANET Portal: pioneering global epidemiological data collection and removing the diagnostic divide

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Haemoglobinopathies are severe monogenic diseases with growing worldwide distribution and significant health-economic impact. Their prevalence in low- and middle-income countries (LMICs) poses an apparently insurmountable challenge to many affected communities through the frequent absence or inadequacy of three key factors: a) an infrastructure for safe and universal treatment of haemoglobinopathy patients, b) a system for carrier screening and disease prevention and c) molecular epidemiological information. Even approximate provision of the latter allows data-guided policies to address all three factors and has thus historically proven to contribute to tremendous savings in health expenditure, disease control and prevention of suffering.

The **Global Globin 2020 Challenge** (www.humanvariomeproject.org/gg2020), initiated by the Human Variome Project, has set as its goal to achieve the comprehensive collection and sharing of variant data in LMICs and to use this information towards more effective disease prevention and management in LMICs. Shared database development and strategic support of screening technology in partner countries will serve as key instruments in achieving these objectives. GG2020 already comprises clinicians, geneticists and bioinformaticians from over 40 countries and in close partnership with the **ITHANET Portal** (www.ithanet.eu) has helped compile an unprecedented resource of national and regional etymological and health care information on IthaMaps (www.ithanet.eu/db/ithamaps). The critical mass of expertise, liaison with ClinGen and envisaged screening activities of GG2020 will further add to variants and their classification in IthaGenes (www.ithanet.eu/db/ithagenes), which with 2590 fully annotated mutations in over 200 genes, regulatory sequences and intergenic regions is already the largest disease-specific mutation database for haemoglobinopathies.

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P18.57A

Circadian genes and differentiated thyroid cancer risk

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Differentiated thyroid carcinoma (DTC) is the most common malignancy of the human endocrine system and its incidence is 3–4 times higher in women than in men. DTC has a familial relative risk higher than others cancers, suggesting a contribution of inherited factors to the disease. However, the susceptibility variants identified to date only account for a small part of genetic predisposition in DTC risk. It has been hypothesized that clock genes may affect cancer susceptibility by impacting on the biological pathways that regulate DNA damage and repair, carcinogen metabolism and/or detoxification and cell-cycle.

We investigated the role of circadian clock gene polymorphisms in DTC risk in women from a population-based

case-control study conducted in France including 463 DTC cases and 482 controls. We estimated thyroid cancer risk associated with each of the 570 SNPs in 23 circadian clock genes. We also used a gene- and pathway-based approach to investigate the overall effect on DTC of circadian clock gene variants that might not be detected in analyses based on individual SNPs. Interactions with suspected risk factors of DTC such as BMI, reproductive factors, alcohol drinking and smoking status were tested at the SNP, gene, and pathway levels.

Overall, we reported no main genetic association at the SNP, gene or pathway level with DTC risk. There was some evidence of an interaction between *PER3* or *RORC* and smoking status in DTC. Our results suggest that circadian clock gene variants may modulate the association between smoking status and DTC risk.

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P18.58B

Annotation of genetic variation within Genome Russia project

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Recent years have seen a large number of national genomic projects highlighting the diversity and similarity of world populations, providing insight into population history and aiding in medical genomics applications. In spite of being the largest country in the world, until recently Russia has not participated in large sequencing projects. To overcome this, Genome Russia project was initiated aiming to generate whole genome sequences of more than 2,000 samples coming from diverse populations throughout Russia. General objectives of Genome Russia include characterization of known and novel genetic variation of different Russian population groups and ethnic minorities; creating a reference database of genetic variation within Russia that can be further used for medical studies; investigating population history and admixture; identifying genetic variants that may affect the frequency of known diseases in Russian populations. Here we present the results obtained on the first batch of 32 samples representing three population groups coming from Novgorod region, Pskov region and Yakutia. We identified more than 4 million SNPs and more than 120,000 short indels in our data. We combined our SNV results with the same mined from 206 recently released Russian genomes samples (Pagani et al. 2016; Mallick et al. 2016) in order to increase the sample size. We annotated the identified variants and investigated the predicted effect of known and novel variants and compared minor allele frequencies with those of populations represented in 1000G data.

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P18.59C

The SweGen project: building a whole-genome reference dataset for the Swedish population

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We are undertaking the largest human whole-genome sequencing (WGS) project in Sweden to date; the generation of a reference database that reflects the genetic structure of the population. 1000 individuals were selected for this project, sampled to cover the genetic variation within Sweden based on results from PCA analysis of available SNP array data. All of these 1000 individuals have been subjected to short-read WGS using Illumina technology at 30X coverage. Two individuals were used to generate high-quality de novo reference genomes for the population.

Assembly of 75X coverage of PacBio data for each of the samples resulted in N50 values of 8.3 and 8.9 Mb, and BioNano optical genome maps increased the N50 to over 50 Mb. Our two Swedish *de novo* genomes are now being used to study population-specific structural variation in some of the regions in the human genome that are difficult to resolve using Illumina technology.

A principal aim of this project is to establish a reference dataset of genetic variants in the Swedish population for use in disease association studies and as a look-up resource for the evaluation genetic variants identified in sequencing of patients. It will also provide a unique dataset for research on the structure of genetic variation within Sweden, as well as for international collaborative projects. The SweGen variant frequency data is made available from the following website: *swefreq.nbis.se*

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P19 Genetic counselling/Education/public services

P19.01A

Reconciling non-directivity and the counselors' preference in prenatal counseling

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Background: In the Netherlands, non-invasive prenatal testing (NIPT) is offered next to prenatal diagnosis (PND) to pregnant women at increased risk of common aneuploidies. Although non-directivity is the guiding principle in prenatal counseling, counselors might inadvertently prefer one option over the other, which may impact reproductive autonomy and/or lead to unequal access to care. This study explored to which extent counselor preference for NIPT/PND affected patients' choice, whether patients were aware of counselor preference and whether there were differences in knowledge scores.

Materials and Methods: 327 women were eligible of whom 158 women were included in the study; 82% opted for NIPT and 18% chose PND. We assessed patients' knowledge scores and perception of counselor test preference. Counselors were either senior obstetricians (SO;1–3) or a senior nurse (SN) (SO1:57; SO2:71; SO3: 23; and SN:23 patients). Counselors participated in a semi-structured interview about the content of their counseling and their own NIPT/PND preferences.

Results: Significant differences in NIPT/PND ratios were observed between counselors, with the largest difference between patients from SO2 and SO3 (38% PND versus 7% respectively). Patients were aware of counselors' preference, and patients' choices were consistent with these preferences, as established through interview analysis. Knowledge scores were equal, however, chi-square tests revealed that counselor preference was associated with more patient knowledge of NIPT or PND.

Conclusion: Patients displayed a tendency to choose in accordance with the personal preference of their counselor. These results are more in line with the paradigm of shared decision-making, than the paradigm of non-directivity.

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P19.02B

Pregnant genetic counselors in the era of advanced genomic tests: What do the experts test prenatally?

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Purpose: Advanced genomic tests in pregnancy, such as chromosomal-microarray-analysis (CMA), provide higher detection rates, yet often produce probabilistic and uncertain information.

This study aimed to understand how knowledgeable patients, i.e., pregnant genetic counsellors, act during their own pregnancies, and by so doing to gain insight into the contribution of patient's knowledge to the diagnostic experience.

Methods: In-depth interviews were conducted with seventeen Israeli genetic counsellors, either pregnant or up to two-years post-pregnancy.

Findings: One third of the participants chose not to have CMA, and two-thirds underwent CMA, even though they had no abnormal-findings during pregnancy. Although knowledge was the main motivation for genomic testing, counsellors varied in the desired degree of knowledge. Two-thirds of those opting for CMA wished to be informed of all findings identified via CMA. About a third asked for a targeted-platform, whereby only large deletions/duplications and those associated with known syndromes are disclosed, wishing to avoid uncertain results.

Being knowledgeable was described as mostly empowering and desired both by counselors opting for CMA, and those avoiding it. Nevertheless, counsellors acknowledged the disadvantages of unlimited information, including a sense of distress and moral confusion.

Conclusion: Our findings suggest that being knowledgeable promotes a sense of control on the one hand, but stress and moral-dilemmas on the other hand. We therefore argue that while the basic premise for informed-consent is crucial, it does not ubiquitously make things easier for educated patients. Consequently, raising levels of patient knowledge is a limited step forward in the strive for best practice.

S. Shkedi-Rafid: None. **Y. Hashiloni-Dolev:** None.

P19.03C

Reproductive experiences, medical concerns and moral attitudes among preimplantation genetic diagnosis (PGD) users: Synthesis of qualitative and quantitative analysis

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PGD may eliminate some of the obstacles related to conservative options of prenatal diagnosis, but can raise personal, social and moral questions. Scant empirical data focused on PGD users' experiences. A combined methodology was used to investigate PGD's implications on its users: Qualitative analysis of semi-structured in-depth face-to-face interviews with 43 PGD users, representing variety of population's sub-groups. On the basis of the interviews, a detailed closed web-based questionnaire was developed. Univariate and multivariate adjustment was performed on data obtained from 155 subjects. PGD is considered a preferable diagnostic procedure for 139 (95%) subjects. Nevertheless, 71 (47 %) reported a complex decision-making process. Perceived advantages are: assurance of the embryo's unaffected status from the beginning of the pregnancy, thus avoiding the need for pregnancy termination and invasive prenatal tests. Perceived disadvantages focused on the medical actions involved, and the delay in time between the first counseling and the PGD procedure itself. Other future needs included improving the communication with medical staff and implementing emotional support. The study indicates special needs of respondents from groups with distinct genetic and socioeconomic backgrounds. Subjects introduce permissive moral stand regarding different PGD uses but made a clear distinction between medically justified applications to unjustified social and aesthetic uses. This interdisciplinary qualitative and quantitative analysis of a large sample of PGD users can form a basis for development of counseling and guidance programs of future PGD users and will also help foster a public debate concerning medical, ethical, sociological and economic aspects of the technology.

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P19.04D

Pooling genomic data into a "national data lake": the first steps towards establishing a UK learning healthcare system

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Introduction: The National Health Service (NHS) recently proposed integrating genomic and other omic data, and data from digitised health records, wearables, and apps, from the general UK population, into a "national data lake". Pseudonymised and identifiable data would be made available for research and analytics by pharma and tech companies (e.g., Google's Deep-Mind). These are the first steps towards establishing a learning healthcare system, a model that hybridises research and clinical care, in the UK. A principle that is professed to underlie the proposed approach is for information-sharing to be based on the local trusted relationship between citizens and healthcare professionals. In light of this, we investigated the acceptability of this proposal to patients and the public.

Method: We analysed online public opinion; surveys with 300 + patients with rare diseases from the UK 100,000 genomes project, and interviews with 25 of these participants.

Results: We will present key themes, furnished with survey results. (1) patients/the public do not know, so cannot be assumed to trust, the commercial actors and government departments with access to their data; (2) broad consent for data-usage is not as acceptable as the genomics literature suggests; (3) patients/the public emphasise the importance of benefit-sharing and equality of access to healthcare; (4) but a discourse of altruism surrounds data sharing practices, which masks this importance.

Conclusions: Meaningful, non-tokenistic, public and patient involvement is needed for any learning healthcare system to be of benefit. We make recommendations to policy and practice on how this should be achieved.

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P19.05A

Educating a community of practice in clinical bioinformatics

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Introduction: At the University of Manchester we are training a masters-level cohort of clinical bioinformaticians, training in the NHS, using a 'flipped teaching' approach focussed on group-centred problem-based learning grounded in clinical case studies. . A new profession to the NHS, many clinical bioinformaticians are geographically isolated preventing rapid sharing of best practices. Therefore, building a strong network or community of practice (CoP) to support this important and innovative profession is critical.

Methodology: In a research study involving Manchester Institute of Education (MIE) students were interviewed using a semi-structured approach regarding their experiences of the new teaching strategy. In order to ascertain whether one or more CoPs were formed, we evaluated against Wenger's key themes characterising CoPs were investigated in the data: (i) mutual engagement of participants; (ii) concepts of the joint enterprise and (iii) shared repertoire.

Results: The results of the interviews aligned to the following main themes:

- Shared best practice in bioinformatics.
- Integration and accountability of a new profession into the NHS.
- Isolation in practice
- Novel teaching approaches
- Continuous course development based upon student feedback
- Cohort maintaining strong ties and ownership of the field.

Conclusions This research showed that via a 'flipped teaching' approach the bioinformatics students were able to develop the three distinct elements of a CoP, i.e. mutual engagement, joint enterprise and shared repertoire, and this CoP extended into their workplace supporting otherwise isolated students.

Wenger, E. (1998). Communities of Practice: Learning, meaning and identity. Cambridge UK, Cambridge University Press.

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P19.06B

BRCA1/BRCA2 mutations in a Mediterranean population with breast or ovarian cancer. A single center experience

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Introduction: Mutation of BRCA1/2 genes are present in 30% of hereditary breast (BC) and ovarian cancers (OC). BRCA1/2 mutations substantially increase the lifetime risk of developing BC and OC. The purpose of our study is to investigate the *percentage* of BRCA mutations in patients (pts) with BC or OC treated in our institution.Materials and Methods: Three hundred twenty-three pts (319 women and 4 men) with BC (82%), OC (16.7%) or BC plus OC (1.2%) were included. We used multiplex ligation-dependent probe amplification (MLPA) to screen mutations in BRCA.

Results: Of the 323 pts analyzed, 58 (18%) were BRCA1/BRCA2 mutated, 34 (10.5%) at BRCA1 gene and 25 (7.8%) at BRCA2; only one subject had both BRCA1 and BRCA2 mutations. Among BC pts, 47 (17.6%) had triple negative (TN) disease and BRCA1/2 mutations were present in 16 (34%) of these: 13 TNBC pts had BRCA1 (81.2%) and 3 (18.7%) BRCA2 mutation. Among BRCA1 mutations, the most frequent was c.5266dupC (67.6%). In TNBC pts, c.5266dupC constitutes about 92% of the BRCA1 mutations (one pt had c.5503C > T).

Conclusions: Different ethnic and geographical Countries have different BRCA1/2 mutation spectrum. The BRCA1 mutation c.5266dupC was originally described as a founder mutation in the Ashkenazi Jewish (AJ) population, but is also present in other Counties. The high incidence of this alteration in our pts may be linked to international migration flows. Understanding genetic predisposition to develop BC and OC may contribute to refine more cost-effective screening measures in a high-risk population.

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P19.07C

Development of cancer risk prediction models into tools for use in clinical settings

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Aim Advances in cancer research have allowed cancer risk prediction models to start incorporating genetic, epidemiological and clinical data. We aim to develop a user-friendly electronic tool to utilise these models in various clinical settings. ***Method*** We held a workshop on cancer risk prediction, model and tool development, and risk communication. All plenary sessions and smaller group work were facilitated, audio-recorded, and transcribed. Data were thematically analysed, and themes compared between clinical settings. **Results** 38 (UK and international) experts in Primary(GPs, public health and social scientists), Secondary (oncology, surgery and radiology) and Tertiary(clinical geneticists and counsellors) healthcare participated. There was general consensus that patients pre-completing parts of the tool prior to their appointment would be valuable. Other themes included challenges of data storage, confidentiality and consent(family and individual) and data transfer across the clinical care levels. Discussions about risk factors were focused on definitions, evidence-base for inclusion and impact on the assessment, and potential impact of behaviour change. There was broad agreement across clinical groups that the risk score should be presented in several formats (graphical, pictogram, expected frequency trees, national guidelines). Most also agreed that such a tool could aid patient-centred risk assessment, communication and management in all clinical settings. **Conclusions** These findings have contributed to the development of the tool prototypes; these will be assessed among clinicians and patients for usability and face validity using mixed methods approaches. Subsequent versions will then be assessed for accuracy and reliable function in the various healthcare settings. *Funding from Cancer Research UK(CRUK)*-C12292/A20861

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P19.08D

Optimal size of pedigrees for risk assessement in cancer genetic counselling using electronic mega-pedigrees

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Introduction: Family history, usually provided by the counsellee and verified by medical records and cancer registry data, is an essential tool to assess risk and guide surveillance in cancer genetic counselling. Guidelines on pedigree size exist, although more data about the correlation of size and informational value is needed. Circumstances in Iceland allow for such evaluation using electronic mega pedigrees constructed from both a population-based genealogy database and cancer registry.

Methods: The Study group comprised 179 women, randomly chosen from the genetics clinic at Landspitali: 89 were positive and 90 were negative for the founder mutation BRCA2:999del5),. Boadicea was used to calculate likelihood of being a *BRCA2* carrier using Icelandic allele frequency 0.3 for the founder mutation BRCA2:999del5, mutation search sensitivity of 0.8 and cancer incidence rates for Iceland. Receiver Operating Curves were used to assess the optimal pedigree size for risk assessment.

Results: Average no. of individuals in pedigrees with 1° relatedness was 8.8, range 3–22, 2° 25.6, range 6–92, 3° 53.8, range 9–220, 4° 102.4, range 15–257, 5° 146.1, range 15–498 and 6° 164, range 15–565. The lowest risk score was 0.1% and highest 92.6%. The optimal pedigree size included three degree of relatedness or up to five generations (AUC 0.801). Larger pedigrees made possible to connect counsellees to known BRCA families.

Conclusions: This study demonstrates the usefulness of basing risk cancer assessment on large pedigrees. Many countries already have the electronic information availability to use genealogy cancer registry data to construct electronic mega-pedigrees, with better risk assessment.

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P19.09A

The impact of cancer genetics on the management of patients with hereditary cancer syndromes

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Introduction: Hereditary cancer syndromes account for at least 5–10% of all cancers. Early detection and/or prevention resulting from appropriate risk management behaviour improve survival rates. This study aimed to: (1)assess the adherence to risk management guidelines among mutation carriers seen by the Cancer Genetics Service(CGS), Singapore; (2)compare the risk management behaviour between affected and unaffected carriers; and (3)describe the cancers subsequently detected among the risk management adherent group.

Method: A retrospective study was conducted to review 70 mutation carriers seen at the CGS. Subjects deemed appropriate for cancer surveillance and/or risk-reducing surgery were identified and their risk management behaviour was recorded. Findings of each procedure were traced, specified with the stage of cancer.

Results: Of the 70 mutation carriers, 38 subjects were appropriate for cancer surveillance and/or risk-reducing surgery(54%). Of the 38 subjects, 27(71%) were fully or partially adherent to risk management recommendations, with unaffected carriers being more likely to exhibit this behaviour. Of 17 BRCA1/2 carriers, 3 underwent risk-reducing mastectomy(18%). Among 7 BRCA1/2 carriers whom risk-reducing bilateral salpingo-oophorectomy (RRBSO) recommendation were appropriate, 4 underwent the surgery(57%). There was 100% adherence to risk-reducing surgery for APC or CDH1 mutation carriers(2/2 and 1/1, respectively). Of 27 risk management adherent individuals, 5 cancers have subsequently been detected.

Conclusion: The risk management adherence rate among mutation carriers is high and the vast majority of cancers were subsequently detected at early stage. Despite small numbers, this study demonstrates the impact of CGS on the management of patients with hereditary cancer syndromes in Singapore.

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P19.10B

It's only one extra sample of blood: Offering prenatal carrier screening for sickle cell disease and thalassemia in a high-risk population

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Background: Carrier screening for hemoglobinopathies (HbPs; sickle cell disease and thalassemia) aims to facilitate autonomous reproductive decision-making. In the Netherlands, in the absence of a national HbP carrier screening program, some primary care midwives offer screening on an ad hoc basis. This study explores how pregnant women perceive an offer of carrier screening for HbP by their midwife.

Methods: Semi-structured interviews ($n = 26$) were conducted with pregnant women at risk of being a HbP carrier, and whom were offered screening at their booking appointment in one of two midwifery practices in Amsterdam.

Results: Half of the respondents were familiar with HbPs. Generally, women perceived the offer of HbP carrier screening as positive, and most women ($n = 19$) accepted screening. Seven declined, of whom two already knew their carrier status. Important reasons to accept screening were obtaining knowledge about their own carrier status and/or health of their unborn child, and the ease of the procedure (It's only one extra sample of blood). A multistep process of decision-making was observed, as many women, while deciding on accepting or declining HbP carrier screening, did not give follow-up testing (e.g. partner, invasive diagnostics) much consideration yet.

Conclusion: Women experienced information overload, and preferred receiving the information at a different moment (e.g. before the intake, or even preconceptionally). While prenatal HbP carrier screening is perceived as positive, informed decision-making seems to be suboptimal, and both the content and timing of the information provided needs improvement.

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P19.11C

We are pretty sure we can never say never: risk and uncertainty communication within complex South African paediatric cases

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Introduction: Risk and uncertainty play a fundamental role in medical genetics. Genetic risk communication is affected by personal perception, formats used to explain risk, and contextual influences. Few studies have focused on the process of genetic risk communication in non-western settings, particularly for paediatric consultations. It is unclear how this is negotiated in South African genetic specialist-patient healthcare interactions. This study aimed to explore how genetic risk and uncertainty is communicated within South African paediatric healthcare interactions between genetic specialists and patients.

Methods: This research is part of an ongoing study which is being conducted at genetic clinics based at three South African tertiary hospitals. Preliminary findings of four video-recorded paediatric genetic consultations will be highlighted. Video-recordings were transcribed and analyzed using principles of conversation analysis and discourse analysis.

Results: Analysis of interactions suggests that South African paediatric genetic consultations present a complex entanglement of micro and macro phenomena. These are created through the presence of multiple individuals, various participant characteristics, the challenges of providing risk explanations and prediction, as well as the social, cultural and clinical setting. Collectively these influence the effectiveness of risk communication.

Conclusion: Results suggest that risk and uncertainty are marked features of South African paediatric genetic consultations. Qualitative interactional methods provide opportunities for reflection of practice and recommendations for more flexible approaches to healthcare provision. This is particularly important for services functioning within diverse and dynamic linguistic and cultural settings.

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P19.12D

The continuous professional medical education for geneticists in Russia: the first experience of the short educational course

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Introduction: The postgraduate education programmes in speciality "genetics" in Russia were created in 1988. Today, after 29 years, we number about 500 certificated geneticists who can work in a variety of settings in multiple specialty areas of human gene. Previously professional certificate "genetics" should be confirmed every 5 years by training 144 hours. But it's hard to keep up with the growth of knowledge, especially in the field of genetics. Since 2016 came into force a law continuing professional medical education according to which every medical specialist must obtain at least 50 credits (hours) per year, as cycles in the department and participation in scientific and practical activities. Our aim was to evaluate the effectiveness of short courses on a number of parameters, including the satisfaction of the trainees. Materials and Methods: We have developed a two-day conference, including the short educational course "New technologies of diagnosis of hereditary diseases". 217 geneticists were interviewed after the course.

Results: We found that the respondents had a high level of satisfaction with the short program of the education: 96% of respondents consider it useful, 89% said that it was available, 98% reported that they were satisfied with the program. At the same time, 76% of respondents correctly answered all test questions.

Conclusions: We conclude that the development of short educational programs for geneticists are effective and deserve special attention when medical knowledge is rapidly changing.

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P19.13A

Danish Cytogenetic Central Register

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Background: The Danish Cytogenetic Central Register (DCCR) is a nationwide Danish register. Since 1968, the results of all prenatal and postnatal chromosome analyses, and results of all prenatal molecular analyses conducted in Denmark are registered in the DCCR. No previous study has measured the quality of these data. We aimed to validate the registration of analyses results in the DCCR. Also, we aimed to describe the number of analyses registered over time.

Method: The quality of the data registration in the DCCR was examined by estimating the positive predictive value (PPV) of the registration of analyses results in the DCCR. We retrieved 300 analysis results from the DCCR registered from 2010 to 2014 and compared them with the analysis results recorded in the local laboratory systems.

Results: The estimated PPV was 98.6% (95% CI 96.6%-99.6%). In four cases, the registration of the sex-chromosomes was missing. The number of chromosomal microarray analyses (CMA) is increasing while traditional karyotyping is decreasing.

	Number of analyses in the period 2001–2015			
Period	Prenatal Karyotyping	Prenatal CMA	Postnatal Karyotyping	Postnatal CMA
2001–2005	29,596	4	17,048	953
2006–2010	18,541	20	17,305	2,792
2011–2015	15,530	2,716	14,707	6,858

Conclusion: The data in the DCCR are reliable and illustrate the shift in diagnostic methods that have occurred over time. Because all analyses are registered with the unique identifier of the person analyzed, data from the DCCR can be accurately matched with data from other Danish national registers. Overall, The DCCR has great potential as a data source for research.

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P19.14B

Finding yourself in front of the mirror: development of a theory regarding presymptomatic genetic testing in young adults

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Introduction: Genetic counselling is a specialist health service provided to those affected by or at risk of a genetic condition. Presymptomatic genetic testing should always involve a considered choice. Young adults are at a key life stage as they may be developing a career, forming partnerships and potentially becoming parents. The aim of this study was to develop a theoretical model regarding the factors involved when young adults undergo genetic counselling for hereditary cancer risk. **Material and Methods:** A mixed-methods sequential explanatory design was used. **Results:** Participants surround themselves with other people who influence their knowledge and awareness. The decision-making process started as a result of the influence of these people and only those young adults who decided to be tested presented for genetic counselling. During genetic counselling they viewed themselves as in front of the mirror. They took distance from themselves and spoke about themselves not in first but in second person, especially when they talked about sensitive situations. Finally, they achieved some autonomy and recognised how integrate the test result into their everyday life. **Conclusions:** Counselling approaches to this population may require modification both for young adults and their parents. Health professionals could have a role in both supporting parents and young adults. It is important to publicise the supportive and educational role of genetic services. The traditional 'wait until they come to us' approach by health services may be failing to meet the educational and emotional needs of this population.

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P19.15C

Couples' experiences of terminating pregnancy following prenatal diagnosis of Down's syndrome. A qualitative study

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Introduction: In Denmark, 95% of pregnant women choose to have combined first trimester screening (cFTS). When diagnostic results show Down's syndrome, the majority choose to terminate the pregnancy. However, little is known about these women's and their partners' experiences following diagnosis and termination. Materials and Methods: Qualitative interviews with 14 women and 9 male partners, 5–8 weeks after termination. All interviews were recorded and transcribed ad verbatim. The material was analyzed using thematic analysis.

Results: Twelve couples had decided to terminate in case of a Downs syndrome diagnosis. Two couples decided in face of the actual diagnosis. All couples reported feeling intense grief and sadness. Uncertainty about severity of cognitive and physical disability was central to the decision to terminate. All couples reported termination to be the 'right' decision, even if it was painful. They grieved not only the loss of a healthy child but also loss of a desired future as family and parents. None felt pressure to terminate, however some felt rushed by the time-limit for surgical abortion. Upon deciding to terminate, some women immediately disconnected from the fetus, while others spent time saying goodbye and/or asking the fetus for forgiveness. All couples felt sensitive to potential negative comments from social networks.

Conclusions: Couples reported that termination was the right decision, but still experienced prenatal diagnosis and termination of pregnancy as painful and difficult. The research was supported by the Health Research Fund of Central Denmark Region.

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P19.16D

An adaptable practitioner and patient visual aid for prenatal aneuploidy screening and testing options

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<The introduction of prenatal chromosomal microarray and Non-invasive prenatal testing (NIPT) as options for patients at increased risk of fetal aneuploidy (on first or second trimester maternal screening) has significantly increased the volume and complexity of prenatal consultations. Increased risk prenatal counselling is particularly challenging, because it is emotionally driven and time restricted. In response to the added volume and complexity and to ensure consistency of information offered across multiple genetic counsellors, we designed a visual aid with dual purpose. Its primary purpose is to assist the genetic counsellors to standardise the application of new technologies and options offered during increased risk prenatal counselling. A secondary purpose is to provide patients with a visual aid to assist their understanding during consultation and enhance informed decision-making. We present this unique, adaptable visual aid, designed to assist both practitioner and patient. The aid is easily adaptable as new technologies become available, for different prenatal scenarios, and for use by different practitioners (e.g. midwives, obstetricians, family practitioners). We have subsequently adapted the "increased risk aid" and developed a "routine prenatal aneuploidy screening aid" and an "advanced maternal age aid". Genetic counsellors at a major Australian maternity hospital have been using the three aids for approximately two years, and we have recently implemented the two latter visual aids for use by midwives at the hospital. We have surveyed the genetic counsellors and midwives using the aid. We present findings from the surveys and a proposal for implementation of these aids into routine prenatal care.>

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P19.17A

Financing regulation for registered genetic counsellors in the United Kingdom & Eire

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In the United Kingdom (UK) the Genetic Counsellor Registration Board (GCRB) was formed in 2001. The purpose of the GCRB is to establish, maintain and improve standards of practice in genetic and genomic counselling to assure public safety.

This elected board is responsible monitoring the conduct and practice of Registered of Genetic Counsellors (RGCs) in the UK which in May 2016 became an Accredited Register verified by the Professional Standards Authority (PSA).

PSA Accredited Registers show that an organisation has met specific standards in key areas including education, training, governance and complaints handling.

As part of the PSA accreditation process the GCRB had to demonstrate financial viability and accountability. The GCRB is a limited not for profit company submitting accounts to Companies House each year. Currently there are 201 registered genetic counsellors each paying Euros 119 annually. Other income sources include registration and course accreditation.

The PSA charged Euros 14 200 for the initial application which included recommendations to the GCRB to meet the criteria for an Accredited Register. These recommendations included revising the GCRB website and setting up a Patient and Public Group to feedback to the board. The GCRB approached regional genetic centres for financial donations to support accreditation.

The PSA reviews Accredited Registers annually and the GCRB will require its registrants to meet and maintain high standards. This annual review will cost Euros 10 638. Financing and maintaining the GCRB Accredited Register will be challenging including handling complaints and hearings.

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P19.18B

The reasons for not choosing prenatal aneuploidy screening in pregnant women receiving genetic counseling for advanced maternal ages

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In Japan, non-invasive prenatal testing (NIPT) has been available as a clinical research since the introduction in 2013. Shinshu University Hospital does not participate in this clinical research, and offers careful genetic counseling solely for the decision whether or not to have amniocentesis in cases of advanced maternal ages (AMA). Our prenatal genetic counseling (GC) for AMA includes (1) pre-counseling by certified genetic counselors (CGC), where reservations are made and family trees are described; (2) main GC by CGC collecting more information and following narratives, and by clinical geneticists specializing in chromosomal or genetic disorders, adding comprehensive medical and social information and discussing freely what the testing truly mean for the women and their husbands; (3) post-counseling by CGC by phone or after prenatal checkups. In total, 151 pregnant women received such GC from April 2013 to December 2016: 70 (46%) had testing (amniocentesis in most) and the rest 79 did not. Among the two groups, mean ages, the percentages of having histories of infertility treatment and delivery were not different. Reasons for not choosing testing are "refusal to terminate their fetuses" and "information that any pregnant women could be mothers of babies with congenital disorders or handicaps and that there is sufficient medical and social support system in Japan". These observations implicate the fundamental value of prenatal GC, both for invasive testing and for NIPT, that appropriate information and fruitful discussion could empower pregnant women to imagine which decision would fit them truly in their lifelong point of view.

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P19.19C

Distance from genetic counseling services as a system-based resource for Young Breast Cancer Survivors

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Introduction: Distance from the closest genetic counseling center can be an important barrier for utilization of genetic services for women diagnosed with breast cancer less than 45 years old (Young Breast Cancer Survivors - YBCS). We calculated the minimum distance between residential location and the closest genetic counseling service in a population of YBCS randomly selected from the Michigan cancer registry and recruited in an efficacy trial, which was initiated in 2012 and concluded in 2014. Materials and Methods: ZIP codes were available for N = 608 YBCS. Residential and genetic counseling center ZIP codes were geocoded in latitude/longitude coordinates using the R package 'zipcode'. Minimum distance (in miles) from YBCS place of residence to the closest board-certified genetic counseling service was calculated using the Great Circle Distance Formula, D=3963.0(arccos[sin(T_1)*sin(T_2) + cos(T_1)*cos(T_2)*cos(G_2-G_1)], where T_i is the latitude and G_i is the longitude of locations 1 and 2 in radians. **Results:** In 2012 there were 21 certified genetic counseling services/centers in the state of Michigan. Since then, approximately 11 additional services began operating. By 2014, N = 194 YBCS had genetic testing (31.9%). Median distance from the closest genetic counseling center in 2012 was 8.3 miles (min = 0, max=147.6, IQR=18.4 miles). There was no statistically significant change in the mean distance availability to genetic counseling services between 2012 and 2017 (t=0.308, p=0.758). **Conclusions:** Given the availability of genetic counseling services, other factors related to accessibility of genetic services (e.g., provider recommendation, barriers accessing care related to cost, etc.) are currently being explored.

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P19.20D

Responding to patient's anxiety: Overall structure of conversation in genetic counselling in Japan

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Introduction: Genetic counselling was introduced to Japan quite recently, about 20 years ago, and is still considered rather 'unfamiliar'. Previously, outpatient services had often been paternalistic in their approach. We have conducted qualitative analyses of genetic consultations focusing on the structural aspects of communication.

Materials and Methods: The data for this pilot study consists of transcripts of the first consultations of three children referred for clinical genetic assessments to one centre in Japan. The sessions were delivered by an experienced clinical geneticist. Our approach is rooted in ethnomethodology and conversation analysis.

Results: In paediatric genetic consultations in Japan, the phases of each session are relatively distinct. The overall structure of each session consists of three phases: (1) Opening and setting the agenda, (2) Assessment/Examination/Explanation, (3) Additional issues and closing. It appeared that the clients often delayed expression of his/her anxieties to the final part, after the main explanation by the clinician. While the basic manner of the geneticist met the expected standards of good genetic counselling, the parents did not express their principal concerns until the closing.

Conclusion: In these three consultations, the structure of the consultation consists of three parts. This is related to the teaching of communication for genetic specialists in Japan. Visiting the genetics clinic and meeting new doctors might impact on a client, inhibiting expression of their concerns. We aim to analyse additional genetic counselling consultations to see if this finding applies more generally.

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P19.21A

Genetic counselling in the EB Centre at University Hospital in Brno, Czech Republic - Changes due to new possibilities in DNA analysis

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Centre for patients with Epidermolysis bullosa congenita (EB) works at the University Hospital in Brno, Czech Republic since 2001 and since 2012 as a highly specialized medical care centre and member of the international network of EB centres and clinical experts. In 2016 our EB Centre was confirmed as a part of European Reference Network-Skin. The Centre cooperates with DEBRA Czech Republic (member of the Czech Association for rare diseases) that workes since 2004 and supports people with EB and their families to engage people with EB to a full life. The centre offers care for all EB patients in Czech Republic (about 300). Previously, we performed the DNA analysis of KRT5 and KRT14 genes for EB simplex and the DNA analysis of the COL7A1 gene for dystrophic EB. We found causal pathological sequence variations in all patients with the recessive dystrophic EB but only in a part of patients with the simplex or dominant dystrofic forms (about 60%). The junctional EB we diagnosed in cooperation with "EB Haus Salzburg - Austria". Since 2014, we have used the NGS analysis for 18 genes associated with EB and we confirmed the diagnosis in next 40 EB patients. In 13 families, we changed the supposed diagnosis EB simplex with AD inheritance to suprabasal form EB (Acral peeling skin syndrome) with AR inheritance. We also changed the prognosis for the recurrence of the disease in these families.

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P19.22B

Genetic counselling in the era of genomics: what's all the fuss about?

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Background: As genomic sequencing becomes more widely available in clinical settings, new counselling issues are arising, raising the question whether genetic counsellors currently possess the necessary skills to support clients through the testing process. The ability to adapt to and manage these new issues will be paramount as genetic and non-genetic healthcare providers navigate the complexities of using genomic technologies to improve diagnosis and patient management. However, care must be taken not to overstate differences between counselling issues in genomics and issues previously established in genetic counselling.

Methods: Counselling issues different to those established in genetic counselling literature prior to the advent of clinical genomics were identified by four genetic counsellors with ten years collective experience providing genetic counselling to individuals undergoing genomic sequencing. These themes were further discussed and refined at a meeting of genetic counsellors working in genomics in Melbourne, Australia. Illustrative cases were selected where pre- and post-test genetic counselling was provided to individuals undergoing singleton or trio whole exome sequencing with targeted analysis through a Melbourne Genomics Health Alliance study. The Alliance is a collaboration between ten research and clinical organisations, conducting research into the integration of genomic sequencing into clinical care.

Outcomes: Identified counselling issues and illustrative cases will be presented, including; managing expectations of genomic sequencing, counselling for secondary use and reanalysis of data, secondary findings and trio sequencing. Considerations discussed contribute to expanding understanding of these issues and highlight the applicability of existing genetic counselling theories and techniques to managing new counselling issues.

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P19.23C

Genetic Counselling the integration of novel educational program

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Genetic Counselling is defined as the procedure applied for helping people and families to understand and adapt to the medical, psychological and familial implications of genetic contributions to the disease of concern. In the National Genetic Centre, the Department of Genetic Counselling and Education has operated a on the job training in genetic Counselling through clinically exposure course work. This course is considered to be the first academic and professional course in genetic counselling to be operated in the region. It has been designed to broaden all candidates with the knowledge about Medical Genetics and the gain insight into the theory that supports the process of genetic counselling. The curriculum consists of nine modules in three days every 2 months, and includes theory, clinical rotations, and laboratory rotation. The total period of the course is 16 months. The course strives to train candidates to be able

to interface between patients, clinicians and medical geneticists. The candidates of the course will gain the skills of genetic counsellors, including technical, ethical and social awareness. There is a need for the graduates of the course in many different areas in the medical profession such as paediatric genetic clinics, cancer medicine, prenatal diagnosis clinics, as well as in laboratory genetics. The aims and objectives of the course together with other related details will be presented in the conference.

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P19.24D

High value genetic services through outcomes-based systems specifications

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Introduction: In 2015 the Personalized pREvention of Chronic DIseases consortium (PRECeDI) obtained funding from the European Commission [1]. The consortium aims to generate multidisciplinary knowledge through exchange of staff involved in training and research on the prevention of chronic diseases. In WP 6, partner institutions collaborated on drafting outcomes-based systems specifications to deliver value-based healthcare for cardiovascular disorders (Familial Hypercholesterolaemia, Long QT), Lynch syndrome, and BRCA-related breast cancer.

Methods: The specifications are based on a format developed by Better Value Healthcare Ltd in Oxford, UK. After a literature search on genetic testing, treatment, and patient pathways for the selected disorders, key performance indicators were selected. These were expected to be relevant to FH, LQT, LS and BRCA-related breast cancer services. The specifications were discussed with clinicians to check appropriateness.

Results: Indicators include: the numbers of patients identified in a specific population, the number of family members informed, the number of patients under surveillance or receiving treatment, and the extent to which patients are involved in decision-making. Criteria for measuring performance and standards are specified, which allow clinical networks to make annual reports to monitor their services.

Conclusion: Clinical networks will be able to improve their services by monitoring their results, taking the population served into account. Interventions that have high technical value (such as cascade screening) should be delivered in ways that optimize personal value. Identifying high value performance allows for reallocating budgets and (human) resources.

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P19.25A

comparison between public and private health care in the state of Rio Grande do Norte, Brazil

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Introduction: Brazil is a country with huge discrepancies, especially in health care. This situation is most pronounced for genetic diseases. While the Ministry of Health, through the National Agency for Supplementary Health, has determined that private health-care providers provide clinical and laboratory care, it is not determined in public attention, even though it has been a constitutional guarantee to the population since 1988. Materials and Methods: The medical records of 1,097 patients from the public health system and 986 private patients, all of whom were attended by the only medical geneticist in the State of Rio Grande do Norte, Northeast Brazil, were reviewed. The study considered the difficulty of access to the professional and the exams requested by him to conclude the case. **Results:** The access to the professional in the private regime was always simple and direct, having occurred even spontaneously in 8%. In the public system, access was only possible by means of referral and prior evaluation of the staff of the service to which the professional is linked. While the patients of the private health sector had quick and easy access to any examination, including exoma, in the public system, patients with Down syndrome had to wait until four years

for a karyotype. **Conclusion:** The results clearly show that an archaic and bureaucratic-based political system becomes the biggest obstacle in access to health, and that situation is more serious how much differentiated the specialty is, especially if an academic background is not common sense in the country.

J.I.C.F. Neri: None.

P19.26B

Impact of early genomic testing on health and reproductive outcomes: a follow up study

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Background Clinical genomic testing is increasingly used in the diagnosis of rare genetic disease but the longer-term impact on patient management, family decision-making, and service provision has not been investigated.

Methods and results We collected follow-up data in a cohort of 80 infants with suspected monogenic disorders who underwent singleton whole exome sequencing (WES) as a first-tier sequencing test. The median duration of follow-up post result was 473 days (interquartile range 411–650). There was no statistical difference in tertiary hospital use between those receiving a diagnosis and those without, although a small number of diagnosed patients (n = 4) had a substantial change in health outcomes such as cessation of hospital admissions. Continued standard-of-care investigations in the undiagnosed group (N = 29) at a cost of AUD $16,975(€12,250) did not result in any additional diagnoses, whereas WES data re-analysis at a cost of AUD$11,350 (€8,191) yielded four additional diagnoses. The parents of 14 diagnosed children and two undiagnosed children accessed reproductive genetic services at a cost of AUD $39,517(€28,519). All couples at high risk of recurrence and achieving a pregnancy utilized either pre-implantation or prenatal genetic diagnosis. One termination of pregnancy occurred in the undiagnosed group, based on uncertainty regarding recurrence risk. Overall, parents of diagnosed children had 8 more ongoing pregnancies compared to those without a diagnosis.

Conclusions These data provide further support for the early use of genomic testing in the diagnostic trajectory, highlighting the value of storage and re-analysis of genomic data, benefits in improved patient management, and restoration of reproductive confidence in families.

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P19.27C

Developing, establishing and supporting specialist roles in genomics across a National Health Service

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The integration of genomics into mainstream healthcare is fast moving and needs a workforce with the right skills and abilities to provide appropriate, high quality and regulated practice at all points in the patient pathway. This requires the development of new specialist roles and care models within existing healthcare professions, together with the establishment of new disciplines. Health Education England's Genomics Education Programme (GEP) is leading the co-ordinated approach to workforce transformation in Genomics in the National Health Service (NHS) across all professional groups. By working with key stakeholders to address educational needs, the GEP is ensuring genomics is represented at appropriate levels throughout training programmes for the NHS workforce. In collaboration with the National School of Healthcare Science, the GEP has facilitated development of accredited training pathways for emergent fields in genomics (bioinformatics) and re-shaped training pathways for established professions (genetic counselling) under the Modernising Scientific Careers framework, a UK-wide education and training strategy for the healthcare science workforce. The GEP has also formed key partnerships with regulators and professional bodies representing the wider clinical workforce and advised on new curricula developments, with a key achievement the inclusion of genomics in the training framework for the newly established Nursing Associate role. In addition, the GEP has supported the development of a Specialist Genetic Nurse network in diabetes, with the aim of developing a transferable model. We will present exemplars of our work, highlighting the impact these roles have played in the development of patient pathways for genomic conditions.

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P19.28D

Evolving Careers in Genetics: Attitudes of Healthcare Scientists

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Genomics has the potential to revolutionise healthcare and as such the science service in the NHS is changing. Studying the specialist workforce in the Genomics Service, particularly in Cytogenetics and Molecular Genetics, offers potential insights into the nature and impact of the changes on their occupational boundaries. Both disciplines have evolved as separate occupations with their own professional bodies in the UK. Recently, the distinction between them has become increasingly 'blurred' through the advancement of shared technology; the need to decrease service costs and find efficiencies; and the Modernising Scientific Careers policy programme which merges two previously separate scientist education and training programmes. As a result, the two specialities are being merged and the services rationalised. This is leading to changes in healthcare science careers and scientists' future working roles and relationships in this clinical domain.

Research into how these changes have affected the two disciplines has been conducted and the recently analysed data based on a national survey of scientists working in NHS Genetics Laboratories will be presented. A response rate of 47% was achieved with the respondent demographic being representative of the workforce population. Statistical analysis showed that there were significant differences, when analysing the scientist responses by occupation, over a number of variables including their intention to remain in the profession. The findings shed light on the nature and potential impact of inter-professional change, and contribute to further policy making in education and training and specialist workforce development.

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P19.29A

An exploratory study among hereditary breast and ovarian cancer patients: the relationship between clients' psychosocial situations and their solo/group visit to genetic counseling

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Background: It is important for genetic counselors to understand the psychosocial background of each client in order to ensure that initial genetic counseling (GC) sessions are successful. In order to help genetic counselors understand clients' backgrounds at the beginning of the session, we attempted to elucidate the relationship between clients' visiting style, whether they visited by solo or group, and their psychosocial situations. **Object:** The object is to explore factors that determine hereditary breast and ovarian cancer (HBOC) clients' solo or group visit before the first session of genetic counseling. **Method**: Semi-structured telephone interviews were conducted. The interviews were analyzed inductively from the viewpoint of "visiting solo factors" and "visiting with companion(s) factors." **Result**: The updated interview data for 17 subjects were analyzed. Most of the subjects were women from, the Clavis Arcus, the HBOC advocacy group in Japan, giving positive reactions to their treatment. In addition to their personalities, behavior patterns, and education levels, considerations and/or conflicts toward their families affected their decisions as "visiting solo factors." Demands of sharing medical information and/or their daily medical practices visit style affected their decisions as "visiting with companion(s) factors." **Conclusion**: This is the first qualitative study focusing on clients' solo visit determination factors (CCDF). From the interview, various CCDF which relate to the clients' psychosocial situations were discovered. These factors could be a sign to understand cancer risk perception levels and/or coping strategy styles of each client, which could help genetic counselors to plan customized sessions and follow-ups.

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P19.30B

Improving follow-up after predictive testing in Huntington's disease: evaluating a narrative group session in a genetic counselling clinic

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Introduction: Recently updated Huntington's disease (HD) predictive testing guidelines emphasise clinicians' responsibility to facilitate emotional support following testing, regardless of the result. Yet there has been little investigation of models of post-test counselling support.

Materials and Methods: In this pilot project, a genetic counsellor (RM) and clinical psychologist (MFD) developed a one-off group session using collective narrative practices for individuals post testing. Four group sessions have been run to date. This evaluation focuses on one group of six people known to the North West family register service, who have tested mutation positive for HD and remain pre-symptomatic. Two partners also attended the session. The aim was to appraise the integration of collective narrative practices within genetic counselling, by inductively exploring experiences of participation. Observations, evaluation forms, and telephone interviews were used in data collection. Qualitative data were analysed using a thematic framework approach.

Results: Participant responses were overwhelmingly positive, emphasising the importance of a specifically arranged time and space to share their experiences with others in a structured way. Typically, this was the first time participants had spoken openly with someone in their situation. Facilitation of group discussion, using the narrative approach, encouraged participants to re-discover their strengths and resilience in the face of dealing with HD, with similar experiences being discovered through connections with others.

Conclusions: The pilot was successful in implementing group narrative interventions as part of the predictive test counselling support for Huntington's disease. Participants suggested that the approach could be adopted for other genetic conditions.

C.L. Stopford: None. **M. Ferrer-Duch:** None. **R.M. MacLeod:** None.

P19.31C

Attitudes toward informed assent for genomic research

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Introduction: The purpose of this study is to determine the attitudes of the general Japanese public toward genetic research on children and informed based on nationwide surveys conducted in 2013 and 2016. Methods: 2,000 people (age = 20–69) from the general Japanese population, were selected using a stratified two-phase sampling method. In a mail survey administered in 2013 and 2016, the participants were surveyed regarding the following topics: (1) their attitudes toward genetic testing; (2) their perspective regarding informed assent; (3) their level of scientific literacy regarding genomics; and (4) their demographic information and socioeconomic status. **Results:** The response rate was 51.2% in 2013 and 50.8% in 2016. Conducting genetic testing on children for disease susceptibilities was favored by 57.2% of participants. Regarding obtaining blood donations from children, 55.2% approved, 11.3% disapproved, and 34.8% were undecided. A higher proportion of participants with high genomic literacy levels approved of obtaining blood donations from children. The multiple logistic analysis odds ratio regarding genomic literacy was 1.40 (95% confidence interval = 1.08–1.52). Regarding whether seeking consent from children is appropriate, 52.0% of people answered that seeking consent from a child is acceptable if the child understands the details of the research. These responses were associated with participants with high genomic literacy. **Conclusions:** The results of this study suggest that people's genomic literacy is related to people's perspective on genomic research on children.

Z. Yamagata: None. **K. Muto:** None. **T. Oooka:** None.

P19.33A

Karyotyping Optimised Online Learning

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Introduction: Karyotyping is one of the skill geneticists should master in their formation. The current form of developing this ability is time consuming for both the fellow and the coordinator. We created an application to fasten the process. **Materials and Methods**: Using images captured by Zeiss Axio Imager.N1 and sorted in karyograms

with the Ikaros v5.2.8 software, with the help of HTML, CSS, JavaScript, SQL, MySQL, PHP scripting language we developed an education software for medical genetics fellows. **Results:** The internet based application aids in the karyotyping learning of new geneticists. It has a theory section, MCQ tests based on it and hands on learning how to recognise the chromosomes and set them into pairs. Using a simple drag and drop system the program stops you from misplacing the chromosomes. It counts the number of false positioning attempts and the time you spend on each test, giving you a progress evaluation feedback. At the end of each test a pop up message appears containing the particularity of the arranged chromosome set(eg: deletion, inversions, duplications etc.). The application can be found at http://optimx.ro/ool/. **Conclusions**: This tool will help the future generation of geneticist develop their skills faster and give them more schedule flexibility. **Acknowledgements:** Some of the research was done at the Center of Genomic Medicine of the "Victor Babeş" University of Medicine and Pharmacy of Timişoara, POSCCE 185/48749, contract 677/09.04.2015.

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P19.34B

The "Monte Santo" experience: a population-based public health care programme to screen, diagnose and manage genetic diseases in Brazil

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Introduction: Monte Santo is a rural area in NE Brazil where several studies have shown high frequencies of different genetic disorders, implicating endogamy and consanguinity as risk factors. In 2014 a public health programme was established to deliver care and education for the detection of genetic diseases, to organize diagnostic facilities, and train local health professionals to offer primary health care linked and coordinated by a reference centre.

Subjects and Methods: Across the population a questionnaire with suitable parameters to detect individuals with suspected hearing impairment (HI), intellectual disability (ID), mental disorder (MD), or congenital disease (CD) is completed by community health staff. Positive cases are evaluated by a multidisciplinary team with confirmed disorders investigated according the most probable genetic cause. Local health professionals have been trained to follow and maintain this systematic routine.

Results: Sensitivity and specificity levels of 59% and 68% were achieved with HI; 78% and 53% with ID; 90% and 95% with MD; and 77% and 70% respectively with CD. Data on 8,445 individuals (16% of the population) were collected, with 1,505 persons evaluated by the multidisciplinary team and 3,150 tests performed to investigate possible genetic diseases. The consanguinity rate of the 1,505 positive cases was 31%, indicating potential vulnerability to recessive genetic diseases besides multifactorial conditions.

Conclusion: The "Monte Santo" experience can provide a feasible and efficient model in the development of public health strategies for the detection and diagnosis of rare diseases via primary care, helping the current Public National Policy of Rare Diseases.

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P19.35C

Too low risk to test?

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Niemann-Pick Type C(NPC) is a rare neurodegenerative, autosomal recessively inherited, lysosomal storage disorder with an incidence of about 1:100,000. There are about 100 known cases in the UK. Genetic Counsellors may see siblings and extended family requesting carrier testing but as the risk of meeting another carrier and having an affected child is so low they may not agree to the test being carried out. The author will examine the reasons why close family may request carrier testing from known family cases. This presentation will look at 2 case studies with very similar family backgrounds and scenarios with very different outcomes. The professionals seem to view only the probability

whilst affected family members see only the impact. Whilst the risk factor may be numerically resolved the issue is one of perception. The main point is that it might bring relief to wider family members knowing that they are not carriers of the disease causing mutation, or, if they are, then forewarned is forearmed. This presentation looks at the psychosocial impact of testing or not testing against the cost to the NHS of familial screening. In looking for a cost effective solution the author will discuss the option of a Genetic Counsellor with good knowledge of the disease working closely with all affected families with a view to initiating testing if it was felt appropriate despite low risk.

J. Imrie: F. Consultant/Advisory Board; Modest; Orphazyme.

P19.36D

European collaborative work to establish standards for Orphanet quality assessment of genetic counsellors

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Introduction: Orphanet, recognized as the largest international rare disease online portal, contains information about 709 expert centres that provide genetic counselling for rare diseases. Unlike medical management centres for rare diseases, which are assessed by criteria established by the European Union Committee of Experts on Rare Diseases, there was no quality assessment of self-declared genetic counselling activity.

Methods: A pilot quality questionnaire was developed by Orphanet Spain regarding patient population, pathways, quality management and teaching. A collaborative work was undertaken with the Genetics Nurses and Counsellors Professional Brach of the European Board of Medical Genetics in order to integrate European Board of Medical Genetics published standards: core competencies for genetic counselling; professional and educational standards; and code of professional practice for genetic counsellors. To ensure cross-country generalizability the questionnaire was also reviewed by two Orphanet Information Scientists who are certified by the Canadian Association of Genetic Counsellors.

Results: The final questionnaire is available for use in all 40 Orphanet countries. Domains queried include: patient population, qualifications and professional registration status of staff providing genetic counselling, multidisciplinary input and access to psychological support, the scope of genetic counselling activities, quality management, commitment to continuing education and teaching.

Conclusion: Existing and newly registered genetic counselling centres on the Orphanet database will undergo national assessment via the new quality standard. Orphanet has once again demonstrated its commitment to quality data for rare diseases, and has made a commitment towards the recognition of quality genetic counselling as a valued resource for rare disease patients.

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P19.37A

Australian public's awareness, use and interest in health-related and recreational personal genomic testing

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Background Individuals can access genetic information relating to their health, as well as 'recreational' personal genomic information such as ancestry and sporting ability, either online or through a health professional. This has predominantly been marketed within the USA, and is accessible to everyone, including Australians. **Aim** To investigate Australians' awareness, interest, and experiences of personal genomics. **Methods** An online survey was made available and advertised through social media, community and professional groups, and researchers' networks. The survey is open until April 2017. **Results** To date, 1,636 Australians have attempted the survey: 75.7% female, 74.1% undertaking/had university education and 22.2% working/had worked in genomics/life sciences. The majority (75.3%) were aware of 'personal genomics', mostly having heard about it through formal studies and media. 283 reported having some type of genetic testing, and testing was greater in females (χ^2=9.8. p=0.007) and those working in genomics/life sciences (χ^2=14.8, p=0.001). Of respondents who had testing, 80 had ordered the test online only, often for multiple purposes, with ancestry most common. 591 had considered but not had testing, while of 418 who had not considered testing, 74.6% had never thought about it. **Conclusion** In this highly educated population, the level of online personal genomic testing was low, with recreational (especially ancestry) testing of greatest interest. While the current cohort of respondents presents a select sampling, efforts are being made to expand recruitment to provide a more representative sample of the Australian population. Findings will contribute to guidelines for policy and education in genomics in Australia.

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P19.38B

Young adults' experiences with risk and with presymptomatic testing for late-onset neurological disorders: findings from a Portuguese qualitative study

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Young adulthood is typically a challenging development transition. Literature recognizes the additional challenges presymptomatic testing may pose at this age, both for young adults and for genetics health professionals. Although young adult's requests of presymptomatic testing for late-onset disorders are increasing, research in this area is scarce. This qualitative exploratory study aimed at exploring experiences of young adults (aged 18–26) with their increased genetic risk for late-onset neurological disorders. We sought to identify key motivators for testing uptake, to understand its anticipated psychosocial impacts, and participants' perspectives on the nature of the information about genetic risks. Psychosocial needs were also explored. After written consent, a semi-structured interview was conducted with twelve participants in our presymptomatic testing protocol, before its results were known. Interviews were recorded, transcribed verbatim and analyzed thematically. Preliminary findings have shown that most participants recognize the psychosocial impact of their increased genetic risk, including feeling more anxious and an augmented sense of vulnerability regarding a potential carrier result. Participants perceive PST as a means to reduce uncertainty about their genetic status, leading to important life-planning decisions (e.g., reproductive choices). Presymptomatic testing has allowed participants to feel more empowered and 'responsible' for the management of their own health status. They also acknowledged genetic information as being of a complex nature, both with a personal and a familial dimension. All participants declared to be willing to share their test results with family members. This has important implications for genetic counselling and potential psycho-educational interventions in this age group. (SFRH/BPD/88647/2012)

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P19.39C

15 years of public engagement: from launch of the biobank to personalised medicine initiative in Estonia

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Introduction: The Estonian Genome Center of the University of Tartu (EGCUT) is a research institute with a population-based, longitudinal biobank representing about 5% of Estonia's adult population. The EGCUT has strongly focused on public engagement in order to explain the potential of biobanks and the field of human genetics, introduce research conducted using biobank's data, and the potential of personalized medicine. By the summer of 2017, the entire EGCUT cohort will be genotyped and we plan to start with return of individual genomic results to biobank's participants.

Methods: Various approaches have been used to inform and engage stakeholders (the public, physicians and decision-makers) as the main goals of the biobank have shifted from recruitment to research, and translation of research to clinical practice. For monitoring public's perspectives, annual surveys on awareness of and attitude towards the biobank have been conducted (2001 - 2014) and perspectives regarding the use of genomic information in healthcare have been surveyed in 2011, 2013, and 2014.

Results: According to the last poll conducted in 2014, 73% of responders were aware of the biobank and 70% of them supported the project while 1% were against it. Reportedly, 77% would be interested in personalized genetic risk predictions.

Conclusions: Stakeholders engagement is of particular importance as the up-coming plans of the EGCUT involve offering return of individual research results and implementation of genomic data in national personalized medicine initiative.

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P19.40D

The Orphanet Knowledge Base - a community-driven knowledge curation platform for rare disorders

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Since 1997, Orphanet maintains a multilingual database of rare disorders (RD), based on literature and on expert advice. Orphanet's scientific data is comprised of a nosology (classification of RD), textual information, epidemiological data, information on natural history, relationships between RD and genes, phenotypes and disabilities. Data are manually curated.

The Orphanet Knowledge Base (OKB), jointly developed by the INSERM and the Garvan Institute, is an expert community-driven knowledge curation platform for RD based on Orphanet scientific content. It is a pilot project that allows users to explore the Orphanet scientific data through an user-friendly web interface.

Each RD is represented as a single web page including the ORPHA number, names and synonyms of the disorder, its semantic typology, a textual description organized into sections, the associated genes (including the type of association) and phenotypes (including frequency of occurrence), and data on natural history and epidemiology, as well as the alignments with other terminological resources.

The platform allows users - the expert community at large – to propose corrections or modifications to the scientific content. Suggestions can be discussed virtually with other experts and with the Orphanet curators. Decisions on changes can therefore be done in a participative, expert-corwd-sourced manner. Changes validated in the OKB are then incorporated to the Orphanet database. The OKB creates an interactive knowledge curation process that enables for input from the RD expert community, significantly reducing the time for curation while maintaining the high quality standard of the Orphanet data in a transparent, traceable way.

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P19.41A

ERN-EYE: the European Reference Network dedicated to European patients with Rare Eye Diseases

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M. Larsen[13], B. P. Leroy[14], P. Liskova[15], B. Lorenz[16], C. Martinho[17], S. Mohand-Saïd[18], A. Petzold[12], M. Preising[16], T. Reinhart[4], T. Wheeler-Schilling[9,19], S. Wong[12], E. Zrenner[9], all the ERN-EYE Members

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Introduction: Rare Eye Diseases (RED) are a diverse group of orphan diseases that display major genetic and phenotypic heterogeneity and represent the leading cause of visual impairment/visual loss in children and young adults in Europe. Significant barriers remain for early diagnosis and patient management. Specific treatments for this highly heterogeneous group of diseases remain challenging reason why RED represent a major socio-economic burden. As for other rare diseases, a multidisciplinary consortium of specialists across the EU has decided to join forces in a European Reference Network (ERN): ERN-EYE.

Methods: ERN-EYE currently consists of 29 healthcare providers from 13 Member States, with important interactions with patients and patient groups (European Patient Advocacy Groups noticeably), and will cover predominantly genetic RED. The general organisation of the ERN as well as mapping of all collaborators across the EU will be presented.

Results: ERN-EYE will address the following genetic fields: retinal diseases, paediatric syndromic and non-syndromic ophthalmic diseases, optic neuropathies, eye movement disorders, anterior segment diseases, and cover domains such as improving genetic counselling, improving genetic diagnosis including organisation of molecular diagnosis on an EU basis, clinical research including boosting of clinical trials and registries.

Conclusion: We anticipate that ERN-EYE network will improve upon existing resources dedicated to patients with RED across the EU and create new facilities to better diagnose, understand and treat patients and families with RED.

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P19.42B

Reporting genomic research results to population biobank participants - participant preferences and response

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Introduction: Returning research results to biobank participants is a topic of ongoing debate. Procedural guidelines are limited, largely due to a dearth of studies on the clinical and psychological impacts of such disclosure. The Estonian biobank legislation gives participants the right to be informed about the data collected and generated and also the corresponding right not to know. Methods: A genotype-first project involving population biobank participants carrying pathogenic variants in three genes associated with familial hypercholesterolemia was initiated. The procedural framework used to offer return of clinically significant genetic findings to participants includes contacting without first disclosing genetic status, project specific consent, independent validation of the finding, disclosure accompanied with genetic counseling, collaboration with cardiologists, and immediate and long-term feedback surveys. **Results:** Of 42 carriers identified, 21 have visited the biobank and expressed interest towards return of results. The 12 participants who have received the results, so far, have

perceived this information valuable and eight have had relatives attend the cascade screening. Although three participants report feeling worry, all appreciate being contacted. **Conclusions:** The choices of participants contacted indicate that there is preference towards disclosure and as per the survey results the information disclosed is perceived valuable. The long-term effect and the impact of the cascade screening are still to be determined. The surveys and follow-up allow us to generate much needed empirical data on the effect of communicating genetic risk information to biobank participants and inform procedural guidelines for future projects involving return of medically actionable findings.

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P19.43C

A randomized controlled trial of return of genomic results shows non-inferiority of web-based compared to counselor return of carrier results

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A critical bottleneck in genomics is the mismatch of large volumes of results to the capacity of the current standard of returning results in-person by a healthcare professional. To test a web-based alternative to in-person results return, we designed a web platform that integrated education regarding carrier results with individualized test results and compared return of results through this platform to the same information returned via a counselor in a randomized controlled non-inferiority trial. Our outcomes included knowledge, test-specific distress, risk worry, decisional conflict, and communication of results to family members and healthcare providers. We randomized 462 participants into web or counselor arms. One to seven carrier results were returned to each participant. The web was non-inferior to the counselor on outcomes assessed six months after return of results including: knowledge (d=0.18; upper limit of 97.5% CI=0.63; non-inferiority margin = 1), distress (d=−0.54; lower limit of 97.5%CI=−1.00; NFM=−1), risk worry (d=−0.10; lower limit of 97.5%CI=−0.32; NFM=−0.5), and decisional conflict (d=−1.18; lower limit of 97.5%CI=−2.66; NFM=−6). There were no significant differences between education arms in disclosure rates to children, siblings, or providers. Participants with children experienced significantly more decisional conflict when educated by the web, but the lower limit did not cross the non-inferiority margin. No other differences in outcomes were found between parents and non-parents. This well-powered trial demonstrates non-inferiority of web-based communication of carrier results and should spur efforts to shift the communication of information about genomic test results from the clinic to the internet to improve efficiency and reduce healthcare costs.

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P19.44D

EGA - future plans

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As of February 2017, the EGA holds 3.5 petabytes of data and has handled some 2,500 help-desk tickets in the last year alone.

Genomic data analysis is no longer for a small number of highly expert scientists but is increasingly a staple of biomedical research. Users from across the world and scientific spectrum wish to access the data held by the EGA and it is important that the services provided by the EGA are accessible and user-friendly so that all researchers, regardless of prior experience, can benefit from the resource.

In the Autumn of 2016 the EGA embarked on its first ever user survey, contacting over 1,000 individuals in order to seek feedback on, and suggestions to, how we might look to improve on and increase our existing services.

In addition to this, the EGA has been involved with the work of Global Alliance for Genomics and Health (GA4GH).In particular, the EGA, in partnership with the Sanger Institute, has set up a small proof-of-concept project to implement "Consent Codes" (SOM Dyke, et al *PLoS Genetics*. 12(1): e1005772), which provide users with an easily understood view of the conditions under which the data may be accessed and used.

Here we present the findings of the EGA survey combined with the Consent Code work in order to provide insight into how the EGA of the future might look.

The EGA is maintained by European Bioinformatics Institute (EMBL-EBI) and the Center for Genomic regulation (CRG) and is available at http://www.ega-archive.org.

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P19.45A

A pilot study of a support group in the follow-up of oncogenetic counseling services for women with *BRCA* mutation

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Introduction: Patients who learn from oncogenetic services that they carry harmful constitutional *BRCA1 or 2* gene mutations experience high levels of psychological distress. In France, additional psychological assistance for these patients has been recommended; however, the optimal conditions for and duration of such assistance need to be clearly defined. To begin addressing these parameters, we proposed a pilot study in which women with *BRCA* mutations would participate in a support group as a follow-up to oncogenetic counseling services at the Centre Paul Strauss, located in Strasbourg, France.

Materials and Methods: In June 2014, we sent by mail a one-page questionnaire to one hundred women, with or without personal history of cancer, and who had been identified within the past two years as carrying a *BRCA* mutation. The questionnaire was designed to survey the potential interest for these women to take part to the support group.

Results: Four month later, 44% answered the questionnaire, and of those responding, 64% expressed interest in participation. Two groups, led by a psychologist and involving jointly a genetic counselor, were established in November 2014. An average of 3 women and 7 women participated in a Wednesday evening group and a Saturday morning group, respectively. Group meetings took place every two months and lasted approximately 2 hours.

Conclusion: After two years in operation, we present our preliminary findings and discuss the effectiveness of post-counselling group support for patients with *BRCA* mutations.

N. Taris: None. **S. Laurent:** None.

P19.46B

A new instrument for quality assessment of genetic counselling practice: the contribution of Portuguese genetics healthcare services

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The importance of appropriate genetic counselling when offering genetic testing has been consensual. Although there are guidelines for practice of genetic counselling, the tools and measures for quality assessment are still insufficient. Recent Portuguese's studies on consultands' and professionals' views highlighted the need for instruments and quality indicators that support them. We present the methodological design, preliminary validation, results and challenges faced along the process of construction of a new instrument for assessing the quality of Portuguese genetic counselling practice. The Reciprocal-Engagement Model of Genetic Counselling Practice served as a theoretical basis for this tool. Five main dimensions were outlined and related to: (1) provision of information; (2) emotional issues as part of the counselling process; (3) counsellor-consultand relationship; (4) effects of genetic counselling on consultands; and (5) standards of care. A first pool of 130 items, resulting from previous national studies and the literature, underwent pre-test validation, through cognitive interviewing with five national experts who assessed clarity, format and content, appropriateness, usefulness and relevance. Currently, the instrument underwent validation through statistical analysis of the items testing accuracy and value of the information collected, which will allow the selection of the best ones. Portuguese professionals of national genetics services are involved in an undergoing process of psychometric validation that should be completed by March. It is expected that this instrument may be useful both in assessing the quality and effectiveness of the genetic counselling practice, and in monitoring improvement of national genetics healthcare services.

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P19.47C

Uncertainty in consultations about genetic testing for cancer. An explorative observational study

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Abstract

Introduction: People seek cancer genetic counseling (CGC) to obtain certainty about their medical situation. Unfortunately, the information provided during CGC involves many uncertainties, e.g., the risk of developing cancer. Communicating about uncertainties is important to maintain patients' wellbeing, enhance patient autonomy and enable shared decision making. Yet, the optimal way of managing and communicating uncertainties remains unknown. To address this problem, we explored whether, how and what types of uncertainty are expressed and discussed during CGC.

Materials and methods: Initial cancer genetic consultations (N = 25) were audiotaped, transcribed and qualitatively analyzed using MAXQDA. A coding scheme identifying all types of uncertainty was developed inductively, in parallel with coding the transcripts. Next, codes were thematically categorized using the literature. All transcripts were double-coded independently and discrepancies were discussed until consensus was reached.

Results: Uncertainty is highly prevalent during CGC in communication by both counselors and counselees. Uncertainties discussed most by counselors relate to scientific issues such as risks and scientific limitations. In contrast, counselees mainly express uncertainty related to personal and practical issues, e.g., their family history, their own and their relatives' health and their knowledge of genetics.

Conclusions: Many types and causes of uncertainty were found. Moreover, a prominent discrepancy appears between uncertainty expressed by counselors compared to counselees. This implies that counselees may lack awareness of uncertainty related to scientific issues. These findings will be used to inform future studies addressing optimal ways of managing and communicating uncertainty in CGC.

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P19.48D

Whole exome sequencing becomes a must in daily practice due to the changing demographic pattern; 100 patients and beyond

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Introduction: Whole-exome-sequencing has rapidly become one of the most successful methods for diagnosis. The main reason for its increasing clinical use is the wide range of clinical phenotypes. This obstacle becomes more obvious than ever, since the great demographic movement occurs in the last decade. Our institute serves as the first-thier, tertiary referral hospital to a very large scale habitat from Mediterranean coast to Middle East borders that are the richest pools of rare disorders with more than 5000 patients per year with most recently established Medical Genetics Department.

Materials and Methods: We set up clinical genetics policlinics for outpatient inclusion and counseling, and a diagnostic laboratory. Our analysis in this report comprises an experience of the changing demographic pattern effect with whole exome sequencing results of 100 patients.

Results: We identified 74 mutated alleles achieving a 74% diagnostic rate. Twentythree of 74(31%) were novel mutations that were likely to be causative and all were confirmed in the parents. 11 of 74(15%) were proved to be de-novo mutations. Among the 74 patients, 27%(n = 20) had autosomal-dominant disease, 70%(n = 52) had autosomal-recessive disease and most interestingly 2 patients(3%) had two different diseases while one of them had both autosomal-recessive, the other had one autosomal-dominant and one autosomal-recessive disease. A total of 10%(n = 2) of the autosomal-dominant mutant alleles occured de-novo.

Conclusion: The standard of clinical practice involves the recognition of specific phenotypic features. However, demographic changes effects the clinical evaluation quality due to the clinical variables. Thus, whole-exome-sequencing becomes the most important diagnostic tool.

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P19.49A

Feasibility of couple-based expanded preconception carrier screening offered by the general practitioner

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Introduction Our previous research showed that Dutch professionals and target population consider the general practitioner (GP) most suitable to provide expanded pre-conception carrier screening (ECS). As part of an implementation study on couple-based ECS offered by GPs, we evaluated feasibility using predefined criteria, explored GPs' experiences and measured patient satisfaction. Beforehand, GPs received training, knowledge-evaluation and supervision (twice).

Methods and materials: Feasibility was defined as (1) < 20% of GPs require additional training or supervision (2) ≥ 80% of consultations occur within 20 minutes (regular double-consultation time) and (3) GPs refer < 20% of average-risk couples to clinical genetics for additional counseling. GPs recorded consultation time. Patient satisfaction with GPs' counselling was measured using a survey. Semi-structured interviews with 10/13 participating GPs were qualitatively analysed to explore test-counseling experiences and views on future implementation.

Results: 130 consultations took place. 1: 0/13 participating GPs required additional training or supervision. 2:63/108 (58%) consultations took place within 20 minutes (median 20, IQR 18–28). 3: No average-risk couples were referred. All GPs felt competent to provide counseling after preparation and with supportive materials provided. GP-training was considered essential for future implementation. Couples' prior knowledge-level and administrative practicalities affected consultation time. 91% of patients were (very) satisfied with GPs' counselling.

Conclusions: During our pilot it was feasible for motivated and trained GPs to provide couple-based ECS. Asking couples to review information about ECS prior to counseling might improve efficient use of GP time. We expect adequately trained GPs will be able to offer couple-based ECS.

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P20 Psychological/Ethical/legal issues

P20.01A

Regulation of direct-to-consumer genetic testing in Europe: a fragmented landscape

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Introduction: Despite the increasing availability of direct-to-consumer (DTC) genetic testing (GT), the way such services are regulated in Europe remains unclear. While EU laws largely cover genetic tests as products, aspects of GT akin to services (e.g. medical supervision, genetic counselling and informed consent) have been regulated mostly on the national level. This has resulted in a potentially heterogeneous and still unmapped European regulatory landscape. Materials and Methods: In order to explore the different ways DTGT may be regulated across Europe, experts in health law and/or regulation of GT from EU and European Free Trade Association countries were contacted and asked a total of eight questions, regarding their national laws pertinent to DTCGT. Each collaborator's answers were analyzed, summarized and grouped into categories. **Results:** Information on the legislation of 26 European countries was collected. These countries are: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, the Netherlands and the UK. Our results indicate that of these countries, 9 prescribe mandatory medical supervision for GT, 16 require

mandatory genetic counselling for some types of GT and 14 provide specific requirements for informed consent in this context. **Conclusion:** Currently there is a wide spectrum of laws regulating GT in Europe. There are countries (e.g. France and Germany) which essentially ban DTCGT, while in others (e.g. Luxembourg and Poland) DTCGT may only be restricted by general laws, usually regarding healthcare services and patients' rights.

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P20.02B

A duty to hunt for pathogenic mutations? Ethical considerations regarding routine screening of genomic data

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Introduction: next-generation sequencing allows for an unprecedented generation of genomic data. This collection of data raises questions regarding the responsibilities of professionals who are dealing with these data. A highly controversial possibility is to routinely check sequencing data for a list of pathogenic mutations (also referred to as routine or opportunistic screening), as has been proposed by the American College of Medical Genetics and Genomics.

Materials and methods: this presentation provides an ethical analysis of arguments pro and contra routine screening. Special attention will be given to the ethically relevant differences between disclosure of unsolicited findings and actively searching for these findings (sometimes called a 'duty to hunt').

Results: reasons for and against routine screening are reviewed. Amongst others, beneficence, iatrogenic harm, costs and fairness are given due consideration. We establish that both sides of the debate have put forward strong arguments. Experts have very different views on the acceptability and desirability of routine screening. Likewise, patients may have different preferences regarding routine screening that should be taken into account. Therefore, patient engagement will be a necessary step to determine whether routine screening should be adopted.

Conclusion: A decent healthcare policy should acknowledge the diversity of judgements regarding routine screening by striving for a stronger engagement of patients, both individually and on a group level. This engagement is needed to make personalized medicine truly personal. The introduction of electronic health records and other IT applications offers unprecedented opportunities for incorporating personal preferences into healthcare policies.

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P20.03C

Which policies work?: Comparative approaches to regulating life insurer use of genetic information from the UK, Canada, and Australia

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Introduction: For over two decades, debates have arisen in many countries over whether life insurers should access and use genetic test results. These debates are sometimes emotionally charged, evoking dire consequences, such as genetic discrimination on the one hand and financial insecurity and industry demise on the other. In response, countries have adopted a variety of policy approaches, from prohibitive bans to maintenance of the status quo to the establishment of an independent body that approves tests available for insurer consideration.Materials and Methods: This presentation examines the effectiveness of the policies of three countries. It reports on a comparative analysis of case-studies in Canada, the United Kingdom, and Australia. Qualitative analysis employs transcribed data from semi-structured interviews with key stakeholders, including policy experts, government officials, advocacy group members, and insurance representatives. The analysis explores whether stakeholders believed their country's policy was effective and how they conceptualized effectiveness. **Results:** This presentation will report on the effectiveness of the chosen policy at addressing public fear of genetic discrimination and industry concerns. Predominately, stakeholders measured effectiveness of the policy by whether it had addressed either fears of discrimination, industry financial concerns, or dually addressed both concerns. **Conclusion:** As additional countries consider and debate policies in this area, measuring the potential effectiveness of options is a necessary element of any debate.Research reported in this presentation is supported by the National Human Genome Research Institute of the National Institutes of Health under Award Number K99HG008819.

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P20.04D

Adolescent attitudes towards genetic testing for adult-onset conditions

A. K. Rahm[1], L. Bailey[1], O. D'Accordo[2], Y. Munishor[2], C. Miller[2], E. Davidson[2], K. Pulliam[3], H. Zhang[3], L. Hercher[2], M. Williams[1], M. J. Dougherty[3,4]

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Introduction: When to test children for adult-onset conditions is an ongoing issue in genetics, and information is lacking on the attitudes and opinions of adolescents themselves.

Materials and Methods: Essays submitted by 9th–12th grade students to the American Society of Human Genetics' 2016 national DNA Day Essay Contest underwent thematic analysis. All submitted student essays were analyzed for adult condition discussed and why testing should be deferred to adulthood or not. Demographic information including gender, grade, school type, and location were analyzed.

Results: 1241 student essays were submitted from 44 U. S. states (87%) and other countries (13%). Over 100 adult-onset conditions were discussed by students; most commonly discussed conditions were Huntington's disease (38%), BRCA-related breast or ovarian cancer (17%), and Alzheimer's disease (10%). Overall, students were evenly split whether they believed testing should be delayed until adulthood or not; however, more agreed to defer testing for Alzheimer's (59%) or Huntington's (57%) than BRCA (42%). Reasons for deferring testing included potential psychological harm and anxiety, while reasons for wanting testing included prevention and life planning. Factors such as family history and personal anxiety were given as reasons for the need for choice. Additional analyses are ongoing and will be presented comparing attitudes towards actionable and non-actionable conditions and perceived psychological risk.

Conclusions: The DNA Day essays provide valuable insights into the attitudes of adolescents regarding genetic testing for adult-onset conditions. This research contributes to understanding the potential psychosocial impact of learning genetic information for adult onset-conditions in healthy adolescents. Funding: 3U01HG8679-02S1

A.K. Rahm: None. **L. Bailey:** None. **O. D'Accordo:** None. **Y. Munishor:** None. **C. Miller:** None. **E. Davidson:** None. **K. Pulliam:** None. **H. Zhang:** None. **L. Hercher:** None. **M. Williams:** None. **M.J. Dougherty:** None.

P20.06B

The use of an electronic health record to facilitate communication ofadditional findings in families

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Introduction: An estimated 20–40% of at-risk relatives are not told about a risk relevant to them. 'Additional findings' (AFs), such as those the UK 100,000 genomes project offers, will likely make this more common: there may be no known family history of, or knowledge about, the risks. How clinicians can best report AFs is debated, and even with primary findings, they perceive several barriers to facilitating communication in the family, such as resource-constraints. Patients also face barriers to communication, such as poor comprehension of risk.

Methods: We are exploring (1) how an electronic health record called My Medical Record (MyMR) might help patients/families understand AFs and (2) whether communication-based web-apps (that MyMR can integrate) could facilitate communication. We hypothesise that the interactivity of web-apps could mitigate some of patients' perceived barriers to communication by providing an efficient and focused view of the content to be communicated and by being more engaging and conducive to identifying and contacting relatives than (what is sometimes) a one-off clinical consultation. As a first step, we are determining patients/families' ($n = 25$) needs and views using mixed-methods research.

Results: Findings so far show that MyMR is under-used. But it has potential to improve understanding of, and increase perceived support for telling relatives about, risk. Crucially, participants underscored the importance of privacy, and transparency around, and control over, who has access to the record.

Conclusion: Based on our findings, we will design and develop web-tools; and pilot and rigorously evaluate them for functionality, usability, perceived impact, and utility.

L. Ballard: None. **A. Fenwick:** None. **A. Lucassen:** None. **A. Lucassen:** None.

P20.07C

ELSI concerning underage subjects in human genome/gene analysis research: the situation in Japan and the issues we face

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Background: Recently it has become possible to perform increasingly rapid and low-cost comprehensive genome sequencing. In Japan, these new technologies have given rise to a variety of clinical research. Purpose: Some of the subjects involved in these studies are minors. Using subjects who lack the ability to consent raises the issue of consent by proxy, as well as other issues that concern the specific nature of genetic information. In this presentation, I would like to consider the issues. Methods: Investigation and analysis of various research papers, guidelines, and legal regulations at home and abroad.

Results: In contrast to western countries, there has been little debate in Japan on the ELSI of minors used in medical research and the legislation concerning these issues has not been fully developed. In the case of human genome/gene analysis research, at present there are only guidelines that have no legal force. Considering the situation in Japan, it is clear that deliberate and binding regulations are needed in the areas of genetic research to protect the rights and interests of underage subjects. On the other hand, we should not ignore the benefits of the research.

Conclusions: Is the subject a healthy minor or one with a condition related to the research? Will the research be beneficial to the subject? How developed is the subjects' ability to give consent?—these kinds of questions should be used to help categorize cases, so that we can better create regulations. This work was supported by KAKENHI Grant Number JP15K08550.

N. Ohashi: None.

P20.08D

Legal and ethical issues for pharmacogenomics: the Ubiquitous Pharmacogenomics state of the art

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The Ubiquitous Pharmacogenomics (U-PGx) project aims at implementing pharmacogenomics testing in European patient care. Beyond scientific challenges, U-PGx must respond to ethical issues and comply with European requirements.

On the regulatory side, U-PGx will be challenged by the implementation of several EU instruments. By 2018, the Clinical Trial Regulation will implement the EU Portal and database, point of entry to submit all information (including genetic information) on clinical trials, which will be stored in the database and made public. At the same time, the General Data Protection Regulation will introduce new legal procedures implying then for U-PGx and all developers of pharmacogenetics drugs to modify data storage and management of the clinical trial. Finally, the in vitro diagnostic medical devices (IVD) Regulation (expected for 2017) will impact on U-PGx activities as it will include genetic testing as IVD in its scope.

From an ethical angle, the return of results in a clinical application project represents a major ethical and legal issue for patients' rights. Genetic data are intrinsically personal, familial and possibly identifying, which raises the issue of communicating results to patients and their families, and particularly of incidental findings. The European Society of Human Genetics recommends communicating unsolicited results in cases where a serious disease is discovered and if this disease can be treated or prevented; but there is no consensus in Europe. In order to achieve European harmonization, guidelines for informed consent need to be developed as a goal of all projects in pharmacogenomics.

A. Constantin: None. **M. Dupui:** None. **E. Rial-Sebbag:** None.

P20.09A

Paternity testing in minors under the cloak of recreational genetics

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M. H. D. Larmuseau, 1983; KU Leuven, Leuven, Belgium

Direct-to-Consumer (DTC) internet companies are selling widely advertised and highly popular genetic ancestry tests to the broad public. These tests are often classified as falling within the scope of so-called 'recreational genetics' but little is known about the impact of using these services. In this talk, a particular focus is whether minors (and under what conditions) should be able to participate in the use of these DTC tests. Current ancestry tests are easily able to reveal whether participants are related and can therefore also reveal misattributed paternity, with implications for the minors and adults involved in the testing. We analysed the

publicly available privacy policies and terms of services of 43 DTC genetic ancestry companies to assess whether minors are able to participate in testing DTC genetic ancestry and also if and how companies ethically account for the potential of paternity inference. Our results indicated that the majority of DTC genetic ancestry testing companies do not specifically address whether minors are able to participate in testing. Furthermore, the majority of the policies and terms of services fail to mention the vulnerability of minors and family members in receiving unexpected information, in particular in relation to (misattributed) paternity. Therefore, recreational genetics carries both the risk of unintentionally revealing misidentified paternity, and also the risk that fathers will deliberately use these services to test their children's paternity without revealing their intentions to the mother or any other third party.

N. Moray: None. **K.E. Pink:** None. **P. Borry:** None. **M. H.D. Larmuseau:** None.

P20.10B

Communicating information about genetic risks within the family: putting the family at the heart of it

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With the application of genome sequencing technologies, more and increasingly complex information is generated and so the need for genetic counselling and intrafamilial circulation of information on genetic risks grow accordingly. This is a great challenge, as families may feel unsure about how, when and whom to tell what. Ethical constraints generally prevent professionals from contacting relatives directly. Drawing on literature on the psychosocial and ethical aspects of disclosing genetic risk information to family members, and on a systematic review we undertook on how genetics professionals address such communication within families, we reflected on sharing information on genetic risks with relatives. Here we present several clinical cases, and our perspective on the main issues raised. Genetics and genomics are indeed "a family affair", because of the distinctive familial character of genetic information. Current ethical perspectives may not be completely appropriate for genomic testing. The framing and "effectiveness" of family communication about inherited conditions often fails to acknowledge the relational and communicative processes that operate within the family. Consultands often feel unsupported in this process and would welcome more support from health care professionals. Help offered by health care professionals is usually information-based and focuses predominantly on the individual, rather than the family unit. Strict adherence to a narrow concept of "non-directiveness" may be an additional obstacle. We propose that family-oriented interventions, such as multifamily discussion-groups, may help professionals to engage with families in this process, and should be included in training programmes for genetic counsellors.

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P20.11C

Diagnostic whole exome sequencing in pediatrics and developmental delay: a model for informed consent and return of incidental findings

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Introduction: Current models for informed consent and return of incidental findings (IFs) are not well-equipped to address the situations of children undergoing whole exome sequencing (WES) for clarifying a developmental delay (DD). We present a model that is tailored to these cases.

Method: An ethical framework was developed aimed at protecting both children's best interests and the interests of parents/relatives. Data from qualitative semi-structured interviews with parents of twenty children with a DD, aged <1–17, undergoing WES (trio-analyses) regarding views about WES and IFs were used to translate the ethical framework into guidelines in the model.

Results: Uncertainty about young children's/infants future cognitive development complicated choices for IFs, prompting some parents to ask whether they should decline certain IFs due to considerations surrounding a child's future autonomy. In contrast, parents of older children had clearer prognoses that their child would remain incompetent; their reasoning for/against receiving IFs was oftentimes related to their child's special needs. Interpreting a child's best interests requires acknowledging future autonomy considerations as a precautionary measure in cases with high uncertainty regarding the child's autonomous development. In cases with low uncertainty, i.e., future autonomy is highly unlikely/impossible, safeguarding the child's welfare should be a central aim.

Conclusion: The model has different disclosure guidelines for IFs (always/never disclose at present, opt-in/-out) based on the degree of certainty regarding the child's future autonomy. Parents should generally have more discretion over IFs when children's future autonomy is unlikely.

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P20.12D

Numberacy, risk information and ethical requirements of informed decision making

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Introduction: With a growing demand in many fields of medicine genetic counselling will become a part of physician patient communication also outside specialized contexts. Especially in such contexts of genetic counselling with physicians who's field of expertise is not genetics (eg physicians with a special 72hours training for genetic counselling according to German law GenDG and GEKO regulations), there is an ongoing controversy on the implications of this for dealing with risk information.

Method: While some experts believe that dealing with uncertainty and probability is something that can be improved by training and by providing information in a way that is not misleading, other experts are not as optimistic and vote for a new type of paternalism (libertarian paternalism). This controversy will be analyzed on two levels. First, based on the results of a review of studies on numberacy in the context of medical information it is discussed what type of professional expertise is needed to enable informed decision making. Second, if counselling is supported by standardized tools to support decision making the question arises if patients should have a choice about different types of information.

Results and conclusion: The paper will argue for a third way of assessing needs for information. It starts with the perspective of patients and their need to transform probabilistic knowledge on risk factors into options for agency, informing family members or life style changes. The knowledge difference between experts and patients can be bridged by better understanding how information gains relevance for patients.

J. Inthorn: None.

P20.13A

Readability of informed consent forms for whole exome/genome sequencing

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Whole genome sequencing (WGS) can generate an unprecedented amount of complex information making the informed consent (IC) process challenging. While the elements of information necessary for IC for WGS and pre-test counselling have been discussed and studied, research has not focused specifically on the way information is presented or worded in IC forms. The aim of our study was to assess the readability of IC forms of English language for clinical whole- exome or genome sequencing. Readability of forms was assessed using SMOG test (based on the number of complex words in sentences). The presence of additional elements such as requisition forms combined with consent forms, sections dedicated to healthcare professionals, and the length of documents were also analysed. We analysed 35 forms, most of which were from US providers. The largest readability grade group was 16 forms with a grade level of 13, indicating that the forms would be completely understandable for a person who completed 13 years of education (first year(s) university in the US system). The lowest and the highest grade levels were 12.7 and 18.4 respectively. All the forms studied seem to fail to meet the

recommended readability grade level of 8 (e.g. by US medical schools) for IC forms. The aspects of effective communication in consent forms are crucial given that in the future, WGS is likely to increasingly be offered by clinicians who are not experts in genomics and who may therefore be more reliant on the consent forms themselves to obtain informed consent.

E. Niemiec: None. **D. Vears:** None. **P. Borry:** None. **H. C. Howard:** None.

P20.14B

Australian Life Insurers use of genetic test results in underwriting decisions

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Since 1999, the Financial Services Council (FSC) has requested its Australian life insurance member companies provide data on applications where a genetic test is disclosed. The FSC provided data collected 2010–2013 to enable repetition of an independent analysis undertaken of applications 1999–2003 (Otlowski et al 2007 Aust J Law and Medicine).

Materials and methods: Data included de-identified insurer; age; gender; genetic condition; reason for testing and result; underwriting decision-maker; and insurance cover. Data was classified as to type of result or other factors relevant to risk, underwriting decisions and, where necessary, FSC-facilitated clarification by insurers.

Results: 340/547 applications were for adult-onset conditions: hereditary haemochromatosis (HH-200); cancer (51); thrombophilia (31); cardiovascular (17), neurodegenerative (13), neuromuscular (9); and other (19). The genetic test result solely influenced the underwriting decision in 170/340 applications: 24 positive, 139 negative, 2 uninformative, 3 pending and 2 unknown. Policies were provided at standard rate for all negative test results and 20/24 positive (HH-14, thrombophilia-5, breast cancer-1) with recognition of screening and risk reduction. Non-standard polices with a broad exclusion of all cancers were provided for positive BRCA2 (2) and Lynch syndrome results; for the two BRCA1/2 uninformative results, breast cancer exclusion and 50% loading were applied respectively. All applications with results pending (cancer-2, Huntington disease-1) were denied outright. There was evidence of reassessment of previous non-standard decisions when a negative result was declared.

Conclusion: No widespread systemic issues regarding genetic discrimination were identified though analysis is limited by the few positive test results disclosed.

M. Liepins: None. **M. Otlowski:** None. **A. Doble:** None. **K.K. Barlow-Stewart:** None.

P20.15C

Genetic testing and life insurance in Canada

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Introduction: Compared with many European jurisdictions, Canada has had no legal protection addressing the use of genetic test results by insurance companies; at present, a potential law regulating this issue is in the final stages of consideration by Canadian parliament. This study is a baseline assessment of Canadian insurance company applications and genetic testing. Materials and Methods: We reviewed primary applications for voluntary life insurance and critical illness insurance from Canadian companies. Applications were assessed for questions related to the applicant's medical history pertaining to genetic testing and counselling, and analyzed for their depth of family medical history assessment.

Results: None of the companies specifically asked about genetic testing, however all assessed if applicants pursued medical testing in the past. The majority assessed whether the applicant had sought consultation from a health professional, including advisement about testing. All applications asked about family medical history, with the majority limiting assessment to the parents and siblings. An average of 11 conditions were assessed for the family medical history. None of the applications asked if family members had sought genetic testing.

Conclusion: This study provides a baseline assessment of Canadian life insurance applications, and may serve as a benchmark for comparison after the probable passage of Canadian legislation. It is imperative that genetics professionals are educated on this topic, as it is a significant concern to many of our patients and their family members.

A.C. Bedard: None. **G. St-Martin:** None. **J.E.J. Bedard:** None.

P20.16D

Is there a legal duty to warn a patient's family about genetic diseases?

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Confidentiality is a cornerstone of medical practice, and is a key legal duty owed by healthcare practitioners (HCPs) to patients. However, because of the familial nature of genetic information, situations arise where HCPs may feel that confidential information about a patient should be disclosed to family members. In such cases, HCPs may find their ethical and legal duties are conflicting.

This paper analyses the English law of the duty of confidentiality and the duty to warn. We argue that the blunt exclusion of any duty to warn a family member in all circumstances in the recent case of ABC v St George's has pushed the balance of rights too far in the direction of the maintenance of confidentiality. As a result, a HCP will never be liable for failure to disclose to a third party, but could be liable for breach of confidence. A prudent HCP will therefore be likely to maintain confidentiality, in spite of ethical arguments in favour of disclosure. This is problematic.

We conclude that, while in most cases there will be no duty owed to family members, in certain limited circumstances, a duty of care should arise. The legal duties of confidence and tort are likely to increasingly compete as genetics becomes part of mainstream clinical practice and it is therefore important that the distinct legal tests for confidentiality and duty to warn result in legally and ethically congruent outcomes.

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N.L. Hawkins: None. **T. Hughes-Davies:** None.

P20.17A

'I would like to discuss it further with an expert': a focus group study of Finnish adults' perspectives on genetic incidental findings

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Introduction: Lowered costs of genomic sequencing facilitate analysing large segments of genetic data and generating many types of incidental findings (IFs). Intense ethical debate has focused on whether and what kind of IFs to report, and how to obtain valid informed consent, whereas what support or resources people need after receiving IFs has received less attention. The aim of this study was to explore Finnish adults' perspectives on the reporting of genetic IFs.

Materials and methods: We performed a qualitative study with four focus group discussions (N = 23). Four vignette letters were used, each reporting an IF predisposing to a different disease that varied in terms of treatability: familial hypercholesterolemia, long QT syndrome, Lynch syndrome and Li–Fraumeni syndrome. Transcribed focus group discussions were analysed through inductive thematic analysis.

Results: Despite a general positive attitude towards receiving IFs, concern of being left alone to deal with them was widely expressed. Empathetic expert support and access to preventive care were seen as essential to coping with the immediate shock and potential prolonged worry, and to being able to appropriately disclose the IF to family.

Conclusions: Professional discussion should focus not only on which IFs to report but also on how to do it in practice, and how to ensure access to relevant support and preventive care for the patient/participant and their family. Distrust towards research and health care may arise if practices of reporting IFs and referral for treatment are perceived as disrespectful, unorganized, or unequal.

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P20.18B

Assessing Next Generation Sequencing Recommendations in Oncogenetics: legal and ethical issues for patients' rights

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Since the discovery of DNA, technologies for sequencing have tremendously grown.leading to an increase of means for explaining physiopathology of diseases, elaborating diagnosis and proposing adequate treatment in particular for cancer. Hence, last generation sequencing permits to generate massive data to be applied for diagnosis to improve preventive measures or the treatment of small groups of patients in the context of the so-called personalised medicine. Several recommendations and initiatives are on-going in France (e.i. Plan France Génomique) and at the international level (International Cancer Genome Consortium, Global alliance for Genomics and Health, European Society of Human Genetics) trying to address the technical and Ethical, legal, social issues (ELSI) of these technological developments from research to their implementation in practice. However, these initiatives are wild and address the

challenges for society at a broad level. Thus, very few has been specifically developed in the area of oncogenetics whereas we hypothesis that there is a need to test and to adapt these recommendations for their implementation oncology services. The change of scale from "traditional" genetic tests to Next Generation Sequencing (NGS) in therapeutic or genetic counseling has raised specific issues for patients in oncology. To this end we surveyed the existing recommendations at the French, European and international level for supporting the use of NGS in oncogenetics. The analysis shows that despite several recommendations adopted from European and French institutions there is a need to reassess them in the specific context of oncogenetics notably regarding patients' rights and for their relatives.

E. Rial-Sebbag: None. **M. Maheu:** None.

P20.20D

Bioethical issues in personalized medicine and pharmacogenomics: Students' awareness and attitudes

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Introduction: Personalized medicine offers the detection of a disease at its earliest stage, the prevention of disease progression, and the optimization of disease treatment. However, there are many challenges in broader clinical implementation of pharmacogenomics. Specific concerns regarding pharmacogenomics are related to potential health disparities, stigma, use of direct-to-consumer genetic tests, genetic data handling, and patient's privacy. Materials and Methods: Here we performed a descriptive, cross-sectional study based on the survey of 559 students from several universities in Bosnia and Herzegovina (BH), including students of the Faculty of Pharmacy, Faculty of Medicine, Genetics and Bioengineering, and Faculty of Health Studies. The statistical analysis was performed by IBM SPSS®23.

Results: A majority of students participating in this survey showed an interest to perform a genetic test (70%) and agreed that personalized medicine represents a promising healthcare model (57%). Interestingly, 44% of the students were worried about the possibility that the results of pharmacogenomic test might be passed to unauthorized persons. Furthermore, the field of study appears to significantly influence the students' wish to continue their postgraduate education in the area of personalized medicine, prior (1.73 CI: 0.99–3.02), $p = 0.056$) and upon adjustment to age and gender (1.79 CI:1.01–3.19, $p = 0.045$) with the level of education particularly affecting the students' opinion (2.4 CI:1.28–4.48, $p = 0.006$).

Conclusions: The results of our survey indicate a positive attitude of biomedical students in BH towards pharmacogenomics and emphasizes its importance in the education of future healthcare professionals.

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P20.21A

Amended Act on the Protection of Personal Information of Japan and its impact to research and direct-to-consumer testing

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In Japan, the Personal Information Protection Act (the 'PIPA') was amended in 2015 and will fully come into force on May 30, 2017. The PIPA includes several important amendments to harmonize with the regulation on personal information in the EU, with respect to issues such as sensitive personal information, traceability and cross-border transfer of personal information. The Personal Information Protection Committee (the 'PPC') was established as an independent supervising authority to protect personal information instead of each Ministry. The new PIPA further clarifies what "personal information' is, in order to protect privacy with certainty. 'Base sequence constituting DNA taken from a cell' is newly included in the scope of personal information. Moreover, the New PIPA classifies personal information into two grades of protection with a new category of 'sensitive information.' This includes race, religion, medical history, and other personal information that could potentially lead to unjustifiable discrimination or prejudice. A prior consent is required to transfer sensitive information to a third party. An opt-out procedure for transferring personal information to third parties is not available for sensitive information. Regarding genomic research, researchers have to comply with Ethical Guidelines for Human Genome/Gene Analysis Research (2001, latest revision in 2013). The Cabinet Order was also amended to enforce the PIPA. We review the impact of its revision to research and direct-to-consumer genetics.

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P20.22B

Non-Invasive Prenatal Test (NIPT): a national study on receiving incidental findings

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Introduction: In the Netherlands NIPT is offered to pregnant women at increased risk for aneuploidy in a national trial. NIPT has a small chance to detect other chromosomal abnormalities than trisomies 13, 18 or 21. Often the incidental finding (IF) is merely present in the placenta, but sometimes also in the fetus. This study examined couples' attitude towards being informed of an IF and its psychological impact. Furthermore, reproductive choices were assessed.

Methods: Women who received an IF from NIPT between 2014 and 2016 were invited for a semi-structured interview (± 30–60 minutes). Seventeen women and 12 partners participated and provided written consent.

Results: Twelve women and ten partners were unaware that NIPT could yield IFs. All participants initially experienced receiving the IF as a shock. In three cases the IF was confirmed with amniocentesis, these couples subsequently chose to end their pregnancy. The other 14 pregnancies were monitored to make sure the fetus' growth was normal and all resulted in the birth of unaffected children. Although in those pregnancies the implications of the IF were not always clear, all couples insisted upon receiving this information to exert their reproductive autonomy. Couples counselled by a clinical geneticist experienced less uncertainty regarding their IF than couples counselled by any other specialist. Sixteen out of 17 women would opt for NIPT again in future pregnancies.

Conclusion: All participants expressed that IFs should be disclosed to enable reproductive autonomy. In pregnancies where NIPT yielded an IF, counselling by a genetic counsellor was psychologically favorable.

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P20.23C

Patient values and perceived utility of incidental genome sequencing results

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Introduction: Genome sequencing (GS) can refine diagnoses and treatments, but may incidentally reveal inherited risks for thousands of other diseases. Guidelines recommend clinicians inform individuals of their incidental GS results (IR). Previous research explores patient preferences using abstract concepts for IR, yet little is known about patient informed and deliberated values towards IR. We describe patient considered values and perceived utility of learning their IR following a deliberative, educational intervention.

Methods: Semi-structured interviews were conducted with 15 cancer patients participating in usability testing of a decision aid (DA) designed to facilitate informed, value-based selection of IR. Patients selected IR from five categories defined as being medically actionable or not. Content analysis was used to analyse the data.

Results: Participants were enthusiastic towards GS itself, and expressed an inherent value for its use in their own healthcare. All participants chose to receive some IR; 9 participants selected all IR. Participants' primary motivation for receiving IR was to inform disease prevention or treatment. When considering IR about non-medically actionable diseases, participants believed this information would encourage actions that could slow or delay onset. Participants also valued learning IR to benefit their relatives' health and to inform their families' future financial or reproductive planning.

Conclusion: Despite this small sample size, results reveal patients' enthusiasm for receiving IR. Patients apply broader definitions of actionability than medical experts, reflecting a key divergence in valuing this incidental

information. Findings can inform service delivery and policy guiding the expected return and anticipated use of IR from GS.

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