

Dr. Mary Public
Organization
Street
City
Country

Patient	Doe, John
ID #	Male (*DD.MM.YYYY)
Sample receipt	DD.MM.YYYY
Material	EDTA blood
Report date	DD.MM.YYYY
Report-ID	R#

Genetic Report – Doe, John (*DD.MM.YYYY)

Indication Spherocytosis

Order Panel Diagnostics: Defects of the erythrocytes and anemia (ExomeXtra® enrichment)

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *ANK1*, which is causative for spherocytosis type 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	Zygoty	Heredity	MAF (%)	Classification
<i>ANK1</i>	c.2559-1G>A; p.? chr8:41555640 C>T (hg19)	het.	AD, AR	-	pathogenic

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

Recommendation

To determine whether the detected variant in gene *ANK1* is *de novo* in your patient, testing of both parents regarding this variant is recommended.

Testing of asymptomatic family members regarding the variant c.2559-1G>A; p.? identified in gene *ANK1* may only be performed following genetic counseling.

Genetic Relevance

Your patient is heterozygous for a pathogenic variant in gene *ANK1*. This may be of relevance for family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring.

Clinical Information and Variant Interpretation

ANK1, NM_000037.4

OMIM / Reference	Phenotype	Heredity
182900	Spherocytosis type 1	AD, AR

The gene **ANK1** encodes a member of the ankyrin protein family, which link the integral membrane proteins to the underlying spectrin-actin cytoskeleton and play key roles in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. Ankyrin 1 has since also been found in brain and muscles (GeneCards®, ANK1). Pathogenic variants in ANK1 have been identified in approximately half of all patients with hereditary spherocytosis (NCBI). The prevalence of the disease is about 1:5000 (ORPHA:822). Hereditary spherocytosis refers to a group of heterogeneous disorders that are characterized by the presence of spherical-shaped erythrocytes (spherocytes) on the peripheral blood smear. The disorders are clinically characterized by anemia, jaundice, and splenomegaly, with variable severity. Common complications include cholelithiasis, hemolytic episodes, and aplastic crises (Perrotta et al., 2008, PMID: 18940465).

ANK1, c.2559-1G>A; p.? (het.)

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 ANK1-associated disease.
PM2	+2	This variant is absent from the gnomAD global population dataset.
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:	Points	Legend
pathogenic	+11	<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <div style="background-color: #0056b3; color: white; padding: 2px 5px; border: 1px solid black;">B</div> <div style="font-size: 8px;">≤ -7</div> </div> <div style="text-align: center;"> <div style="background-color: #00a0e3; color: white; padding: 2px 5px; border: 1px solid black;">LB</div> <div style="font-size: 8px;">-6 - -1</div> </div> <div style="text-align: center;"> <div style="background-color: #00c0e3; color: white; padding: 2px 5px; border: 1px solid black;">VUS (Ice Cold)</div> <div style="font-size: 8px;">0</div> </div> <div style="text-align: center;"> <div style="background-color: #00d0e3; color: white; padding: 2px 5px; border: 1px solid black;">VUS (Cold)</div> <div style="font-size: 8px;">1</div> </div> <div style="text-align: center;"> <div style="background-color: #00e0e3; color: white; padding: 2px 5px; border: 1px solid black;">VUS (Cool)</div> <div style="font-size: 8px;">2</div> </div> <div style="text-align: center;"> <div style="background-color: #fff9c4; color: black; padding: 2px 5px; border: 1px solid black;">VUS (Tepid)</div> <div style="font-size: 8px;">3</div> </div> <div style="text-align: center;"> <div style="background-color: #fff176; color: black; padding: 2px 5px; border: 1px solid black;">VUS (Warm)</div> <div style="font-size: 8px;">4</div> </div> <div style="text-align: center;"> <div style="background-color: #ff9800; color: black; padding: 2px 5px; border: 1px solid black;">VUS (Hot)</div> <div style="font-size: 8px;">5</div> </div> <div style="text-align: center;"> <div style="background-color: #ff5722; color: white; padding: 2px 5px; border: 1px solid black;">LP</div> <div style="font-size: 8px;">6 - 9</div> </div> <div style="text-align: center;"> <div style="background-color: #c00000; color: white; padding: 2px 5px; border: 1px solid black;">P</div> <div style="font-size: 8px;">≥ 10</div> </div> </div>

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:

ABCB7, ABCG5, ABCG8, AK1, ALAS2, ALDOA, ANK1, ATP11C, BPGM, CD59, CDAN1, CDIN1, COL4A1, CYB5R3, DHFR, DNASE2, EGLN1, EPAS1, EPB41, EPB42, EPO, EPOR, G6PD, GATA1, GCLC, GLRX5, GPI, GSR, GSS, HBB, HBG2, HK1, HSPA9, JAK2, KCNN4, KIF23, KLF1, LARS2, LCAT, LPIN2, MT-ATP6, MTHFD1, NDUFB11, NT5C3A, PFKM, PGK1, PIEZO1, PKLR, PUS1, RHAG, SEC23B, SH2B3, SLC11A2, SLC19A2, SLC25A38, SLC2A1, SLC30A10, SLC4A1, SPTA1, SPTB, TMPRSS6, TP11, TRNT1, UMPS, VHL, XK, YARS2 (Defects of the Erythrocytes and Anemia)

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 degree of heteroplasmy of mitochondrial variants can vary remarkably between different tissues (Wallace & Chalkia 2013; PMID: 24186072). Therefore, it is possible that disease causing variants, deletions and duplications are not detectable in the mtDNA from leucocytes, but present in other tissues. 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, flanking intronic regions and additional disease-relevant non-coding regions of the nuclear encoded genes, as well as the mitochondrial DNA 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 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. 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 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) of the nuclear encoded genes and in the mitochondrial DNA with a minor allele frequency (MAF) $< 1.5\%$ are evaluated. Known disease-causing variants (according to HGMD and MITOMAP) 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, MITOMAP) 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, 97.86% 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.