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

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

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

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

Indication	Diamond-Blackfan anemia, positive family history
Order	Panel Diagnostics: Diamond-Blackfan anemia Genes, Defects of the erythrocytes and anemia (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a pathogenic variant in gene *RPL35A*, which confirms the diagnosis of Diamond-Blackfan anemia type 5 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
RPL35A	**c.82_84delCTT; p.Leu28del**	**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

***RPL35A*, c.82_84delCTT; p.Leu28del (het.), NM_000996.4, NM_001316311.2, rs116840807**

OMIM / Reference	Phenotype	Heredity
612528	Diamond-Blackfan anemia 5	AD

The ***RPL35A*** gene encodes a component of the ribosomal 60S subunit and mediates the initialization and elongation of protein biosynthesis between the tRNA and the ribosome. Heterozygous alterations leading to haploinsufficiency of the *RPL35A* gene are causative for Diamond-Blackfan anemia, type 5 (DBA5). DBA is a disease of congenital bone marrow failure characterized in its classic form by severe macrocytic anemia with normal leukocyte and platelet counts. In addition to growth retardation, affected individuals often have congenital malformations, including craniofacial and skeletal dysmorphisms. In addition, ocular, urogenital, and cardiac abnormalities may be present. There is also an increased risk of myelodysplastic syndromes (MDS) and solid tumors (Clinton, updated 2021, PMID: 20301769). Congenital diaphragmatic hernias can occur but are rarely observed in association with DBA (Yoo et al., 2019, PMID: 31574871). The expression of Diamond-Blackfan anemia can be clinically variable, and subclinical presentation in some patients has been reported (OMIM #612528).

The variant **c.82_84delCTT; p.Leu28del** in gene *RPL35A* has been identified in your patient in a heterozygous state. This variant is absent from the gnomAD global population dataset. It has previously been described in several publications in association with Diamond-Blackfan anemia type 5 (Farrar et al., 2008, PMID: 18535205; described as c.78_80del; p.26_27del in table S4 in Gálvez et al., 2021, PMID: 33718801; table S3 in Muramatsu et al., 2017, PMID: 28102861).

Based on the current data, the identified pathogenic in-frame deletion in *RPL35A* confirms the diagnosis of Diamond-Blackfan anemia type 5 in your patient.

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. Further the degree of heteroplasmy of mitochondrial variants can vary remarkably between different tissues (Wallace & Chalkia 2013; PMID: 24186072). Therefore, it is possible that disease causing variants, deletions and duplications are not detectable in the mtDNA from leucocytes, but present in other tissues. 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 *RPL35A*. 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.

RECOMMENDATION

We recommend further clinical evaluation, management, and treatment of manifestations according to the current guidelines for Diamond-Blackfan Anemia (Clinton, updated 2021, PMID: 20301769, GeneReviews).

Testing of asymptomatic family members regarding the variant c.82_84delCTT; p.Leu28del identified in gene *RPL35A* may only be performed following genetic counseling.

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

Requested Regions

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

ABCB6, ***ABCB7***, ***ABCG5***, ***ABCG8***, ***AK1***, ***ALAS2***, ***ALDOA***, ***ANK1***, ***ATP11C***, ***BPGM***, ***CD59***, ***CDAN1***, ***CDIN1***, ***COX4I2***, ***CYB5R3***, ***EGLN1***, ***EPAS1***, ***EPB41***, ***EPB42***, ***EPO***, ***EPOR***, ***G6PD***, ***GATA1***, ***GCLC***, ***GLRX5***, ***GPI***, ***GSR***, ***GSS***, ***HK1***, ***HSPA9***, ***JAK2***, ***KCNN4***, ***KIF23***, ***KLF1***, ***LPIN2***, ***MT-ATP6***, ***NDUFB11***, ***NT5C3A***, ***PFKM***, ***PGK1***, ***PIEZO1***, ***PKLR***, ***PUS1***, ***RHAG***, ***SEC23B***, ***SH2B3***, ***SLC11A2***, ***SLC19A2***, ***SLC25A38***, ***SLC2A1***, ***SLC30A10***, ***SLC4A1***, ***SPTA1***, ***SPTB***, ***TMPRSS6***, ***TPI1***, ***TRNT1***, ***VHL***, ***XK***, ***YARS2*** (Defects of the erythrocytes and anemia)

RPL11, ***RPL15***, ***RPL18***, ***RPL26***, ***RPL27***, ***RPL35***, ***RPL35A***, ***RPL5***, ***RPS10***, ***RPS15A***, ***RPS17***, ***RPS19***, ***RPS24***, ***RPS26***, ***RPS27***, ***RPS28***, ***RPS29***, ***RPS7*** **and** ***TSR2*** (Diamond-Blackfan anemia Genes)

Methods

Sequencing: Protein-coding regions, flanking intronic regions and additional disease-relevant non-coding regions of the nuclear encoded genes, as well as the mitochondrial DNA were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq6000 system. In order to provide a second, independent validation, one or more rare variant(s) identified within the NGS data were additionally sequenced using conventional Sanger sequencing.

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.

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) of the nuclear encoded genes and in the mitochondrial DNA with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD and MITOMAP) 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, MITOMAP) 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, 99.06% 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.

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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.

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