REFERENCE LABORATORY GENETICS

ENAC ISO 15189 Nº 1065/LE2112

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GENETIC STUDY OF HEREDITARY HEARING LOSS (OtoRef Global®) BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	58175		
Patient Name:	**xxx**		
Date of Birth:	17/05/1998	**Patient Ref.:**	xxx
Gender:	Female	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: A 21-year-old patient with hypoacusis for 6 years and occasional tinnitus. No dizziness, no pain. An audiometry was performed that revealed a mild/moderate bilateral hypoacusis. Cranial MRI: temporal arachnoid cyst. No alterations at the level of internal ears. Subjectively her clinical condition remains the same, except for headaches about twelve episodes a month. Normal otoscopy in both ears,

RESULT AND INTERPRETATION

No pathogenic or likely pathogenic variants have been detected in the sequence of the genes analysed.

The presence of four heterozygous variants of uncertain clinical significance (VUS) has been identified. (See Recommendations)

The complete list of studied genes is available in Annex 1. (Methodology)

List of reported genes and coverage details is available in Table 1. (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
OTOF	NM_194248.2:**c.2498A>T p.(Gln833Leu)**	Heterozygosis	Autosomal Recessive	**VUS**
OTOF	NM_194248.2:**c.1640C>T p.(Thr547Met)**	Heterozygosis	Autosomal Recessive	**VUS**
OTOGL	NM_173591.3:**c.1796G>A p.(Ser599Asn)**	Heterozygosis	Autosomal Recessive	**VUS**
LOXHD1	NM_144612.6:**c.2874_2891dup p.(Ser960_Ser965dup)**	Heterozygosis	Autosomal Recessive	**VUS**

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

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The ***OTOF*** variant **c.2498A>T p.(Gln833Leu)** is a *missense* that predicts an amino acid change from Glutamine to Leucine at position 833 of the protein. It is described in the ClinVar database (ID: 178511) as a variant of uncertain clinical significance/likely benign, associated with an autosomal recessive deafness type 9 and in the *Deafness Variation Database* as a variant of uncertain clinical significance. The variant appears in the dbSNP database (rs191568463) and in the gnomAD population frequency database (0.052%). The bioinformatic predictor MutationTaster estimates that the change would have a pathogenic effect while two other predictors (SIFT, Polyphen-2) estimate that the change would have a tolerated effect. This variant is not described in the scientific literature consulted.

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

The ***OTOF*** variant **c.1640C>T p.(Thr547Met)** is a *missense* that predicts an amino acid change from Threonine to Methionine at position 547 of the protein. It is described in the ClinVar database (ID: 335450) as a variant of uncertain clinical significance/likely benign, associated with an autosomal recessive nonsyndromic deafness and in the *Deafness Variation Database* as a benign variant. The variant appears in the dbSNP database (rs200191563) and in the gnomAD population frequency database (0.054%). The bioinformatic predictors SIFT, Mutation Taster and Polyphen-2 estimate that the change would have a pathogenic effect. This variant is not described in the scientific literature consulted.

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

The *OTOF* gene (OMIM: 603681) encodes the Otoferlin protein. Pathogenic variants in the *OTOF* gene are associated with an autosomal recessive deafness type 9 (OMIM: 601071), with an autosomal recessive inheritance pattern.

The ***OTOGL*** variant **c.1796G>A p.(Ser599Asn)** is a *missense* that predicts an amino acid change from Serine to Asparagine at position 599 of the protein. It is described in the *Deafness Variation Database* as a benign variant. The variant appears in the dbSNP database (rs202156673) and in the gnomAD population frequency (0.062%). The bioinformatic predictors SIFT and Mutation Taster estimate that the change would have a tolerated effect. This variant is not described in the scientific literature consulted.

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

The *OTOLG* gene (OMIM: 614925) encodes the *Otogelin-like* protein. Pathogenic variants in the *OTOGL* gene are associated with a autosomal recessive deafness type 84B (OMIM: 614944), entity with an autosomal recessive inheritance pattern.

The ***LOXHD1*** variant **c.2874_2891dup p.(Ser960_Ser965dup)** is a duplication of 5 amino acids at position 960 of the protein. t is described in the ClinVar database (ID: 228821) as a variant of uncertain clinical significance/likely benign. The variant appears in the dbSNP database (rs759237437) and in the gnomAD population frequency database (0.11%). The bioinformatic predictor Mutation Taster estimates that the change would have a pathogenic effect. The variant is not described in the scientific literature consulted.

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

The *LOXHD1* gene (OMIM: 613072) encodes the *Lipoxygenase homology domain-containing* type 1 protein. Pathogenic variants in the *LOXHD1* gene are associated with a autosomal recessive deafness type 77 (OMIM: 613079), entity with an autosomal recessive inheritance pattern.

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Given the type of autosomal recessive inheritance of phenotypes associated with the *OTOF*, *OTOGL and LOXHD1* genes, two pathogenic variants in trans configuration (one in each allele) are necessary to obtain a diagnostic confirmation. Regardless of the classification, in the case of *OTOGL and LOXHD1* genes, the identification of a single variant could not, by itself, explain the disease studied. Therefore, co-segregation studies with the disease of a variant in *OTOGL* and *LOXHD1* associated with an autosomal recessive inheritance are not informative, regardless of variant classification.

RECOMMENDATIONS

In order to establish the configuration *cis* (same allele) or *trans* (different allele), co-segregation with the disease and thus the possible pathogenicity of the variants of uncertain clinical significance in the *OTOF* gene, it is necessary to study them in parents. If the trans configuration of the variants identified in the OTOF gene were confirmed, the evidence for their possible causality would increase.

If the segregation study is inconclusive, we recommend completing the study with the identification of large deletions/duplications. (*Copy Number Variant* ; CNV) (code 25232).

Genetic counselling should be offered to the patient by the prescriber physician. If additional information regarding the results or genetic counselling is required, the physician can contact our team at 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 was 110,80x being > 20x in 98,10% of the regions analysed.

The reported INDEL variants are confirmed 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

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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.

Annex 1. List of genes studied

ABHD12, ACTB, ACTG1, ADCY1, ADGRV1, AIFM1, ALMS1, ANKH, ATP6V1B1, BCS1L, BSND, BTD, CABP2, CACNA1D, CATSPER2, CCDC50, CD151, CD164, CDH23, CDKN1C, CEACAM16, CHD7, CHSY1, CIB2, CLDN14, CLIC5, CLPP, CLRN1, COCH, COL11A1, COL11A2, COL2A1, COL4A3, COL4A4, COL4A5, COL4A6, COL9A1, COL9A2, COL9A3, CRYL1, CRYM, DCAF17, DCDC2, DIABLO, DIAPH1, DIAPH3, DLX5, DNMT1, DSPP, EDN3, EDNRB, ELMOD3, EPS8, ERCC2, ERCC3, ESPN, ESRRB, EYA1, EYA4, FGF3, FGFR1, FGFR2, FGFR3, FOXI1, GATA3, GIPC3, GJA1, GJB2, GJB3, GJB6, GPSM2, GRHL2, GRXCR1, GRXCR2, GSDME, HARS, HARS2, HGF, HOMER2, HOXB1, HSD17B4, ILDR1, KARS, KCNE1, KCNJ10, KCNQ1, KCNQ4, KIT, KITLG, LARS2, LHFPL5, LHX3, LOXHD1, LRP2, LRTOMT, MAN2B1, MANBA, MARVELD2, MCM2, MET, MGP, MIR96, MITF, MSRB3, MYH14, MYH9, MYO15A, MYO1C, MYO3A, MYO6, MYO7A, NARS2, NDP, NF2, NLRP3, OPA1, OSBPL2, OTOA, OTOF, OTOG, OTOGL, P2RX2, PAX3, PCDH15, PDZD7, PEX1, PEX26, PEX6, PJVK, PMP22, PNPT1, POLR1C, POLR1D, POU3F4, POU4F3, PRPS1, PTPRQ, RDX, RMND1, RPS6KA3, SALL1, SALL4, SEMA3E, SERPINB6, SIX1, SIX5, SLC12A1, SLC17A8, SLC19A2, SLC26A4, SLC26A5, SLC29A3, SLC33A1, SLC4A11, SLC52A2, SLC52A3, SLITRK6, SMAD4, SMPX, SNAI2, SOX10, SOX2, STRC, SUCLA2, SUCLG1, SYNE4, TBC1D24, TBL1X, TBX1, TCOF1, TECTA, TFAP2A, TIMM8A, TJP2, TMC1, TMIE, TMPRSS3, TMPRSS5, TNC, TPRN, TRIOBP, TRMU, TSPEAR, TWNK, TYR, USH1C, USH1G, USH2A, VCAN, WFS1, WHRN.

Table 1. List of genes reported and coverage

Gene	NM	10x %	Exons with coverage < 100%*
LOXHD1	NM_144612	100,00	-
OTOF	NM_194248	100,00	-
OTOGL	NM_173591	100,00	-

***Due to the current intrinsic limitations associated with massive sequencing technology, some gene exons analysed may be insufficiently covered. If it is considered appropriated by a medical specialist, it would be possible to sequence exons with coverage below 100% using the Sanger method or other alternative molecular technique.

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.

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