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## STATEMENT

### CLINICAL HISTORY

Patient is a newborn baby with neutropenia, anemia, and reticulocytopenia since birth. A bone marrow biopsy showed pronounced erythroid and granulocytic hypoplasia with evidence of reticulin fibrosis and megakaryocytic hyperplasia. There is no family history of similar disease.

### CLINICAL REPORT

The Del/Dup (CNV) Analysis using the Blueprint Genetics (BpG) Comprehensive Hematology Panel identified a heterozygous 284 kb deletion on chr3: 197677738 - 197961930 (exact breakpoints were not determined). This deletion involves several genes including all the coding exons of *RPL35A*. [Decipher](#) database contains several copy-number variants covering this region. Typically, they are copy-number gains and larger in size than the deletion observed in this patient. We have previously detected a 4-year-old boy with a 2.3Mb heterozygous deletion involving the *RPL35A* gene. His clinical picture was compatible with Diamond-Blackfan anemia.

The *RPL35A* gene (MIM [\\*180468](#)) encodes ribosomal protein L35A. This protein is required for the normal proliferation and viability of hematopoietic cells and it is one of the several proteins needed to make up the 60S subunit of the ribosomes (UniProt [P18077](#)). It can bind both initiator and elongator transfer-RNAs.

Dominant pathogenic variants of the *RPL35A* cause Diamond-Blackfan anemia (DBA, MIM [#612528](#)). DBA is a red blood cell aplasia with the main clinical features of profound normochromic macrocytic anemia, reticulocytopenia and low levels or absence of erythroid progenitors in the bone marrow. Many patients with DBA have congenital malformations and growth retardation but clinical picture may be heterogenous among patients and even within patients in one family (GeneReviews [NBK7047](#)). Most of the patients have hematologic complications during the first year of life and patients have an increased risk for acute myelogenous leukemia, myelodysplastic syndrome and solid tumors.

Some 3% of DBA cases are attributed to pathogenic variants in *RPL35A*. Farrar et al found pathogenic variants of *RPL35A* in 5/150 DBA families studied (PMID: [18535205](#)). Two of the families with sporadic patients had the whole *RPL35A* gene deleted, while the rest had one amino acid in-frame deletion, a nonsense variant and a frameshift variant, respectively. The presenting age of the patients was 2 to 4 months in four families but 14 months in a patient with the in-frame deletion. Smetanina et al analyzed 57 patients with DBA (PMID [25946618](#)). They found one sporadic patient with a 1-bp deletion in exon 3 of *RPL35A* leading to frameshift and a premature termination of translation. The HGMD mutation database lists 12 (August 2017) mutations in *RPL35A* causing DBA. Only two of these are missenses, while the rest are more radical. 50% of the listed mutations are gross deletions of the entire gene.

### CONCLUSION

Considering the current literature and the well-established role of the *RPL35A* gene deletions as a disease causing mechanism, we classify the identified heterozygous 284 kb deletion on chr3: 197677738 - 197961930 as pathogenic. Disease caused by *RPL35A* mutations is inherited in an autosomal dominant manner. Thus, each child of an affected individual has a 50% risk of inheriting the mutation. A proband with autosomal dominant Diamond-Blackfan anemia may have the disorder as a result of a de novo mutation. Genetic counselling and family member testing is recommended. BpG offers mutation testing for the family if requested.

### CONFIRMATION

The heterozygous 284 kb deletion was confirmed by two independent assays in qPCR.

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**STEP**

**DATE**

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Order date

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Sample in analysis

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Reported

(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

*Signature*

Name

Title

## APPENDIX 5: SUMMARY OF METHODS

### OS-SEQ (SEQUENCE ANALYSIS)

**Sequencing.** Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.

**Data analysis.** Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (eg, 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (eg, ClinVar) as well as by searching from an in-house curated database of previously reported variants.

**Interpretation.** The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.

**Confirmation.** Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.

**Reporting.** Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

**Notes.** This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

**Accreditation.** This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing and digital PCR confirmation).

### DEL/DUP (CNV) ANALYSIS

**Data analysis.** Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. Expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).

**Interpretation.** The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.

**Confirmation.** Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).

**Reporting.** Reporting was done using an HGNC-approved gene nomenclature.

**Notes.** This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by

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#### **GLOSSARY OF USED ABBREVIATIONS:**

**POS** = genomic position of the variant in the format of chromosome:position

**ID** = rsID in dbSNP

**Transcript** = GenBank accession for reference sequence used for variant nomenclature

**Nomenclature** = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

**ExAC AC/AN** = allele count/allele number in the Exome Aggregation Consortium Database

**AD** = autosomal dominant

**AR** = autosomal recessive

**OMIM** = Online Mendelian Inheritance in Man®

**ExAC** = Exome Aggregation Consortium Database (>60,000 unrelated individuals)

**het** = heterozygous

**hom** = homozygous

**Del/Dup** = Deletion and Duplication

**CNV** = copy number variation

**gnomAD** = genome Aggregation Database (reference population database; >138,600 individuals)

**gnomAD AC/AN** = allele count/allele number in the genome Aggregation Database

PolyPhen, SIFT and MutationTaster are *in silico* prediction tools used to evaluate the significance of identified amino acid changes.

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