CeGaT

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

Dr. Saskia Biskup
Paul-Ehrlich-Str. 23
72076 Tübingen
GERMANY

Patient	Doe, John
ID 112233	Male (*01.01.2018)
Sample receipt	01.10.2023
Material	EDTA blood
Report date	10.10.2023
Report-ID	R123456

Genetic Report – Doe, John (*01.01.2018)

Indication Global developmental delay, motor development delay, poor head control, fixed gaze, intellectual disability, lack of speech

Order Single exome analysis

Result: Report with Significant Findings

- **Detection of a homozygous pathogenic variant in gene *EXOSC3*, which is causative for pontocerebellar hypoplasia type 1B 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
EXOSC3	**c.395A>C; p.Asp132Ala** **chr9:37783990 T/G**	**homo.**	**AR**	**0.08**	**pathogenic**

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

Recommendation

We recommend further clinical evaluation, management, and treatment of manifestation according to the current guidelines for *EXOSC3*-associated pontocerebellar hypoplasia (Baas and van Dijk, updated 2020, PMID: 25144110, GeneReviews).

Carrier testing of both parents regarding the identified variant in gene *EXOSC3* in your patient may be performed in order to determine their carrier status and to determine the risk of reoccurrence for further offspring of the parents of your patient.

Genetic Relevance

Your patient is homozygous for a pathogenic variant in gene *EXOSC3*. This may be of relevance for at-risk family members.

One altered *EXOSC3* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

Our sequencing data indicated that both gene copies of *EXOSC3* are present. The data did not show evidence for a deletion of one allele, which would mimic homozygosity.

Clinical Information and Variant Interpretation

EXOSC3, NM_016042.4

OMIM / Reference	Phenotype	Heredity
614678	Pontocerebellar hypoplasia, type 1B (PCH1B)	AR

The ***EXOSC3*** gene encodes the protein exosome component 3, which is a non-catalytic subunit of the human RNA exosome complex. This protein complex plays an important role in RNA processing. The activity of EXOSC3 has a critical role in the normal development and growth of specific areas of the brain (Mukherjee et al., 2002, PMID: 11782436). Biallelic pathogenic variants in this gene result in pontocerebellar hypoplasia type 1B (PCH1B) a severe autosomal recessive neurologic disorder characterized by a combination of cerebellar and spinal motor neuron degeneration beginning at birth. Patients present with hypotonia at birth and poor feeding. Spasticity, dystonia, and seizures become evident in patients with prolonged survival. Within the first year of life respiratory insufficiency and swallowing difficulties are common. Intellectual disability is severe. Life expectancy ranges from a few weeks to adolescence. PCH1B can be divided into mild, moderate, and severe subgroups that vary in age at onset, progression, clinical and neuroradiologic severity, and survival. There are clear genotype-phenotype correlations for certain *EXOSC3* pathogenic variants (Halevy et al., 2014, PMID: 25149867; Baas and van Dijk et al., updated 2020, PMID: 25144110).

EXOSC3, c.395A>C; p.Asp132Ala (homo.)

ACMG/ACGS Criterion	Points	Description
PM2	+2	This variant is listed in the gnomAD global population dataset with very low frequency.
PM3 (very strong)	+8	The variant has already been detected *in trans* with a pathogenic variant or in a homozygous state. Rudnik-Schöneborn et al, 2013, PMID: 23284067
PM5	+2	The variant results in the change of an amino acid residue, for which a different amino acid change (p.Asp123Tyr) has already been described as pathogenic. Mitani et al, 2021, PMID: 34582790
PP1 (strong)	+4	Cosegregation of the variant with disease in multiple affected family members.
ACMG/ACGS Classification: Pathogenic	+16	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: Scientist 1

Proofread by: Scientist 2

Validated by: Physician 1

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup
Dr. med. Friedmar Kreuz, M.A.
Dr. med. Lisa Dudler

Consultant for Human Genetics

Additional Information

Requested Regions

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

ANKLE2, ***ASNS***, ***ASPM***, ***ATR***, ***CDC45***, ***CDC6***, ***CDK5RAP2***, ***CDK6***, ***CDT1***, ***CENPE***, ***CENPF***, ***CENPJ***, ***CEP135***, ***CEP152***, ***CEP63***, ***CIT***, ***COPB2***, ***CTNNA2***, ***DNA2***, ***DONSON***, ***DYRK1A***, ***EXOSC3***, ***GMNN***, ***KIF11***, ***KIF14***, ***KIF2A***, ***KIF5C***, ***KNL1***, ***LMNB1***, ***LMNB2***, ***MCM5***, ***MCPH1***, ***MFSD2A***, ***NBN***, ***NCAPD2***, ***NCAPD3***, ***NCAPH***, ***NIN***, ***NSMCE2***, ***NUP37***, ***ORC1***, ***ORC4***, ***ORC6***, ***PCNT***, ***PHC1***, ***PHGDH***, ***PLK4***, ***PNKP***, ***PSAT1***, ***PSPH***, ***RBBP8***, ***RNU4ATAC***, ***RRP7A***, ***RTTN***, ***SASS6***, ***STIL***, ***TRAIP***, ***TRAPPC14***, ***TUBA8***, ***TUBB***, ***TUBB2A***, ***TUBB2B***, ***TUBB3***, ***TUBG1***, ***TUBGCP4***, ***TUBGCP6***, ***WDFY3***, ***WDR62***, ***ZNF335*** (Primary Microcephaly and Differential Diagnoses)

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. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered. Positions refer to the hg19 genome build. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: pathogenic, likely pathogenic, and uncertain significance. **All variants for which the clinical relevance cannot be conclusively confirmed or excluded are referred to as variants of unknown clinical significance.**

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 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.

Variants identified through single exome analysis were evaluated with reference to the indicated phenotype. Therefore, single heterozygous variants in genes associated with autosomal recessive inheritance may not have been reported.

In this case, 97.89% 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.