REFERENCE LABORATORY GENETICS

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Pablo Iglesias, 57 – Polígono Gran Via Sur
08908 L'Hospitalet de Llobregat (Barcelona)
Tel. 932 593 700 – Fax. 932 845 000

GENETIC STUDY OF CLINICAL EXOME BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	25955		
Patient Name:	xxx		
Date of Birth:	03/09/2006	**Patient Ref.:**	xxx
Gender:	Female	**Sample Type:**	Blood EDTA
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information : Patient with generalized epilepsy, cognitive retardation, hamartoma in the central nervous system (CNS) and behavioral problems. She has a poor academic performance despite of curricular adaptation and a good behavior at school. The next course, she would have to go to a specialized center but the girl does not agree. Suspicion of neurofibromatosis.

Previous studies: The suspicion of neurofibromatosis. The suspicion of neurofibromatosis was genetically ruled out in 90% (study in another laboratory).

VARIANTS ASSOCIATED WITH PATIENT´S CLINICAL CONDITION

RESULT AND INTERPRETATION

The presence, in heterozygosis, of :

- a pathogenic variant in the *METTL23* gene
- a likely pathogenic variant and another variant of uncertain clinical significance in the *ACSF3* gene, has been identified. (See Recommendations)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
METTL23	NM_001080510.4: **c.169_172del p.(His57Valfs*11)**	Heterozygosis	Autosomal Recessive	**Pathogenic**
ACSF3	NM_174917.4: **c.667-2A>G p.?**	Heterozygosis	Autosomal Dominant/Recessive	**Likely pathogenic**
ACSF3	NM_174917.4: **c.1568G>A p.(Arg523Gln)**	Heterozygosis	Autosomal Dominant/Recessive	**VUS**

* Nomenclature according to HGVS v15.11
^ Based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)*

The ***METTL23*** variant **c.169_172del p.(His57Valfs*11)** is a *frameshift* that predicts an amino acid change from Histidine to Valine at position 57 of the protein and causes a premature STOP codon 10 aminoacids downstream. It is described in the HGMD (CD147298) and ClinVar (ID: 144023) databases as a pathogenic variant. It appears in the dbSNP (rs1175461719) and gnomAD (0.0097%) population frequency databases. In the scientific literature, it was reported in homozygosis, in a consanguineous Arabian family which the affected individuals have severe cognitive impairment, autistic features and variable dysmorphic characteristics (PMID: 24501276).

Based on these data, the variant is classified as a Pathogenic variant.

Pathogenic variants in *METTL23* gene (OMIM: 615262) have been associated with the autosomal recessive mental retardation-44 (OMIM: 615942).

The ***ACSF3*** variant **c.667-2A>G p.?** is located at position -2 of intron 3, and predicts an involvement of the splicing acceptor site. It is not described in clinical databases or in the scientific literature consulted. It is reported population frequency database dbSNP (rs373322510). All splice site predictors (MaxEnt, NNSPLICE and HSF) estimate a change of the splice acceptor site.

Based on these data, the variant is classified as a Likely Pathogenic variant.

The ***ACSF3*** variant **c.1568G>A p.(Arg523Gln)** is a *missense* that predicts an amino acid change from Arginine to Glutamine at position 523 of the protein. It is not described in clinical databases or in the scientific literature consulted. It appears in the population frequency databases dbSNP (rs369726475) and gnomAD (0.00080%). The bioinformatic predictor SIFT estimates that the variant would have a tolerated effect.

Based on these data, the variant is classified as a Variant of Uncertain Clinical Significance.

Pathogenic variants in *ACSF3* gene(OMIM: 614245) have been associated with combined malonic and methylmalonic aciduria, with no inheritance pattern reported in *Online Mendelian Inheritance in Man* (OMIM: 614265). In Orphanet, this pathology has been associated with a both, autosomal dominant and recessive inheritance pattern (ORPHA: 289504). (ORPHA:289504).

VARIANTS WITH RELEVANT CLINICAL SIGNIFICANCE / ACTIONABLE WITHOUT APPARENT ASSOCIATION WITH THE PATIENT´S CLINICAL CONDITION (*SECONDARY FINDINGS)**

*Incidental or secondary findings (I*SFs*) actionables in clinical exomes or genome sequencing based on the recommendation of *American College of Medical Genetics and Genomics (ACMG)* (Kalia et al., 2016). (The list of genes is described in the Annex 1).

The analysis of variants in genes related to actionable pathologies without apparent association with the patient´s clinical condition is only provided with prior informed consent.

RECOMMENDATIONS

Variants in the *METTL23* gene have been associated with intellectual disability type 44 with an autosomal recessive inheritance pattern, so two pathogenic variants in trans configuration (one in each allele) are necessary to obtain a diagnostic confirmation. The identification of a single variant could not explain, by itself, the studied disease.

The frequency of large deletions/duplications (Copy Number Variant; CNV) as a possible cause of the disease in the *METTL23* gene is unknown. However, given the pattern of autosomal recessive inheritance of the disease, it is possible to conclude the study with the identification of large deletions/duplications in this gene (code 25231).

Since pathogenic variants in *ACSF3* have been associated with combined malonic and methylmalonic aciduria, and this pathology has been associated with a dominant and recessive autosomal inheritance pattern; it is recommended to study these variants in parents and/or in relatives affected and not affected in order to establish a co-segregation with the disease and thus the possible pathogenicity of the variants.

Genetic counselling should be offered to the patient by the specialist who treats the patient. If the physician needs additional information about the results or genetic counselling, please contact genetics@referencelaboratory.es

METHODOLOGY

DNA extraction and quantitative and qualitative evaluation of the DNA obtained.

Capture and enrichment of exonic regions and flanking intronic areas of genes contained in the REFLAB Cancer (Roche) sequencing panel with the Roche NimbleGen SeqCap EZ HyperCap Library™ technology.

Massive sequencing with the NextSeq™(Illumina) sequencer.

Identification of the variants of interest in regard to the reference genome (hg19) after filtering, according to specific quality criteria. Annotation of the obtained variants with a specific bioinformatic software: Alamut Visual™ (Interactive Biosoftware), Ingenuity Variant Analysis™ (QIAGEN), Variant interpreter™ (Illumina) and VarAFT™. The used reference databases have been the population databases dbSNP, 1000genomes, EXAC and gnomAD; the clinical databases Human Gene Mutation Database (HGMD version 2019.3), ClinVar and LOVD; the disease specific databases, if applicable, and Reference Laboratory Genetics´own databases. The bioinformatic analysis to evaluate the possible impact of the variants of interest on the structure and functionality of the protein has been carried out with the bioinformatic programs Mutation Taster, SIFT and PolyPhen-2. These analyses are only a predictive tool; they were not experimentally proven.

The nomenclature used to define the variants follows the criteria of the *Human Genome Variation Society (HGVS)* (http://www.HGVS.org/varnomen).

Classification of variants based on the recommendations of the *American College of Medical Genetics and Genomics (ACMG)* (Richards S. *et al*., 2015). Only those variants that, based on current information, are considered pathogenic, likely pathogenic or of uncertain clinical significance, are reported. (The complete list of identified variants is available upon request).

The obtained average reading depth was 152,90x being > 20x in 99,00% of the regions analysed.

The table 1 specifies the list of partially covered genes and most relevant to the patient's clinical condition (if it is considered that the phenotype associated with these genes correlates with that of the patient, it is possible to complete the analysis by Sanger sequencing).

LIMITATIONS: The results obtained do not exclude variants outside the analysed regions of the genome or genetic anomalies not detectable by massive sequencing such as large rearrangements, large deletions/duplications (Copy Number Variant; CNV), insertions / deletions of> = 10 nucleotides, variants in repetitive regions or with a high percentage of GC, and variants in genes with pseudogenes with highly homologous sequences.
It is not possible to rule out the presence of variants in other unanalysed genes.

Table 1. Partially covered genes and most relevant genes related to the patient's clinical condition

The most relevant genes related to the patient's clinical condition have been fully covered.

Annex 1.

ACTA2, ACTC1, APC, APOB, ATP7B, BMPR1A, BRCA1, BRCA2, CACNA1S, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RET, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM43, TNNI3, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, WT1

IMPORTANT NOTE

The information contained in this report is based on current scientific knowledge and the results obtained from the application of the technology in this report, are detailed. Due to continuous advances, the documented information may be modified in the future as a result of the emergence of new scientific evidence.
The genetic/genomic studies carried out by Reference Laboratory S.A. are exclusively intended for qualified health professionals for their interpretation. The results obtained are not, per se, a medical consultation, diagnosis or treatment, nor should they be interpreted as such. Only a specialized professional can correctly interpret the results and offer a diagnosis or prescribe a treatment to a patient based on these. Consequently, no information obtained from our studies can be used to replace the advice and diagnosis of a specialized professional.

Signed: Cristina Camprubí, PhD

Head of Diagnosis and Genetic Counseling

License number 21841-C

College of Biologists of Catalonia

Accredited by AEGH

Signed: Irina Royo, MSc

Head of Molecular Genetics

License number 22078-C

College of Biologists of Catalonia

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