Blueprint Genetics

Marfan Syndrome Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
26 Female

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
DNA

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *FBN1* c.4930C>T, p.(Arg1644*), which is 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
FBN1	15:48757777	NM_000138.4	c.4930C>T, p.(Arg1644*)	stop_gained	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs140630	0/0	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Acromicric dysplasia, Geleophysic dysplasia, MASS syndrome, Marfan syndrome		AD	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Marfan Syndrome Panel	30	869	142462	142422	171	99.97

TARGET REGION AND GENE LIST

The Blueprint Genetics Marfan Syndrome Panel (version 4, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ABL1, ADAMTS10, ADAMTS17, ADAMTSL4, BGN, CBS, COL11A1, COL11A2, COL1A1, COL1A2, COL2A1, COL3A1, COL5A1, COL5A2, EFEMP2, FBN1, FBN2, MAT2A*, MED12, PLOD1, SKI, SLC2A10, SMAD3, SMAD6, TGFB2, TGFB3, TGFBR1, TGFBR2, UPF3B and VCAN. 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 sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 26-year-old female with suspected Marfan syndrome (no more info from referring doctor).

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Marfan Syndrome Panel identified a heterozygous nonsense variant c.4930C>T, p.(Arg1644*) in *FBN1*. It causes a premature stop codon and loss of normal protein function either through protein truncation (1643 out of 2871 aa) or nonsense-mediated mRNA decay. It has been observed in one heterozygous individual in the Exome Aggregation Consortium (ExAC) but is absent in the Genome Aggregation Database (gnomAD). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. Loss-of-function of FBN1 is a well established disease mechanism in Marfan syndrome (MfS).

The *FBN1* c.4930C>T, p.(Arg1644*) has been identified in over 14 patients with MfS (PMID 11700157, 19293843, 16222657, 17657824, 19839986, 14695540, 12068374, 15241795, 19159394, 17663468, ClinVar 200186) and it has occurred as *de novo* in at least four of these patients (PMID 11700157, 16222657, 14695540, 12068374). We have previously identified this variant in a female patient with MfS (BpG unpublished observations).

FBN1 gene on chromosome 15q21.1 encodes a member of the fibrillin family of proteins. The encoded preproprotein is proteolytically processed to generate two proteins including the extracellular matrix component fibrillin-1 and the protein hormone asprosin. Fibrillin-1 is an extracellular matrix glycoprotein that serves as a structural component of calcium-binding microfibrils. These microfibrils provide force-bearing structural support in elastic and nonelastic connective tissue throughout the body. It has only the canonical transcript (NM_000138.4) consisting 2,871 amino acids within 66 exons. Autosomal dominant *FBN1* mutations cause a broad spectrum of phenotypes, often called “type I fibrillinopathies”, of which the most important are Marfan syndrome (MfS, OMIM **154700*), familial ectopia lentis (OMIM **129600*), and phenotypes from acromelic dysplasia short stature disease group including acromicric dysplasia (PMIM **102370*) and geleophysic dysplasia 2 (OMIM **614185*).

There are altogether >3,000 reported variants in UMD-FBN1 mutation database and >1,550 *FBN1* variants associating with complete MfS phenotype listed in the HGMD mutation database. Of the HGMD variants, 52.4% are missense, 15.2% small deletions, 11.8% splice, 11.1% nonsense, 5.3% small insertions, 2.8% gross deletions or insertions and 1.4% small indel variants. In 2003, Collod-Béroud *et al.* published data on 563 *FBN1* mutations submitted to UMD-*FBN1* mutation database (PMID *12938084*). *De novo* mutations were observed in 47.2% (188/398) of the patients with parents available for transmission mode evaluation. Among all missense mutations, 78.0% (263/337) located in the calcium-binding modules. Of the mutation in these regions, 56.7% (149/263) either introduced or substituted cysteine residues potentially implicated in disulfide bonding. Functional studies have shown that these mutations often result in a delay in secretion or intracellular retention of profibrillin-1 (PMID *8111384*, *10647894*, *10486319*). Since three disulfide bonds are required to maintain the native cbEGF-like module fold, suppression or addition of cysteine residues would result in cbEGF-like module misfolding, which impairs trafficking (PMID *11031247*, *10906272*, *12938084*). In-line with previous, 63.9% of missense *FBN1* variants in the HGMD lead to substitution or insertion of cysteine residue. One per 188 individuals in gnomAD reference population (n>135,000) carries a unique *FBN1* missense variant (not present in anybody else in this cohort) but only one per 5,909 have unique missense variant substituting or introducing cysteine residue. One per 8,020 individuals carry a high-quality truncating *FBN1* variant. Loss of function variants in *FBN1* have pLI value of 1.00 (maximum) in ExAC indicating that they are extremely intolerant.

The clinical spectrum associated with *FBN1* mutations varies widely from features limiting to one system (eg. isolated thoracic aortic aneurysm, ectopia lentis or skeletal features) to complete presentation of classic MfS (PMID *21742617*). Every patient with a *FBN1* mutation is at risk for developing severe cardiovascular, skeletal, and ophthalmologic complications (PMID *21742617*). Cysteine affecting variants have higher probability for ocular complications, whereas premature terminations rather than inframe mutations were associated with severe skeletal and skin anomalies (PMID *25863307*). The two major allelic type I fibrillinopathies are mitral valve prolapse syndrome and MASS phenotype (mitral valve prolapse, borderline and non-progressive *a*ortic enlargement, and nonspecific *s*kin and *s*keletal findings (*MfS - GeneReviews*). Mutation’s location in exons 24-32 of the *FBN1* gene associates with a more severe and complete phenotype including younger age at diagnosis and higher probability for aortic dissection and elective aortic surgery. Over 90% of patients with neonatal MfS has mutation in exons 24-32. (PMID *19117906*).

Comprehensive management of MfS by a multidisciplinary team is recommended. Surgical repair of the aorta should be considered when the maximal diameter approaches 4.0-5.0 cm in adults or older children, the rate of increase of the aortic root diameter approaches 1.0 cm per year, or progressive and severe aortic regurgitation occurs (The American College of Cardiology Foundation / American Heart Association / American Association for Thoracic Surgery guidelines; PMID *20359588*) and slightly different criteria are applied for younger children. Severe mitral valve regurgitation with concomitant LV dysfunction is the leading indication for cardiovascular surgery in children with MfS. When mitral or aortic valve regurgitation is present, endocarditis prophylaxis is indicated. Annual echocardiography is recommended to monitor aorta when aortic dimensions are small and/or the rate of aortic dilation is slow but more frequent examinations are indicated when the aortic root diameter is clearly abnormal or aortic dilation exceed approximately 0.5 cm per year. Medications that reduce hemodynamic stress on the aortic wall, such as beta blockers, are generally initiated at diagnosis. Contact and competitive sports, and isometric exercise should be avoided as well as agents causing vasoconstriction. (*MfS - GeneReviews*). Follow-up strategies and indications for operative treatment vary substantially between hospitals and countries, and thus these clinical decisions should be done by experts who have access to all relevant patient information.

Mutation nomenclature is based on GenBank accession NM_000138.4 (*FBN1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and the well-established role of *FBN1* c.4930C>T, p.(Arg1644*) as a disease-causing variant, we classify it as pathogenic. Genetic counseling and family member testing are recommended. Disease caused by *FBN1* 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 MfS may have the disorder as a result of a *de novo* event. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

FBN1 c.4930C>T, p.(Arg1644*) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Aug 29, 2018
Sample received	Aug 29, 2018
Reported	Sep 20, 2018

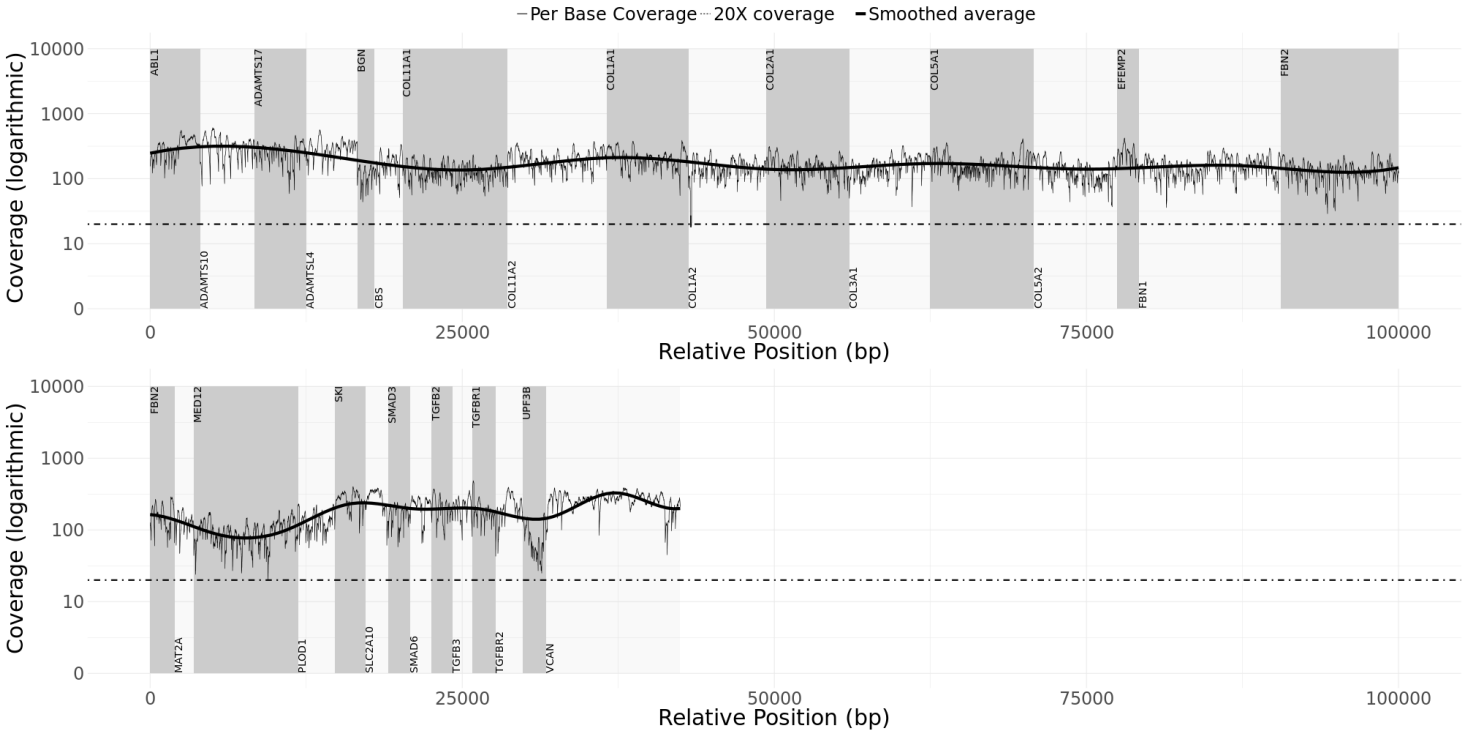
On Sep 20, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Johanna Tommiska, Ph.D.

Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer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Smoothed average
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10
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ADAMTS17
BGN
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COL1A1
COL2A1
COL5A1
EFEMP2
FBN2
ADAMTS10
ADAMTSL4
CBS
COL11A2
COL1A2
COL3A1
COL5A2
FBN1
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Relative Position (bp)

Coverage (logarithmic)
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1000
100
10
0
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MED12
SKI
SMAD3
TGFB2
TGFBR1
UPF3B
MAT2A
PLOD1
SLC2A10
SMAD6
TGFB3
TGFBR2
VCAN
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_080629.2(COL11A1):c.3744+437T>G
NM_080629.2(COL11A1):c.1027-24A>G
NM_080629.2(COL11A1):c.781-450T>G
NM_000088.3(COL1A1):c.2451+94G>T
NM_000088.3(COL1A1):c.2451+77C>T
NM_000088.3(COL1A1):c.2343+31T>A
NM_000088.3(COL1A1):c.1354-12G>A
NM_000088.3(COL1A1):c.904-14G>A
NM_000089.3(COL1A2):c.70+717A>G
NM_001844.4(COL2A1):c.1527+135G>A
NM_000090.3(COL3A1):c.3256-43T>G
NM_000093.4(COL5A1):c.2647-12A>G
NM_000093.4(COL5A1):c.2701-25T>G
NM_000093.4(COL5A1):c.5137-11T>A
NM_000138.4(FBN1):c.8051+375G>T
NM_000138.4(FBN1):c.6872-14A>G
NM_000138.4(FBN1):c.6872-961A>G
NM_000138.4(FBN1):c.5672-87A>G
NM_000138.4(FBN1):c.5672-88A>G

NM_000138.4(FBN1):c.863-26C>T
NM_001999.3(FBN2):c.3974-24A>C
NM_001999.3(FBN2):c.3974-26T>G
NM_001999.3(FBN2):c.3725-15A>G
NM_003239.2(TGFB3):c.*495C>T
NM_003239.2(TGFB3):c.-30G>A
NM_001024847.2(TGFBR2):c.-59C>T

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.