



HAL
open science

The diagnostic rate of inherited metabolic disorders by exome sequencing in a cohort of 547 individuals with developmental disorders

Julian Delanne, Ange-Line Bruel, Frédéric Huet, Sébastien Moutton, Sophie Nambot, Margot Grisval, Nada Houcinat, Paul Kuentz, Arthur Sorlin, Patrick Callier, et al.

► To cite this version:

Julian Delanne, Ange-Line Bruel, Frédéric Huet, Sébastien Moutton, Sophie Nambot, et al.. The diagnostic rate of inherited metabolic disorders by exome sequencing in a cohort of 547 individuals with developmental disorders. *Molecular Genetics and Metabolism Reports*, 2021, 29, pp.100812. 10.1016/j.ymgmr.2021.100812 . hal-04453181

HAL Id: hal-04453181

<https://hal.science/hal-04453181v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

BRIEF COMMUNICATION

The diagnostic rate of inherited metabolic disorders by exome sequencing in a cohort of 547 individuals with developmental disorders

Julian Delanne^{1,2}, Ange-Line Bruel^{1,3}, Frédéric Huet⁴, Sébastien Moutton^{1,2}, Sophie Nambot^{1,2}, Margot Grisval⁴, Nada Houcinat², Paul Kuentz^{1,3,5}, Arthur Sorlin^{1,2,3}, Patrick Callier^{1,6}, Nolwenn Jean-Marçais², Anne-Laure Mosca-Boidron⁶, Frédéric Tran Mau-Them^{1,3}, Anne-Sophie Denommé-Pichon^{1,3}, Antonio Vitobello^{1,3}, Daphné Lehalle², Salima El Chehadeh², Christine Francannet⁷, Marine Lebrun⁸, Laetitia Lambert⁹, Marie-Line Jacquemont¹⁰, Marion Gerard-Blanluet¹¹, Jean-Luc Alessandri¹², Marjolaine Willems¹³, Julien Thevenon^{1,2,3}, Mondher Chouchane⁴, Véronique Darmency⁴, Clémence Fatus-Fauconnier⁴, Sébastien Gay¹⁴, Marie Bournez⁴, Alice Masurel², Vanessa Leguy⁴, Yannis Duffourd^{1,3}, Christophe Philippe^{1,3}, François Feillet¹³, Laurence Faivre^{1,2,3#}, Christel Thauvin-Robinet^{1,3,15#}.

1. INSERM – University of Bourgogne Franche-Comté, UMR 1231 GAD team, Genetics of Developmental disorders, FHU TRANSLAD, CHU Dijon Bourgogne, France;
2. CHU Dijon, Centre de référence maladies rares Anomalies du Développement et Syndromes Malformatifs, Centre de Génétique, FHU TRANSLAD, CHU Dijon Bourgogne, France;
3. Unité Fonctionnelle d'Innovation diagnostique dans les maladies rares, Laboratoire de Génétique chromosomique moléculaire, CHU Dijon Bourgogne, France ;
4. Centre de Compétence Maladies Héritaires du Métabolisme, CHU Dijon Bourgogne, France ;
5. Biologie moléculaire, CHU Besançon, Besançon, France ;
6. Laboratoire de cytogénétique et génétique moléculaire, CHU Dijon Bourgogne, France ;
7. Service de Génétique Médicale, Centre de Référence Déficiences Intellectuelles de causes rares, CHU Clermont Ferrand, France.;
8. Laboratoire de génétique, CHU de Saint-Etienne, Saint-Etienne, France ;
9. Service de Génétique Clinique, CHRU Nancy, France ;
10. Unité de Génétique Médicale, Pole Femme-Mère-Enfant, Groupe Hospitalier Sud Réunion, CHU de La Réunion, La Réunion, France ;
11. APHP, Department of Genetics, Robert Debré Hospital, Paris, France;
12. Service de Réanimation Néonatale, Pole Femme-Mère-Enfant, CH Felix Guyon, CHU de La Réunion, Saint-Denis, La Réunion, France ;
13. Department of Medical Genetics, Reference Center for Rare Diseases, Developmental Disorders and Multiple Congenital Anomalies, Arnaud de Villeneuve Hospital, Montpellier, France;
14. Service de Pédiatrie, CH William Morey, Chalon-Sur-Saône, France ;
15. Centre de référence maladies rares Déficiences Intellectuelles de causes rares, Centre de Génétique, FHU TRANSLAD, CHU Dijon Bourgogne, France

These authors equally contributed to the work

Abstract: 74 words

Wordcount: 1146 words

Number of table: 1

Keywords: Inherited Metabolic Disorders, Exome sequencing, Intellectual disability, Developmental delay, Genotype first.

Synopsis: Exome sequencing and diagnostic rate of inborn errors of metabolism in individuals with developmental disorders

Corresponding author:

Pr Christel Thauvin-Robinet, MD, PhD

Centre de Génétique, Hôpital d'Enfants, CHU Dijon Bourgogne

Phone: +33 (0)3.80.29 53 13 /Fax: +33 (0)3.80.29.32.66

e-mail: christel.thauvin@chu-dijon.fr

Conflict of interest statement

The authors declare no conflicts of interest.

Abstract

Considering that some Inherited Metabolic Disorders (IMDs) can be diagnosed in patients with no distinctive clinical features of IMDs, we aimed to evaluate the power of exome sequencing (ES) to diagnose IMDs within a cohort of 547 patients with unspecific developmental disorders (DD). IMDs were diagnosed in 12% of individuals with causative diagnosis (177/547). There are clear benefits of using ES in DD to diagnose IMD, particularly in cases where biochemical studies are unavailable.

Introduction

Inherited Metabolic Disorders (IMDs), which affect 1/500 live-born infants, harbor a great phenotypical and genetic heterogeneity^{1,2}. When an IMD is suspected without any obvious clinical diagnosis, a first-line biochemical screening is generally proposed (lactate and pyruvate levels, plasma amino acids, urine organic acids, acylcarnitines, ketone bodies and very long chain fatty acids). Usually, these initial results drive specific secondary investigations, mainly based on enzymatic studies and/or targeted genetic analyses. This strategy offers an overall diagnostic yield around 50%, when clinical features are highly suggestive of IMDs (i.e. encephalopathy, coma, hypotonia or organomegaly) and are associated with biological marker elevation^{3,4}.

Exome/genome sequencing (ES/GS) has revolutionized translational research and diagnosis in rare diseases in a diagnostic genotype-first approach, followed by reverse phenotyping⁵. Harboring a high diagnostic yield (40-70%) in suspected IMDs⁶, ES has also appeared efficient in individuals with intellectual disability (ID) and unexplained metabolic anomalies (diagnostic yield 68%)⁷. Some authors therefore suggested updating the diagnosis strategy for IMDs in different steps, bringing together first-line biochemical screening and targeted next-generation panels⁴.

For years, biochemical screening has been indicated in first-line etiological investigations for individuals with global developmental delay (DD) or ID^{8,9}. However, in isolated ID, the diagnostic yield of first-line biochemical screening is extremely low, around 1%, increasing to 5% in the presence of specific neurological features¹⁰. ES now appears to be one of the most cost-effective and powerful tools for the diagnosis of ID, with a mean diagnostic yield of 36%,^{5,11-16}. It has dramatically improved the diagnosis of unspecific or atypical phenotypes and has led to the discovery of hundreds of unknown genes¹⁷.

Here, we aim to evaluate the power of ES to diagnose IMDs in a cohort of 547 patients with non-specific developmental disorders.

Patients and methods

Over a five-year period (2015-2019), we recruited 547 individuals affected with a wide variety of developmental disorders. The local ethics committee approved the study (DC 2011-1322). They presented with multiple congenital anomalies or syndromic ID (56%), non-syndromic ID (20%), seizures or epileptic encephalopathy (9%), abnormal neurologic features without seizure (5%) or other presentations (10%). Seventeen were the offspring of consanguineous parents. The majority of patients had solo or trio ES after different genetic tests selected according to their phenotype, particularly array-CGH. A minority of patients had ES as a first diagnostic test.

ES was performed from DNA obtained from blood samples. A solo strategy was used in 506/547 individuals (92%), following protocols previously described^{5,18,19} and American College of Medical Genetics and Genomics guidelines²⁰. All candidate or pathogenic variants were verified by a second genetic technique, as well as familial segregation. If available, biomarkers were retrospectively checked to confirm ES results.

Results

In the overall cohort, 177/547 individuals (32%) had a positive diagnosis identified by ES⁵. Within this cohort, 21/177 individuals (12%) were diagnosed with 15 different IMDs (Figure 1, Table 1). No dual diagnosis was found. Therefore, the diagnostic yield for IMDs included 3.8% of the total cohort. Nineteen of these 21 individuals were live-born (9 males and 10 females), ranging from 6 days to 44 years of age. Two were fetuses (1 male and 1 female),

aged 27 and 33 weeks of gestation, presenting multiple congenital anomalies. Ten individuals had disorders of organelle biogenesis, dynamics and interactions, five neurotransmitter disorders, two congenital disorders of glycosylation (CDG), two disorders of mitochondrial cofactor biosynthesis, one disorder of mitochondrial DNA maintenance and replication, and one disorder of amino acid metabolism (Table 1). Five individuals have already been published in the literature²¹⁻²⁴. Eight of the 17 IMDs did not have known specific biomarkers (*DNML1*, *ADCK3*, *ALDH18A1*, *ST3GAL5*, *SLC13A5*, *SLC6A1*, *NGLY1*, *PIGN*), although two of them display non-specific elevated lactates (*DNML1*, *ADCK3*) (figure 1B). Within this cohort, two treatable diseases were diagnosed, leading to a direct benefit for the affected individuals (*GLUT1*, *SPR*) (details in supplemental data).

Twelve of the of these 21 individuals (57%) benefited of variable biochemical investigation. Eighteen were alive when the ES results were returned to the physicians. Specific treatments or diet were given to 5/18 individuals (28%).

Discussion

ES identified a diagnosis of IMDs in 3.6% of cohort of individuals with non-specific developmental disorders, accounting for 12% of the causal diagnoses. Panel and ES showed similar results (13%) in a smaller cohort of individuals with childhood epilepsy.²⁵ However, considering the prevalence of IMD (1 in 500 live born infants), this rate appears low because most individuals affected with IMDs did not present developmental disorders.

In 11/19 live-born individuals, the presence of seizures associated with DD/ID (*DNMIL*, *CLN3*, *COQ8A*, *PPT1*, *ST3GAL5*, *SLC2A1*, *SLC13A5*, *SLC6A1*, *NGLY1*, 10 individuals), or abnormal movements (*SPR*, 1 individual), could have led to informative biochemical screening. However, in the majority of individuals, no specific biochemical biomarker could

have led to the diagnosis of the IMD (*DNM1L*, *COQ8A*, *ST3GAL5*, *SLC13A5*, *SLC6A1*, *NGLY1*, *PIGN*) (8/12 individuals). Indeed, ES made it possible to obtain an early diagnosis for non-specific IMD phenotypes, which is of particular interest seeing as certain of these diseases are treatable. In addition, obtaining a diagnosis is particularly important for genetic counseling and prenatal diagnosis, especially since most IMDs follow an autosomal recessive inheritance, and the risk of recurrence in siblings is 25%. Parents may therefore be eligible for early prenatal diagnosis or even preimplantation diagnosis.

The literature already reports the unexpected diagnosis of IMDs using non-targeted tests such as ES. For example, the diagnosis of PGM1-CDG was reported in a 13-year-old girl with short stature and cleft palate, who died of sudden cardiac arrest, which revealed severe cardiomyopathy²⁶. ES made it also possible to diagnose IMDs in fetuses with unspecific symptoms. For example, ES performed the diagnosis of glutaric acidemia type 2 in a fetus with enlarged hyperechoic kidneys²⁷ and of COG8-CDG in a fetus with facial dysmorphism, Dandy-Walker malformation and arthrogyriposis multiplex congenita²⁸. In our series, ES identified extreme fetal presentations of IMDs that would not have been suspected clinically²². *ALDH18A1* pathogenic variants are usually associated with autosomal recessive spastic paraplegia 9B (MIM # 616586) and *NPCI* pathogenic variants with Niemann-Pick disease type C1 (MIM # 257220).

In addition to the clinical analysis focused on OMIM-morbid genes, ES is a well-known powerful tool for the discovery of new genes in a translational research setting³¹. In our series, ES identified the first individual affected by an autosomal recessive epileptic encephalopathy with early seizures linked to *SLC13A5* variants (MIM # 615905)²⁹. In the specific case of IMDs, the identification of novel causal genes can also uncover new metabolic pathways. This could also lead to the development of new therapeutic approaches or the use of well-known therapeutics through drug repositioning³⁰.

Overall, this study demonstrates that ES is a powerful tool that can be used for the earlier diagnosis of IMDs, especially in the case of unspecific developmental disorders without specific biomarkers. This implicates a result delivery time compatible with patient care. When biochemical confirmation is available, it should be proposed as part of reverse phenotyping.

Acknowledgements

We thank the families for taking part in the study. We thank the University of Burgundy Centre de Calcul (CCuB) for technical support and management of the informatics platform, and the Centre de Ressources Biologique (CRB) of Dijon University Hospital. Most authors of this publication are members of the European Reference Network for Developmental Anomalies and Intellectual Disability (ERN-ITHACA). This work was supported by grants from Dijon University Hospital, the ISITE-BFC (PIA ANR) and the European Union through the FEDER programs. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Legends to table and figure

Table 1: Summary of the clinical and genetic features of the 21 individuals with IMD diagnosed using ES.

References

1. Saudubray J-M, Garcia-Cazorla A. An overview of inborn errors of metabolism affecting the brain: from neurodevelopment to neurodegenerative disorders. *Dialogues Clin Neurosci*. 2018;20:301–25.
2. Ezgu F. Inborn Errors of Metabolism. *Adv Clin Chem*. 2016;73:195–250.
3. Tarailo-Graovac M, Wasserman WW, Van Karnebeek CDM. Impact of next-generation sequencing on diagnosis and management of neurometabolic disorders: current advances and future perspectives. *Expert Rev Mol Diagn*. 2017;17:307–9.
4. Ghosh A, Schlecht H, Heptinstall LE, et al. Diagnosing childhood-onset inborn errors of metabolism by next-generation sequencing. *Arch Dis Child*. 2017;102:1019–29.
5. Nambot S, Thevenon J, Kuentz P, et al. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. *Genet Med*. 2018;20:645–54.
6. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. *Nat Rev Genet*. 2018;19:253–68.
7. Tarailo-Graovac M, Shyr C, Ross CJ, et al. Exome Sequencing and the Management of Neurometabolic Disorders. *N Engl J Med*. 2016;374:2246–55.
8. Bélanger SA, Caron J. Evaluation of the child with global developmental delay and intellectual disability. *Paediatr Child Health*. 2018;23:403–19.
9. Michelson DJ, Shevell MI, Sherr EH, Moeschler JB, Gropman AL, Ashwal S. Evidence report: Genetic and metabolic testing on children with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*. 2011;77:1629–35.
10. Shevell M, Ashwal S, Donley D, et al. Practice parameter: evaluation of the child with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society. *Neurology*. 2003;60:367–80.
11. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. *Nature*. 2015;519:223–8.
12. Iglesias A, Anyane-Yeboa K, Wynn J, et al. The usefulness of whole-exome sequencing in routine clinical practice. *Genet Med Off J Am Coll Med Genet*. 2014;16:922–31.
13. Monroe GR, Frederix GW, Savelberg SMC, et al. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. *Genet Med Off J Am Coll Med Genet*. 2016;18:949–56.
14. Clark MM, Stark Z, Farnaes L, Tan TY, White SM, Dimmock D, Kingsmore SF. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*. 2018;3:16.
15. Vissers LELM, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet*. 2016;17:9–18.

16. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014;312:1870–9.
17. Hayeems RZ, Boycott KM. Genome-wide sequencing technologies: A primer for paediatricians. *Paediatr Child Health*. 2018;23:191–7.
18. Thevenon J, Duffourd Y, Masurel-Paulet A, Lefebvre M, Feillet F, El Chehadeh-Djebbar S, St-Onge J, Steinmetz A, Huet F, Chouchane M, Darmency-Stamboul V, Callier P, Thauvin-Robinet C, Faivre L, Rivière JB. Diagnostic odyssey in severe neurodevelopmental disorders: Toward clinical whole-exome sequencing as a first-line diagnostic test. *Clin Genet*. 2016;89:700–7.
19. Garret P, Bris C, Procaccio V, Bonneau PA, Vabres P, Houcinat N, Tisserant E, Feillet F, Bruel A, Quéré V, Philippe C, Sorlin A, Mau-Them FT, Vitobello A, Costa J, Boughalem A, Trost D, Faivre L, Thauvin-Robinet C, Duffourd Y. Deciphering exome sequencing data: bringing mitochondrial DNA variants to light. *Hum Mutat*. 2019; 40:2430-2443.
20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–24.
21. Thevenon J, Milh M, Feillet F, et al. Mutations in SLC13A5 cause autosomal-recessive epileptic encephalopathy with seizure onset in the first days of life. *Am J Hum Genet*. 2014;95:113–20.
22. Lefebvre M, Beaufriere A-M, Francannet C, et al. Extending the ALDH18A1 clinical spectrum to severe autosomal recessive fetal cutis laxa with corpus callosum agenesis. *Am J Med Genet A*. 2018;176:2509–12.
23. Lehalle D, Colombo R, O'Grady M, et al. Hearing impairment as an early sign of alpha-mannosidosis in children with a mild phenotype: Report of seven new cases. *Am J Med Genet A*. 2019;179:1756-1763.
24. Alessandri JL, Gordon CT, Jacquemont ML, et al. Recessive loss of function PIGN alleles, including an intragenic deletion with founder effect in La Réunion Island, in patients with Fryns syndrome. *Eur J Hum Genet*. 2018;26:340-349.
25. Costain G, Cordeiro D, Matviychuk D, Mercimek-Andrews S. Clinical Application of Targeted Next-Generation Sequencing Panels and Whole Exome Sequencing in Childhood Epilepsy. *Neuroscience*. 2019;418:291-310.
26. Fernlund E, Andersson O, Ellegård R, et al. The congenital disorder of glycosylation in PGM1 (PGM1-CDG) can cause severe cardiomyopathy and unexpected sudden cardiac death in childhood. *Forensic Sci Int Genet*. 2019;43:102111.
27. Kucinska-Chahwan AM, Roszkowski T, Geremek M, et al. Prenatal diagnosis of glutaric acidemia type 2 with the use of exome sequencing - an up-to-date review and new case report. *Ginekol Pol*. 2021;92:51-56.
28. Arora V, Puri RD, Bhai P, et al. The first case of antenatal presentation in COG8-congenital disorder of glycosylation with a novel splice site mutation and an extended phenotype. *Am J Med Genet A*. 2019;179:480-485.
29. Bruel A-L, Nambot S, Quéré V, et al. Increased diagnostic and new genes identification outcome using research reanalysis of singleton exome sequencing. *Eur J Hum Genet*. 2019;27:1519–31.

30. Graham E, Lee J, Price M, et al. Integration of genomics and metabolomics for prioritization of rare disease variants: a 2018 literature review. *J Inherit Metab Dis*. 2018;41:435-445.

Class of IMDs	Gene name	OMIM-related disease (MIM number)	Biochemical Pathway / Mechanism	Number of index cases diagnosed	Age at ES	Clinical presentation	Biochemical and genetic investigations performed prior to ES	Solo /trio ES	Variant(s) (cDNA or CNV)	Variant(s) (protein)	ACMG variant classification	Biochemical markers performed after ES results for reverse phenotyping	Specific treatments	
Disorders of mitochondrial DNA maintenance and replication	<i>DNM1L</i>	Encephalopathy, lethal, due to defective mitochondrial peroxisomal fission 1 (MIM # 614388)	Mitochondrial/ peroxisomal fission	1	10.5 years	ID, microcephaly, ataxic gait, seizures, insensitivity to pain	Normal carbohydrate deficient transferrin, array-CGH, telomeric MLPA, DM1/DM2 amplification, <i>MECP2</i> , <i>FOXP1</i> , targeted gene panel sequencing (9 genes implicated in encephalopathy)	Solo	NM_005690.4:c.1085G>A	p.Gly362Asp	V	None	-	
Disorders of mitochondrial cofactor biosynthesis	<i>COQ8A / ADCK3</i>	Coenzyme Q10 deficiency, primary, 4 (MIM # 612016)	Coenzyme Q10 metabolism	2	7 years	Cerebellar ataxia, motor delay	Normal albumin, total cholesterol, array-CGH	Solo	NM_020247.4:c.638G>A NM_020247.4:c.1471T>A	p.Arg213Gln p.Trp491Arg	IV III	None	Coenzyme Q10	
					3 years	Status epilepticus, global DD, walking disability	Normal plasmatic ammonia, aminoacid chromatography, carnitine level, blood/CSF glucose level, CPK, carbohydrate deficient transferrin, urinary organic acid chromatography, muscle mitochondrial respiratory chain, array-CGH, <i>POLG</i> sequencing Moderate elevated lactates	Solo	NM_020247.4:c.811C>T NM_020247.4:c.1625_1626del	p.Arg271Cys p.Ile542Argfs*31	III IV	None	Ketogenic diet Coenzyme Q10	
Disorders of amino acid metabolism	<i>ALDH18A1</i>	Spastic paraplegia 9B, autosomal recessive (MIM # 616586)	Biosynthesis of proline, ornithine, and arginine	1	Foetus (27 WG)	Corpus callosum agenesis, hypoplastic cerebellum IUGR short long bones and ribs, cutis laxa	Normal standard chromosomal analysis, array-CGH	Solo	NM_002860.3 :c.1273C>T NM_002860.3 :c.177del	p.Arg425Cys p.Lys59Asnfs*9	V IV	None	NA*	
Disorders of organelle biogenesis, dynamics and interactions	<i>PPT1</i>	Ceroid lipofuscinosis, neuronal, 1 (MIM # 256730)	Catabolism of lipid-modified proteins	2	5 years	Progressive myoclonic encephalopathy	Normal tripeptidyl peptidase I and palmitoyl-protein thioesterase 1 in leucocytes, standard chromosomal analysis, array-CGH, telomeric MLPA, SNRPN methylation, <i>ARX</i> duplication, <i>MECP2</i> , <i>CDKL5</i> , <i>CLN5</i> , <i>CLN6</i> and <i>CLN8</i> sequencing Skin biopsy: autofluorescent ceroid lipopigments	Solo	NM_000310.3:c.541G>A NM_000310.3:c.471del	p.Val181Met p.His158Thrfs*10	V IV	-	-	
					2 years	Microcephaly, global DD, neurological regression, myoclonic epilepsy	Normal plasmatic lactate, pyruvate, ammonia, phytanic, pristanic and pipecolic acids, guanidoacetate aminoacid chromatography, very long chain fatty acid tests, lymphocyte vacuoles, blood/CSF glucose level, CPK, uric acid, carbohydrate deficient transferrin, urinary organic acid chromatography, lysosomal storage disease explorations, array-CGH, <i>FRAXA</i> Skin biopsy: autofluorescent ceroid lipopigments	Solo	chr1:40558255-40562842del hmz	NA	V	Leucocyte enzyme deficiency	-	
	<i>CLN3</i>	Ceroid lipofuscinosis, neuronal, 3 (MIM # 204200)	N-glycosylation	2	44 years	Microcephaly, retinitis pigmentosa, late onset cerebellar ataxia with cerebellar atrophy, axonal and myelinic neuropathy	Normal total cholesterol, alpha foetoprotein, vitamin E, CPK, plasmatic aminoacid chromatography, carbohydrate deficient transferrin, very long chain fatty acid tests, urinary organic acid chromatography, muscle mitochondrial respiratory chain, mitochondrial DNA, <i>LAMP2</i> and <i>ASPM</i> sequencing	Solo	NM_000086.2:c.883G>A chr16:28497282_28498403del	p.Glu295Lys NA	V V	None	-	
					10 years	Retinitis pigmentosa, seizures	-	Solo	chr16:28495668_28498500del hmz	NA	V	NA	-	
	<i>HEXA</i>	Tay-Sachs Disease (MIM # 272800)	GM2-gangliosidosis	1	4.5 years	Epileptic encephalopathy	Normal albumin, alpha foetoprotein, vitamins E, B1 and B2, CPK, ammonia, plasmatic aminoacid chromatography, very long chain fatty acid tests Moderate elevated lactate	Solo	NM_000520.4:c.533G>A hmz	p.Arg178His	V	Leucocyte enzyme deficiency	-	
	<i>NPCI</i>	Niemann-Pick disease, type C1 (MIM # 257220)	Regulation of intracellular cholesterol trafficking	1	Fetus (33 WG)	Hydrops, hepatosplenomegaly	Normal array-CGH, prenatal explorations for lysosomal storage disease	Solo	NM_000271.4:c.2819C>T hmz	p.Ser940Leu	V	microvacuolization in some macrophage cells in fetal spleen slides	NA*	
	<i>ST3GALS</i>	Salt and pepper developmental regression syndrome (MIM # 609056)	GM3 synthase deficiency	2	6 years	Epileptic encephalopathy, deafness, microcephaly	Normal plasmatic lactate, pyruvate, ammonia, aminoacid chromatography, acylcarnitine profile, CPK, copper level and ceruloplasmin, urinary organic acid chromatography, oligosaccharides, AICAR-SAICAR, array-CGH, <i>FRAXA</i> , <i>MECP2</i> , <i>FOXP1</i> , and <i>UBE3A</i> sequencing	Solo	NM_003896.3:c.740G>A hmz	p.Gly247Asp	V	None	-	
					7.5 years	ID, seizures, stature and weight delay, cerebral atrophy	Normal plasmatic aminoacid chromatography, very long chain fatty acid tests, acylcarnitine profile, CPK, copper level and ceruloplasmin, urinary organic acid chromatography, AICAR-SAICAR, array-CGH,	Solo	NM_003896.3:c.740G>A hmz	p.Gly247Asp	V	None	-	
<i>MAN2B1</i>	Mannosidosis, alpha-, types I and II (MIM # 248500)	N-glycosylation	2	8 years	ID, marfanoid habitus, deafness, dysmorphism	Normal array-CGH	Solo	NM_000528.3:c.2402dup NM_000528.3:c.1645-1G>A	p.Ser802Glnfs*129 p.?	IV IV	IV IV	Mannose-rich oligosacchariduria and leucocyte alpha-mannosidase deficiency	-	
				8.5 years	Deafness, learning disabilities, abnormal teeth enamel, dysmorphism	Normal array-CGH, <i>GJB2</i> sequencing	Solo	NM_000528.3:c.418C>T NM_000528.3:c.2864_2879del	p.Arg140* p.Thr955Serfs*73	IV IV	IV IV	Mannose-rich oligosacchariduria and leucocyte alpha-mannosidase deficiency	-	
Neurotransmitter disorders	<i>SLC2A1 / GLUT1</i>	GLUT1 deficiency syndrome I, infantile onset, severe (MIM # 606777)	Cerebral glucose transport	1	1 year	Global DD, infantile spasms, microcephaly (< - 3 SD), hypotonia	Normal array-CGH, <i>MECP2</i> , <i>CDKL5</i> , and <i>FOXP1</i> sequencing	Solo	NM_006516.2:c.102T>G	p.Asn34Lys	IV	Abnormal blood/CSF glucose level	Ketogenic diet	
	<i>SLC13A5</i>	Developmental and epileptic encephalopathy 25, with amelogenesis imperfecta (MIM # 615905)	Cerebral citrate transport	1	4 years	Early epileptic encephalopathy, global DD	-	Solo	NM_177550.3:c.1463T>C hmz	p.Leu488Pro	IV	None	Citrate	
	<i>SLC6A1</i>	Myoclonic-ataxic epilepsy (MIM# 616421)	GABA transport	2	2 years	Global DD, hand stereotypies, seizures with abnormal EEG pattern	Normal array-CGH and SNRPN methylation, targeted panel sequencing (9 genes implicated in encephalopathy)	Solo	NM_003042.3:c.801delC	p.Ile268Serfs*36	IV	IV	None	-
					17 years	Learning disabilities, marfanoid habitus	Normal standard chromosomal analysis, plasmatic and urinary homocysteine	Trio	NM_003042.3:c.1377C>A	p.Ser459Arg	IV	IV	None	--
<i>SPR</i>	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency (MIM # 612716)	NADPH-dependent reduction of various carbonyl substances	1	5 years	Microcephaly, global DD, abnormal movements,	Normal plasmatic lactate, pyruvate, ammonia, aminoacid chromatography, very long chain fatty acid tests, acylcarnitine profile, blood/CSF lactate level, CPK, carbohydrate deficient transferrin, urinary organic acid chromatography, array-CGH, <i>FRAXA</i> , <i>SMN1</i> deletion, SNRPN methylation and DM1 amplification analyses Mitochondrial respiratory chain in muscle and fibroblasts	Solo	NM_003124.4:c.18_19insGGGCG GGCTG hmz	p.Arg7Glyfs*37	IV	Elevated lactates, abnormal CSF neurotransmitter profile	L-DOPA		

Congenital disorder of glycosylation	<i>NGLY1</i>	Congenital disorder of deglycosylation (MIM # 615273)	Protein deglycosylation	1	5.5 years	Epileptic encephalopathy, severe global DD, dyskinesia, (alacrimia)***	Normal plasmatic ammonia, guanidoacetate, aminoacid chromatography, very long chain fatty acid tests, copper level blood/CSF glucose level, CPK, AICAR/SAICAR, urinary copper level and organic acid chromatography, lysosomal storage disease explorations, standard chromosomal analysis, array-CGH, ARX duplication and SNRPN methylation analysis, <i>STXBP1</i> targeted gene panel sequencing 220 genes implicated in intellectual disability)	Solo	NM_001145293.1:c.1427_1434del NM_001145293.1:c.931G>A	p.His476Leufs*14 p.Glu311Lys	IV III	None	-
	<i>PIGN</i>	Multiple congenital anomalies-hypotonia-seizures syndrome 1 (MIM # 614080)	Glycosylphosphatidylinositol anchor biosynthesis	1	6 days	Congenital bilateral cataract, club feet, cleft lip and palate, congenital cardiopathy	-	Solo	chr18 :59819883_59824941del hmz	NA	V	None	NA**

ACMG: american college of medical genetics; AICAR/SAICAR:aminoimidazole carboxamide ribotide / succinylaminoimidazole-carboxamide riboside; CSF: cerebrospinal fluid; CGH: comparative genomic hybridization CNV: copy number variation; CPK: creatine phosphokinase; DD: developmental delay; DM1/DM2: dystrophic myotony types 1 and 2; cDNA: complementary DNA; ES: exome sequencing; GABA: gamma-aminobutyric acid; ID: intellectual disability; hmz: homozygous; IMD: inherited metabolic disorders; WG: weeks of gestation; IUGR: intrauterine growth retardation; MIM: mendelian inheritance in man; MLPA: multiplex ligation-dependent probe amplification ; NA: not available; NADPH: nicotinamide adenine dinucleotide phosphate; OMIM: online mendelian inheritance in man; SD: standard deviation; *foetal case; ** death at 8 days of life, *** noted in reverse phenotyping..