Blueprint Genetics

Primary Immunodeficiency Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0	Male	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood	2017-03-17	

SUMMARY OF RESULTS

TEST RESULTS

Patient is homozygous for *RFX5* c.234-1G>A, which is classified as pathogenic.

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

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
RFX5	1:151317324	NM_000449.3	c.234-1G>A	splice_acceptor	HOM	**Pathogenic**
	ID	**EXAC AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs748270285	10/121034	N/A	N/A	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Bare lymphocyte syndrome		AR	-	

SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Primary Immunodeficiency Panel	232	3182	543282	541802	324	99.7

TARGET REGION AND GENE LIST

Blueprint Genetics Primary Immunodeficiency Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with autoinflammatory disorders, combined immunodeficiencies, complement deficiencies, congenital defects of phagocytes, defects in innate immunity, diseases of immune dysregulation and immunodeficiencies with antibody defects: ACP5, ACTB*, ADA, ADAR, ADIPOQ, ADIPOR1*, ADIPOR2, AICDA, AIRE, AK2, AP3B1, ARMC4*, ATM, BLM, BTK, C1QA, C1QB, C1QBP, C1QC, C1R, C1S, C2*, C3, C3AR1, C4A*, C4B*, C4BPA, C4BPB, C5, C5AR1, C5AR2, C6, C7, C8A, C8B, C8G, C9, CARD11, CARD14, CASP8, CASP10, CCDC39, CCDC40, CCDC65, CCDC103, CCDC114, CCNO, CD3D, CD3E, CD3G, CD8A, CD19, CD27, CD40, CD40LG, CD46*, CD55, CD59, CD93, CD247, CECR1, CFB, CFD, CFH*, CFHR1*, CFHR3*, CFI, CFP, CIITA, CLU, COLEC11, CORO1A*, CR1*, CR2, CRP, CSF2RA*, CTC1, CTLA4, CTSC, CYBA, CYBB, DCLRE1C*, DDX58, DGKE, DKC1, DNAAF1, DNAAF2, DNAAF3, DNAAF5, DNAH5, DNAH11*, DNAI1, DNAI2, DNAL1, DNMT3B, DOCK2, DOCK8, DRC1, DYX1C1, ELANE, FAS, FCN1, FCN2, FCN3, FERMT3, FOXP3, G6PC3, G6PD, GATA2, HAX1, HYDIN*, IFIH1, IFNGR1, IFNGR2, IGHM, IGLL1*, IKBKG*, IL1RN, IL2RA, IL2RG, IL7*, IL7R, IL10RA, IL10RB, IL12RB1, IL36RN, ISG15, ITGB2, ITK, JAGN1, JAK3, LCK, LIG4, LPIN2, LRBA, LRRC6, LYST, MAGT1, MALT1, MASP1, MASP2, MAT2A*, MEFV, MRE11A, MVK, NBN, NCF1*, NCF2, NCF4, NFKB1, NFKB2, NFKBIA, NHEJ1, NHP2, NLRP3, NLRP12, NME8, NOD2, NOP10, NRAS, OFD1, ORAI1, PIGA*, PIK3CD*, PIK3R1, PLCG2, PMS2*, PNP, PRF1, PRKDC, PSMB8, PSTPIP1, PTPRC, PTX3, RAB27A, RAG1, RAG2, RFX5, RFXANK, RFXAP, RHOH, RMRP, RNASEH2A, RNASEH2B, RNASEH2C, RPGR, RSPH1, RSPH4A, RSPH9, RTEL1, SAMHD1, SBDS*, SERPING1, SH2D1A, SLC37A4, SMARCAL1, SP110, SPAG1, SPINK5, STAT1, STAT2, STAT3, STAT4, STAT5B*, STIM1, STK4, STXBP2, TAP1, TAP2, TAPBP, TBX1, TCIRG1, TERC, TERT, THBD, TINF2, TMEM173, TNFRSF1A, TNFRSF4, TNFRSF13B, TRAC, TREX1, TYK2, UNC119, USB1, VSIG4, VTN, WAS, WRAP53, XIAP*, ZAP70 and ZMYND10. 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 autoinflammatory disorders, combined immunodeficiencies, complement deficiencies, congenital defects of phagocytes, defects in innate immunity, diseases of immune dysregulation and immunodeficiencies with antibody defects mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Primary Immunodeficiency 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 (e.g. 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 recognise 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

This patient is a 7-month-old baby boy. He has *Pneumocystis jirovecii* pneumonia diagnosed and no immunoglobulins in the peripheral blood. Also T cell levels are low, but B cell levels are normal. His leukocytes lack MHC II antigens. Stem cell transplantation is being planned.

CLINICAL REPORT

Blueprint Genetics (BpG) Primary Immunodeficiency Panel Sequence Analysis identified a homozygous splice acceptor variant c.234-1G>A in the *RFX5* gene.

The *RFX5* c.234-1G>A variant has been observed in 10 heterozygous individuals in the Exome Aggregation Consortium (ExAC) data set, comprised of over 60,000 unrelated individuals (*http://exac.broadinstitute.org/*). This variant has previously been reported in association with bare lymphocyte syndrome (BLS, PMID: 7744245). This variant was shown to lead to the use of a cryptic splice acceptor site situated 5 nucleotides downstream of the one used normally and, hence, gives rise to the RFX5 mRNA containing the 5-nucleotide deletion. We have also seen this variant previously in a compound heterozygous state in a Finnish baby patient with severe pneumonia, small thymus, no immunoglobulins and lymphopenia.

Bare lymphocyte syndrome (BLS, MIM #209920) is an autosomal recessive, severe-combined immunodeficiency that can result from mutations in four different transcription factor genes *RFX5* (MIM *601863), *RFXAP* (MIM *601861, *CIITA* (MIM *600005) and *RFXANK* (MIM *603200) that regulate the expression of the major histocompatibility complex (MHC) class II molecules. BLS is a very rare disorder with a prevalence <1:1,000,000. The International Union of Immunological Societies Expert Committee for Primary Immunodeficiency categorizes BLS into a category of combined immunodeficiencies generally less profound than severe combined immunodeficiency (PMID: 26482257). In BLS, CD4+ T cells are typically decreased, while the levels of circulating B cells are normal. T cells fail to express MHC II. Serum immunoglobulins may be normal or decreased. Associated features include failure to thrive, diarrhea, respiratory tract infections and liver/biliary tract diseases.

There are six *RFX5* variants associated with BLS reported in the HGMD mutation database. Five of them are truncating (two nonsense, three splicing) and only one is missense.

Mutation nomenclature is based on GenBank accession NM_000449.3 (*RFX5*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and the well-established role of *RFX5* c.234-1G>A as a disease causing mutation, we classify it as pathogenic. Disease caused by *RFX5* mutations is inherited in an autosomal recessive manner. This patient is homozygous for the variant, which is in line with autosomal recessive inheritance. If both parents are found to be heterozygous for this variant, each sibling of an affected individual has a 25% risk of being a homozygous for the variant and thus being affected, a 50% risk of being an asymptomatic carrier, and a 25% chance of not having the variant. Genetic counseling and family member testing is recommended. BpG offers mutation testing for the family if requested.

CONFIRMATION

The *RFX5* c.234-1G>A was confirmed by bidirectional Sanger sequencing.

STEP	DATE
Order date	Mar 17, 2017
Sample received	Mar 17, 2017
Reported	Apr 06, 2017

On Apr 06, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Hannu Turpeinen, Ph.D.

Geneticist

Juha Koskenvuo, MD, Ph.D.

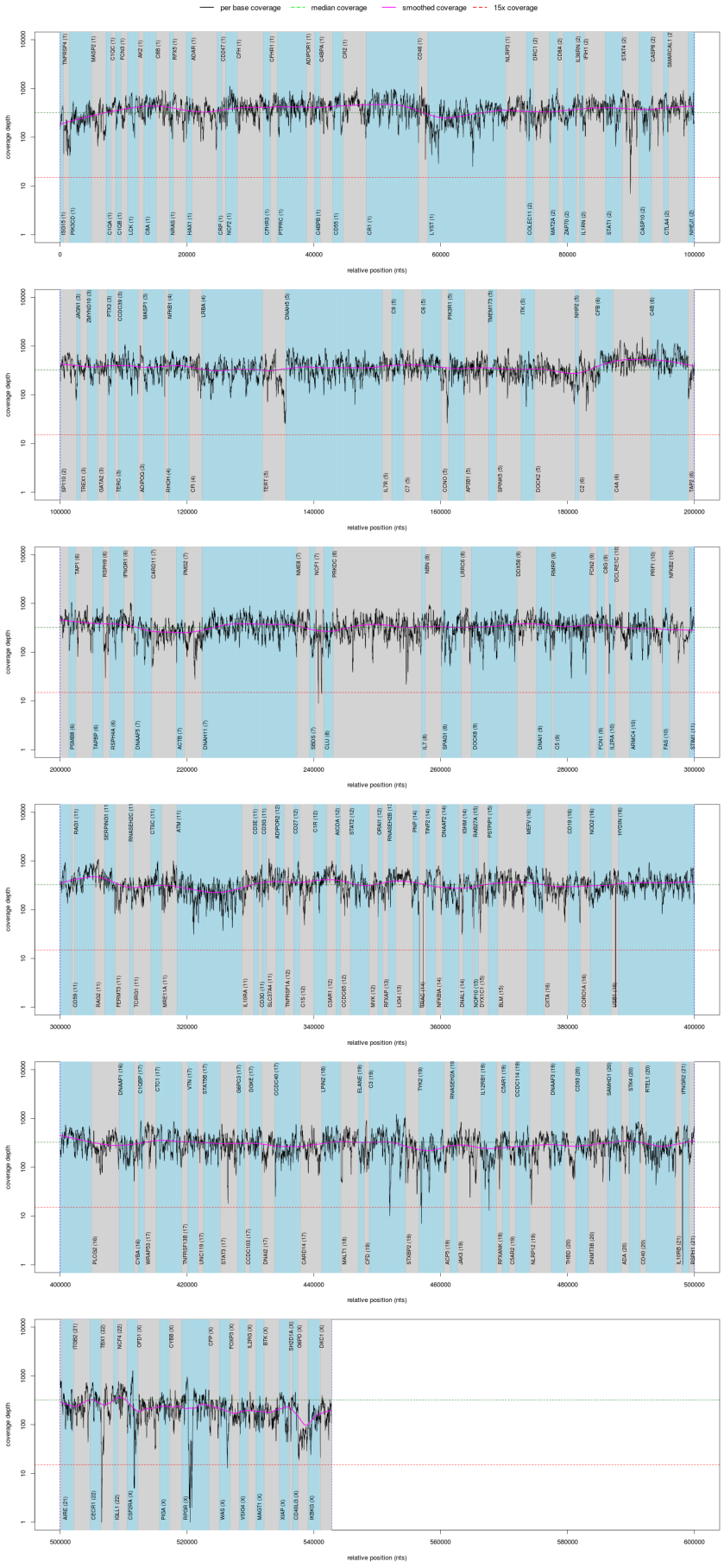
Lab Director, Chief Medical Officer

Seppo Meri, MD, Ph.D.

Clinical Consultant

Coverage plot for sample 11429

Panel type: Primary Immunodeficiency Panel
Number genes: 232
Median coverage: 324
Mean coverage: 349
Percent above 15x coverage: 99.7



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 (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. 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.

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 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.
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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

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