REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF X-LINKED FAMILIAL HYPOSPADIAS BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	41649		
Patient Name:	**xxx**		
Date of Birth:	18/03/2010	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Whole blood
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: Patient with hypospadias.

RESULT AND INTERPRETATION

The presence of a heterozygous likely pathogenic variant 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^
AR	NM_000044.4:**c.2612C>T p.(Ala871Val)**	Hemizygosis	X-linked	**Likely Pathogenic**

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

The ***AR*** variant **c.2612C>T p.(Ala871Val)** is a *missense* that predicts an amino acid change from Alanine to Valine at position 871 of the protein, affecting several functional domains. It is described in the HGMD (CM980115) and ClinVar (ID: 492801) databases as a pathogenic variant associated with androgen insensitivity syndrome. This variant appears in the dbSNP database (rs143040492) and in the the gnomAD population frequency database (0,0011%). The bioinformatic predictors MutationTaster and Polyphen-2 estimate that the change has a pathogenic effect, while the SIFT predictor estimates a tolerated effect. In the scientific literature, it has been identified in patients with hypospadias (PMID: 20305676, 28261839 31219235). In addition, in the same position, the variants c.2612C>A p.(Ala871Glu) and c.2612C>G p.(Ala871Gly) are also described as pathogenic.

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

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Pathogenic variants in the *AR* gene (OMIM: 313700 are associated with an androgen insensitivity syndrome (OMIM: 300068, 312300), hypospadias (OMIM: 300633) and Spinal and bulbar muscular atrophy of Kennedy (OMIM: **313200**), with an autosomal recessive inheritance pattern.

RECOMMENDATIONS

In order to confirm the segregation and causality of a likely pathogenic variant, it is necessary to study it in unaffected and affected relatives through the mother.

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 143,90x 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

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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%*
AR	NM_000044	100,00	-
MAMLD1	NM_005491	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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