REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF TRIO WHOLE EXOME IN BLOOD BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	25953		
Patient Name:	xxx		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	paraffin-embedded miscarriage tissue sample
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: Legal Termination of Pregnancy (LTP) due to agenesis of the corpus callosum and ventriculomegaly. A second LTP with the same clinical picture. The trio clinical exome sequencing is requested from the parents' samples and the fetus sample 1.

BIOLOGICAL SAMPLES ANALYSED

Reference	Name	Sample Type
000	**miscarriage tissue from fetus 1 (Index case)**	paraffin-embedded tissue
000	**xxx** (Father)	Blood EDTA
000	**xxx** (Mother)	Blood EDTA

For the Trio Clinical Exome sequencing, parental samples are used to filter out the variants found in the index case. The segregation analysis allows a better identification and interpretation of the variants detected.

VARIANTS ASSOCIATED WITH PATIENT´S CLINICAL CONDITION

RESULT AND INTERPRETATION

The presence of a hemizygous likely pathogenic variant in *L1CAM* gene, that could explain the phenotype of suspicion in fetus 1, has been detected.

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
L1CAM	NM_000425.4: **c.551G>T p.(Arg184Leu)**	Hemizygosis	X-linked	**Likely Pathogenic**

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

The ***L1CAM*** variant **c.551G>T p.(Arg184Leu)** is a *missense* that predicts an amino acid change from Arginine to Leucine at position 184 of the protein affecting two functional domains. It is not described in clinical databases, population frequency databases dbSNP and gnomAD or in the scientific literature consulted. In HGMD database, other variants (p.Arg184Gln, p.Arg184Gly, p.Arg184Trp) have been reported in the same position as pathogenic. All bioinformatic predictors (SIFT, MutationTaster, PolyPhen-2) estimate a pathogenic effect of the change.

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

Pathogenic variants in the *L1CAM* gene (OMIM: 308840) have been associated with partial agenesis of the corpus callosum (OMIM: 304100), CRASH syndrome (OMIM: 303350), MASA syndrome (OMIM: 303350), Hydrocephalus due to aqueductal stenosis (OMIM: 307000), Hydrocephalus with congenital idiopathic intestinal pseudo-obstruction (OMIM: 307000) and Hydrocephalus with Hirschsprung disease (OMIM: 307000); all diseases with an inheritance pattern linked to the X chromosome.
In isolated cases of diseases associated with pathogenic variants in the L1CAM gene (grouped as Syndrome L1) and specifically in the case of MASA disease, variability in phenotypic expression or involvement in heterozygous women has been described as a consequence of X chromosome inactivation (PMID: 8062435).

In the trio clinical exome sequencing, the parents' samples are used to filter out the variants found in the index case, and therefore, can reveal their possible carrier status. In the present study, it has been identified that the mother presents the variant detected in the fetus, confirming her carrier status. Therefore, it is possible to consider that the clinical condition of both fetuses is caused by the identified variant inherited from the mother. However, a molecular confirmation is required with the study of the variant c.551G> T p. (Arg184Leu) in the *L1CAM* gene from the Fetus 2 sample.

RECOMMENDATIONS

The study of the likely pathogenic variant c.551G> T p. (Arg184Leu) in the *L1CAM* gene from the fetus 2 sample would increase the causality of the variant if such variant was identified. This study is available in our laboratory upon request.

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 MedExome (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 for the sample with reference 000 was 138,7x being >20x in 90% of the regions analysed. (Coverage information for each gene is available upon request)

The obtained average reading depth for the sample with reference 000 was 271,8x being >20x in 98,9% of the regions analysed. (Coverage information for each gene is available upon request)

The obtained average reading depth for the sample with reference 000 was 229,5x being >20x in 98,4% of the regions analysed. (Coverage information for each gene is available upon request)

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.

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

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Accredited by AEGH

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