Blueprint Genetics

Severe Combined Immunodeficiency Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**
Blood

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for two variants in *RAG1:* c.256_257del, p.(Lys86Valfs*33) and c.1186C>T, p.(Arg396Cys). Both of these are classified as 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**
RAG1	11:36595109	NM_000448.2	c.256_257del, p.(Lys86Valfs*33)	frameshift_variant	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		8/276810	N/A	N/A	N/A	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Alpha/beta T-cell lymphopenia with gamma/delta T-cell expansion; severe cytomegalovirus infection and autoimmunity, Combined cellular and humoral immune defects with granulomas, Omenn syndrome, T cell-negative; B cell-negative; natural killer cell-positive severe combined immunodeficiency		AR	-	
GENE	**POS**	**TRANSCRIPT**	**NOMENCLATURE**	**CONSEQUENCE**	**GENOTYPE**	**CLASSIFICATION**
RAG1	11:36596040	NM_000448.2	c.1186C>T, p.(Arg396Cys)	missense_variant	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs104894289	2/245680	probably damaging	deleterious low confidence	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Alpha/beta T-cell lymphopenia with gamma/delta T-cell expansion; severe cytomegalovirus infection and autoimmunity, Combined cellular and humoral immune defects with granulomas, Omenn syndrome, T cell-negative; B cell-negative; natural killer cell-positive severe combined immunodeficiency		AR	-	

Please see APPENDIX 2: Additional Findings

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Severe Combined Immunodeficiency Panel	79	1280	235725	235205	162	99.78

TARGET REGION AND GENE LIST

The Blueprint Genetics Severe Combined Immunodeficiency Panel (version 3, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ADA, AK2, ATM, BCL11B, BLM, CARD11, CD247, CD27, CD3D, CD3E, CD3G, CD40, CD40LG, CD8A, CIITA, CORO1A*, DCLRE1C*, DNMT3B, DOCK8, EPG5, FOXN1, IFNGR1, IKBKB, IL12RB1, IL2RA, IL2RG, IL7R, IRF8, ITGB2, ITK, JAK3, LAT, LCK, LIG4, LRBA, MAGT1, MALT1, MAP3K14, MSN*, NHEJ1, NSMCE3, ORAI1, PARN*, PGM3, PIK3CD*, PMS2*, PNP, POLE, POLE2, PRKDC, PTPRC, RAG1, RAG2, RFX5, RFXANK, RFXAP, RHOH, RMRP, RTEL1, SH2D1A, SMARCAL1, SP110, SPINK5, STAT1, STAT2, STAT3, STAT5B*, STIM1, STK4, TAP1, TAP2, TAPBP, TBX1, TFRC, TNFRSF4, TYK2, UNC119, WAS and ZAP70. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: CORO1A (11) and PMS2 (15). 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

This patient is a 2-month-old baby girl suspected to have SCID. She has eosinophilia, hyperbilirubinemia and hydronephrosis. She also has rash and nonspecific skin eruption and worsening crusting on face and other parts of body.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Severe Combined Immunodeficiency Panel identified a heterozygous frameshift variant *RAG1* c.256_257del, p.(Lys86Valfs*33) and a heterozygous missense variant *RAG1* c.1186C>T, p.(Arg396Cys). Due to the large genomic distance between these variants, NGS-based methods are unable to determine whether they occur in the same or different alleles.

There are 8 and 2 individuals heterozygous for these variants, respectively, in 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 *RAG1* c.256_257del, p.(Lys86Valfs*33) variant deletes 2 base pairs and generates a frameshift leading to a premature stop codon. It is predicted to cause loss of normal protein function either through protein truncation (111 out of 1044 aa in a reference protein) or nonsense-mediated mRNA decay. The variant has been reported in ClinVar (by two submitters, variant seen in clinical testing, classified pathogenic, ID285045) and in the literature in homozygous and compound heterozygous states in several patients affected either with Omenn syndrome, severe combined immunodeficiency (SCID) or combined immunodeficiency (CID) (PMID: 9630231, 25516070, 24418478, 22424479, 28083621). The *RAG1* c.256_257delAA, p.(Lys86Valfs*33) was for the first time described in a compound heterozygous state together with another variant *RAG1* A1398G, p.(Asp429Gly) in a girl with non-consanguineous parents. She developed generalized erythrodermia in the neonatal period and chronic diarrhea and failure to thrive at two months of age. At that time, she had eosinophilia (2024 cells/mm3) and increased serum IgE (500 U/ml). Lymphocyte subpopulations were as follows: CD3 11%, CD19 1% and CD16 57%. Virtually all CD3+ cells coexpressed CD45R0, a marker for primed T cells, and 93% of them were also DR+. In vitro lymphocyte proliferative responses to phytohemagglutinin (PHA) and anti-CD3 monoclonal antibody were absent. The remaining VDJ-recombination activity for the variant *RAG1* c.256_257delAA, p.(Lys86Valfs*33) has been measured as 2.7% of the wild type by using a flow cytometry–based assay (PMID: 24290284).

The *RAG1* c.1186C>T (p.Arg396Cys) variant is predicted deleterious by all three *in silico* tools used. This variant was originally described by Villa et al in one homozygous and one compound heterozygous patient with typical signs of Omenn syndrome (PMID 9630231). Lee et al described further patients and showed greatly reduced (<1 % of wild type) recombination activity for the variant (PMID 24290284, 28783691). The variant has an entry in the ClinVar (seen in clinical testing, classification pathogenic, ID13144). In addition, other variants affecting the same amino acid have been described in patients with Omenn syndrome (c.1187G>A, p.(Arg396His) (PMID 9630231, 11971977, 21131235, 24290284), c.1187G>T, p.(Arg396Leu) (PMID 11133745, 11971977, 24290284)).

RAG1 gene (MIM *179615) encodes V(D)J recombination-activating protein 1. RAG complex is a multiprotein complex that mediates the DNA cleavage phase during V(D)J recombination. RAG1mediates the DNA-binding to the conserved recombination signal sequences and catalyzes the DNA cleavage activities by introducing a double-strand break between the recombination signal sequences and the adjacent coding segment (UniProt P15918). Pathogenic recessive mutations in *RAG1* cause severe combined T-, B-, NK+ immunodeficiency (MIM #601457) and Omenn syndrome (MIM #603554). Some 20-30% of all SCID patients are T-, B-, NK+ and some 50% of these have mutations either in *RAG1* or *RAG2* (MIM *179616). Kutukculer et al studied 11 Turkish patients with RAG1-related SCID (PMID: 22424479). Five patients had T-B-NK+ SCID, four patients had T+B-NK+ SCID and two patients had T+B+NK+ SCID. Variants p.(Arg394Gln) (6 alleles, all in T-B-NK+ SCID patients) and p.(His249Arg) (6 alleles, one homozygous T-B-NK+ SCID patient, two compound heterozygous + one heterozygous T+B-NK+ SCID patients and one heterozygous T+B+NK+ SCID patient) were the most common variants observed. In the HGMD mutation database, there are almost 200 *RAG1*-variants associated with immunological phenotypes. Some 2/3 of them are missense variants, 10% are nonsense and 20% are small deletions/insertions/indels. Notarangelo et al reviewed recently the human *RAG1* mutations (PMID: 26996199). They stated that 80% of the known RAG1-mutations are located in the core domain, while the remaining 20% fall in the non-core region. Missense mutations are found predominantly in the zinc-binding region, but, when normalized to length of domains, the nonamer-binding domain and carboxy-terminal domain have the highest number of mutations.

Mutation nomenclature is based on GenBank accession NM_000448.2 (*RAG1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

RAG1 c.256_257del, p.(Lys86Valfs*33) and *RAG1* c.1186C>T, p.(Arg396Cys) are classified as pathogenic, considering the current literature and their well-established role as disease-causing variants. Disease caused by *RAG1* variants is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants in this patient occur in *cis* (the same copy of the gene) or in *trans* (different copies of the gene). Compound heterozygosity of the identified variants (variants in *trans*) would explain the patient's disease. If each of the parents of the affected individual has one pathogenic variant, then each sibling of the affected individual has a 25% risk of being compound heterozygous and affected, a 50% chance of being an asymptomatic carrier of one of the variants, and a 25% chance of not having the variants. Genetic counseling and family member testing are recommended. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

RAG1 c.256_257del, p.(Lys86Valfs*33) and *RAG1* c.1186C>T, p.(Arg396Cys) have been confirmed with bi-directional Sanger sequencing.

STEP	DATE

STEP	DATE
Order date	Aug 10, 2018
Sample received	Aug 10, 2018
Reported	Sep 05, 2018

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

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Clinical Consultant

APPENDIX 2: ADDITIONAL FINDINGS

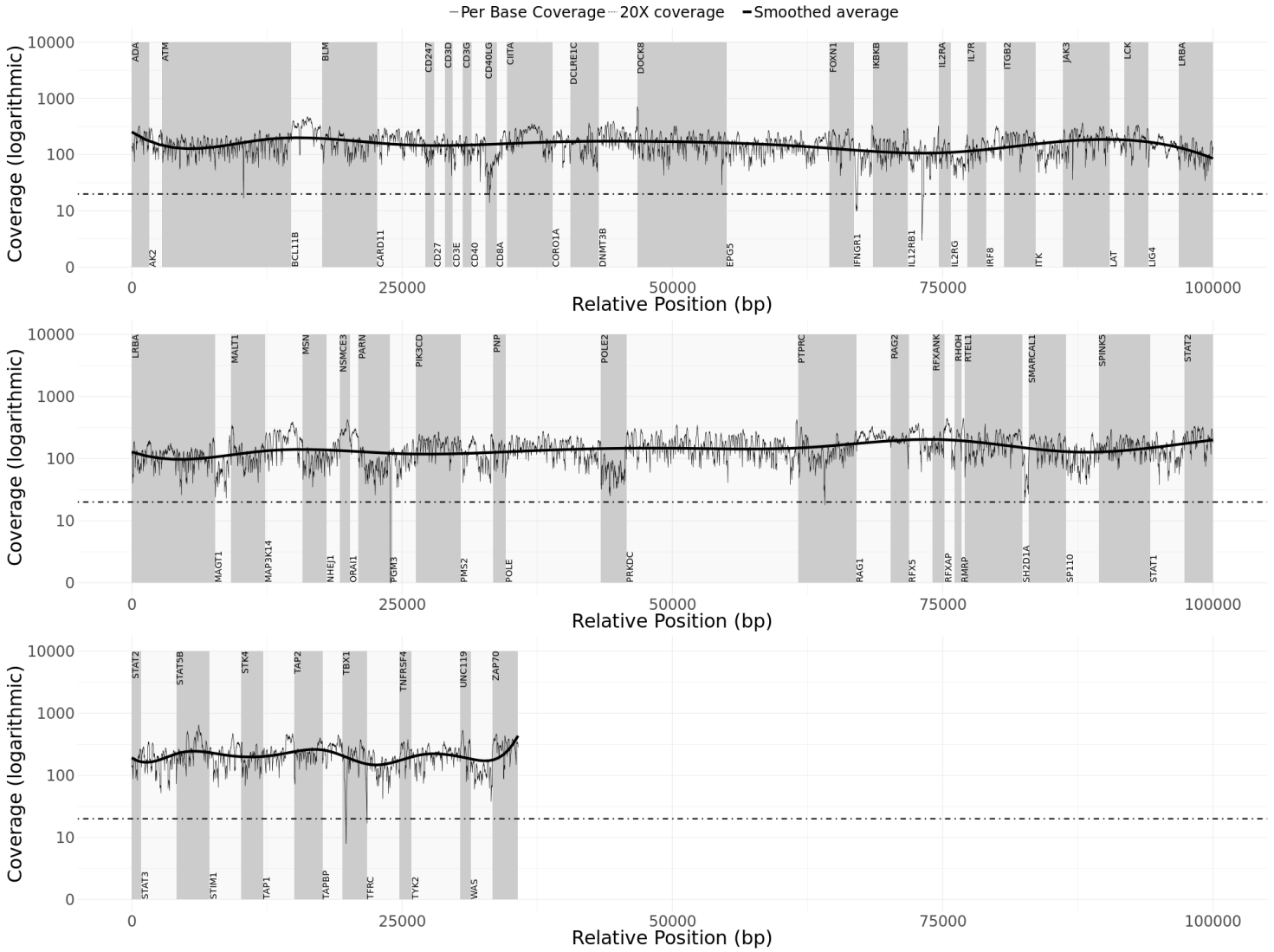
This table includes variants that either are not thought to be the likely cause for patient's phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient's phenotype), are secondary findings potentially relevant to patient's medical care (risk variants, heterozygous pathogenic or likely pathogenic variants for autosomal dominant disorders not related to patient's current phenotype) or carrier status for pathogenic or likely pathogenic variants for autosomal recessive or X-linked disorder not suspected in the patient.

VARIANT TABLE: ADDITIONAL GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
DOCK8	9:414863	NM_203447.3	c.3612A>T, p.(Lys1204Asn)	missense_variant	HET	**Variant of uncertain significance**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		39/277228	benign	deleterious	disease causing	
	OMIM	**PHENOTYPE**		**INHERITANCE**	**COMMENT**	
		Hyper-IgE recurrent infection syndrome, Mental retardation autosomal dominant 2		AR	-	

NOTES REGARDING ADDITIONAL FINDINGS

Patient is heterozygous for this variant in a gene causing recessively inherited disease. No other known pathogenic or rare, potentially disease-causing variant was observed in this gene.


Per Base Coverage
20X coverage
Smoothed average
Coverage (logarithmic)
10000
1000
100
10
0
ADA
ATM
BLM
CD247
CD3D
CD3G
CD40LG
CIITA
DCLRE1C
DOCK8
FOXN1
IKBKB
IL2RA
IL7R
ITGB2
JAK3
LCK
LRBA
AK2
BCL11B
CARD11
CD27
CD3E
CD40
CD8A
CORO1A
DNMT3B
EPG5
IFNGR1
IL12RB1
IL2RG
IRF8
ITK
LAT
LIG4
0
25000
50000
75000
100000
Relative Position (bp)
LRBA
MALT1
MSN
NSMCE3
PARN
PIK3CD
PNP
POLE2
PTPRC
RAG2
RFXANK
RHOH
RTEL1
SMARCAL1
SPINK5
STAT2
MAGT1
MAP3K14
NHEJ1
ORAI1
PGM3
PMS2
POLE
PRKDC
RAG1
RFX5
RFXAP
RMRP
SH2D1A
SP110
STAT1
STAT2
STAT5B
STK4
TAP2
TBX1
TNFRSF4
UNC119
ZAP70
STAT3
STIM1
TAP1
TAPBP
TFRC
TYK2
WAS

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_000022.2(ADA):c.1079-15T>A
NM_000022.2(ADA):c.976-34G>A
NM_000051.3(ATM):c.-174A>G
NM_000051.3(ATM):c.-31+595G>A
NM_000051.3(ATM):c.-30-1G>T
NM_000051.3(ATM):c.1236-404C>T
NM_000051.3(ATM):c.2639-384A>G
NM_000051.3(ATM):c.2839-579_2839-576delAAGT
NM_000051.3(ATM):c.3403-12T>A
NM_000051.3(ATM):c.3994-159A>G
NM_000051.3(ATM):c.5763-1050A>G
NM_000074.2(CD40LG):c.289-15T>A
NM_000074.2(CD40LG):c.347-915A>T
NM_001033855.1(DCLRE1C):c.973-1777G>C
NM_006892.3(DNMT3B):c.2421-11G>A
NM_203447.3(DOCK8):c.54-1G>T
NM_203447.3(DOCK8):c.742-18C>G
NM_203447.3(DOCK8):c.742-15T>G
NM_203447.3(DOCK8):c.1797+61A>C

NM_000206.2(IL2RG):c.*308A>G
NM_000206.2(IL2RG):c.270-15A>G
NM_000206.2(IL2RG):c.-105C>T
NM_002185.3(IL7R):c.379+288G>A
NM_000211.3(ITGB2):c.742-14C>A
NM_000211.3(ITGB2):c.500-12T>G
NM_000215.3(JAK3):c.2680+89G>A
NM_000215.3(JAK3):c.1915-11G>A
NM_001134477.2(PARN):c.-165+2C>T
NM_000270.3(PNP):c.286-18G>A
NM_006904.6(PRKDC):c.1777-710dupA
NM_000536.3(RAG2):c.-28G>C
chr9:g.35657745-35657745
chr9:g.35657746-35657746
NM_006846.3(SPINK5):c.283-12T>A
NM_006846.3(SPINK5):c.1431-12G>A
NM_006846.3(SPINK5):c.1820+53G>A
NM_080647.1(TBX1):c.-777C>T
NM_080647.1(TBX1):c.-620A>C
NM_001079.3(ZAP70):c.838-80G>A
NM_001079.3(ZAP70):c.1624-11G>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.