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

Dr. Jane Doe
Paul-Ehrlich-Str. 23
72076 TÜBINGEN

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

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

Indication	Cerebral palsy, atopic-astatic form, speech development delay, large high forehead, internal compensated hydrocephalus, hypoplastic chest muscles, dysarthria of the cranial nerves, pathological foot reflexes, joint hypermobility, general muscle hypotension, ataxic gait
Order	Single exome analysis

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *ZBTB20*, which is causative for Primrose syndrome 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
ZBTB20	**c.1832G>A; p.Cys611Tyr** **chr3:114058246 C>T (hg19)**	**het.**	**AD**	**-**	**pathogenic**

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

Recommendation

We recommend further clinical evaluation and management according to the current guidelines for *ZBTB20*-associated Primrose syndrome (Arora et al., 2021, PMID: 33956417, GeneReviews).

To determine whether the detected variant in gene *ZBTB20* is *de novo* in your patient, testing of both parents regarding this variant is recommended.

Genetic Relevance

Your patient is heterozygous for a pathogenic variant in gene *ZBTB20*. This may be of relevance for future 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

ZBTB20, NM_001164342.2

OMIM / Reference	Phenotype	Heredity
259050	Primrose syndrome (PRIMS)	AD

The gene ***ZBTB20*** belongs to a family of transcription factors with an N-terminal BTB/POZ domain and a C-terminal DNA-binding zinc finger domain. It acts as a transcriptional repressor and plays a role in many processes including neurogenesis, glucose homeostasis, and postnatal growth (GeneCards *ZBTB20).* Pathogenic *de novo* variants in *ZBTB20* are associated with autosomal dominant Primrose syndrome. Various microdeletions, missense, nonsense and frameshift variants have already been described (HGMD professional). Primrose syndrome is characterized by macrocephaly, hypotonia, developmental delay, intellectual disability with expressive speech delay, behavioral issues, a recognizable facial phenotype (amongst others brachycephaly, deeply set eyes, downslanted palpebral fissures, ptosis and large ears with small or absent lobes) radiographic features, and altered glucose metabolism. Additional features seen in adults include sparse body hair, distal muscle wasting, and contractures. Further features include hearing impairment, ocular anomalies, cryptorchidism, and nonspecific findings on brain MRI (Arora et al., 2021, PMID: 33956417, GeneReviews).

ZBTB20, c.1832G>A; p.Cys611Tyr (het.)

ACMG/ACGS Criterion	Points	Description
PS2	+4	The variant has already been detected *de novo* in a patient with the disease, and without a positive family history. The number of *de novo* cases and other factors such as confirmed parenthood influence the strength of evidence. Alby et al., 2018, PMID: 29681083
PS4 (supporting)	+1	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Alby et al., 2018, PMID: 29681083
PM2	+2	This variant is absent from the gnomAD global population dataset.
PM5	+2	The variant results in the change of an amino acid residue, for which a different amino acid change p.Cys611Ser has already been described as pathogenic. Martínez et al., 2017, PMID: 27620904
PP2	+1	Fewer than expected missense variants are present within the gene *ZBTB20* in the general population, which suggests poor tolerance for missense variation in this gene.
PP3	+1	The variant was given a pathogenic prediction.
ACMG/ACGS Classification: pathogenic	+11	

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

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

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:

AKT1, ***AKT2***, ***AKT3***, ***APC2***, ***ASPA***, ***ASXL2***, ***BRWD3***, ***CCND2***, ***CDKN1C***, ***CHD3***, ***CHD4***, ***CHD8***, ***CRADD***, ***DIS3L2***, ***DNMT3A***, ***EED***, ***EZH2***, ***GCDH***, ***GFAP***, ***GLI3***, ***GPC3***, ***H1-4***, ***HEPACAM***, ***HERC1***, ***HRAS***, ***HUWE1***, ***KIF7***, ***KPTN***, ***L1CAM***, ***MITF***, ***MLC1***, ***MPDZ***, ***MTOR***, ***NF1***, ***NFIA***, ***NFIB***, ***NFIX***, ***NONO***, ***NSD1***, ***PAK1***, ***PHF6***, ***PIGA***, ***PIK3CA***, ***PIK3R2***, ***PPP1CB***, ***PPP2R5B***, ***PPP2R5C***, ***PPP2R5D***, ***PTCH1***, ***PTEN***, ***RAB39B***, ***RIN2***, ***RNF125***, ***SETD2***, ***SOS1***, ***STRADA***, ***SUFU***, ***SUZ12***, ***TBC1D7***, ***TRIO***, ***UPF3B***, ***ZBTB20*** (Macrocephaly)

The following differential diagnoses were also taken into account in the evaluation of our sequencing data: Intellectual disability, X-linked non-syndromic; Intellectual disability, autosomal dominant; Intellectual disability, autosomal recessive

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 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, 97.72% 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.