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Dr. James Public
Model Company
Model Street 1
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COUNTRY

| | |
|---|---|
| **Patient** | XXX, XX |
| ID # | Male (*DD.MM.YYYY) |
| **Sample receipt** | xxx |
| **Material** | EDTA blood |
| **External ID** | # |
| **Report date** | xxx |
| **Report-ID** | R9999999999 |

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

**Indication** Suspected bile acid metabolism disorder; markedly elevated bile acids (>10 times the upper normal limit in blood), elevated GLDH, mildly elevated AST/ALT.

**Order** Panel Diagnostics: *ABCB11*, *ABCB4*, *ATP8B1*, *KIF12*, *NR1H4*, *MYO5B*, *TJP2*, *USP53*, *VPS33B*, *ZFYVE19* and *SLC10A1* (ExomeXtra® enrichment)

## Result: Report with Significant Findings

- **Detection of one homozygous likely pathogenic variant in gene *SLC10A1*, which is consistent with Familial hypercholanemia 2 (FHCA2) 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 |
|---|---|---|---|---|---|
| ***SLC10A1*** | **c.755G>A; p.Arg252His**<br>**chr14:70245238 C>T (hg19)** | **homo.** | **AR** | **0.04** | **likely pathogenic** |

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

## Recommendation

To determine the probability of reoccurrence for future family planning, we recommend analysis of the patient's parents with regard to this variant.

Testing of asymptomatic family members regarding the variant in gene *SLC10A1* may only be performed following genetic counseling.

## Genetic Relevance

Your patient is homozygous for a likely pathogenic variant in gene *SLC10A1*. This may be of relevance for family planning and at-risk family members.

One altered *SLC10A1* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

Our sequencing data indicates that both gene copies of *SLC10A1* are present. The data does not show evidence for a deletion of one allele, which would mimic homozygosity.

## Clinical Information and Variant Interpretation

***SLC10A1*, NM_003049.4**

| OMIM / Reference | Phenotype | Heredity |
|---|---|---|
| 619256 | Familial hypercholanemia 2 (FHCA2) / NTCP deficiency | AR |

The gene ***SLC10A1*** encodes for the sodium taurocholate cotransporting polypeptide (NTCP), which is the major transporter of conjugated bile salts from the plasma compartment into the hepatocyte and thereby maintaining the enterohepatic circulation of bile salts between liver and intestine (Vaz et al., 2015, 24867799).

Biallelic pathogenic variants in the gene *SLC10A1* have been associated with autosomal recessive familial hypercholanemia 2 (FHCA2), an inborn error of metabolism leading to elevated plasma levels of conjugated bile salts. Most patients are asymptomatic and have no liver dysfunction. However, some neonates were reported having a variable phenotype including jaundice, cholestasis, hyperbilirubinemia, hepatomegaly (Qui et al., 2017, PMID: 29290974). Information about the laboratory and clinical presentations of patients with NTCP deficiency remains limited. FHCA2 usually manifests in neonatal age or early childhood and abnormalities improve with age, but also adults with FHCA2 were reported.

***SLC10A1*, c.755G>A; p.Arg252His (homo.), exon 4, ClinVar ID: 501945**

| ACMG/ACGS Criterion | Points | Description |
|---|---|---|
| **PS3** (supporting) | +1 | Functional studies support the pathogenicity of this variant.<br>Vaz et al., 2015, PMID: 24867799 |
| **PM2** | +2 | This variant is listed in the gnomAD global population dataset with a very low frequency. |
| **PM3** | +2 | The variant was detected *in trans* with a diagnostically relevant variant and/or in a homozygous state. The co-occurrence of the variant with a (likely) pathogenic variant, without determining the allelic configuration, can also be used as evidence.<br>Vaz et al., 2015, PMID: 24867799 |
| **PP4** | +1 | The phenotype or family history of patients with this variant is highly specific for a disease with a limited genetic etiology. |
| **ACMG/ACGS Classification:** likely pathogenic | +6 | B (≤ -7) \| LB (-6 – -1) \| VUS (Ice Cold) (0) \| VUS (Cold) (1) \| VUS (Cool) (2) \| VUS (Tepid) (3) \| VUS (Warm) (4) \| VUS (Hot) (5) \| **LP (6 - 9)** \| P (≥ 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,

Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

## Additional Information

**CeGaT ExomeXtra® based diagnostics**

The genetic analysis was performed using CeGaT's in-house designed ExomeXtra® enrichment (version 6), which includes:

- all exons of the approximately 20,000 protein-coding genes
- more than 46,000 intergenic and intronic positions associated with genetic diseases
- the entire mitochondrial genome at high coverage
- selected clinically relevant RNA genes, including all snRNAs of the spliceosome
- genome wide CNV calling with an array-like resolution of approximately 50 kb in intergenic regions and higher resolution of CNVs in coding regions
- high evidence pharmacogenetic variants
- genomic regions associated with repeat expansion disorders
- screening for infections of human papillomavirus (HPV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Herpes simplex virus (HSV) 1 and 2, Toxoplasma gondii (Toxoplasmosis), Varicella Zoster virus, Parvovirus B19 (Fifth disease), and Treponema pallidum (Syphilis)

Please note that data evaluation is based on the provided phenotype. Therefore, not all of the above-mentioned characteristics are relevant for every case and reporting is restricted to phenotypically relevant variants. Further methodological details on the specific diagnostics for this patient are provided below.

**Requested Regions**

The analysis focused on the following gene regions:

***ABCB11*** (NM_003742.4), ***ABCB4*** (NM_000443.4), ***ATP8B1*** (NM_005603.6), ***KIF12*** (NM_138424.1), ***NR1H4*** (NM_005123.4), ***MYO5B*** (NM_001080467.3), ***TJP2*** (NM_004817.4), ***USP53*** (NM_019050.2), ***VPS33B*** (NM_018668.5), ***ZFYVE19*** (NM_001077268.2), ***SLC10A1*** (NM_003049.4)

**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-2024v1.2, and ClinGen variant classification guidelines (Richards et al., 2015, PMID: 25741868; Durkie et al., 2024, Association for Clinical Genomic Science). If applicable, the following approach is used. 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 (Durkie et al., 2024, 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 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 copy number neutral structural variants such as balanced translocations, inversions, uniparental heterodisomy or low-level mosaicism. Aberrations within 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. 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 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-2024v1.2 guidelines (Richards et al., 2015, PMID: 25741868, Durkie et al., 2024, Association for Clinical Genomic Science).

Only variants (single-nucleotide 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 and ClinVar) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Possible exceptions include risk factors and hypomorphic alleles. 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, 97.8% 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.**