REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF RHEUMATOID TYPE OSTEOARTHROPATHY AND RELATED DISORDERS BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	58065		
Patient Name:	**xxx**		
Date of Birth:	N/A	**Patient Ref.:**	xxx
Gender:	Female	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: N/A.

RESULT AND INTERPRETATION

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

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

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

Gene	Variant*	Zygosity	Inheritance pattern	Classification^
COL2A1	NM_001844.4:**c.3173G>A p.(Arg1058His)**	Heterozygosis	Autosomal Dominant	**VUS**

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

The ***COL2A1*** variant **c.3137G>A p.(Arg1058His)** is a *missense* that predicts an amino acid change from Arginine to Histidine at position 1058 of the protein. It is not described in the clinical database or in the scientific literature consulted. The variant appears in the dbSNP database (rs774603840) and in the gnomAD population frequency database (0.0012%). 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 *COL2A1* gene (OMIM: 120140) encodes collagen type 2 alpha-1. Pathogenic variants in the *COL2A1* gene are associated with multiple phenotypes with autosomal dominant inheritance pattern:

Achondrogenesis, type II or hypochondrogenesis (OMIM: 200610)

Czech dysplasia (OMIM: 609162)

Avascular necrosis of the femoral head (OMIM: 608805)

Epiphyseal dysplasia, multiple, with myopia and deafness (OMIM: 132450)

Kniest Dysplasia (OMIM: 156550)

Legg-Calve – Perthes Disease (OMIM: 150600)

Osteoarthritis with mild chondrodysplasia (OMIM: 604864)

Platyspondylic lethal skeletal dysplasia,Torrance type (OMIM: 151210)

SED congenita (OMIM: 183900)

SMED Strudwick type (OMIM: 184250)

Spondyloepiphyseal dysplasia, Stanescu type (OMIM: 616583)

Spondyloperipheral dysplasia (OMIM: 271700)

Stickler sydrome, type I, nonsyndromic ocular (OMIM: 609508)

Stickler sydrome, type I, (OMIM: 108300)

They have also been associated with vitreoretinopathy with phalangeal epiphyseal dysplasia with unknown inheritance pattern.

RECOMMENDATIONS

In order to establish co-segregation with the disease and thus the possible pathogenicity of the variants of uncertain clinical significance identified, it is necessary tio study them in patient´s parents. If any of them were *de novo*, it would increase the evidence of its possible involvement in the clinical suspicion.

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 126,40x being > 20x in 98,70% 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.

Table 1. STUDIED GENES AND COVERAGE DETAILS

Gene	NM	10x %	Exons with coverage < 100%*
ACAN	NM_013227	92,23	12
COL2A1	NM_001844	100,00	-
HPGD	NM_000860	100,00	-
IL1RN	NM_173841	100,00	-
LPIN2	NM_014646	100,00	-
TRPV4	NM_021625	100,00	-
WISP3	NM_003880	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.

Signed: Cristina Camprubí, PhD

Head of Diagnosis and Genetic Counseling

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