REFERENCE LABORATORY GENETICS

ENAC ISO15189 Nº 1065/LE2112

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

GENETIC STUDY OF CARDIOVASCULAR DISEASES (CardioRef Global®) BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	14970		
Patient Name:	xxx		
Date of Birth:	-	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	fetal skin culture
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: The sample comes from cells of fetal skin culture. After intrauterine death, a study related to sudden death is requested.

Previous studies: Genetic study of Ventricular Arrhythmia and Sudden Cardiac Death by massive sequencing panel (NGS) identified the presence of a heterozygous variant of uncertain clinical significance c.11791G> A (p.Glu3931Lys) in the *ANK2* gene (see report with request number 5941851 issued on 10/27/2016).

RESULT AND INTERPRETATION

The presence of a heterozygous pathogenic variant in the *TRDN* gene, associated with a Catecholaminergic polymorphic ventricular tachycardia, has been identified. Given the type of autosomal recessive inheritance of the disease for this gene, a second pathogenic variant would be required for the result to be compatible with the clinical diagnosis of suspicion.

The presence of five heterozygous variants of uncertain clinical significance (VUS) has been detected.

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)

The list of partially covered genes is available in Table 2. (Methodology)

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
TRDN	NM_006073.3: c.1996del (p.Ala666LeufsTer78)	Heterozygosis	Autosomal Recessive	**Likely pathogenic**

TRPM4	NM_017636.3:c.2289C>G (p.Cys763Trp)	Heterozygosis	Autosomal Dominant	VUS
DSP	NM_004415.2:c.740C>T (p.Ala247Val)	Heterozygosis	Autosomal Recessive/Dominant	VUS
TTN	NM_001256850.1:c.5479G>T (p.Ala1827Ser)	Heterozygosis	Autosomal Recessive/Dominant	VUS
TTN	NM_001256850.1:c.97348C>T (p.Arg32450Trp)	Heterozygosis	Autosomal Recessive/Dominant	VUS
TTN	NM_001256850.1:c.101017G>A (p.Ala33673Thr)	Heterozygosis	Autosomal Recessive/Dominant	VUS

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

The ***TRDN*** variant **c.1996del (p.Ala666LeufsTer78)** is a *frameshift* that predicts an amino acid change from Alanine to Leucine at position 666 of the protein and causes a premature STOP codon 79 amino acids downstream. It is not described in clinical and population frequency databases or in the scientific literature consulted.

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

The *TRDN* gene (OMIM: 603283) is associated with a Catecholaminergic polymorphic ventricular tachycardia (OMIM: 615441), with an autosomal recessive inheritance pattern.
Given the type of autosomal recessive inheritance of *TRDN* gene for the associated disease, two pathogenic variants in trans configuration (one in each allele) are necessary to obtain a diagnostic confirmation. Regardless of the classification, the identification of a single variant could not explain, by itself, the disease studied. Therefore, co-segregation studies with the disease of a variant in a gene associated with an autosomal recessive inheritance are not informative, regardless of the variant classification.

The ***TRPM4*** variant **c.2289C>G (p.Cys763Trp)** is a *missense* that predicts an amino acid change from cysteine to tryptophan at position 763 of the protein. It is not described in clinical database or in the scientific literature consulted. This variant appears in the dbSNP database (rs200760537) and in the gnomAD population frequency database (0,0012%). Two bioinformatic predictors (SIFT, Polyphen-2) estimate that the change would have a pathogenic effect while a third predictor (MutationTaster) estimates that the change would have a tolerated effect.

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

The *TRPM4* gene (OMIM: 606936) is associated with the progressive familial heart block disorder (OMIM: 604559), with an autosomal dominant inheritance pattern.

The ***DSP*** variant **c.740C>T (p.Ala247Val)** is a *missense* that predicts an amino acid change from Alanine to Valine at position 247 of the protein, affecting a functional domain. It is not described in the clinical database or in the scientific literature consulted. This variant appears in the dbSNP database (rs544360130) and in the the gnomAD population frequency database (0,024%). 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.

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

The *DSP* gene (OMIM: 125647) is associated with the arrhythmogenic right ventricular dysplasia (OMIM: 607450), with an autosomal dominant inheritance pattern.

The ***TTN*** variant **c.5479G>T (p.Ala1827Ser)** is a *missense* that predicts an amino acid change from Alanine to Serine at position 1827 of the protein, affecting a functional domain. It is described in the ClinVar database (ID 47184) as a variant of uncertain clinical significance, probably benign or benign associated with different entities such as dilated cardiomyopathy, hypertrophic cardiomyopathy, hereditary myopathy, Salih myopathy, distal myopathy or limb-girdle muscular dystrophy. This variant appears in the dbSNP database (rs141213991) and in the gnomAD population frequency database (0,084%). Two bioinformatic predictors (SIFT, MutationTaster) estimate that the change would have a pathogenic effect while a third predictor (Polyphen-2) estimates that the change would have a tolerated effect.

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

The ***TTN*** variant **c.97348C>T (p.Arg32450Trp)** is a *missense* that predicts an amino acid change from Arginine to Tryptophan at position 32450 of the protein, affecting several functional domains. It is described in the HGMD database (CM057411) as a variant of uncertain clinical significance associated with myopathy and in ClinVar (ID 178157) as a variant of uncertain clinical significance or probably benign associated with cardiomyopathy and other cardiovascular diseases. This variant appears in the gnomAD population frequency database (0,10%), but not in the dbSNP database. All bioinformatic predictors (SIFT, MutationTaster, Polyphen-2) estimate that the change would have a pathogenic effect. In the scientific biliography the variant has been reported as polymorphism associated with hereditary myopathy with early respiratory failure but by itself, it would not cause this disease (PMID: 24231549)

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

The ***TTN*** variant **c.101017G>A (p.Ala33673Thr)** is a *missense* that predicts an amino acid change from Alanine to Threonine at position 33673 of the protein, affecting several functional domains. It is described in the ClinVar database (ID 203102) as a variant of uncertain clinical significance associated with dilated cardiomyopathy. This variant appears in the dbSNP database and in the gnomAD population frequency database (0,0082%). 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.

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

The *TTN* gene (OMIM: 188840) is associated with Familial isolated dilated cardiomyopathy 1G (OMIM: 604145), Hereditary myopathy with early respiratory failure (OMIM: 603689) and Familial hypertrophic cardiomyopathy 9 (OMIM: 613765), entities with autosomal dominant inheritance pattern and with Salih Myopathy (OMIM: 611705), an entity with autosomal recessive inheritance pattern. The *TTN* gene is the largest gene in the genome, so it is more likely that new variants can be identified, so many of them are classified as uncertain. In addition, the *TTN* gene encodes the titin protein, a large muscle protein expressed in cardiac and skeletal muscles with a key role in muscle assembly. Therefore, pathogenic variants in the *TTN*

gene are associated with different phenotypes in both, cardiology and neurology, with different types of inheritance and incomplete penetrance. These facts increase the complexity of interpreting variants of unconfirmed pathogenicity, which must be interpreted in the family´s clinical context, depending on the family tree, in order to reach diagnostic conclusions.

RECOMMENDATIONS

Genetic studies in cardiology are of complex interpretation, there is no direct relationship between the presence of a variant and the development of the disease (incomplete penetrance). The genetic result indicates a risk and not a cause of disease. They are very useful and informative in families for monitoring and prevention in cases where a clear pathogenic variant can be identified in a related gene, but a very complex interpretation when unclear variants are identified in cases where clinical information is not available either. The extent of the study of variants of uncertain clinical significance to relatives related to heart diseases should be assessed according to the family tree, the number of affected/unaffected individuals in the family and therefore assessed on a case-by-case basis. In the case of the *TTN* gene, considering or excluding the involvement of the identified variants depends on the genotype-phenotype correlation that the medical specialist evaluates and that, as reported in reference to the *TTN* gene, the interpretation of variants of unconfirmed pathogenicity in this gene is complex.

It is recommended to evaluate whether any of the diseases associated with the *DSP, TRDN, TRPM4* and *TTN* genes can be correlated with the fetal clinical conditions and, if considered appropriate, expand the study with large deletion/duplication analysis (Copy Number Variant; CNV) in the *TRDN* gene and/or with family tree-based co-segregation studies in the case of variants detected in the *DSP, TRPM4* and *TTN* genes.

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 136,8x being > 20x in 98,7% 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

AARS2, ABCA1, ABCB1, ABCC9, ABCG1, ABCG5, ABCG8, ACAD9, ACADM, ACADVL, ACTA1, ACTA2, ACTC1, ACTN2, ACVR1, ACVR2B, ACVRL1, ADAMTS2, ADAMTSL4, AGK, AGL, AGPAT2, AKAP9, AKT2, ALMS1, AMPD1, ANGPTL3, ANK2, ANK3, ANKRD1, ANO5, APOA1, APOA5, APOB, APOC2, APOC3, APOE, ASPH, ATP5E, ATP7A, ATPAF2, B3GAT3, B4GALT7, BAG3, BLK, BMP10, BMPR1A, BMPR1B, BMPR2, BRAF, BSCL2, CACNA1C, CACNA1D, CACNA2D1, CACNB2, CALM1, CALM2, CALM3, CALR3, CAPN3, CASQ2, CAV1, CAV3, CAVIN1, CAVIN4, CBL, CBS, CEL, CETP, CFC1, CHD7, CHRM2, CHST14, CIDEC, CITED2, COA5, COA6, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL7A1, COQ2, COX15, COX6B1, CPT2, CREBBP, CRELD1, CRYAB, CSRP3, CTF1, CTNNA3, CTNNB1, CYP2D6, CYP3A4, CYP3A5, DES, DLD, DMD, DNAJC19, DNM1L, DOLK, DSC2, DSG2, DSP, DTNA, EFEMP2, EHMT1, EIF2AK3, EIF2AK4, ELAC2, ELN, EMD, ENG, EP300, EVC, EYA4, FAH, FBN1, FBN2, FGF12, FHL1, FHL2, FHOD3, FKBP14, FKRP, FKTN, FLNA, FLNC, FOXC1, FOXD4, FOXF1, FOXH1, FOXP1, FOXP3, FOXRED1, FXN, GAA, GATA4, GATA5, GATA6, GATAD1, GCK, GDF1, GDF2, GFM1, GJA1, GJA5, GLA, GLB1, GLIS3, GNPTAB, GPD1, GPD1L, GPIHBP1, GUSB, HAND2, HCN4, HFE, HNF1A, HNF1B, HNF4A, HRAS, IDH2, IER3IP1, ILK, INS, INSIG2, INSR, IRX4, ISL1, JAG1, JPH2, JUP, KANSL1, KCNA5, KCND2, KCND3, KCNE1, KCNE2, KCNE3, KCNE5, KCNH2, KCNJ11, KCNJ2, KCNJ5, KCNJ8, KCNK3, KCNQ1, KLF10, KLF11, KMT2D, KRAS, LAMA2, LAMA4, LAMP2, LCAT, LDB3, LDLR, LDLRAP1, LEFTY2, LEP, LIAS, LIPA, LIPC, LMF1, LMNA, LPA, LPL, LRP6, LZTR1, MAP2K1, MAP2K2, MED12, MED13L, MEF2A, MFAP5, MIB1, MLYCD, MRPL3, MRPL44, MRPS22, MTO1, MTTP, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLIP, MYLK, MYLK2, MYOM1, MYOT, MYOZ2, MYPN, NEBL, NEUROD1, NEUROG3, NEXN, NF1, NKX2-5, NKX2-6, NNT, NODAL, NOS1AP, NOTCH1, NOTCH2, NOTCH3, NPC1L1, NPHP4, NPPA, NRAS, OBSCN, OBSL1, OPA3, PAX4, PCDH15, PCSK9, PDGFRA, PDHA1, PDLIM3, PDX1, PHKA1, PITX2, PKP2, PKP4, PLIN1, PLN, PLOD1, PLTP, PMM2, PNPLA2, PPARA, PPARG, PRDM16, PRKAG2, PRKG1, PSEN1, PSEN2, PTF1A, PTPN11, PYGM, RAF1, RANGRF, RASA1, RASA2, RBM20, RFX6, RIT1, RRAS, RYR1, RYR2, SALL4, SAR1B, SCARB1, SCN10A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SCO2, SDHA, SGCA, SGCB, SGCD, SHOC2, SKI, SLC22A5, SLC25A3, SLC25A4, SLC2A10, SLC2A2, SLC39A13, SLCO1B1, SLMAP, SMAD1, SMAD3, SMAD4, SMAD6, SMAD9, SNTA1, SOS1, SOS2, SPEG, SPRED1, SURF1, SYNE1, SYNE2, TAB2, TAZ, TBC1D4, TBX1, TBX20, TBX5, TCAP, TDGF1, TFAP2B, TGFB2, TGFB3, TGFBR1, TGFBR2, TMEM43, TMEM70, TMPO, TNNC1, TNNI3, TNNI3K, TNNT2, TOPBP1, TOR1AIP1, TPM1, TRDN, TRIB1, TRIM63, TRPM4, TSFM, TTN, TTR, TXNRD2, UPF3B, VCL, WFS1, XK, ZDHHC9, ZFHX3, ZFPM2, ZIC3, ZMPSTE24.

Table 1. List of reported genes and coverage details

Gene	NM	10x %	Exons with coverage < 100%*
DSP	NM_004415	100,00	-
TRDN	NM_006073	98,90	28
TRPM4	NM_017636	100,00	-
TTN	NM_001256850	100,00	-

Table 2 List of partially covered genes

Gene	NM	10x %	Exons with coverage < 100%*
ADAMTS2	NM_014244	96,18	1
ASPH	NM_004318	98,55	10
CTF	NM_001330	85,31	3
DMD	NM_004006	99,59	64, 65
EHMT1	NM_024757	99,46	1
EIF2AK4	NM_001013703	98,36	5
EVC	NM_153717	94,16	1
FOXC1	NM_001453	98,92	1
GATA6	NM_005257	97,2	2
GUSB	NM_000181	96,73	9, 10
INSR	NM_000208	99,28	1
KMT2D	NM_003482	99,69	39
MED12	NM_005120	97,32	42, 43
NF1	NM_000267	97,97	16, 17, 23, 24, 35
NOTCH3	NM_000435	99,47	1
NPC1L1	NM_013389	98,01	15
OBSCN	NM_052843	99,93	62
PHKA1	NM_002637	99,89	5
PKP2	NM_004572	97,09	6
PTF1A	NM_178161	99,59	1
RYR1	NM_000540	99,13	91
SCN1B	NM_199037	98,51	1
SKI	NM_003036	99,86	1
TBX1	NM_080647	85,42	3, 9
TGFBR1	NM_004612	97,49	1
TRDN	NM_006073	98,9	28
TSFM	NM_001172696	93,95	5
UPF3B	NM_080632	99,17	2

*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.

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

RESPONSIBILITY EXEMPTION CLAUSE: Reference Laboratory S.A. is not responsible for the use made by the contractor of the results obtained through their studies, nor for the possible harmful consequences arising from this use, making express reservation to exercise the appropriate legal actions in the event of improper use of them.
The contractor of the studies referred to above made by Reference Laboratory S.A. may not modify, reduce, expand or, in any way, alter the content of this report. Therefore, the contractor irrevocably exonerates Reference Laboratory S.A. of any responsibility or eventual harmful consequence derived, directly or indirectly, from the breach of this obligation.