Blueprint Genetics

Sample report as of Nov 1st, 2020. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at ***blueprintgenetics.com/certifications***

Comprehensive Hematology Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0		

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for 284kb deletion on chr3: 197677738 - 197961930. This deletion contains the *RPL35A* gene and is classified as pathogenic.

VARIANT TABLE: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
RPL35A	DELETION	1	HET	Whole gene	UCSC	**Pathogenic**
	OMIM	**PHENOTYPE** Diamond-Blackfan anemia			**COMMENT** -	

SEQUENCING PERFORMANCE METRICS OS-

SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Comprehensive Hematology Panel	175	2568	463372	463089	236	99.9

TARGET REGION AND GENE LIST

Blueprint Genetics Comprehensive Hematology Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with hereditary anemia, inherited bleeding disorder, inherited bone marrow failure syndrome and leukemia: ABCA3, ABCB7, ABCG5, ABCG8, ACTB*, ACTN1, ADAMTS13, AK2, ALAS2, AMN, ANK1, ANKRD26, AP3B1, ATM, ATR, ATRX, BLM, BLOC1S3, BLOC1S6, BRCA2, BRIP1,

C15ORF41, CDAN1, CDKN2A, CEBPA, CSF2RA*, CTC1, CTSC, CUBN*, CXCR4, CYCS*, DKC1, DTNBP1, ELANE, EPB42, ERCC4, F2, F5, F7, F8*, F9, F10, F11, F12, F13A1, FANCA, FANCB, FANCC, FANCD2*, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FGA, FGB, FGG, FLNA, G6PC3, G6PD, GATA1, GATA2, GGCX, GP1BA, GP1BB, GP9, GPI, GPR143, GSS, HAX1, HBA1*, HBA2*, HBB, HFE, HOXA11, HPS1*, HPS3, HPS4, HPS5, HPS6, HRAS, IFNGR2, ITGA2, ITGA2B, ITGB3, ITK, JAGN1, KLF1, KRAS*, LMAN1, LPIN2, LYST, MAGT1, MASTL, MLH1, MPL, MSH2, MSH6, MTR, MYH9, MYO5A, NBEAL2, NBN, NF1*, NHP2, NOP10, NRAS, OCA2, P2RY12, PALB2, PC, PDHA1, PDHX, PKLR, PMS2*, PRF1, PROC, PROS1*, PTPN11, PUS1, RAB27A, RAD51C, RBM8A*, RECQL4, RPL5, RPL11, RPL15*, RPL35A, RPS7, RPS10, RPS17*, RPS19, RPS24, RPS26, RPS29, RTEL1, RUNX1, SBDS*, SEC23B, SERPINC1, SFTPB, SFTPC, SH2D1A, SLC4A1, SLC19A2, SLC45A2, SLFN14, SLX4, SPTA1, SPTB, STX11, STXBP2, TBXA2R, TCIRG1, TERC, TERT, THBD, TINF2, TMPRSS6, TP53, TPI1, TUBB1, TYR*, TYRP1, UNC13D, USB1, VKORC1, VWF*, WAS, WRAP53, XIAP*, XRCC2 and YARS2. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of hereditary anemia, inherited bleeding disorder, inherited bone marrow failure syndrome and leukemia mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Comprehensive Hematology Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (eg, copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

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.

STEP	DATE

STEP	DATE
Order date	
Sample in analysis	
Reported	

On November 1, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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Senior Geneticist

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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.