Blueprint Genetics

Thrombocytopenia Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
5 Male

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
Blood

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *RUNX1* c.838del, p.(Ser280Profs*31), which is likely pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
RUNX1	21:36171726	NM_001754.4	c.838del, p.(Ser280Profs*31)	frameshift_variant	HET	**Likely pathogenic**

ID	gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER
	0/0	N/A	N/A	N/A

OMIM	PHENOTYPE	INHERITANCE	COMMENT
OMIM	Platelet disorder, familial, with associated myeloid malignancy	AD	-

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Thrombocytopenia Panel	34	496	102056	102056	308	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Thrombocytopenia Panel (version 3, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ABCG5, ABCG8, ACTN1, ADAMTS13, ANKRD26, ARPC1B, CYCS*, ETV6, FLI1, FLNA, FYB, GATA1, GFI1B, GP1BA, GP1BB, GP9, HOXA11, ITGA2, ITGA2B, ITGB3, MASTL, MECOM, MPL, MYH9, NBEAL2, PRKACG, RBM8A*,#, RUNX1, SLFN14, SRC, THBD, TUBB1, WAS and WIPF1. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: RBM8A (3). This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) up to 220 bps and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
#The gene has suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads.
The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#).

STATEMENT

CLINICAL HISTORY

This patient has persistent thrombocytopenia and abnormal bruising since birth. His platelet count is low and mean platelet volumes within normal range.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Thrombocytopenia Panel identified a heterozygous frameshift variant c.838del, p.(Ser280Profs*31) in *RUNX1*.

The variant has not been observed in the large reference population cohorts of the Genome Aggregation Database, (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. The variant deletes 1 bp and generates a frameshift leading to a premature stop codon at position 31 in a new reading frame. It is predicted to cause loss of normal protein function either through protein truncation (out of 480 aa) or nonsense-mediated mRNA decay. To our knowledge, the variant has not been published in the relevant medical literature or reported in the disease-related variation databases such as ClinVar or HGMD. There are nonsense variants in adjacent codon leading to premature stop codon reported: *RUNX1* c.836G>A, p.(Trp279*) in three patients affected with familial platelet disorder (PMID: 26800764, 28659335) and *RUNX1* c.837G>A, p.(W279*) in one patient affected thrombocytopenia (PMID: 25893166).

Runt-related transcription factor 1 (*RUNX1*) is a part of the RUNX gene family and is encoding a part of the RUNX transcription factor. *RUNX1* is crucially required for normal hematopoiesis, is required for CD8 T-cell development, plays a supportive role in bone formation, is important in the development of the neurons that transmit pain and can produce oncogenic transformation to acute myelogenous leukemia (MIM *151385 GeneCards – RUNX1). Germline mutations in *RUNX1* are associated with familial platelet disorder with associated myeloid malignancy (FPDMM; MIM #601399). FPDMM is a very rare autosomal dominant disorder that is characterized by moderate thrombocytopenia, abnormal platelet function and the propensity to develop myeloid malignancies, in particular acute myeloid leukemia (AML) (ORPHA: 71290, MIM# 601399). The clinical presentation of FPDMM is highly variable, patients usually presenting with mild to moderate bleeding symptoms which first appear during childhood, and which are characterised by quantitative and/or qualitative platelet defects, and mild thrombocytopenia with normal sized platelets. However, many patients have no bleeding tendency and thrombocytopenia is not always a feature (PMID: 21606161, 27112265).

Somatic mutations and chromosomal rearrangements involving *RUNX1* are frequently observed in myelodysplastic syndrome (MDS) and leukemias of myeloid and lymphoid lineages, i.e. acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelomonocytic leukemia (CMML) (PMID 28179279).

HGMD professional includes 85 variants (October 2018) in *RUNX1,* and most of them have been identified in the familial platelet disorder. Majority of the variants are missense variants, but also more radical variants (nonsense, splicing, small deletion/insertions) have been reported. Specifically, also some 20 gross deletions including some or all exons are listed in the HGMD.

Mutation nomenclature is based on GenBank accession NM_001754.4 (*RUNX1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

RUNX1 c.838del, p.(Ser280Profs*31) is classified as likely pathogenic considering the current evidence of the variant (established association between the gene and the patient's phenotype, rarity in control populations, variant type (frameshift)). However, additional information is still needed to confirm the pathogenicity of the variant. Genetic counseling and family member testing are recommended. Disease caused by *RUNX1* variants is inherited in an autosomal dominant manner, and thus each child of an affected individual has a 50% chance of inheriting the variant. A proband with autosomal dominant *RUNX1* disease may have the disorder as a result of a *de novo* event. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

RUNX1 c.838del, p.(Ser280Profs*31) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Aug 24, 2018
Sample received	Sep 11, 2018
Reported	Oct 05, 2018

On Oct 05, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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Geneticist

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Lab Director, Chief Medical Officer

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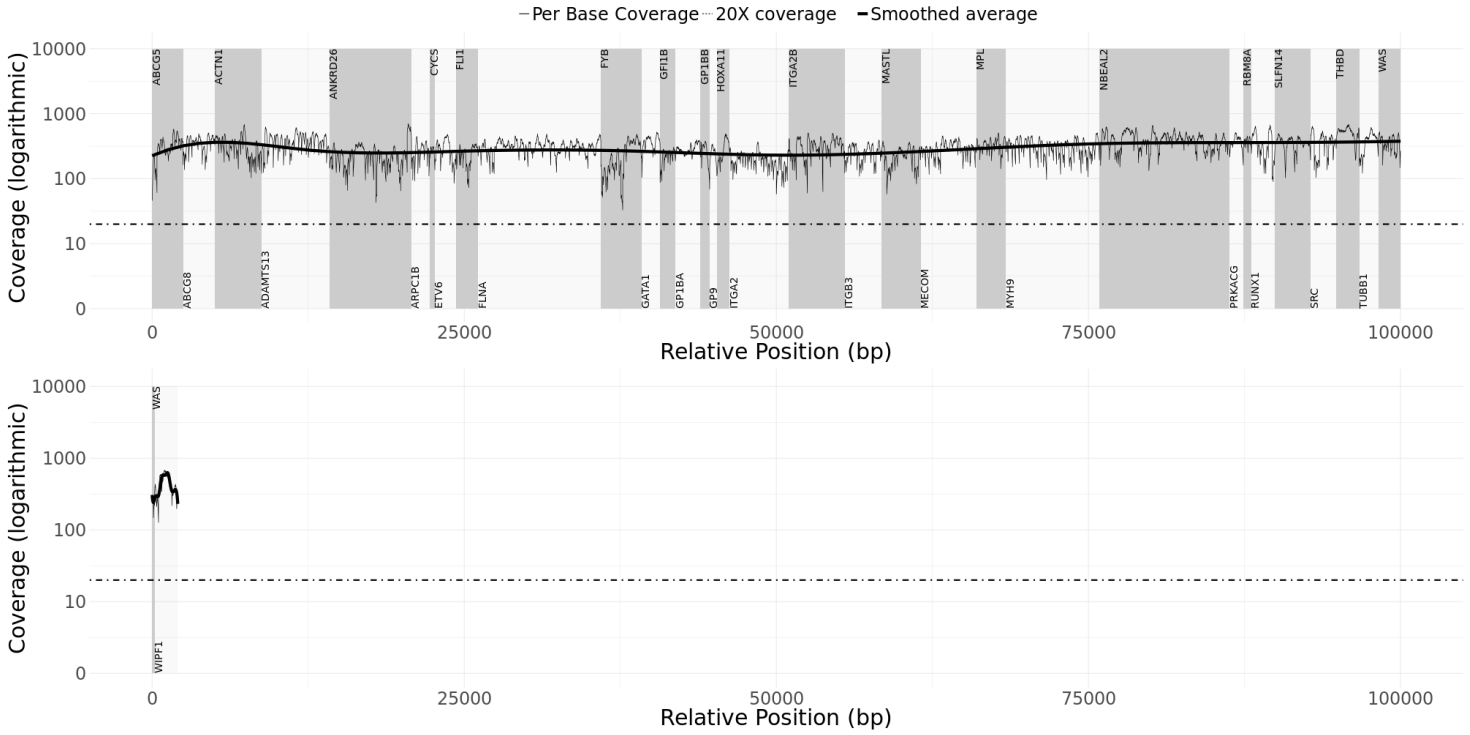
Senior Geneticist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20X coverage
Smoothed average
Coverage (logarithmic)
10000
1000
100
10
0
ABCG5
ABCG8
ACTN1
ADAMTS13
ANKRD26
ARPC1B
CYCS
ETV6
FLI1
FLNA
FYB
GATA1
GFI1B
GP1BA
GP1BB
GP9
HOXA11
ITGA2
ITGA2B
ITGB3
MASTL
MECOM
MPL
MYH9
NBEAL2
PRKACG
RBM8A
RUNX1
SLFN14
SRC
THBD
TUBB1
WAS
0
25000
50000
75000
100000
Relative Position (bp)

Coverage (logarithmic)
10000
1000
100
10
0
WAS
WIPF1
0
25000
50000
75000
100000
Relative Position (bp)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads. 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. The 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.
Interpretation: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited, to the 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_014915.2(ANKRD26):c.-134G>A
NM_002049.3(GATA1):c.-19-2A>G
NM_000407.4(GP1BB):c.-160C>G
NM_000419.3(ITGA2B):c.*165T>C
NM_000419.3(ITGA2B):c.2095-19T>A
NM_000419.3(ITGA2B):c.1211-78A>G
NM_000419.3(ITGA2B):c.408+11C>A
NM_000419.3(ITGA2B):c.-4082G>A
NM_000361.2(THBD):c.-151G>T
NM_000361.2(THBD):c.-302C>A

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AR = autosomal recessive
CNV = Copy Number Variation e.g. one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)
gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)

HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MutationTaster = in silico prediction tools used to evaluate the significance of identified amino acid changes.
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.