X CeGaT

CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

Patient	XXX, XX
ID #	Male (*DD.MM.YYYY)
Sample receipt	xxx
Material	DNA
External ID	#
Report date	xxx
Report-ID	R#

Genetic Report – XXX, XX (*DD.MM.YYYY)

Indication   Hypogonadotropic hypogonadism, cryptorchidism, sense of smell intact

Order   Panel Diagnostics: Hypogonadotropic hypogonadism with or without anosmia (whole exome enrichment)

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *ANOS1*, which is causative for hypogonadotropic hypogonadism 1 in your patient.**

- Based upon current scientific knowledge, we did not identify any reportable copy number variants which are likely to be causative for your patient's disease.

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
ANOS1	**c.67_92dup; p.Ala32Trp*fs**32** chrX:8699985 Ains26	**hemi.**	**XL**	**-**	**pathogenic**

Information for the interpretation of this table can be found in section *Additional Information*.

Recommendation

We recommend further clinical management and treatment according to the current guidelines for hypogonadotropic hypogonadism (Balasubramanian and Crowley, updated 2022, PMID: 20301509, GeneReviews). In order to determine whether this variant occurred *de novo* or is inherited, we recommend testing of the patient's mother.

Genetic Relevance

The patient is hemizygous for a pathogenic variant in gene *ANOS1*. This may be of relevance for family planning and at-risk family members.

This variant will be passed on to every female offspring of your patient, who will each be heterozygous carriers of this variant and may show clinical features of disease in some cases. Male offspring of your patient will not inherit the variant.

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Clinical Information and Variant Interpretation

ANOS1, NM_000216.4

OMIM / Reference	Phenotype	Heredity
308700	Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1)	XL

The **ANOS1** gene encodes the anosmin-1 protein, which plays a key role in the migration of GNRH neurons and olfactory nerves to the hypothalamus. Pathogenic variants in *ANOS1* lead to hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1), which is characterized by absent or incomplete sexual maturation by the age of 18 years, in conjunction with low levels of circulating gonadotropins and testosterone and no other abnormalities of the hypothalamic-pituitary axis. The majority of patients are diagnosed during puberty due to their lack of sexual development. Both sexes can present a partial or total impairment of the sense of smell (anosmia); however, Kallmann syndrome affects predominantly males, who can also show e.g. cryptorchidism, micropenis, or azoospermia. Females may present with primary amenorrhea.

ANOS1, c.67_92dup; p.Ala32Trp*fs**32 (hemi.)

ACMG/ACGS Criterion	Points	Description
PVS1	+8	The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *ANOS1* associated disease.
PM2	+2	This variant is absent from the gnomAD global population dataset.
ACMG/ACGS Classification: Pathogenic	+10	

B	LB	VUS (Ice Cold)	VUS (Cold)	VUS (Cool)	VUS (Tepid)	VUS (Warm)	VUS (Hot)	LP	P
≤ -7	-6 – -1	0	1	2	3	4	5	6 – 9	≥ 10
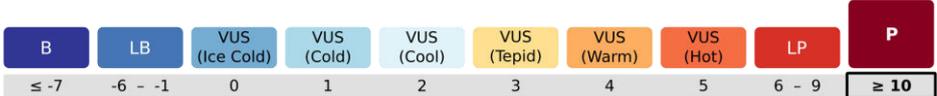
Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,

Saskia Biskup

Consultant for Human Genetics

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Additional Information

Requested Regions	The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

AMH, *AMHR2*, *ANOS1*, *CHD7*, *CPE*, *DUSP6*, *FEZF1*, *FGF17*, *FGF8*, *FGFR1*, *FLRT3*, *FSHB*, *GNRH1*, *GNRHR*, *HAMP*, *HESX1*, *HFE*, *HS6ST1*, *HSD3B2*, *IGSF10*, *IL17RD*, *KISS1R*, *LAS1L*, *LHB*, *LHCGR*, *NDNF*, *NR0B1*, *NSMF*, *PROK2*, *PROKR2*, *PRORP*, *SEMA3A*, *SLC40A1*, *SOX10*, *SOX2*, *SOX3*, *SPRY4*, *TAC3*, *TACR3*, *TCF12*, *TFR2*, *WDR11* (Hypogonadotropic hypogonadism with or without anosmia)

General Remarks

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Information for the interpretation of the tables

Heredity: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

MAF: The *minor allele frequency* describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot*, *warm*, *tepid*, *cool*, *cold*, and *ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

Methods

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Computational Analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small

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insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. If an acceptable sequencing-depth per base is not achieved by high-throughput sequencing, our quality guidelines demand local re-sequencing using classical Sanger-technology. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

In this case, 97.73% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. In silico predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

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