Blueprint Genetics

Retinal Dystrophy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		16	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *ABCA4* c.4539+2028C>T, which is pathogenic.
Patient is heterozygous for *ABCA4* c.5413A>G, p.(Asn1805Asp), 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
ABCA4	1:94492973	NM_000350.2	c.4539+2028C>T	intron_variant	HET	**Pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
		2/30964	N/A	N/A	N/A	
		PHENOTYPE				
	OMIM	Cone rod dystrophy, Fundus flavimaculatus, Retinal dystrophy, early-onset severe, Retinitis pigmentosa, Stargardt disease		**INHERITANCE** AR	**COMMENT** -	

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
ABCA4	1:94480146	NM_000350.2	c.5413A>G, p.(Asn1805Asp)	missense_variant	HET	**Likely pathogenic**
	ID	**gnomAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**	
	rs61753029	8/277206	probably damaging	deleterious	disease causing	
		PHENOTYPE				
	OMIM	Cone rod dystrophy, Fundus flavimaculatus, Retinal dystrophy, early-onset severe, Retinitis pigmentosa, Stargardt disease		**INHERITANCE** AR	**COMMENT** -	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Retinal Dystrophy Panel	266	4318	854993	854286	337	99.92

TARGET REGION AND GENE LIST

The Blueprint Genetics Retinal Dystrophy Panel (version 4, Mar 01, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ABCA4, ABHD12, ACO2, ADAM9, ADAMTS18, ADGRV1, ADIPOR1*, AGBL5, AHI1, AIPL1, ALMS1*, ARHGEF18, ARL13B, ARL2BP, ARL6, ARMC9, ATF6, ATOH7, B9D1, B9D2, BBIP1#, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BEST1, C1QTNF5, C21ORF2, C2ORF71, C5ORF42, C8ORF37, CA4, CABP4, CACNA1F, CACNA2D4, CAPN5, CC2D2A, CDH23, CDH3, CDHR1, CEP104, CEP120, CEP164, CEP290*, CEP41, CEP78, CERKL, CHM, CIB2, CISD2*, CLN3, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL11A1, COL11A2, COL18A1, COL2A1, COL9A1, COL9A2, COL9A3, CRB1, CRX, CSPP1, CTC1, CTNNA1, CTNNB1, CWC27, CYP4V2, DFNB31, DHDDS, DHX38, DRAM2, DTHD1, EFEMP1, ELOVL4, EMC1, EYS*, FAM161A, FLVCR1, FRMD7, FZD4, GNAT1, GNAT2, GNB3, GNPTG, GPR179, GRM6, GUCA1A, GUCY2D, HARS*, HGSNAT, HK1, HMX1, IDH3B, IFT140, IFT172, IFT81, IMPDH1, IMPG1, IMPG2, INPP5E, INVS, IQCB1, JAG1, KCNJ13, KCNV2, KIAA0556, KIAA0586, KIAA0753, KIF11, KIF7, KIZ, KLHL7, LCA5, LRAT, LRIT3, LRP2, LRP5*, LZTFL1, MAK, MERTK, MFN2, MFRP, MFSD8, MKKS, MKS1, MMACHC, MTTP, MVK, MYO7A, NDP, NEK2, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NR2F1, NRL, NYX, OAT, OFD1, OPA1, OPA3, OTX2, P3H2, PANK2, PAX2, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6D, PDE6G, PDE6H, PDZD7, PEX1, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX2, PEX26, PEX3, PEX5, PEX6, PEX7, PHYH, PITPNM3, PLA2G5, PNPLA6, POC1B, PRCD, PRDM13, PRKCG, PROM1#, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, PRPS1*, RAB28, RAX2, RBP3, RBP4, RD3, RDH11, RDH12, RDH5, REEP6, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROM1, RP1, RP1L1, RP2, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, RTN4IP1, SAG, SAMD11, SDCCAG8, SEMA4A, SLC24A1, SLC25A46, SLC7A14, SNRNP200, SPATA7, SPP2, TCTN1, TCTN2, TCTN3, TEAD1, TIMM8A*, TIMP3, TMEM107, TMEM126A, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TOPORS, TRAF3IP1, TREX1, TRIM32, TRPM1, TSPAN12, TTC21B, TTC8, TTLL5, TTPA, TUB, TULP1, USH1C, USH1G, USH2A, VCAN, VPS13B, WDPCP, WDR19, WFS1, ZNF408, ZNF423 and ZNF513. 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

Patient is a 16-year-old female with pigmentary retinal dystrophy.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Retinal Dystrophy Panel identified two heterozygous variants in *ABCA4*: a deep intronic variant c.4539+2028C>T and a missense variant c.5413A>G, p.(Asn1805Asp). Due to the large genomic distance between these variants, we were unable to determine whether they occur in the same or in different alleles.

There are two individuals heterozygous for the *ABCA4* c.4539+2028C>T variant in the Genome Aggregation Database (gnomAD, n >15,000 genomes). The variant is located in intron 30, 2028 bp downstream from the last coding nucleotide of exon 30 (c.4539). Initially, Braun et al identified the variant c.4539+2028C>T (referred to as V5) together with another ABCA4 variant in seven patients with Stargardt disease (PMID: 23918662). It was found in a compound heterozygous state in five patients: together with c.5461-10T>C in three patients from two families, together with p.(Arg1108Cys) in one patient and together with p.(Thr1526Met) in one patient. In addition, the c.4539+2028C>T was reported together with p.(Thr1526Met) and p.(Gly65Glu) variants, respectively, in two patients, however, the phase of the variants was not determined. This variant c.4539+2028C>T was found to strengthen an acceptor splice signal within a minor exon that was observed in the original RNA sequencing data. Schulz et al identified the c.4539+2028C>T variant in one patient but no other probable mutant *ABCA4* allele (PMID: 28118664). They concluded that based on bioinformatics, variant c.4539+2028C>T (V5) is not predicted to affect splicing. In addition, Albert et al described a STGD patient with the *ABCA4* c.4539+2028C>T variant in cis with a deep-intronic variant c.302+68C>T and a genomic deletion, c.6148 −698_6670delinsTGTGCACCTCCCTAG on the other allele. They generated photoreceptor precursor cells (PPCs) from fibroblasts obtained from this individual and showed that the variant results in a retina-specific inclusion of a 345-nt pseudoexon in a proportion of *ABCA4* transcripts. This pseudoexon, which is predicted to lead to protein truncation (p.Arg1514Leufs∗36), was found as a low-abundance alternative splice form of *ABCA4* when performing deep RNA sequencing of human macula RNA (PMID: 23918662).

There are eight individuals heterozygous for the *ABCA4* c.5413A>G, p.(Asn1805Asp) variant in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). *In silico* tools PolyPhen, SIFT, and MutationTaster predict the variant as deleterious. The variant *ABCA4* c.5413A>G, p.(Asn1805Asp) has been reported in a homozygous state in one patient with Stargardt disease (PMID: 11385708).

ABCA4 encodes a transmembrane protein that is almost exclusively expressed in outer segment disk edges of rod photoreceptors. Pathogenic variants in ABCA4 have been associated with various autosomal recessive retinal dystrophies, including Stargardt disease (STGD1), cone-rod dystrophy (CRD), retinitis pigmentosa (RP), and fundus flavimaculatus (OMIM: *601691). Variants in ABCA4 were initially shown to cause the autosomal-recessive STGD, which is a hereditary juvenile macular degeneration disease causing progressive loss of photoreceptor cells (PMID: 9054934; OMIM: #248200). STGD is characterized by reduced visual acuity and color vision, loss of central (macular) vision, delayed dark adaptation, and accumulation of autofluorescent RPE lipofuscin. Of note, retinal disease caused by variants in ABCA4 covers a wide spectrum of severity (PMID: 19074458). A diagnosis of STGD is considered to represent a less severe disease than CRD or atypical RP because of the retina-wide involvement implicit in the latter diagnoses. However, many individuals with STGD can also show retina-wide involvement, and a wide variation is observed in the clinical severity within each diagnostic category. According to the Human Gene Mutation Database (HGMD), more than 1000 disease-causing variants in ABCA4 have been found, of which over 800 are associated with STGD (HGMD Professional 2018.2). The majority of variants are missense, but also frameshift, nonsense, and splice-site variants are common. Truncating variants have been associated with a more severe phenotype (PMID: 15942264, 23949494, 22229821). Each mutated ABCA4 allele produces either no protein or a functionally abnormal protein (PMID: 19074458). The ABCA4 mutated alleles' residual function can range from normal to absent. The clinical severity of ABCA4-related disease is thought to be inversely associated with the residual ABCR function. ClinVar lists 289 pathogenic/likely pathogenic ABCA4 variants that have been identified through clinical testing to date (July, 2018).

Mutation nomenclature is based on GenBank accession NM_000350.2 (*ABCA4*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

Considering the current literature and well-established role of *ABCA4* c.4539+2028C>T as a disease-causing variant, we classify it as pathogenic. We classify the identified *ABCA4* c.5413A>G, p.(Asn1805Asp) as likely pathogenic, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, in silico predicted pathogenicity, identification of the variant in an individual with Stargardt disease). However, additional information is still needed to confirm the pathogenicity of the variant. Disease caused by *ABCA4* variants is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants 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 most likely explain the patient's disease. If both parents are found to be carriers of one of the mutations, each sibling of an affected individual has a 25% chance of being a compound heterozygous for the mutations and thus being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Genetic counseling and family member testing are recommended. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

ABCA4 c.4539+2028C>T and *ABCA4* c.5413A>G, p.(Asn1805Asp) have been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	
Sample received	
Reported	

On Jul 11, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Sari Tuupanen, Ph.D.

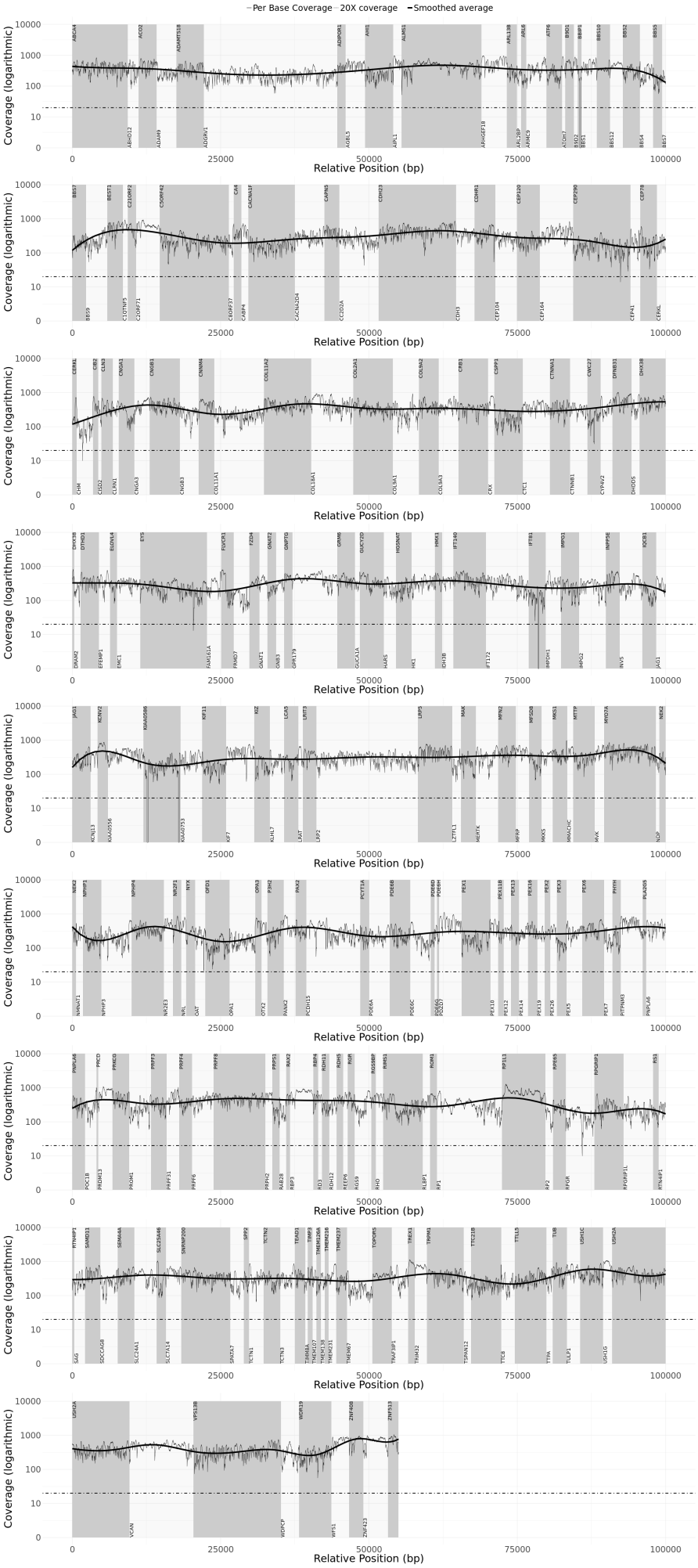
Senior Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

Jennifer Schleit, Ph.D. FACMG

Senior Geneticist


Per Base Coverage
20X coverage
Smoothed average
Coverage (logarithmic)
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_000350.2(ABCA4):c.6148-471C>T
NM_000350.2(ABCA4):c.5196+1137G>A
NM_000350.2(ABCA4):c.5196+1137G>T
NM_000350.2(ABCA4):c.5196+1056A>G
NM_000350.2(ABCA4):c.4539+2064C>T
NM_000350.2(ABCA4):c.4539+2028C>T
NM_000350.2(ABCA4):c.4539+2001G>A
NM_000350.2(ABCA4):c.4539+1928C>T
NM_000350.2(ABCA4):c.4539+1729G>T
NM_000350.2(ABCA4):c.3050+370C>T
NM_000350.2(ABCA4):c.2160+584A>G
NM_000350.2(ABCA4):c.1938-619A>G
NM_000350.2(ABCA4):c.570+1798A>G
NM_000350.2(ABCA4):c.302+68C>T
NM_000350.2(ABCA4):c.67-16T>A
NM_024649.4(BBS1):c.951+58C>T
NM_024649.4(BBS1):c.1110+329C>T
NM_033028.4(BBS4):c.77-216delA
NM_152384.2(BBS5):c.619-27T>G

NM_001139443.1(BEST1):c.-29+1G>T
NM_001139443.1(BEST1):c.-29+5G>A
NM_001271441.1(C21ORF2):c.1000-23A>T
NM_000717.3(CA4):c.*89G>A
NM_022124.5(CDH23):c.1135-1G>T
NM_025114.3(CEP290):c.6012-12T>A
NM_025114.3(CEP290):c.2991+1655A>G
NM_000390.2(CHM):c.315-1536A>G
NM_000390.2(CHM):c.315-4587T>A
NM_000086.2(CLN3):c.461-13G>C
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_001844.4(COL2A1):c.1527+135G>A
NM_024887.3(DHDDS):c.441-24A>G
NM_001142800.1(EYS):c.-448+5G>A
NM_194277.2(FRMD7):c.285-118C>T
NM_005272.3(GNAT2):c.461+24G>A
NM_032520.4(GNPTG):c.610-16_609+28del
NM_000180.3(GUCY2D):c.-9-137T>C
NM_033500.2(HK1):c.-390-3838G>C
NM_033500.2(HK1):c.-390-3818G>C
NM_033500.2(HK1):c.27+14901A>G
NM_000883.3(IMPDH1):c.402+57G>A
NM_000214.2(JAG1):c.1349-12T>G
NM_000253.2(MTTP):c.619-5_619-2delTTTA
NM_000253.2(MTTP):c.1237-28A>G
NM_000260.3(MYO7A):c.-48A>G
NM_000260.3(MYO7A):c.3109-21G>A
NM_000266.3(NDP):c.-207-1G>A
NM_000266.3(NDP):c.-208+5G>A
NM_000266.3(NDP):c.-208+2T>G
NM_000266.3(NDP):c.-208+1G>A
NM_022787.3(NMNAT1):c.-70A>T
NM_022787.3(NMNAT1):c.-69C>T
NM_003611.2(OFD1):c.935+706A>G
NM_003611.2(OFD1):c.1130-22_1130-19delAATT
NM_130837.2(OPA1):c.610+360G>A
NM_130837.2(OPA1):c.610+364G>A
NM_130837.2(OPA1):c.2179-40G>C
NM_001142763.1(PCDH15):c.-29+1G>C
NM_006204.3(PDE6C):c.481-12T>A
NM_000287.3(PEX6):c.2301-15C>G
NM_000287.3(PEX6):c.2300+28G>A
NM_000288.3(PEX7):c.-45C>T
chr6:g.100040906-100040906
chr6:g.100040987-100040987
chr6:g.100041040-100041040
NM_006017.2(PROM1):c.2077-521A>G
NM_015629.3(PRPF31):c.1374+654C>G
NM_000322.4(PRPH2):c.829-4C>G
NM_000329.2(RPE65):c.246-11A>G
NM_001034853.1(RPGR):c.1059+363G>A
NM_020366.3(RPGRIP1):c.2711-13G>T
NM_001077416.2(TMEM231):c.824-11T>C
NM_206933.2(USH2A):c.14583-20C>G
NM_206933.2(USH2A):c.9959-4159A>G
NM_206933.2(USH2A):c.8845+628C>T
NM_206933.2(USH2A):c.7595-2144A>G
NM_206933.2(USH2A):c.5573-834A>G
NM_206933.2(USH2A):c.-259G>T
NM_006005.3(WFS1):c.-43G>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.