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

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

Index	XXX, XX (*DD.MM.YYYY)
Sex	Female
Patient-ID	#
Mother	XXX, XX (*DD.MM.YYYY)
Patient-ID	#
Father	XXX, XX (*DD.MM.YYYY)
Patient-ID	#
Sample receipt	xxx
Material	DNA
External ID	#
Report date	xxx

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

Indication	Developmental problems but making progress, poor growth, microcephaly, ataxic gait, delayed speech and language, MRI normal, metabolic screening normal
Order	1) Panel Diagnostics: Dystonia (see report dated DD.MM.YYYY)
	2) Trio exome analysis

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a pathogenic variant in gene *ZBTB18*, which is causative for autosomal dominant mental retardation 22 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
		Index	Mother	Father			
ZBTB18	**c.491dupT; p.Leu164Phe*fs***4**	**het.**	**-**	**-**	**AD**	**-**	**pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene variant at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows the **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene variant at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
mitochondrial: gene encoded in the mitochondrial DNA. Information on degree of heteroplasmy (heteropl. or homopl.), frequency and read count (variant/total reads).
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. 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 clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION

ZBTB18, c.491dupT; p.Leu164Phe*fs**4 (het.), NM_205768.3

OMIM / Reference	Phenotype	Heredity
612337	Mental retardation, autosomal dominant 22 (MRD22)	AD

The gene ***ZBTB18*** encodes a zinc finger protein (also known as *ZNF238*), which acts as a transcriptional repressor in the regulation of neuronal developmental genes (Xiang et al., 2012, PMID: 22095278). The gene is in the critical region of the 1q43q44 microdeletion syndrome, which is associated with mental retardation, microcephaly, epilepsy, and agenesis of the corpus callosum (Ballif et al., 2012, PMID: 21800092). Patients with missense or truncating variants in *ZBTB18* typically have mental retardation, developmental delay, including speech development disorder, hypotonia, poor growth, microcephaly, behavioural abnormalities, epilepsy, ataxia, and agenesis or hypoplasia of the corpus callosum. Phenotypic expression is, however, very variable (de Munnik et al., 2014, PMID: 24193349; Cohen et al., 2017, PMID: 27598823; Depienne et al., 2017, PMID: 28283832).

The variant **c.491dupT; p.Leu164Phe*fs**4** in gene *ZBTB18* has been identified in your patient in a heterozygous state. It was not identified in the analyzed DNA samples of the parents, and therefore likely occurred *de novo* in your patient. The variant creates a shift in the reading frame, which will most likely result in a truncated protein, which is a known pathomechanism for *ZBTB18*-associated disease. This variant is absent from the gnomAD global population dataset and has not been described in the scientific literature, nor is it present within the ClinVar dataset.

Based upon the available data, the detected variant in *ZBTB18* was classified as pathogenic and is causative for MRD22 in your patient.

Variants in regions not analyzed (e.g. introns, untranslated regions (UTRs), promotors, or enhancers), and in regions with repeat expansions cannot be reliably detected, and therefore their potential involvement in disease cannot be excluded. Furthermore, mosaic variants that occur at a low frequency in the sampled tissue cannot be reliably detected, and therefore, likewise cannot be excluded. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

Your patient is heterozygous for a pathogenic variant in gene *ZBTB18* that likely arose *de novo*. This may be of relevance for future family planning.

The possibility of parental germline mosaicism has to be taken into consideration as the variant in gene *ZBTB18* has not been detected in DNA extracted from leukocytes from the parents of your patient. The likelihood of further offspring of the parents inheriting this variant is difficult to determine due to a lack of scientific data, however the probability of reoccurrence for parents with a child carrying the pathogenic variant is statistically increased (Human Genetics: From Molecules to Medicine - Schaaf, Zschocke & Potocki 2011).

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

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. Following the identification of the molecular cause of a genetic disease, genetic counseling has to be offered.

Medical report written by: Scientist 1

Proofread by: Scientist 2

With kind regards,

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

Consultant for Human Genetics

Dr. rer. nat. Heinz-Dieter Gabriel
PD Dr. biol. hum. Christiane Maier
Dr. rer. nat. Christian Wilhelm
Dr. rer. nat. Martin Ritthaler

Diagnostics

ADDITIONAL INFORMATION

Analyzed Regions

Trio whole exome analysis was performed for the three individuals described above.

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 NovaSeq6000 system.

NGS based CNV-Calling: (only applicable for nuclear encoded genes) Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. 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 can not 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% 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. X-chromosomal variants that are listed in public databases equal to or greater than 50 times in a hemizygous state and are not disease-causing variants according to HGMD are excluded from analysis. 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.

Trio analysis: Variants found in the patient and in the patient's parents were compared and filtered for the following cases: *de novo* in the patient, patient is compound heterozygous, patient is homozygous and the parents are heterozygous.

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.

96.34%, 95.36%, and 96.27% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base for the index, mother, and father, respectively.

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).

CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany
Tel: + 49 7071 565 44 55 | Fax: + 49 7071 565 44 56 | info@cegat.de | www.cegat.de
Court District Stuttgart - HRB 729958 | VAT No: DE265504070
VR Bank Tübingen eG | IBAN: DE20 6406 1854 0021 2890 00 | SWIFT / BIC: GENODES1STW
Managing Directors: Dr. Dr. Saskia Biskup, Dr. Dirk Biskup, Dr. Detlef Schumann