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### ► 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

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## BRIEF COMMUNICATION

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

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**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

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**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<sup>1,2</sup>. 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<sup>3,4</sup>.

Exome/genome sequencing (ES/GS) has revolutionized translational research and diagnosis in rare diseases in a diagnostic genotype-first approach, followed by reverse phenotyping<sup>5</sup>. Harboring a high diagnostic yield (40-70%) in suspected IMDs<sup>6</sup>, ES has also appeared efficient in individuals with intellectual disability (ID) and unexplained metabolic anomalies (diagnostic yield 68%)<sup>7</sup>. Some authors therefore suggested updating the diagnosis strategy for IMDs in different steps, bringing together first-line biochemical screening and targeted next-generation panels<sup>4</sup>.

For years, biochemical screening has been indicated in first-line etiological investigations for individuals with global developmental delay (DD) or ID<sup>8,9</sup>. 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 features<sup>10</sup>. 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%,<sup>5,11-16</sup>. It has dramatically improved the diagnosis of unspecific or atypical phenotypes and has led to the discovery of hundreds of unknown genes<sup>17</sup>.

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<sup>5,18,19</sup> and American College of Medical Genetics and Genomics guidelines<sup>20</sup>. 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 ES<sup>5</sup>. 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 literature<sup>21-24</sup>. 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.<sup>25</sup> 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 cardiomyopathy<sup>26</sup>. 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 kidneys<sup>27</sup> and of COG8-CDG in a fetus with facial dysmorphism, Dandy-Walker malformation and arthrogyriposis multiplex congenita<sup>28</sup>. In our series, ES identified extreme fetal presentations of IMDs that would not have been suspected clinically<sup>22</sup>. *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 setting<sup>31</sup>. In our series, ES identified the first individual affected by an autosomal recessive epileptic encephalopathy with early seizures linked to *SLC13A5* variants (MIM # 615905)<sup>29</sup>. 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 repositioning<sup>30</sup>.



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.

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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 hnz	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 hnz	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 hnz	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 hnz	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 hnz	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 hnz	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 hnz	p.Leu488Pro	IV	None	Citrate	
	<i>SLC6A1</i>	Myoclonic-astatic 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 hnz	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..