REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF OCULAR DISORDERS (OphtalmoRef Global®) BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	57450		
Patient Name:	**xxx**		
Date of Birth:	28/04/1998	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: 21-year-old patient with mitochondrial myopathy diagnosed on follow-up. Continuous bilateral palpebral ptosis along with muscle weakness with exercises. FH: Uncle of 20 years with muscle weakness in study. Paternal grandfather with palpebral ptosis and blindness attributed by DMID.

RESULT AND INTERPRETATION

The presence of a heterozygous likely pathogenic variant in *POLG* gene has been detected.

In addition, the presence of a heterozygous variant of uncertain clinical significance (VUS) in the same gene, has been identified. (See Recommendations)

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

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

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
POLG	NM_002693.2:**c.3139C>T p.(Arg1047Trp)**	Heterozygosis	Autosomal Recessive Autosomal Dominant	**Likely pathogenic**
POLG	NM_002693.2:**c.803G>C p.(Gly268Ala)**	Heterozygosis	Autosomal Recessive Autosomal Dominant	**VUS**

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

The *POLG* variant **c.3139C>T p.(Arg1047Trp)** is a *missense* that predicts an amino acid change from Arginine to Tryptophan at position 1047 of the protein. It is described in the HGMD database (CM087768) as a pathogenic variant associated with Alpers syndrome and ClinVar database (ID: 206548) as a pathogenic/likely

pathogenic variant/of uncertain clinical significance. The variant appears in the dbSNP database (rs181860632) and in the gnomAD population frequency database (0.0046%). The bioinformatic predictors (SIFT, Mutation Taster and Polyphen-2)stimate that the change would have a pathogenic effect. In the scientific literature consulted, the variant is reported in patients with axonal neuropathy who presented ophthalmoplegia and ptosis, along with another pathogenic variant (PMID: 19251978, 22189570, 18195149).
Based on these data, the variant is classified as a **Likely Pathogenic Variant**

The ***POLG*** variant **c.803G>C p.(Gly268Ala)** is a *missense* that predicts an amino acid change from Glicine to Alanine at position 268 of the protein, affecting several functional domains. It is described in the HGMD database (CM033442) as a variant of uncertain clinical significance and in ClinVar database (ID: 196354) as a benign, likely benign variant, and of uncertain clinical significance, associated with different diseases related to *POLG*. The variant appears in the dbSNP database (rs61752784) and in the gnomAD population frequency database (0,34%) with 4 homozygotes and 1000Genomes (0,3%). The bioinformatic predictors (SIFT, Polyphen-2 and MutationTaster) estimate that the change has a pathogenic effect, In the scientific literature, it has been reported in patients with external ophthalmoplegia and ptosis. Different functional studies show an increase in polymerase dysfunction, this increase being lower than that of pathogenic variants known in *POLG*; and also a decrease in exonuclease activity. Some authors classify it as a polymorphic modifier or an ecogenetic variant (PMID: 16940310, 21880868, 27987238).
Based on these data, the variant is classified as a **Variant of Uncertain Clinical Significance.**

The *POLG* gene encodes the DNA polymerase gamma protein. Pathogenic variants in the *POLG* gene (OMIM: 174763) are associated with Mitochondrial DNA depletion syndrome 4A (Alpers type) (OMIM: 203700), Mitochondrial DNA depletion syndrome 4B (MNGIE type) (OMIM: 613662),Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE) (OMIM: 607459), with an autossomal recessive inheritance pattern; Progressive external ophthalmoplegia, autosomal recessive 1 (OMIM: 258450) and autosomal dominant progressive external ophthalmoplegia (OMIM: 157640).

RECOMMENDATIONS

In order to establish co-segregation with the disease and thus the possible pathogenicity of likely pathogenic variant and a variant of uncertain clinical significance, it must be studied in parents and/or affected and unaffected relatives. As two variants were identified in the same gene, the study of parental variants is specifically recommended. It would confirm whether both variants were inherited and the cis (both variants in the same allele) or trans configuration (one variant in each allele). If the trans configuration is confirmed, depending on the genotype-phenotype correlation, the evidence of the possible causality of these variants would be increased.

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 was 205,00x being > 20x in 99,50% 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 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 studied genes

ABCA4, ABCB6, ABCC6, ABCD1, ABHD12, ACBD5, ACO2, ACTB, ACVR1, ADAM9, ADAMTS18, ADAMTSL4, ADGRV1, ADIPOR1, AFG3L2, AGBL1, AGBL5, AGK, AHI1, AIPL1, ALDH1A3, ALMS1, AMACR, ANKS6, ANTXR1, AP3B1, ARHGEF18, ARL13B, ARL2BP, ARL6, ARMC9, ASB10, ATF6, ATOH7, ATXN7, AUH, B3GLCT, B9D1, B9D2, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BCOR, BEST1, BFSP1, BFSP2, BLOC1S3, BLOC1S6, BMP4, BMP7, C10orf11, C12orf57, c12orf65, C19ORF12, C1QTNF5, C21orf2, C2CD3, C5orf42, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CANT1, CAPN5, CAV1, CC2D2A, CDH23, CDH3, CDHR1, CEP104, CEP120, CEP164, CEP290, CEP41, CEP83, CERKL, CFH, CHD7, CHM, CHMP4B, CHN1, CHST6, CIB2, CISD2, CLDN19, CLN3, CLN5, CLN6, CLN8, CLPB, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL11A1, COL11A2, COL17A1, COL18A1, COL2A1, COL4A1, COL4A3, COL4A4, COL4A5, COL5A1, COL8A2, COL9A1, COL9A2, COL9A3, CRB1, CRX, CRYAA, CRYAB, CRYBA1, CRYBA2, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGB, CRYGC, CRYGD, CRYGS, CSPP1, CTDP1, CTNNA1, CTNNB1, CTSD, CTSF, CYP1B1, CYP27A1, CYP4V2, DCN, DHDDS, DNAJC5, DNM1L, DTNBP1, EDN3, EDNRB, EFEMP1, ELOVL4, EPG5, EPHA2, ERCC1, ERCC2, ERCC5, ERCC6, EYA1, EYS, FAM126A, FAM161A, FBN1, FGFR1, FLVCR1, FOXC1, FOXE3, FOXL2, FRAS1, FREM1, FREM2, FRMD7, FSCN2, FTL, FYCO1, FZD4, GALK1, GALT, GCNT2, GDF3, GDF6, GFER, GIPC3, GJA1, GJA3, GJA8, GJB2, GJB6, GLI2, GLIS2, GNAT1, GNAT2, GNB3, GNPTG, GPR143, GPR179, GRIP1, GRK1, GRM6, GRN, GSN, GUCA1A, GUCA1B, GUCY2D, HARS, HCCS, HCN1, HESX1, HGSNAT, HK1, HMCN1, HMX1, HOXA1, HOXB1, HPS1, HPS3, HPS4, HPS5, HPS6, HSF4, IARS2, IDH3B, IFT140, IFT172, IFT27, IFT43, IFT81, IGBP1, IMPDH1, IMPG1, IMPG2, INPP5E, INVS, IQCB1, IRX5, ISPD, ITM2B, JAG1, JAM3, KCNJ13, KCNV2, KCTD7, KERA, KIAA0556, KIAA0586, KIF11, KIF21A, KIF7, KIZ, KLHL7, KRT12, KRT3, LAMA1, LCA5, LCAT, LEMD2, LEPREL1, LIM2, LMX1B, LOXHD1, LOXL1, LRAT, LRIT3, LRP5, LSS, LTBP2, LYST, LZTFL1, MAB21L2, MAF, MAK, MC1R, MERTK, MFN2, MFRP, MFSD8, MIP, MIR184, MITF, MKKS, MKS1, MLPH, MMACHC, MSMO1, MTPAP, MTTP, MVK, MYO5A, MYO7A, MYOC, NAA10, NDP, NDUFS1, NEK2, NEK8, NEUROD1, NGLY1, NHS, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NR2F1, NRL, NTF4, NYX, OAT, OCA2, OCRL, OFD1, OPA1, OPA3, OPN1SW, OPTN, OTX2, OVOL2, P3H2, PANK2, PAX2, PAX3, PAX6, PCARE, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6D, PDE6G, PDE6H, PDZD7, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PGAP1, PGK1, PHOX2A, PHYH, PIGL, PIKFYVE, PITPNM3, PITX2, PITX3, PLA2G5, PLG, PLK4, PNPLA6, POC1B, POLG, POMGNT1, POMT1, PORCN, PPT1, PQBP1, PRCD, PRDM5, PRKCG, PROKR2, PROM1, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, PRPS1, PRSS56, PXDN, RAB18, RAB27A, RAB28,

RAB3GAP1, RAB3GAP2, RARB, RAX, RAX2, RB1, RBP3, RBP4, RCBTB1, RD3, RDH11, RDH12, RDH5, RECQL4, REEP6, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROBO3, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RPGRIP1L, RRM2B, RS1, RTN4IP1, SAG, SALL2, SALL4, SBF2, SCN2A, SDCCAG8, SEMA3E, SEMA4A, SH3PXD2B, SHH, SIL1, SIPA1L3, SIX3, SIX6, SLC16A12, SLC24A1, SLC24A5, SLC25A46, SLC33A1, SLC38A8, SLC45A2, SLC4A11, SLC4A3, SLC4A4, SLC7A14, SMOC1, SNAI2, SNRNP200, SOX10, SOX2, SOX3, SOX5, SPATA7, SPG7, SRD5A3, STRA6, SUFU, TACSTD2, TBC1D20, TBK1, TCF4, TCTN1, TCTN2, TCTN3, TDRD7, TEAD1, TEK, TENM3, TFAP2A, TGFBI, TGIF1, TIMM8A, TIMP3, TMEM107, TMEM126A, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TMEM98, TMPRSS4, TOPORS, TPP1, TRAF3IP1, TREX1, TRIM32, TRNT1, TRPM1, TSPAN12, TTC21B, TTC8, TTLL5, TTPA, TTR, TUBB3, TUBGCP4, TUBGCP6, TULP1, TYR, TYRP1, UBIAD1, UCHL1, UNC119, UNC45B, USH1C, USH1G, USH2A, VAX1, VCAN, VHL, VIM, VPS13B, VSX1, VSX2, WDPCP, WDR19, WDR36, WFS1, WHRN, WRN, YAP1, ZEB1, ZIC2, ZNF408, ZNF423, ZNF469, ZNF513, ZNF644.

Table 1. List of reported genes and coverage details

Gene	NM	10x %	Exons with coverage < 100%*
POLG	NM_002693	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.

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