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

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

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

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

Indication Global developmental delay (speech and motor milestones), multiple subcortical-periventricular T2 hyperintense small infarcts, low set ears, pulmonary flow murmur

Order Trio exome analysis

Result: Report with Significant Findings

- **Detection of a *de novo* pathogenic variant in gene *BRAF*, which is causative for a *BRAF*-associated RASopathy 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
		Index	Mother	Father			
BRAF	**c.722C>T; p.Thr241Met** **chr7:140501350 G>A (hg19)**	**het.**	**-**	**-**	**AD**	**< 0.01**	**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 *BRAF*-associated RASopathy syndromes (GeneReviews: Roberts, updated 2022, PMID: 20301303 – Noonan syndrome; Rauen, updated 2023, PMID: 20301365 – CFC syndrome).

Genetic Relevance

Your patient is heterozygous for a pathogenic variant in gene *BRAF* that most 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 *BRAF* 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.

Clinical Information and Variant Interpretation

***BRAF*, NM_004333.6**

OMIM / Reference	Phenotype	Heredity
115150	Cardiofaciocutaneous syndrome 1 (CFC1)	AD
613707	LEOPARD syndrome 3 (LPRD3)	AD
613706	Noonan syndrome 7 (NS7)	AD

The gene ***BRAF*** encodes a protein kinase which is a part of the RAS/MAPK pathway. Pathogenic variants in gene *BRAF* are associated with Cardiofaciocutaneous syndrome 1, LEOPARD syndrome 3, and Noonan syndrome 7. All three syndromes show clinical overlap, and it is under debate whether these *BRAF*-associated diseases represent distinct entities (GeneReviews, Rauen, updated 2023, PMID: 20301365). Cardiofaciocutaneous syndrome is characterized by cardiac abnormalities, which are present in 75-80% of cases, craniofacial and cutaneous abnormalities. Additionally, patients may present with abnormal brain imaging, speech delay, muscular hypotonia, optic nerve hypoplasia and renal malformations including hydronephrosis. Cognitive impairment is common, as are seizures, and failure to thrive (GeneReviews, Rauen, updated 2023, PMID: 20301365). Noonan syndrome is typically characterized by short stature, congenital heart defects, facial dysmorphism, pectus deformity, and variable developmental delay (GeneReviews, Roberts, updated 2022, PMID: 20301303). LEOPARD syndrome (also known as Noonan syndrome with multiple lentigines) is additionally characterized by lentigines, hearing loss, and skeletal abnormalities (GeneReviews, Gelb and Tartaglia, updated 2022, PMID: 20301557).

***BRAF*, c.722C>T; p.Thr241Met (het.), ClinVar ID: 29805**

ACMG/ACGS Criterion	Points	Description
PS2	+4	The variant has already been detected *de novo* in a patient with the disease, and no family history. Strength level of call is based on factors such as disease specificity and number of previously reported *de novo* findings for this variant.
PS4 (supporting)	+1	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Battaglia et al., 2021, PMID: 34573299; Okuzono et al., 2019, PMID: 30414707; Sarkozy et al., 2009, PMID: 19206169
PM2	+2	This variant is listed in the gnomAD global population dataset with very low frequency.
PM5	+2	The variant results in the change of an amino acid residue, for which a different amino acid change (p.Thr241Arg) has already been described as pathogenic. Sarkozy et al., 2009, PMID: 19206169; Battaglia et al., 2021, PMID: 34573299
PP2	+1	Fewer than expected missense variants are present within gene *BRAF* in the general population, which suggests poor tolerance for missense variation.
PP3	+1	The variant was given a pathogenic prediction by *in silico* tools.
ACMG/ACGS Classification: pathogenic	+11	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: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,

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

Consultant for Human Genetics

Additional Information

Analyzed Regions	Trio whole exome analysis was performed for the three individuals described above.
General Remarks	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. 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% 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.

97.69%, 97.74%, and 97.77% 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).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

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