REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF DERMATOLOGICAL DISEASES (DermaRef Global®) BY MASSIVE SEQUENCING (NGS)

Request No.:	000		
Client:	-		
Analysis code:	17035		
Patient Name:	xxx		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Blood EDTA
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: Patient with a personal history of blisters with a predominance of friction with minimal trauma. At the time of exploration, no blisters, scars or *millium* cysts are observed. He presents with residual dyschromia. No dental alterations. FHx: healthy ancestors; 2 affected siblings in children's age; 2 children and 5 grandchildren without involvement.

RESULT AND INTERPRETATION

The presence of a heterozygous pathogenic variant in the COL7A1 gene, associated with Epidermolysis bullosa, has been identified.

Additionally, the presence of three heterozygous variants of uncertain clinical significance (VUS) has been identified. (See recommendations)

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

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

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
COL7A1	NM_000094.3: **c.6527dup p.(Gly2177Trpfs*113)**	Heterozygosis	Autosomal Dominant Autosomal Recessive	**Pathogenic**
COL7A1	NM_000094.3: **c.6341G>A p.(Gly2114Asp)**	Heterozygosis	Autosomal Dominant Autosomal Recessive	**VUS**
COL7A1	NM_000094.3: **c.4782+9A>T**	Heterozygosis	Autosomal Dominant	**VUS**

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	p.?		Autosomal Recessive	
ITGA6	NM_000210.3: c.3001G>A NP_000201: p.(Val1001Met)	Heterozygosis	Autosomal Recessive	VUS

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

The ***COL7A1*** variant **c.6527dup p.(Gly2177Trpfs*113)** is a duplication of 1 nucleotide which causes a *frameshift* that predicts an amino acid change from Glycine to Tryptophan at position 2177 of the protein and causes a premature STOP codon 113 amino acids downstream. It is described in clinical databases HGMD (CI972584) and ClinVar (ID: 372345) as a pathogenic variant associated with dystrophic epidermolysis bullosa. The variant appears in the dbSNP database (rs768128088) and in the gnomAD population frequency database(0,0068%). In the scientific literature, it has been reported in people with recessive dystrophic epidermolysis bullosa, in homozygosis or compound heterozygosis. With another pathogenic variant. In addition, studies for future therapies show that this variant causes a protein expression deficiency. (PMID: 20920254, 23947675, 24577406, 27498345, 29272047).

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

The ***COL7A1*** variant **c.6341G>A p.(Gly2114Asp)** is a *missense* that predicts an amino acid change from Glycine to Aspartic acid at position 2114 of the protein, affecting a functional domain. It is not described in the clinical and population frequency databases consulted or in the scientific literature. The bioinformatic predictors (Mutation Taster and Polyphen-2) estimate that the change would have a pathogenic effect while the predictor SIFT 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 ***COL7A1*** variant **c.4782+9A>T p.?** is a change located in the +9 position of the *splicing* donor site. It is described in clinical database ClinVar (ID:345844) as a variant of uncertain clinical significance. The variant appears in the dbSNP database (rs369635501) and in the gnomAD population frequency database (0,0088%). Bioinformatic splicing predictors (NNSPLICE and MaxEnt) estimate that the change would have a tolerated effect. It is not described in the scientific literature consulted

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

The *COL7A1* gene (OMIM: 120120) is associated with dominant dystrophic epidermolysis bullosa (OMIM: 131750), recessive dystrophic epidermolysis bullosa (OMIM: 226600) and other types of epidermolysis bullosa with dominant and recessive inheritance pattern.

The ***ITGA6*** variant **c.3001G>A p.(Val1001Met)** is a *missense* that predicts an amino acid change from Valine to Methionine at position 1001 of the protein. It is not described in the clinical databases or in the scientific literature consulted. The variant appears in the dbSNP database (rs567133568) and in the gnomAD population frequency database (0,088%). The bioinformatic predictors (SIFT, Mutation Taster and Polyphen-2) estimate that the change would have a pathogenic effect.

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

The *ITGA6* gene (OMIM:147556) is associated with epidermolysis bullosa junctionalis with pyloric atresia (OMIM:226730), entity with autosomal recessive inheritance pattern.

Given the type of autosomal recessive inheritance of the 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

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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.

RECOMMENDATIONS

The *COL7A1* can have an autosomal dominant or autosomal recessive inheritance pattern. Given the finding of a heterozygous pathogenic variant, it is recommended to establish the segregation of the pathogenic variant and the two variants of uncertain clinical significance identified in the COL7A1 gene in affected and unaffected first-degree relatives. This segregation study would allow to analyse the possible pathogenicity of the different variants of uncertain clinical significance together with the inheritance pattern. The causality of the variants should be interpreted in conjunction with the patient´s clinical condition and based on their family tree by a medical specialist.

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).

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 117,3x being > 20x in 98,4% 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.

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Annex 1. List of studied genes

AAGAB, ABCA12, ABCB6, ABCC9, ABHD5, ACD, ACTA2, ACVRL1, ADAM10, ADAM17, ADAR, AGPAT2, ALDH18A1, ALDH3A2, ALOX12B, ALOXE3, ANTXR1, AP1S1, AP3B1, APCDD1, AQP5, ARSE, ATP2A2, ATP2C1, ATP6V0A2, ATP7A, ATR, AXIN2, B4GALT7, BANF1, BLM, BLOC1S3, BLOC1S6, BRAF, BSCL2, C10ORF11, C1R, C1S, CARD14, CAST, CAV1, CAVIN1, CBL, CCBE1, CCM2, CDH3, CDSN, CECR1, CERS3, CLDN1, COL17A1, COL3A1, COL7A1, CSTA, CTC1, CTSC, CYP4F22, DCLRE1C, DDB2, DKC1, DLX3, DSC2, DSC3, DSG1, DSG4, DSP, DST, DTNBP1, DYNC2LI1, EBP, EDA, EDA2R, EDAR, EDARADD, EDN3, EDNRB, ELOVL4, ENG, ENPP1, EPG5, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, EVC, EVC2, EXPH5, FAT4, FBN1, FERMT1, FGF10, FGFR2, FGFR3, FLG, FLT4, FOXC2, GATA2, GDF2, GJA1, GJB2, GJB3, GJB4, GJB6, GJC2, GPR143, GRHL2, GTF2E2, GTF2H5, GUCY1A3, HOXC13, HPS1, HPS3, HPS4, HPS5, HPS6, HR, IFT122, IFT43, IFT52, IKBKG, ITGA3, ITGA6, ITGB4, JAG1, JUP, KANK2, KCNJ6, KCTD1, KIF11, KIT, KITLG, KRIT1, KRT1, KRT10, KRT14, KRT16, KRT17, KRT2, KRT5, KRT6A, KRT6B, KRT6C, KRT74, KRT81, KRT83, KRT85, KRT86, KRT9, LAMA3, LAMB3, LAMC2, LIPH, LIPN, LMNA, LOR, LPAR6, LRP6, LTBP3, LYST, LZTR1, MAP2K1, MBTPS2, MC1R, MITF, MLPH, MPLKIP, MSX1, MVK, MYO5A, NF1, NF2, NFKBIA, NHP2, NIPAL4, NOP10, NSDHL, OCA2, OFD1, PARN, PAX3, PAX9, PDCD10, PEX7, PHYH, PIEZO1, PIK3R1, PKP1, PLEC, PLIN1, PNPLA1, PNPLA2, POC1A, POFUT1, POGLUT1, POLD1, POLH, POMP, PORCN, PPARG, PSENEN, PSMB8, PTDSS1, PTEN, PTPN11, PVRL1, PVRL4, PYCR1, RAB27A, RAF1, RAG1, RAG2, RASA1, RECQL4, RHBDF2, RIN2, RNF213, RTEL1, SAMHD1, SERPINB7, SHOC2, SLC24A5, SLC27A4, SLC29A3, SLC45A2, SLURP1, SMAD4, SMARCAD1, SMARCB1, SNAP29, SNRPE, SOX10, SOX18, SPINK5, SPRED1, ST14, ST3GAL5, STK11, STS, SULT2B1, SUMF1, TERC, TERT, TGM1, TGM5, TINF2, TP63, TRPS1, TRPV3, TWIST2, TYR, TYRP1, UBR1, USB1, UVSSA, VEGFC, VPS33B, WDR19, WDR35, WNT10A, WNT10B, WRAP53, WRN, XPA, XPC, ZMPSTE24.

Table 1. List of reported genes and coverage details

Gene	NM	10x %	Exons with coverage < 100%*
COL7A1	NM_000094	100,00	-
ITGA6	NM_000210	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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