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GENETIC STUDY OF SKELETAL DYSPLASIAS BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	20284		
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: 15-year-old patient with a suspected bone dysplasia.

RESULT AND INTERPRETATION

The presence of a h*omozygous* pathogenic variant i*n the SLC26A2* gene that confirms the Skeletal Dysplasia hypothesis, 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^
SLC26A2	NM_000112.3: **c.835C>T** NP_000103: **p.(Arg279Trp)**	Homozygosis	Autosomal recessive	**Pathogenic**

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

The identification of a homozygous variant should be interpreted with care and consider an apparent homozygosis, especially in cases where the parents are not consanguineous. In these cases, it is important to study the variant in the parents to rule out:

-a possible large deletion (*Copy Number Variant; CNV*) in an allele
-a possible uniparental disomy of the chromosome in which the identified variant is found

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Note that these situations are unlikely to occur. Even so, they cannot be ruled out and must be considered. If the zygosity is confirmed by studying the variant in the parents, the finding of a homozygous pathogenic variant in a gene with autosomal recessive inheritance pattern, confirms the diagnosis of suspicion.

The **homozygous *SLC26A2* variant c.835C>T p.(Arg279Trp)** is a *missense* that predicts an amino acid change from Arginine to Tryptophan at position 279 of the protein, affecting two functional domains. It is described in the HGMD (CM960498) and ClinVar (ID: 4089) databases as a pathogenic variant associated with Atelosteogenesis type 2, trophic dysplasia and multiple epiphyseal dysplasia. The variant is described in the dbSNP database (rs104893915) and in the gnomAD population frequency database (0,098%). The bioinformatic predictors (*SIFT, Mutation Taster and Polyphen-2*) estimate that the change would have a pathogenic effect. In the scientific literature, it has been reported in cases of diastrophic dysplasia and Atelosteogenesis type 2 in patients with compound heterozygous variant and in homozygosis (PMID:8571951, 8931695, 9342225, 29024831). In functional studies, it has been observed that the variant has a reduced sulfate transport rate of 32% (15294877). Cases have been reported in homozygous patients who have a milder clinical condition than the compound heterozygotes. (10465113,11241838).

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

The *SLC26A2* gene (OMIM:606718) is associated with Atelosteogenesis type 2 (OMIM:256050), with Diastrophic dysplasia (OMIM:222600) and with multiple epiphyseal dysplasia (OMIM:226900), entities with autosomal recessive inheritance pattern.

RECOMMENDATIONS

Being the patient homozygous for variant c.835C>T p.(Arg279Trp) in *SLC26A2* gene, parents have a high probability of being asymptomatic carriers. It is recommended to study the variant in parents to confirm their carrier status and to provide appropriate genetic counselling to the family.

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

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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 176,10x being > 20x in 99,10% 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 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.

Table 1. STUDIED GENES AND COVERAGE DETAILS

Gene	NM	10x %	Exons with coverage < 100%*
ALPL	NM_000478	100,00	-
ARSE	NM_000047	98,7	2
COL10A1	NM_000493	100,00	-
COL11A1	NM_001854	100,00	-
COL11A2	NM_080680	100,00	-
COL1A1	NM_000088	100,00	-
COL1A2	NM_000089	100,00	-
COL2A1	NM_001844	100,00	-
COL9A1	NM_001851	100,00	-
COL9A2	NM_001852	100,00	-
COL9A3	NM_001853	99,9	1
COMP	NM_000095	100,00	-
DDR2	NM_006182	100,00	-
DYNC2H1	NM_001080463	100,00	-
EBP	NM_006579	100,00	-
EVC	NM_153717	94,16	1

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EVC2	NM_147127	100,00	-
FGFR1	NM_023110	100,00	-
FGFR2	NM_000141	100,00	-
FGFR3	NM_000142	100,00	-
FLNB	NM_001457	100,00	-
HSPG2	NM_005529	99,81	1
IFT122	NM_052985	100,00	-
IFT140	NM_014714	100,00	-
IFT172	NM_015662	100,00	-
IFT43	NM_052873	100,00	-
IFT80	NM_020800	100,00	-
LBR	NM_002296	100,00	-
LIFR	NM_002310	100,00	-
MATN3	NM_002381	100,00	-
MMP13	NM_002427	100,00	-
MMP9	NM_004994	100,00	-
NEK1	NM_012224	100,00	-
NKX3-2	NM_001189	100,00	-
NSDHL	NM_015922	100,00	-
PEX7	NM_000288	100,00	-
PTH1R	NM_000316	100,00	-
RMRP	NR_003051	100,00	-
SBDS	NM_016038	100,00	-
SLC26A2	NM_000112	100,00	-
SLC35D1	NM_015139	100,00	-
SOX9	NM_000346	100,00	-
TCTN3	NM_015631	100,00	-
TRIP11	NM_004239	100,00	-
TRPV4	NM_021625	100,00	-
TTC21B	NM_024753	100,00	-
WDR19	NM_025132	100,00	-
WDR34	NM_052844	100,00	-
WDR35	NM_001006657	100,00	-
WDR60	NM_018051	100,00	-

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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

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Signed: Irina Royo, MSc

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