REFERENCE LABORATORY GENETICS

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GENETIC STUDY OF HYPOGONADOTROPIC HYPOGONADISM (KALLMANN SYNDROME) BY MASSIVE SEQUENCING (NGS)

Request No.:	**000**		
Client:	-		
Analysis code:	45988		
Patient Name:	**xxx**		
Date of Birth:	26/04/1986	**Patient Ref.:**	xxx
Gender:	Male	**Sample Type:**	Blood EDTA
Sample Arrival Date:	DD/MM/AAAA	**Date of Result:**	DD/MM/AAAA

Clinical information: Isolated hypogonadotropic hypogonadism. Suspicion of Kallmann syndrome.

RESULT AND INTERPRETATION

The presence of a hemizygous pathogenic variant in *ANOS1* gene and a heterozygous pathogenic variant in *GNRHR* gene, both associated with Kallmann syndrome, have 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^
ANOS1	NM_000216.2: **c.1369C>T p.(Arg457Ter)**	Hemizygosis	X-linked	**Pathogenic**
GNRHR	NM_000406.2: **c.317A>G p.(Gln106Arg)**	Heterozygosis	Autosomal Recessive	**Pathogenic**

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

The ***ANOS1*** variant **c.1369C>T p.(Arg457Ter)** is a *nonsense* that predicts an amino acid change from Arginine to a premature stop codon at position 457 of the protein. It is described in the HGMD (CM015287) and ClinVar (ID: 180157) clinical databases a pathogenic variant and a likely pathogenic variant, associated with Kallmann syndrome. This variant appears in the dbSNP database (rs727505374) , but not in the population frequency database. In the scientific literature, it has been identified in patients with hypogonadotrotic hypogonadism (Kallmann syndrome) (PMID: 11297579, 15605412, 23643382).

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

Pathogenic variants in the *ANOS1* gene (OMIM: 300836) are associated with hypogonadotropic hypogonadism with or without anosmia (Kallmann syndrome) (OMIM: 308700), with a X-linked inheritance pattern.

The ***GNRHR*** variant **c.317A>G p.(Gln106Arg)** is a *missense* that predicts an amino acid change from Glutamine to Arginine at position 106 of the protein, affecting several functional domains. It is described in the HGMD (CM970686) and ClinVar (ID: 16023) databases as a pathogenic and likely pathogenic variant, associated with hypogonadotropic hypogonadism. This variant appears in the dbSNP database (rs104893836); in the gnomAD population frequency database (0,27%) and 1000Genomes (0,1%). The bioinformatic predictors (SIFT, MutationTaster and Polyphen-2) estimate that the change has a pathogenic effect. In the scientific literature, it has been identified in compound heterozygosis and homozygosis in individuals affected by hypogonadotropic hypogonadism. In addition, *in vitro* studies show that it affects the function of the protein. (PMID: 12364481, 12574221, 28348023, 28611058, 29182666)

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

Pathogenic variants in the *GNRHR* gene (OMIM: 138850) are associated with hypogonadotropic hypogonadism 7 without anosmia (OMIM: 146110), with an autosomal recessive inheritance pattern.

In the literature consulted, pathogenic variants have been described in two genes in the same patient with Kallmann syndrome/Isolated hypogonadotropic hypogonadism (IHH). The prevalence of digenic inheritance is unknown, but is estimated to be 2,5% (PMID: 22035731). The result obtained confirms the diagnosis of Kallmann/IHH syndrome in the patient.

RECOMMENDATIONS

The study of the variant c.1369C> T p (Arg457Ter) in the *ANOS1* gene in the patient's mother and the variant c.317A> G p (Gln106Arg) in the *GNRHR* gene in both parents is recommended to establish the segregation and provide an appropriate genetic counseling 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 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 185,8x being > 20x in 99,4% of the regions analysed..

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%*
ANOS1	NM_000216	100,00	-
CHD7	NM_017780	100,00	-
DUSP6	NM_001946	100,00	-
FEZF1	NM_001024613	100,00	-
FGF17	NM_003867	100,00	-
FGF8	NM_033163	100,00	-
FGFR1	NM_023110	100,00	-
FLRT3	NM_198391	100,00	-
FSHB	NM_000510	100,00	-
GNRH1	NM_000825	100,00	-
GNRHR	NM_000406	100,00	-
HESX1	NM_003865	100,00	-
HS6ST1	NM_004807	100,00	-
IL17RD	NM_017563	100,00	-
KISS1	NM_002256	100,00	-
KISS1R	NM_032551	100,00	-
LHB	NM_000894	83,57	2
NR0B1	NM_000475	100,00	-
NSMF	NM_015537	100,00	-
POLR3B	NM_018082	100,00	-
PROK2	NM_001126128	100,00	-
PROKR2	NM_144773	100,00	-
SEMA3A	NM_006080	100,00	-
SPRY4	NM_030964	100,00	-
TAC3	NM_013251	100,00	-

TACR3	NM_001059	100,00	-
WDR11	NM_018117	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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