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Application of SNP array for rapid prenatal diagnosis – implementation, genetic counselling and diagnostic flow

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Abstract

We report on the validation and implementation of the HumanCytoSNP-12 array (Illumina) (HCS) in prenatal diagnosis. 64 samples were used to validate the Illumina platform (20 with a known (sub) microscopic chromosome abnormality, 5 with known maternal cell contamination and 39 normal control samples). There were no false positive or false negative results. In addition to the diagnostic possibilities of arrayCGH, the HCS allows detection of regions of homozygosity (ROH), triploidy and helps recognizing maternal cell contamination (MCC). Moreover, in 2 cases of maternal cell contamination, a deletion was correctly detected. Furthermore we found out that only about 50 ng of DNA is required, which allows a reporting time of only 3 days.

We also present a prospective pilot study of 61 foetuses with ultrasound abnormalities and a normal karyotype tested with HCS. In 4/61 (6,5%) foetuses a clinical relevant abnormality was detected. We designed and present pre-test genetic counselling information on categories of possible test outcomes. On the basis of this information about 90% of the parents chose to be informed about adverse health outcomes of their future child at infancy and childhood and 55% also about outcomes at an adult stage. The latter issue concerning the right of the future child itself to decide whether or not to know this information needs to be addressed.

Keywords: genomic SNP array, rapid genomic array testing, whole genome screening, pre-test counselling, prenatal diagnosis

Introduction

Array based comparative genomic hybridization (arrayCGH) has already been successfully employed in prenatal diagnosis.[1-7] Although arrayCGH cannot detect regions of homozygosity (ROH) and has a limited value for detecting triploidy and maternal cell contamination (MCC), single nucleotide polymorphism (SNP) arrays are still not routinely used for prenatal diagnosis in ongoing pregnancies. There are only two studies using a whole genome SNP array on foetal material. Tyreman et al.[8] have performed a retrospective analysis on 106 karyotypically normal referrals with abnormal ultrasound findings using the GeneChip 6.0 array from Affymetrix. Faas et al.[9] have also shown results of a SNP array (Affymetrix 250k NspI) on prenatal material. In both studies all analyses were performed after 24th week of pregnancy, termination of pregnancy (TOP), intrauterine foetal death (IUFD) or birth on DNA isolated from foetal or neonatal material, so rapid analysis was not required.

Prenatal genetic diagnosis after ultrasound detection of foetal abnormalities prefers a fast diagnostic technique, especially when there is a limited legal time frame for termination of pregnancy (TOP). The standard cytogenetic prenatal diagnosis includes rapid detection of specific abnormalities (most common aneuploidies and known microdeletions) and time-consuming karyotyping. Decisions regarding pregnancy management are best made taking into account both ultrasonographic and genetic results. Implementing rapid microarray technology in routine prenatal diagnosis will allow the detection of unbalanced chromosomal abnormalities in a shorter time frame with much better resolution than conventional karyotyping.

We report on the validation and implementation of a genomic SNP array (HumanCytoSNP-12, Illumina – HCS) in prenatal diagnosis. To our best knowledge it

is the first report describing the implementation of rapid whole genome SNP array analysis in ongoing pregnancies.

Material and methods

Pilot study design: From May 2009 till November 2010 the HumanCytoSNP-12 (HCS) microarray from Illumina (San Diego, USA) was performed on 125 prenatal samples. The 12-sample HumanCytoSNP-12 BeadChip (HCS) is a whole-genome scanning panel. It includes ~300,000 markers - genome-wide tag SNPs and markers targeting all regions of known cytogenetic importance. This includes dense coverage of around 250 genomic regions commonly screened in cytogenetic laboratories, including subtelomeric regions, pericentromeric regions, sex chromosomes, and targeted coverage in around 400 additional disease-related genes (www.illumina.com).

Validation was done on 64 prenatal samples which all were karyotyped before (table 1). Normal controls were patients referred because of advanced maternal age or abnormal first trimester screening. In these cases DNA was isolated from ~4 ml amniotic fluid and also used for RAD (rapid aneuploidy detection). DNA remaining after routine diagnostics was used to perform the array assay. That is the reason why some samples had very low DNA input. Samples with low DNA input were not excluded from validation to test whether HCS can be performed on patients with very low DNA concentrations. In the control cases parental DNA was unavailable.

In order to determine whether HCS can detect maternal cell contamination (MCC), 5 blood stained uncultured AF samples were tested (suspected MCC, including a sample fraction of one index patient not used for array testing). Suspected MCC was not routinely measured as those samples were cultured and not used for RAD.

61 index foetuses with ultrasound abnormalities (table in Supplemental Material) that were referred for array testing were examined with HCS. 14 patients were referred after termination of pregnancy or after birth (retrospective testing) and 47 foetuses were tested prospectively in an ongoing pregnancy. Some of them terminated the pregnancy before receiving the array results. The mean gestational age of the foetuses was 19 weeks (range 11–30 weeks). In all cases of ultrasound abnormalities detected in the second trimester, 30 ml AF was sampled for prenatal testing (20 ml was used for culturing and 10 ml was used for DNA isolation).

Only in 3 cases parental DNA was not available: case 4 – the parents refused to be tested; case 5 – a pathogenic deletion unlikely inherited from a parent was found; case 11 – the father refused to be tested.

In the index group only clear amniotic fluid samples were used for array testing, green/brown or blood stained samples were excluded due to possible MCC.

Sample preparation: 4-10 ml of uncultured amniotic fluid (uAF) was used for DNA isolation with the Chemagic Magnetic Separation Module I (Chemagen, BaesWeiler, Germany). The DNA yield from uncultured AF ranged between 10 and 500 ng. DNA from uncultured amniotic fluid was first used for RAD.

For DNA-isolation of cultured cells the midi kit from Qiagen (Hilden, Germany) was used, resulting in about 7-26 µg DNA per sample.

DNA concentration was measured by using a Quant-it DNA assay kit (broad range 2-1000 ng) (Invitrogen, Paisley, UK). Depending on the DNA concentration 4-10 µl of patient DNA was used for the array-assay.

SNP Array, data analysis and interpretation: During validation 1-200 ng of DNA was used as an input for a single array. DNA amplification, tagging and hybridization were performed according to the manufacturer's protocol. The array slides were scanned on an iScan Reader (Illumina). Data analysis was performed using Genome Studio version 2010.1, Karyo Studio version 1.2 (Illumina, standard settings) and Nexus Copy Number 5.0 (BioDiscovery, El Segundo, USA). The HapMap control set provided by the manufacturer was used as a control. After validation 50-200 ng of DNA was used (if possible) and the samples were only analysed by using Nexus.

Standard settings for SNP arrays in Nexus were adjusted: a cut-off value of 0.15, Homozygous Frequency Threshold of 0.95 and minimum LOH (region with loss of heterozygosity) length of 5000 (kb) were set (SNP-FASST Rank Segmentation). QC measurement in Nexus was used as a measure of the array profile quality. Samples with QC<0.13 were further analysed.

The B allele frequency (BAF) of HCS helps to recognise and exclude false-positive calls. The BAF is a value between 0 and 1 and represents the proportion contributed by one SNP allele (B) to the total copy number. A BAF value of 0.5 indicates a heterozygous genotype (AB), whereas 0 and 1 indicate homozygous genotypes (AA and BB, respectively). For example a region with a deletion in all cells will show homozygosity – bands at 1 and 0. In other words a loss should always be accompanied by a LOH visible in the BAF plot. A region of single copy number gain in all cells will, in addition to the two bands of homozygous SNPs at BAF = 0 (AAA) and BAF = 1 (BBB), also show two bands: one at BAF = 0.33 with SNPs having genotype AAB and one at BAF = 0.67 with SNPs having genotype ABB.

The following resolution was generally applied: losses ≥150 kb and gains ≥200 kb.

UCSC built Hg18 (Human Mar. 2006 (NCBI36/hg18) Assembly) was used to analyse the data. Copy number variations (CNVs) were checked in the local databases, DGV (http://projects.tcag.ca/variation/), CHOP database (http://cnv.chop.edu) and literature.

Parental DNA, when available, was analysed to identify inherited CNVs. Rare CNVs not associated with an increased risk or presence of abnormal phenotype, inherited from a healthy parent, were assumed to be benign. If a similar gain or loss was found in ≥3 healthy individuals (preferably in different studies) the call was recognized as a polymorphism (POL). The most important criterion for classification as MUT is association with a known abnormal phenotype. There was no cut-off for the size of the array abnormality. CNVs associated with an abnormal phenotype were assumed to be mutagenic (MUT). Also CNVs associated with an abnormal phenotype of variable penetrance or with a known increased risk of an abnormal phenotype were classified as MUT. ROHs (regions of homozygosity) are almost always of unknown clinical relevance except the cases where they indicate (segmental or whole chromosome) uniparental disomy of imprinted regions. A *de novo* (or of unknown inheritance) gain and loss (CNV) or copy neutral ROH of unknown (absence of a known disease causing gene) or uncertain clinical importance were defined as unclassified variants (UVs).

All MUTs were reported to the referring clinical geneticist. UVs were not reported, but in some cases (uncertain relevance) discussed with the referring clinical geneticist. Common polymorphisms (POL) and inherited (benign) rare CNVs were not reported. In this study, only clinically relevant CNVs were confirmed with another technique in order to validate HCS calls. We also followed recommendations of The Association for Clinical Cytogenetics (Constitutional Array CGH Best Practice Guidelines, http://www.cytogenetics.org.uk/prof_standards/professional_standards.htm).

After validation we decided to use an alternative technique to determine whether a specific imbalance is a true positive call only in cases where the possibility existed that an imbalance is not a true CNV. Clear imbalances do not require confirmatory studies.[10] Parental arrays were only used to determine the origins of foetal CNVs. Parental whole genome analysis was therefore not done to avoid unexpected findings.

Pre-test counselling: The gynaecologist referred pregnant women and their partner (= parents) with abnormal foetal ultrasound findings to the clinical geneticist. SNP array analysis was offered when a submicroscopic chromosomal aberration was included in the differential diagnosis. We designed a form in which categories of possible outcomes of the foetal microarray were described (table 2). Pre-test genetic counselling included general information about cytogenetics (RAD and conventional karyotyping), molecular genetics, a tailored description of the array test, benefits and limitations of the offered tests, and finally discussion of all possible test outcomes. The potential detection of variations of unknown or uncertain clinical relevance (UVs) was mentioned as well. Parents were told that these results would not be revealed to them. After genetic counselling they decided which test results they wish to receive (see table 2). During pre-test genetic counselling we discuss the very small chance of a genetic finding affecting the health of the parents / family members. For this validation study we did not include this as a separate category in the informed consent form, since parental arrays were only used in a targeted way to determine the origin of foetal CNVs. In future editions we will include this choice to have a more complete record. The parents could decide whether they wished to be contacted if clinical significant information about their child would become available in the future. A signed informed consent with the parents' choices was archived. Parental blood for determination of *de novo* versus

inherited variations was preferably sampled on the day of counselling or at the time of prenatal sampling.

Results

Figure 1 shows our current diagnostic flow. Now we choose to perform genomic array testing directly after rapid aneuploidy diagnosis (RAD) on uncultured amniotic fluid samples. Parental and foetal DNA are simultaneously analysed. The whole array assay (from DNA isolation to an array result) can be performed within 72h (3 days) if required. In general it takes 1 week mainly depending on the day of sampling.

During validation 18/20 known chromosome abnormalities were detected using HCS. The results of the validation samples are summarized in table 1. As expected the samples with a balanced inversion and AZFc microdeletion showed normal results. Triploidy was correctly identified (Fig. 2 upper panel). All blood stained samples with suspected maternal cell contamination were identified by HCS (compare a normal profile on Fig. 2 middle panel and a profile with MCC on Fig. 2 lower panel). Moreover, we tested two samples in which a deletion and maternal cell contamination were present (validation sample with a 10,2Mb del(13)(q22q31), and a blood stained fraction of a patient's sample with a 2,7Mb del(17)(q24.3q24.3)). Despite MCC, which was easy to recognise in the B-allele frequency (BAF)-plot in both samples, the deletions were correctly detected by Nexus 5.0 (see Fig. 3 for 17q deletion). Since both deletions were not called by Genome Studio and KaryoStudio, Nexus 5.0 was chosen as a primary analysis tool after we have validated it in a prenatal setting.

According to the manufacturer, the HCS requires input of 200 ng DNA. However, we have seen that samples with a very low DNA input can also show a good quality array

profile (see Supplemental Material). In general we estimate that samples with DNA input <50 ng are at risk of failure, but we do not exclude them from array analysis.

Eight normal control samples showed a low quality (QC>0.13) array profile probably due to low DNA input (<30 ng). There were no pathogenic abnormalities found in the normal control group. In 5/31 normal control samples unclassified variants (UVs) were detected. 2 (6,45%) of them were CNVs en 3 ROH >5 Mb (see table 1). The inheritance of the CNVs is unknown, since parental samples were not available for these normal validation samples.

61 index patients were tested with HCS. The results of index patients are summarized in Supplemental Material. In all cases, a normal karyotype was found during routine chromosome analysis. Most of the samples were karyotyped before the array was requested. Since September 2010 we perform a genomic array immediately after sampling. Only one index patient (case 2) showed no results probably due to low DNA input (40 ng).

There were 4/61 (6,5%) pathogenic abnormalities found:

Case 4: A 930 kb deletion in band 15q11.2, associated with an increased risk for seizures and behavioural disturbances, was detected.[11-13] The deletion was confirmed with MLPA kits 181 and 182 (data not shown). Parental DNA was unavailable. This finding belongs to category 3 - abnormal result that does not explain the ultrasound abnormality, however, an adverse effect of the CNV on seizures and behavioural disturbances could be expected.

In this case the array was requested after karyotyping was completed and before receiving the array results, the parents decided to terminate of the pregnancy based on

the ultrasound findings (NT 5.5 mm, atrio-ventricular defect (ASVD), microcephaly). The parents were counselled that this finding did not explain this combination of foetal ultrasound anomalies, but it is a risk factor for seizures and behavioural disturbances. The father and paternal uncle were known with seizures. A further investigation in the parents was not performed on their request. Presumably this 15q11.2 deletion is paternal.

Case 5: A 2,7 Mb deletion in band 17q24.3 associated with campomelic dysplasia was found.[14] The deletion was confirmed with Agilent 105K array (data not shown). Parental DNA was not tested because a pathogenic deletion unlikely inherited from a parent was found. This finding belongs to category 2 - abnormal result that explains the ultrasound abnormality (femoral bowing, possible skeletal dysplasia).

Case 51: An atypical 591 kb deletion (LCRC-LCRD) in band 22q11 associated with DiGeorge syndrome was found. The deletion was confirmed by MLPA kit P250 (data not shown). Exactly the same deletion was found in the maternal DNA sample. A patient with CATCH22 syndrome and a similar deletion has been published by Kurahashi et al.[15] This finding belongs to category 3 - abnormal result that does not explain the ultrasound abnormality, however, an adverse effect can be expected.

In this case the array was requested after karyotyping and before receiving the arrays results, the parents decided to terminate of the pregnancy based on the clinical severity of the ultrasound findings (plexus choroïdeus cyst, increased NT, echodens focus heart, unilateral pes equinovarus, intrauterine growth retardation (IUGR), oligohydramnion). The parents were counselled that this finding did not explain all of the ultrasound anomalies. This finding is associated with DiGeorge syndrome; therefore the mother

will be investigated for features of DiGeorge syndrome. Further investigations to the aetiology of the ultrasound abnormalities are still pending.

Case 60: A 2,1 Mb *de novo* deletion (overlapping with the commonly deleted region LCRA-LCRB-LCRC) in band 22q11 associated with DiGeorge syndrome was found in a foetus with single umbilical artery, extreme IUGR, echodens bowel, and pericardial effusion. The deletion was confirmed by MLPA kit P250 (data not shown). This finding belongs to category 3 - abnormal result that does not explain this combination of ultrasound anomalies, however, an adverse effect can be expected.

HSC showed 15, 4, and 6 >5 Mb ROHs in three foetuses of consanguineous parents (cases 10, 52 and 61). In three other cases a ROH was detected (cases 45, 55 and 58), but the parents were not consanguineous.

In 6/61 (9,8%) samples a CNV of unknown clinical importance (UV) was detected (see Supplemental Material cases 6, 11, 13, 50, 55, 61).

Because of the complexity concerning possible outcomes of microarrays in a prenatal diagnostic setting, we designed a consent form in which they are categorised and discussed with the parents during pre-test genetic counselling (table 2). Currently, due to this complexity, we only accept referrals from clinical geneticists for prenatal microarray analysis. Evaluation of the parents' choices showed that most of them were interested in results that (most probably) have an adverse health effect in infancy and childhood (~90%). About half of the couples (~55%) chose to be informed about findings that affect the future health of their unborn child at an adult stage as well (table 2). We considered the a priori chance of finding a genetic imbalance with clinical consequences for the parent(s) /family member(s) to be very small, since parental arrays

are only screened for foetal CNVs in a targeted way. In the present study we did not find any CNV of this kind.

Discussion

In The Netherlands a second trimester foetal ultrasound examination is offered to all pregnant women around the 20th week of gestation, generally performed by a midwife. Parents are referred to a gynaecologist if abnormalities are detected. Subsequent amniocentesis is mostly performed at about 20-22 weeks of gestation. The legal time frame for TOP in The Netherlands is before the 24th week of gestation. Before the implementation of HCS we used the 105K/180K oligo-array from Agilent for whole genome analysis in prenatal diagnosis (validation on 20 samples and after implementation 85 index patients tested - data not published). Because of the need for AF cell culturing, it was often not feasible to produce a result before the 24th week of gestation. At that time (2007-2009) we did not choose to perform pre-test whole genome amplification of DNA from uncultured AF cells due to the chance of selective amplification bias.[16] We noticed that in many cases genomic array results did not contribute to the parents' decision about termination or continuation of the pregnancy, because the reporting time was too long, or the array was requested after karyotyping appeared to be normal (for example case 4 and 51). As parents confronted with an abnormal result need time to consider the continuation of their pregnancy, only a 1-2 week reporting time of genetic testing is acceptable and the array should be requested immediately after amniocentesis. Since HumanCytoSNP-12 (Illumina) requires only 50 ng DNA, implementing HCS in prenatal diagnostics allowed using uncultured AF cells

for direct array analysis, which shortened the reporting time to one week or even 3 days if required.

In our experience HCS allows detecting unbalanced chromosome abnormalities with a resolution of 150-200 kb. We demonstrated that the HCS detected all known unbalanced abnormalities (inclusive triploidy) except for an AZFc microdeletion. Samples with MCC could be identified and deletions could be correctly detected in such samples. However, we are aware that a gain in a sample with MCC might be missed due to the BAF imbalance already present because of MCC. For that reason we try to exclude blood stained samples from the diagnostic array assay. ROHs can be detected, which can help to detect uniparental disomy. Despite the high failure rate (20%) during the validation of the normal control samples (many samples with low DNA input), we decided to conduct our pilot on 61 index foetuses since we would have enough DNA in pregnancies tested prospectively. In pregnancies with ultrasound abnormalities, our target for array analysis, 30 ml of amniotic fluid, is sampled in our centre instead of the standard 20 ml which will yield enough DNA in most cases.

A clinically relevant chromosome abnormality was found in 6,5% (4/61) of our index patients with a normal karyotype, which is concordant to the data in the literature.[8,17-19]

In prenatal settings genomic regions of clinical importance can be overlooked by targeted arrays designed for postnatal studies.[8,20] Coppinger et al.[21] demonstrated that whole genome analysis does not increase the number of results of unclear significance when compared to targeted screening. We have carefully chosen a platform that allows for prenatal whole genome screening on uncultured amniotic fluid cells without increasing the number of calls, allowing a quick analysis. HCS is a whole genome panel designed to detect cytogenetic abnormalities and therefore, probes for some (highly

polymorphic) regions are not included. We found about 9,8% (6/61) CNVs of unknown significance in the index patients group, whereas Tyreman et al. found 12% (13/106) of them.[8] They tested a similar group, however they did not test the parental DNA and the array they used (Affymetrix 6.0) has a different (higher) probe coverage, which may explain the differences. Moreover, we have also chosen not to analyse deletions <150 kb and duplication <200 kb, because the capacity to reliably detect single CNVs below 100 kb, appeared to be limited for ~300 k platforms[22] , and performing high-throughput higher resolution array analysis in such a short time frame might be not feasible. Our approach is a compromise of a rapid screening with a resolution that is much better than conventional karyotyping.

The arguments for implementing HCS as a primary diagnostic tool for foetuses with ultrasound abnormalities were:

- ~50 ng DNA input; no need for culturing amniotic fluid cells (most patients are referred in the second trimester), no extra amplification is necessary before the array assay.
- Higher resolution screening results on uncultured AF is faster than karyotyping
- No need to take along a control (like in case of 105/180K Agilent).
- Detection of CNV, ROH and polyploidy, and easier recognition of false positive calls (due to extra information from the BAF).
- Whole genome analysis does not increase the number of results of unclear significance when compared to targeted screening[21]
- Low level of mosaicism is easier to determine due to information from the BAF[22]

After pre-test counselling the parents had an opportunity to decide which test results they wish to receive (table 2). None of the couples disagreed on our policy that abnormalities of uncertain or unknown clinical significance (UVs) would not be revealed to them. We decided not to inform the parents about UVs, because these findings may be clinically irrelevant for the ongoing pregnancy and may cause anxiety. Although we did not notify the parents about UVs, it is important to collect these data and detailed clinical follow-up of the patients because in the future an association may be found.

Most of the parents were interested in genetic abnormalities influencing the health and development of their unborn child in infancy and childhood. However, about half of the parents also chose to be informed about genetic abnormalities affecting the health of their future child in adulthood. So far we didn't detect a CNV that probably has an adverse health effect in adulthood. In case we find one, the parents will receive extensive genetic counseling on possible consequences and will be offered psychological counseling. In the Netherlands the foetus is legally considered to be part of its mother and therefore not protected by the right not to know. The disclosure of genetic information involving the health in adult life of an unborn individual could be regarded as a violation of its autonomy. Moreover, this information may have implications on the health of one of the parents and possibly also on his or her family. Naturally, these issues are addressed in the pre-test counselling and it will be included in our new informed consent form. More research needs to be done on the motivations of the parents in choosing which test results they wish to receive. In addition we have to investigate whether future parents can truly make informed decisions concerning these complex matters in such a small time frame. [24]

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

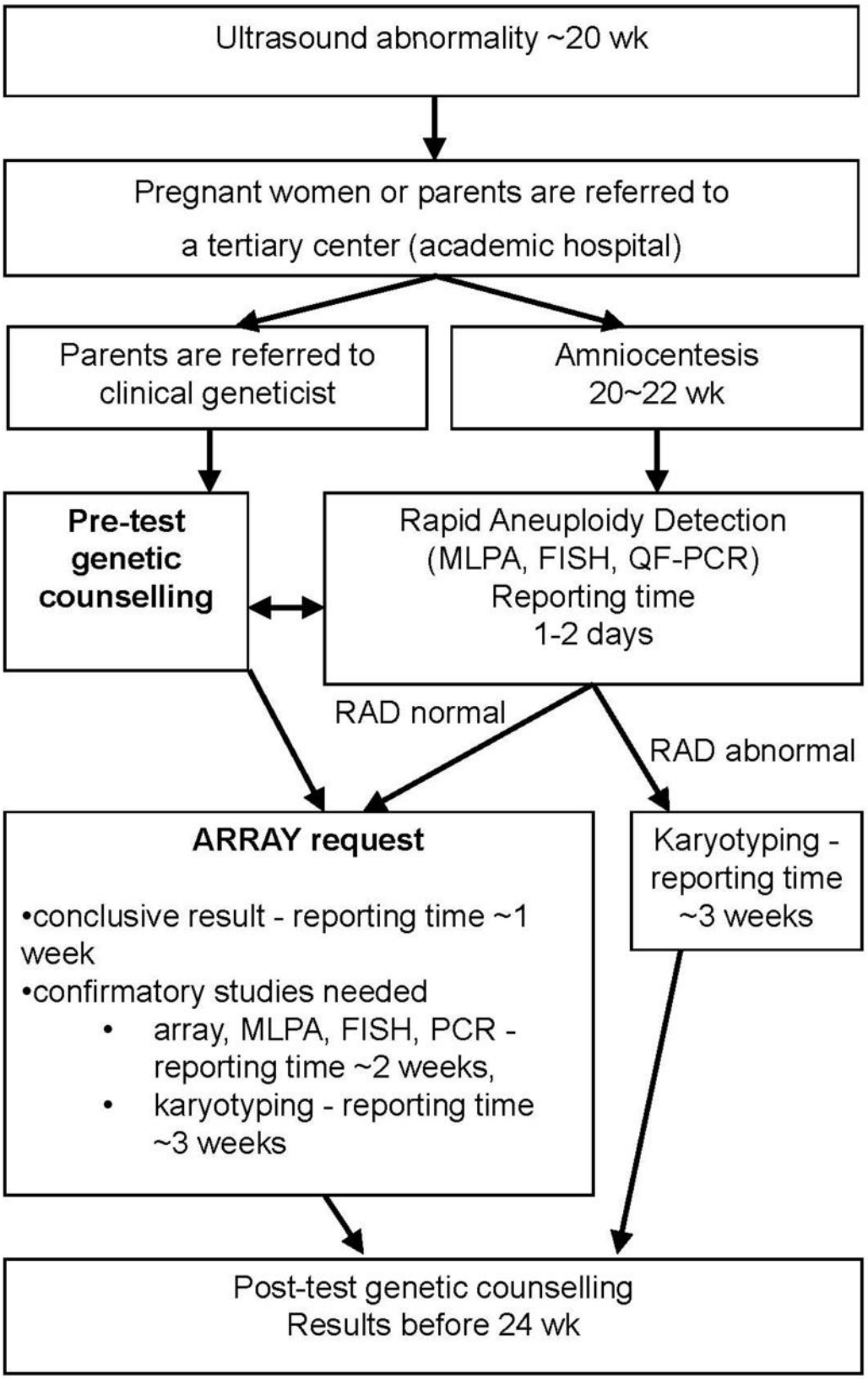
Figure 1. Current diagnostic flow.

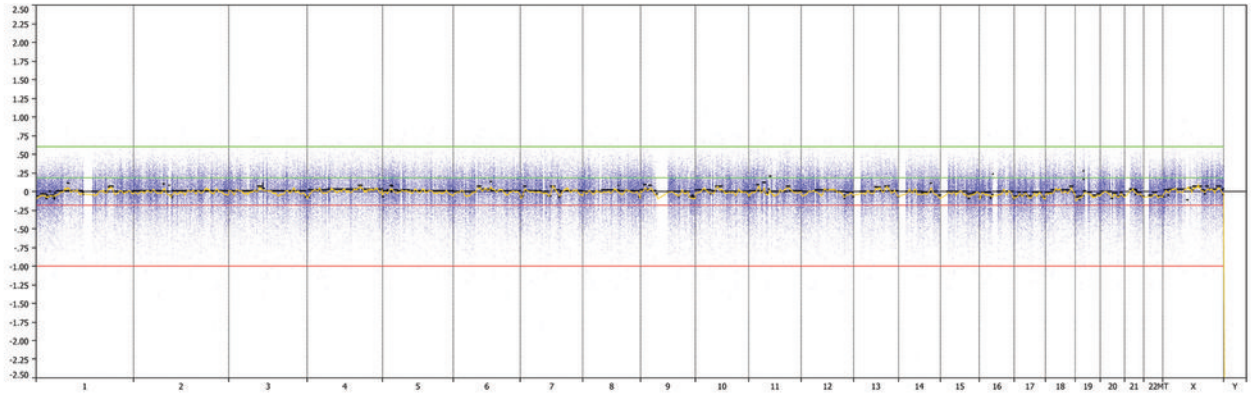
Figure 2. Whole genome view of Human CytoSNP-12 profiles in Nexus 5.0.

Both Log Ratios (above) and B-Allele Frequencies (BAF) (below) are shown. The upper panel shows a profile of triploidy 69,XXX. The LogRatio is normal; however, the BAF indicates a duplication of all chromosomes. There is no normal frequency at ~0.5, but two at ~0,4 and ~0,6. The DNA input was 117 ng, the QC=0.071. The middle panel shows a normal male profile. The Log Ratio is about 0 (except for X) and the BAF shows three lines (upper and lower (~1.0 and ~0.0) – homozygosity, middle (~0.5) - heterozygosity). The DNA input was 164 ng, the QC= 0.038. The lower panel presents a profile with an indication for maternal cell contamination. BAF shows additional B allele frequencies at ~0,1 and ~0,9 on all chromosomes. The DNA input is 202 ng, the QC=0.047.

Figure 3. The 17q deletion in a sample with maternal cell contamination (MCC) (case 5 Supplemental Material) shown on a chromosome plot from Nexus 5.0. The deletion in 17q24.3 was correctly identified by Nexus 5.0 in both clear and blood stained AF. The upper panel shows the chromosome 17 plot of a sample with a 17q deletion and with MCC (uncultured blood stained AF, DNA input 118 ng, QC=0.067). The lower panel shows the chromosome 17 plot of the same patient sample with a 17q deletion, but the array assay is performed on uncultured clear AF (DNA input 54 ng, QC=0.07).

Diagnostic flow

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-.25
-.50
-.75
-1.00
-1.25
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-2.50
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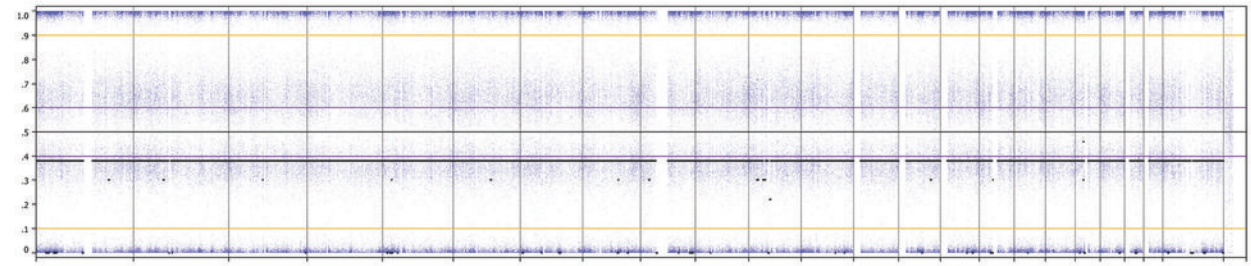
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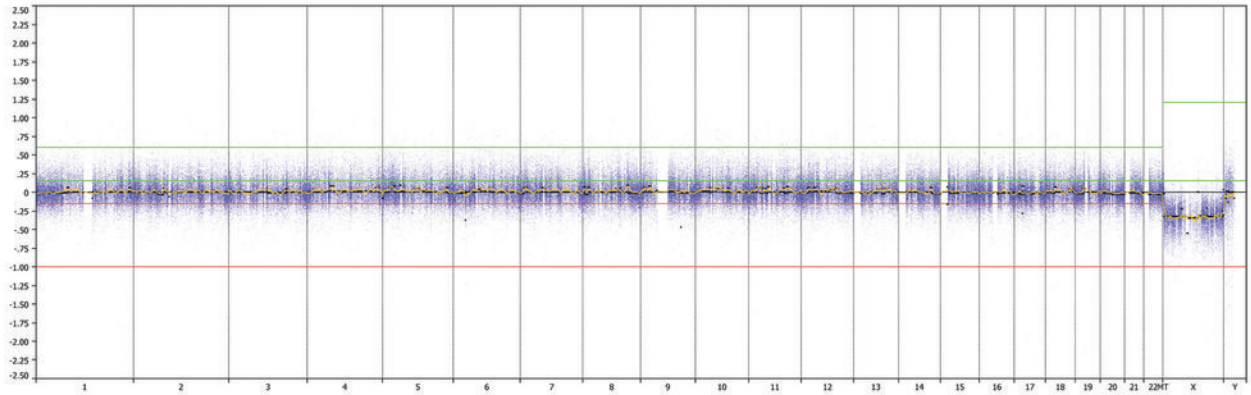
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-2.50
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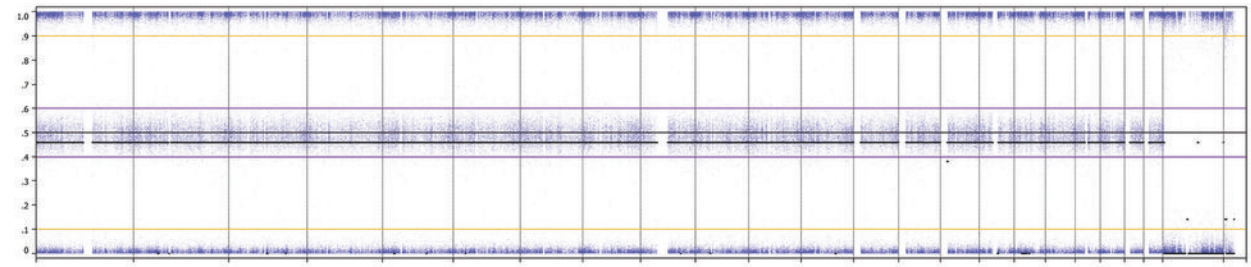
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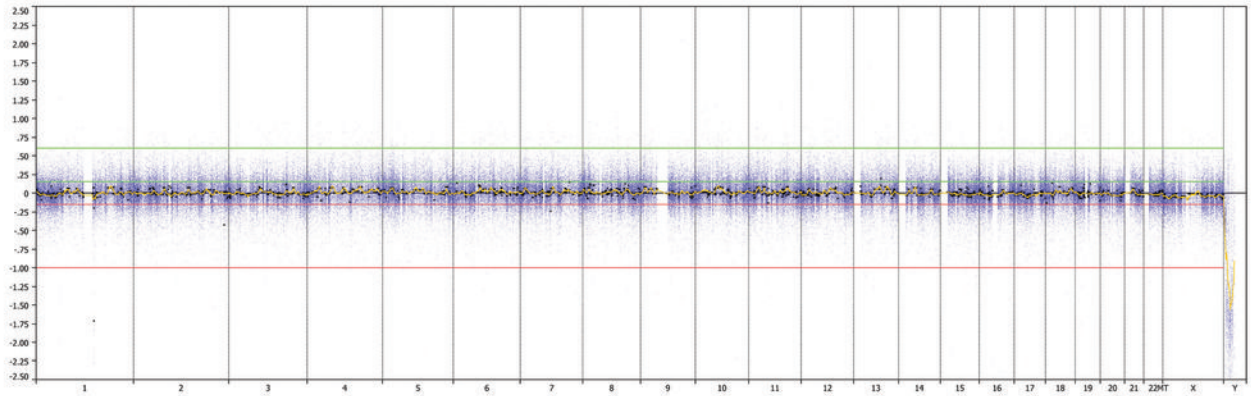
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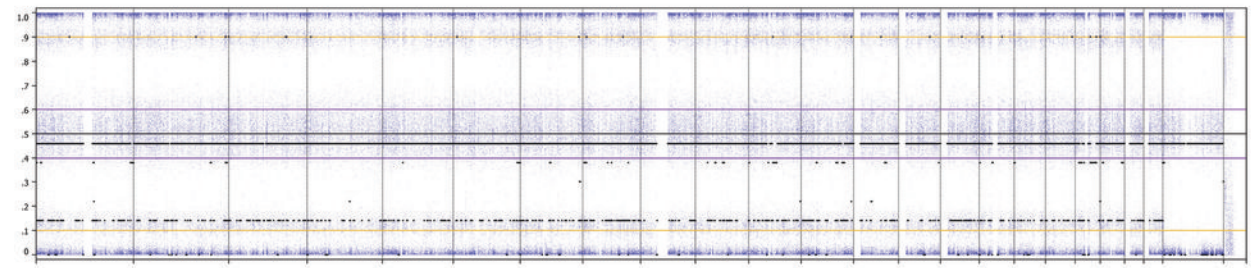
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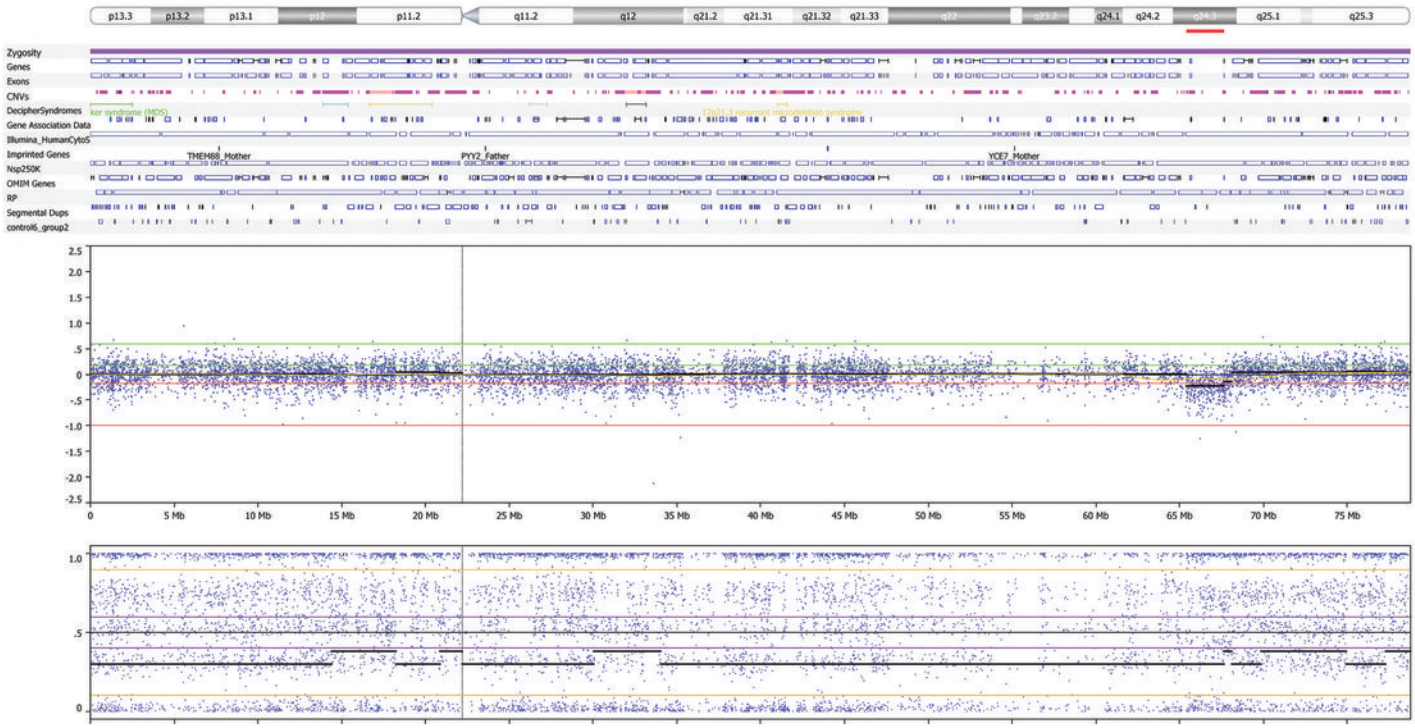
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q12
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q21.31
q21.32
q21.33
q22
q23.2
q24.1
q24.2
q24.3
q25.1
q25.3
Zygosity
Genes
Exons
CNVs
DecipherSyndromes
Gene Association Data
Illumina_HumanCytoS
Imprinted Genes
Nsp250K
OMIM Genes
RP
Segmental Dups
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TMEM88_Mother
PIY2_Father
YCE7_Mother
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15 Mb
20 Mb
25 Mb
30 Mb
35 Mb
40 Mb
45 Mb
50 Mb
55 Mb
60 Mb
65 Mb
70 Mb
75 Mb

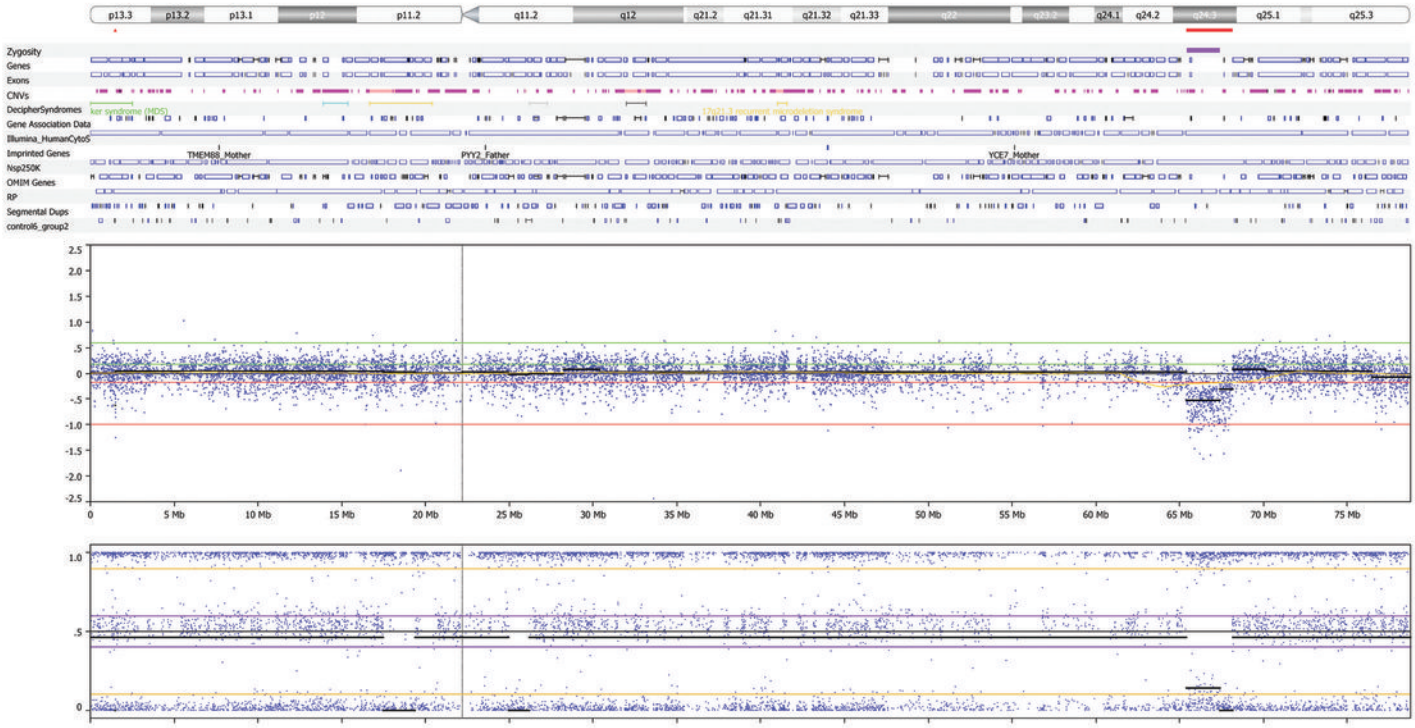
p13.3
p13.2
p13.1
p12
p11.2
q11.2
q12
q21.2
q21.31
q21.32
q21.33
q22
q23.2
q24.1
q24.2
q24.3
q25.1
q25.3
Zygosity
Genes
Exons
CNVs
DecipherSyndromes
Gene Association Data
Illumina_HumanCytoS
Imprinted Genes
Nsp250K
OMIM Genes
RP
Segmental Dups
control6_group2
TMEM88_Mother
PIY2_Father
YCE7_Mother
0
5 Mb
10 Mb
15 Mb
20 Mb
25 Mb
30 Mb
35 Mb
40 Mb
45 Mb
50 Mb
55 Mb
60 Mb
65 Mb
70 Mb
75 Mb

Table 1. The HCS validation results.

Group	N	Chromosome constitution	HCS results
Known abnormal samples	**25**		
Known autosomal chromosome aberration cases (targeted analysis)	13 (uAF, cAF or LTC)	T13; T18; T21; del(13)(q22q31); del(18)(p11.32p11.23); del(9)(q34); del(22)(q11q11); der(18)t(16;18)(p13.3;p11); del(7)(q3?5); del(13)(q21.1q21.3); inv(13)(q13q21.3), 69,XXX; 69,XXY.	All confirmed, except for the balanced inversion (12/13)
Known sex chromosome aberration cases (targeted analysis)	7 (uAF, cAF or LTC)	47,XXY; 47,XXX, 45,X; 46,X,i(X); 47,XYY; 46,X,t(X;Y); microdeletion Y (AZFc)	All confirmed, except for microdeletion Y (no probes overlapping with AZFc) (6/7)
Blood stained samples with suspected MCC	5 (uAF)	17q24 2,7Mb loss; del(13)(q22q31); 1x 46,XX, 2x 46,XY	MCC correctly identified (5/5), both deletions called by Nexus
Normal control samples	**39**		
Normal control patients (AMA and/or FTS) (whole genome analysis)	39 (uAF)	normal karyotypes	No abnormalities associated with abnormal phenotype were found (8 failed due to too low DNA input: 2x 1ng, 2ng, 2,5ng, 9ng, 10ng, 11ng, 30ng) 26/31 normal 2/31 (6,45%) CNVs of unknown/uncertain clinical significance (inheritance unknown, parental samples unavailable 1) arr **12p13.33**(1,652,754-2,453,037)**x3** 2) arr **7q32.2q32.3**(130,022,769-130,495,753)**x3**, **11p11.12**(50,951,547-51,398,585)**x3**

			3 samples with ROH of unknown clinical significance: 1) arr **8q12.1q12.3**(57,056,403-62,782,107)x2 hmz 2) arr **8q13.1q21.3**(67,128,593-89,067,187)x2 hmz, **11q13.3q13.5**(69,634,104-76,058,460)x2 hmz 3) arr **2q32.1q32.2**(183,889,086-189,468,291)x2 hmz

NB. Physical positions of the breakpoints utilize the hg18 build of the human genome sequence

AMA – Advanced Maternal Age
FTS – Abnormal First Trimester Screening
MCC – Maternal Cell Contamination
POL – Polymorphism
T - Trisomy
UV - Unknown Variants
cAF – cultured Amniotic Fluid
uAF – uncultured Amniotic Fluid
LTC – Long Term Culture chorion villi
ROH - Region of Homozygosity

Table 2. Pre-test counselling: possible outcomes of the genomic array examination and choices of the parents (after pre-test counselling and before array analysis).

Possible outcomes and the meaning of an abnormality seen in array analysis:		
1. No evidence of known disease-causing abnormality 2. Abnormal result that explains the ultrasound abnormality 3. Abnormal result that does not explain the ultrasound abnormality, however, an adverse effect on the growth and / or development of the child is (most probably) expected. Sometimes the nature or severity of the defects / disease cannot be predicted 4. Abnormal array results that (most probably) have an adverse health effect in adulthood 5. Inherited DNA variation of yet unknown significance (~10% of results): little cause for concern unless the parent has a congenital anomaly / developmental disorder 6. New DNA variation (not inherited) of unknown significance (~10% of results) Sometimes the meaning of it becomes clear in the future		
Choices of the parents		
More than one option is possible		% of parents who have chosen the particular option
1	Only an abnormal result that explains ultrasound abnormality	11,1%
2	Results that probably have an adverse health effect in infancy and childhood	88,9%
3	Results that probably have an adverse health effect in adulthood	55,5%
4	I would like to be informed later when clinically important information becomes available	61,1%
5	Results that probably have an adverse effect on my own health	Discussed during pre-test counselling together with category 3, but not yet included as a separate category in the informed consent form.