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

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

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

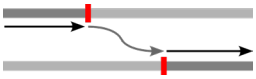
Genetic Report – XXX, XX (*DD.MM.YYYY)

Indication History of prematurity and intrauterine growth restriction (IUGR), delayed motor milestones, generalized seizures, encephalopathy, clonic tonic seizures associated with sleep, dystonia, epicanthus, atrial septal defect, heart failure, tachycardia, increased electrical activity of left ventricle, incomplete right bindle branch block, pulmonary arterial hypertension, anemia, muscular hypotonia

Order Whole-Genome Diagnostics

Result: Report with Significant Findings

- **An unbalanced translocation between 3p25.3 and 5p15.32 was detected, which results in a pathogenic terminal deletion of approx. 10.1 Mb on 3pterp25.3 (including genes *BRPF1* and *SETD5*) and a pathogenic terminal gain of approx. 6.3 Mb (73 genes*) of 5pterp15.32. Deletions on 3p25 are associated with 3p-syndrome which is consistent with your patient's clinical phenotype.**

Type		Chromosomal Breakpoints / ISCN (2024)	Affected Genes / Associated Syndrome
Unbalanced translocation		**(chr5:6250193, chr3:10127305)** **seq[hg19] der(3)t(3;5)(p25.3;p15.32) NC_000003.11:g.pter_10127305delins [NC_000005.9:g.pter_6250193]**	-

Chromosomal Region	Type of Imbalance	Size	ISCN (2024)	Microdeletion Syndrome / Affected Genes
3p26.3p25.3	**deletion (1 copy)**	**10.1 Mb**	**seq[hg19] 3p26.3p25.3(1_10127305)×1**	**3p-syndrome**
5p15.33p15.32	**gain (3 copies)**	**6.3 Mb**	**seq[hg19] 5p15.33p15.32(1_6250193)×3**	**47 genes***

* Additional genes within the described chromosomal aberration/copy number variation that are not associated with the clinical phenotype of the patient based upon current scientific knowledge are not discussed further in this report.

Recommendation

We recommend a chromosomal or molecular cytogenetic analysis of the parents to determine whether the unbalanced translocation has occurred in the patient as a result of a parental balanced translocation.

Genetic Relevance

Your patient is a carrier of a pathogenic unbalanced translocation. 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

Unbalanced translocation

with chromosomal breakpoints: (chr5:6250193, chr3:10127305)

Evidence
The unbalanced translocation between chr5:6250193 and chr3:10127305 results in a derivative chromosome 3 with a terminal 3p26.3–p25.3 deletion and a 5p15.33–p15.32 duplication, affecting multiple dosage-sensitive genes and causing a pathogenic genomic imbalance.

Classification: pathogenic

3p26.3p25.3 (10.1 Mb, het. deletion)

OMIM / Reference	Phenotype
613792	3p-syndrome

Distal monosomy 3p is a rare chromosomal anomaly syndrome, resulting from a partial deletion of the short arm of chromosome 3, with a highly variable phenotype typically characterized by pre- and post-natal growth retardation, intellectual disability, developmental delay and craniofacial dysmorphism (microcephaly, trigonocephaly, downslanting palpebral fissures, telecanthus, ptosis, micrognathia). Postaxial polydactyly, hypotonia, renal anomalies and congenital heart defects (e.g. atrioventricular septal defect) may be associated. The size of the deletion varies among affected individuals, ranging from approximately 150 kb to 11 Mb. It can include between 4 and 71 known genes. Some of the critical genes in this region are *SETD5*, *BRPF1*, and *VHL*. It is suggested that deletion of the *SETD5* gene is most likely the largest contributor to the core phenotype in 3p25 deletion syndrome. A genotype/phenotype comparison of patients with deletions affecting either *BRPF1* or *SETD5* and those with deletions affecting both genes suggested that disruption of either gene causes mild to moderate intellectual disability, whereas disruption of both genes causes more severe intellectual disability. Features enriched in patients with *BRPF1* disruptions include ptosis and/or blepharophimosis, strabismus, short stature, and small head size (OMIM #613792; ORPHA: 1620; Keuchler et al., 2015, PMID: 25138099).

Evidence
Deletions in this chromosomal region are associated with a microdeletion syndrome.
This aberration is either not present in population databases, occurs at a very low allele frequency within the general population, or with an allele frequency which is consistent with reduced penetrance (gnomAD, DGV).

Classification: pathogenic

5p15.33p15.32 (6.3 Mb, het. duplication)

Evidence
The chromosomal region affected by this aberration contains many protein coding genes.
This aberration is either not present in population databases, occurs at a very low allele frequency within the general population, or with an allele frequency which is consistent with reduced penetrance (gnomAD, DGV).

Classification: pathogenic

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

Whole-Genome Diagnostics

The genetic analysis was performed using whole-genome sequencing (WGS), generating the following diagnostically relevant data for evaluation:

- protein-altering sequence variants (SNVs and small indels) in all known genes
- sequence variants in non-protein-coding RNA genes
- changes in intronic and untranslated (UTR) regions affecting splicing
- sequence variants in the entire mitochondrial genome
- copy number changes (deletions & duplications) that affect gene activity
- structural variants (translocations, inversions, insertions) that interrupt genes or affect their activity, or lead to the formation of new fusion genes
- high evidence pharmacogenetic variants
- changes in 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.

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Managing Directors: Dr. Dr. Saskia Biskup, Dr. Dirk Biskup

Requested Regions	The whole genome of the individual above was targeted and sequenced.
General Remarks	Additional variants may be present within regions which were not analyzed (e.g. distant enhancer regions). 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
Methods	**Sequencing:** Whole-genome sequencing was performed using the Illumina NovaSeq 6000/NovaSeq X Plus system. **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 Illumina DRAGEN Bio-IT Platform v4.4. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine genome-wide sequence variants (single nucleotide changes and small insertions/deletions), copy-number variants, as well as structural variants. NGS based CNV Calling will not detect low-level mosaicism. Aberrations within the pseudoautosomal region (PAR) cannot be detected with high accuracy. Sequence variants were analyzed within transcripts including all intronic regions as well as up to 1000 bp upstream resp. downstream. **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, as well as variants with a predicted splice-altering effect in all introns and UTR sequences (including 1kb downstream and upstream of the UTRs), and variants affecting upstream ORFs. Known disease-causing variants (according to HGMD and ClinVar) are evaluated 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. Candidate CNV calls are evaluated manually. Copy number variants are named according to current ISCN guidelines. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis. All variants identified were evaluated with reference to the indicated phenotype. Single heterozygous variants in genes associated with autosomal recessive inheritance may not have been reported. In this case, 86.85% of the genome was covered by a minimum of 20 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).

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