Blueprint Genetics

My Retina Tracker – IRB Study Protocol Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL
Christine Kay, M.D.	USA - FL - Gainesville - Vitreoretinal Associates

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
Ye, Jonathan	2001-05-11	20	Male	171692

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
Blood	2021-11-12	

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *RP1* c.4052_4053insAlu, which is pathogenic.
The patient is heterozygous for *RP1* c.6181del, p.(Ile2061Serfs*12), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
RP1	NM_006269.2	c.4052_4053insAlu	HET	AluIns	AD,AR	Pathogenic
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	8:55540494	C/insAlu		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	N/A	N/A	N/A	Retinitis pigmentosa	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
RP1	NM_006269.2	c.6181del, p.(Ile2061Serfs*12)	HET	frameshift_variant	AD,AR	Pathogenic
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	8:55542620	GA/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	8/282624	N/A	N/A	N/A	Retinitis pigmentosa	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
My Retina Tracker – IRB Study Protocol Panel	314	4998	989155	986087	207	99.69

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	5971	100

TARGET REGION AND GENE LIST

The Blueprint Genetics My Retina Tracker – IRB Study Protocol Panel (version 5, Oct 30, 2021) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABCA4*, *ABCC6**, *ABCD1**, *ABHD12*, *ACO2*, *ADAM9*, *ADAMTS18*, *ADGRV1*, *ADIPOR1**, *AGBL5*, *AHI1*, *AIPL1*, *ALMS1**, *AMACR*, *ARHGEF18*, *ARL13B*, *ARL2BP*, *ARL3*, *ARL6*, *ARMC9*, *ARR3*, *ARSG*, *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*, *CEP19*, *CEP250*, *CEP290**, *CEP41*, *CEP78*, *CEP83*, *CERKL*, *CHM*#, *CIB2*, *CISD2**, *CLN3*, *CLN5*, *CLN6*, *CLN8*, *CLRN1*, *CNGA1*#, *CNGA3*, *CNGB1*, *CNGB3*, *CNNM4*, *COL11A1*, *COL11A2*, *COL18A1*, *COL2A1*, *COL9A1*, *COL9A2*, *COL9A3*, *COQ2*, *CPE*, *CRB1*, *CRX*, *CSPP1*, *CTC1*, *CTNNA1*, *CTNNB1*, *CTSD*, *CWC27*, *CYP4V2*, *DFNB31*, *DHDDS*, *DHX38*, *DNAJC5*, *DRAM2*, *DTHD1*, *DYNC2H1*, *EFEMP1*, *ELOVL4*, *EMC1*, *ESPN**, *EXOSC2*, *EYS**, *FAM161A*, *FDXR*, *FLVCR1*, *FRMD7*, *FZD4*, *GNAT1*, *GNAT2*, *GNB3*, *GNPTG*, *GPR143*, *GPR179*, *GRK1*, *GRM6*, *GUCA1A*, *GUCY2D*, *HARS*, *HGSNAT*, *HK1*#, *HMX1*, *IDH3A*, *IDH3B*, *IFT140*, *IFT172*, *IFT27*, *IFT81*#, *IMPDH1*, *IMPG1*, *IMPG2*, *INPP5E*, *INVS*, *IQCB1*, *ISPD*, *JAG1*, *KCNJ13*, *KCNV2*, *KIAA0556*, *KIAA0586*#, *KIAA0753*, *KIAA1549*, *KIF11*, *KIF7*, *KIZ*, *KLHL7*, *LAMA1*, *LCA5*, *LRAT*, *LRIT3*, *LRP2*, *LRP5**, *LZTFL1*, *MAK*, *MERTK*, *MFN2*, *MFRP*, *MFSD8*, *MKKS*, *MKS1*, *MMACHC*, *MT-ATP6*, *MT-ATP8*, *MT-CO1*, *MT-CO2*, *MT-CO3*, *MT-CYB*, *MT-ND1*, *MT-ND2*, *MT-ND3*, *MT-ND4*, *MT-ND4L*, *MT-ND5*, *MT-ND6*, *MT-RNR1*, *MT-RNR2*, *MT-TA*, *MT-TC*, *MT-TD*, *MT-TE*, *MT-TF*, *MT-TG*, *MT-TH*, *MT-TI*, *MT-TK*, *MT-TL1*, *MT-TL2*, *MT-TM*, *MT-TN*, *MT-TP*, *MT-TQ*, *MT-TR*, *MT-TS1*, *MT-TS2*, *MT-TT*, *MT-TV*, *MT-TW*, *MT-TY*, *MTTP*, *MVK*, *MYO7A*, *NAGLU*, *NDP*, *NEK2*#, *NMNAT1*#, *NPHP1*, *NPHP3*, *NPHP4*, *NR2E3*, *NR2F1*, *NRL*, *NYX*, *OAT*, *OCA2*, *OFD1*, *OPA1*, *OPA3*, *OPN1SW*, *OTX2*, *P3H2*, *PANK2*, *PAX2*, *PCDH15*, *PCYT1A*, *PDE6A*, *PDE6B*, *PDE6C*, *PDE6D*, *PDE6G*, *PDE6H*, *PDSS1*#, *PDSS2*, *PDZD7*#, *PEX1*, *PEX10*, *PEX11B*, *PEX12*, *PEX13*, *PEX14*, *PEX16*, *PEX19*, *PEX2*, *PEX26*, *PEX3*, *PEX5*, *PEX6*, *PEX7*, *PHYH*, *PISD*, *PITPNM3*, *PLA2G5*, *PLK4*, *PNPLA6*, *POC1B*, *POMGNT1*, *PPT1*, *PRCD*, *PRDM13*, *PROM1*, *PRPF3*, *PRPF31*, *PRPF4*, *PRPF6*, *PRPF8*, *PRPH2*, *PRPS1**, *RAB28*, *RAX2*, *RBP3*, *RBP4*, *RCBTB1*, *RD3*, *RDH11*, *RDH12*, *RDH5*, *REEP6*, *RGR*, *RGS9*, *RGS9BP*, *RHO*, *RIMS1*, *RLBP1*, *ROM1*, *RP1*, *RP1L1*, *RP2*, *RPE65*, *RPGR*, *RPGRIP1*, *RPGRIP1L*#, *RS1*, *RTN4IP1*, *SAG*, *SAMD11*, *SCAPER*, *SCLT1*#, *SDCCAG8*, *SEMA4A*, *SGSH*, *SLC24A1*, *SLC25A46*, *SLC45A2*, *SLC7A14*, *SNRNP200*, *SPATA7*, *SPP2*, *SRD5A3**, *TCTN1*#, *TCTN2*, *TCTN3*, *TEAD1*, *TIMM8A**, *TIMP3*, *TMEM107*, *TMEM126A*, *TMEM138*, *TMEM216*, *TMEM231*, *TMEM237*, *TMEM67*, *TOPORS*, *TPP1*, *TRAF3IP1*, *TREX1*, *TRIM32*, *TRPM1*, *TSPAN12*, *TTC21B*, *TTC8*, *TTLL5*, *TTPA*, *TUB*, *TUBB4B*, *TUBGCP4*, *TUBGCP6*, *TULP1*, *TYR**, *TYRP1*, *USH1C*, *USH1G*, *USH2A*, *VCAN*, *VPS13B*, *WDPCP*, *WDR19*, *WFS1*, *YME1L1**, *ZNF408*, *ZNF423* and *ZNF513*. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: CC2D2A (NM_020785:7), CHM (NM_001145414:5), CNGA1 (NM_001142564:2), HK1 (NM_001322365:5), IFT81 (NM_031473:12), KIAA0586 (NM_001244189:6, 33), NEK2 (NM_001204182:8), NMNAT1 (NM_001297779:5), PDSS1 (NM_014317:2), PDZD7 (NM_024895:10), RPGRIP1L (NM_015272:23), SCLT1 (NM_001300898:6) and TCTN1 (NM_001173976:2;NM_024549:6).
*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 a mapping quality score of 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 20-year-old male with dystrophies of the retinal pigment epithelium OU. Flat ffERG OU, constricted III4e VF OU, RPE changes and atrophy OU, and blunted foveal reflex OU are reported. Maternal aunt with significant peripheral vision loss, diagnosed with retinitis pigmentosa or retinal degeneration.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) My Retina Tracker – IRB Study Protocol Panel identified a heterozygous Alu element insertion *RP1* c.4052_4053insAlu and a heterozygous frameshift variant *RP1* c.6181del, p.(Ile2061Serfs*12). Due to the large genomic distance between these variants, NGS-based methods cannot determine whether they occur on the same (in *cis*) or different (in *trans*) parental alleles.

RP1 c.4052_4053insAlu

Sequence analysis detected a heterozygous Alu element insertion *RP1* c.4052_4053insAlu locating to the last exon (exon 4) of *RP1*. This Alu insertion was detected based on two high quality breakpoints in the NGS data and a duplicated target sequence between them. Nucleotide sequence detected from the insertion indicates that the insertion belongs to the AluYd8 subfamily of Alu elements (Dfam database). However, the exact size and sequence of the insertion cannot be determined by the current assay. This Alu element insertion, described as *RP1* c.4052_4053ins328, p.(Tyr1352Alafs*9) in the literature, has been reported as a founder variant for autosomal recessive retinal dystrophy in the Japanese population (PMID: 31253780, 30913292, 32193659, 32627106). The variant introduced 328 additional nucleotides in exon 4 of the *RP1* gene, leading to a frameshift and a premature termination codon in the canonical *RP1* coding sequence. As the premature stop codon occurs in the last exon, the transcript is expected to escape nonsense mediated decay. The variant is, however, predicted to lead to the loss of 796 C-terminal amino acids. *RP1* c.4052_4053ins328, p.(Tyr1352Alafs*9) was initially identified through whole-genome sequencing as homozygous in a Japanese patient with hereditary retinal degeneration (PMID: 31253780). The variant was detected in a homozygous state also in the index patient's affected brother and as heterozygous in the unaffected mother of the siblings. Subsequently, the Alu insertion *RP1* c.4052_4053ins328, p.(Tyr1352Alafs*9) has been identified in several patients with inherited retinal dystrophy, either as homozygous or as compound heterozygous with another disease-causing variant in *RP1* locating outside the defined region in exon 4 in which truncating variants have been suggested to exert a dominant negative effect (PMID: 30913292, 32193659, 32627106).

RP1 c.6181del, p.(Ile2061Serfs*12)

There are 8 individuals heterozygous for this variant in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. The variant deletes one base pair in the last exon of *RP1* (exon 4) and generates a frameshift, leading to a premature stop codon 11 amino acids downstream. Of note, as the premature stop codon occurs in the last exon, the truncated transcript is expected to escape nonsense mediated decay. The variant is, however, predicted to lead to the loss of 96 C-terminal amino acids. The variant has been reported as homozygous or together with another disease-causing *RP1* variant in several patients with autosomal recessive retinitis pigmentosa (RP); as homozygous in one patient with disease-onset at the age of 16, as compound heterozygous with *RP1* c.1498_1499del, p.(Met500Valfs*7) (each variant inherited from a healthy parent) in a Japanese patient, and together with *RP1* c.4905_4906del, p.(Tyr1636Argfs*2) in the proband and her affected brother from a Chinese family (PMID: 29425069, 30027431, 31960602). The variant has also been reported together with *RP1* c.1934_1937del, p. (Ile646Metfs*8) in a 22-year-old female with cone rod dystrophy (PMID: 33090715). Furthermore, the variant has been reported as heterozygous in two Japanese patients with RP, one of which was subsequently identified to harbour the recurrent pathogenic Alu insertion *RP1* c.4052_4053ins328, p.(Tyr1352Alafs*9) in the other allele of the gene (PMID: 32193659). *RP1* c.6181del, p.(Ile2061Serfs*12) has been detected by another laboratory in the context of clinical testing and submitted to ClinVar (variation ID 1031616).

RP1

The *RP1* gene (OMIM *603937) encodes a member of the doublecortin family of proteins, and is associated with the photoreceptor microtubules. It plays a role in the organization of the outer segment of rod and cone photoreceptors ensuring the correct orientation and higher-order stacking of outer segment disks along the photoreceptor axoneme. Pathogenic variants in *RP1* have been associated with autosomal dominant and autosomal recessive retinitis pigmentosa (RP; OMIM #180100). Recently, bi-allelic *RP1* variants have also been described in patients with autosomal recessive macular and cone-rod dystrophy (PMID: 30029497, 30913292).

RP is the most common form of inherited retinal degeneration with a prevalence of 1 in 4000 individuals. RP is characterized by

primary rod degeneration leading to night blindness, the development of tunnel vision, and slowly progressive decrease in central vision (GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1417/). Pathogenic variants in *RP1* have been shown to account for 4–7% of patients with autosomal dominant retinitis pigmentosa (adRP). In a cohort of 215 adRP families, variants in *RP1* were found in 8 families (3.7%: PMID: 18188946) and in another cohort of 250 families, *RP1* variants appeared in 7% of the cases (17/250) (PMID: 10484783). Variation in the severity of disease is observed even in the patients with the same primary disease-causing variant (PMID: 22131869). Furthermore, pathogenic variants in *RP1* have been found in families with autosomal recessive RP (PMID: 15863674, 19933189, 22917891). For instance, homozygous missense variants *RP1* c.607G>C p.(Gly203Arg) and *RP1* c.679T>G p.(Phe227Val) have been described in patients with RP (PMID: 24265693, 2649737).

The vast majority of pathogenic variants in *RP1* are truncating variants clustered in the last exon of *RP1*, exon 4, which encodes amino acid residues 263-2156. It has been suggested that adRP *RP1* variants are clustered in a relatively small region in exon 4, between amino acid residues 500 and 1053, and result in the production of a truncated protein with a presumed dominant-negative activity (PMID: 23077400, 22927954, 30913292). In contrast, most variants located more toward the N- or C-terminus of RP1 result in autosomal recessive RP (arRP). Wang et al (2021) performed a systemic analysis on *RP1* truncating variants using WES data from 7,092 individuals and 185 *RP1* variants from published literature (PMID: 33681214). An overall evaluation of *RP1* causative variants suggested three separate regions: the N-terminal (c.1-1837, p.1-613), the middle region (c.1981-2749, p.661-917), and the C-terminal (c.2816-6471, p.939-2157), where truncations in the middle portion were associated with adRP, while those in the N- and C-terminals were responsible for arRP (PMID: 33681214). However, 13 variants showed reversed phenotype correlation: 7 located in the middle region were reported in arRP rather than adRP, while 6 located in the N- and C-terminals were reported in adRP rather than arRP. Four of the 13 were involved in both adRP and arRP. Finally, the c.2391_2392del (p.Asp799*) located in the middle portion was reported to cause arCRD in homozygous status, and in a heterozygous carrier with no sign of RP (PMID: 25692139, 33681214). Twelve single heterozygous truncation variants were identified in a cohort of 7,092 individuals with various forms of eye conditions and were considered non-pathogenic, due to the occurrence in multiple individuals who were not affected with RP (n=36) and/or carried other genetic RP diagnoses (n=5). Among 38 missense variants found in the literature, 22 variants were involved in recessive retinal degeneration, and all of them were located at the N- and C-terminals. Fifteen missense variants reported to cause adRP were distributed scattered across the whole coding region of *RP1*, but segregation information was not available. None of the heterozygous missense variants predicted to be damaging were associated with adRP in the cohort of 7,092 individuals.

Patients with autosomal recessive macular dystrophy (arMD) or autosomal recessive cone-rod dystrophy (arCRD) are likely to carry a heterozygous variant that is expected to have a mild effect on protein function [*RP1* c.539T>G p.(Phe180Cys), *RP1* c.569T>G p.(Val190Gly), or *RP1* c.5797C>T p.(Arg1933*)] in combination with a more severe nonsense or frameshift variant, or a combination of two predicted mild variants (PMID: 30913292).

HGMD Professional 2021.3 lists 240 disease-causing variants in *RP1,* of which the majority are truncating variants (87%). Over 35 of the variants have been associated with autosomal recessive RP. No homozygous truncating variants in *RP1* are reported in the control cohorts of gnomAD, suggesting that biallelic truncating variants are not tolerated.

Mutation nomenclature is based on GenBank accession NM_006269.2 (*RP1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

RP1 c.4052_4053insAlu and *RP1* c.6181del, p.(Ile2061Serfs*12) are both classified as pathogenic, based on currently available evidence supporting their disease-causing roles. Disease caused by these variants is expected to be inherited in an autosomal recessive manner. Testing of parental/offspring samples is needed to determine whether the variants occur in *cis* (on the same allele) or in *trans* (on different alleles). Compound heterozygosity of the variants (in *trans*) would explain the patient's clinical presentation. If both of these variants are parentally inherited, any siblings of the patient will have a 25% chance of being compound heterozygous and thus affected, a 50% chance of being an unaffected carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

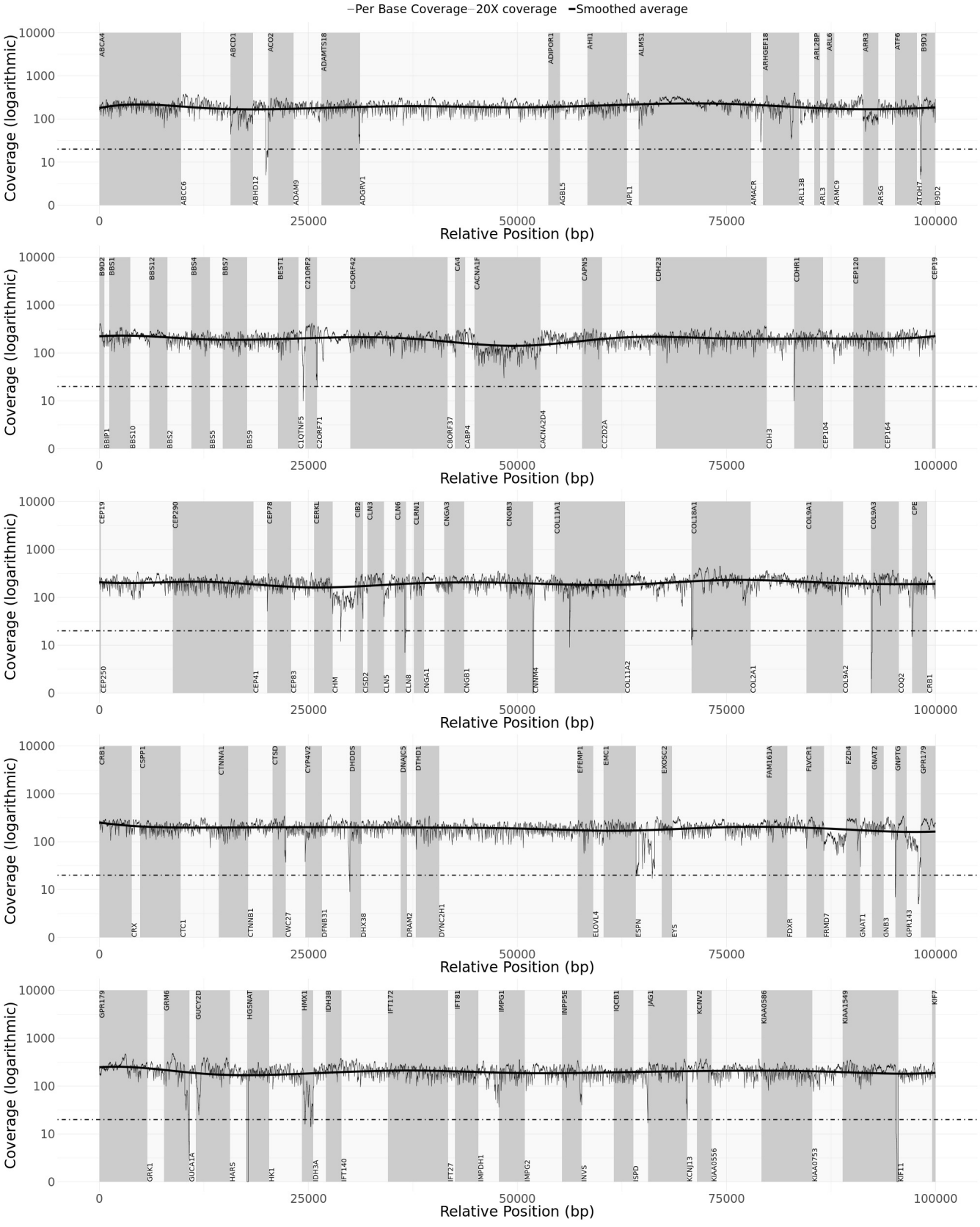
STEP	DATE
Order date	Nov 12, 2021
Sample received	Nov 16, 2021
Sample in analysis	Nov 16, 2021
Reported	Dec 11, 2021

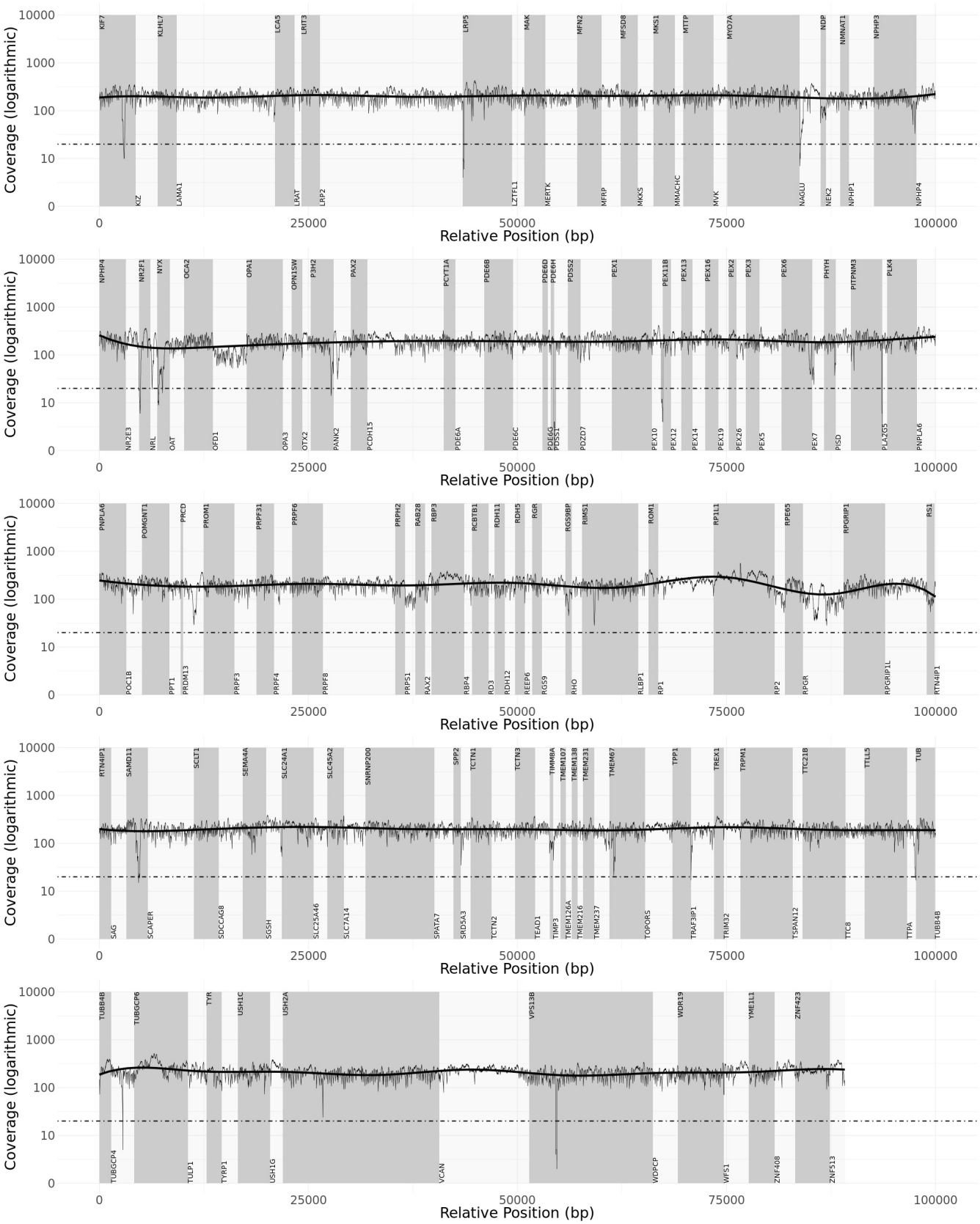
Reviewed and approved on Dec 11, 2021 by:

Jennifer Schleit, Ph.D., FACMG

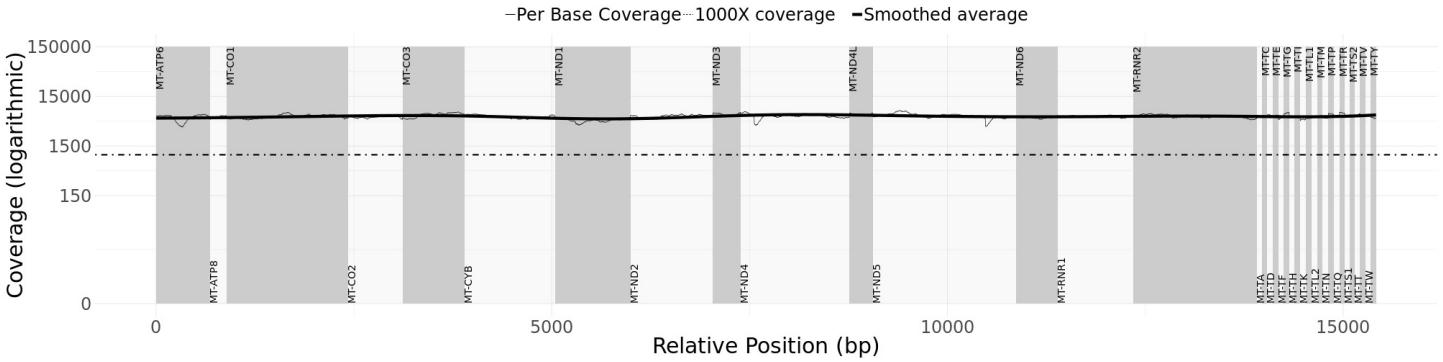
Senior Geneticist

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.





Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.


Per Base Coverage
1000X coverage
Smoothed average
Coverage (logarithmic)
150000
15000
1500
150
0
MT-ATP6
MT-CO1
MT-CO3
MT-ND1
MT-ND3
MT-ND4L
MT-ND6
MT-RNR2
MT-ATP8
MT-CO2
MT-CYB
MT-ND2
MT-ND4
MT-ND5
MT-RNR1
0
5000
10000
15000
Relative Position (bp)

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. DNA quality and quantity were assessed using electrophoretic methods at Blueprint Genetics, Inc. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.These steps were performed at Quest Diagnostics Nichols Institute.

Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). 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) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. 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 including assessments for contamination and sample mix-up. 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. 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. Bioinformatics and quality control processes were performed by Blueprint Genetics.

Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported. The interpretation was performed at Blueprint Genetics.

Variant classification: 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. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com

Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the 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 gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic

Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.
Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.The confirmation of sequence alterations was performed at Blueprint Genetics, Inc.
Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.The confirmation of copy number variants was performed at Blueprint Genetics, Inc.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.
Test restrictions: 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.
Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

This test was developed and its analytical performance characteristics have been determined by Blueprint Genetics, Inc. It has not been cleared or approved by the US Food and Drug Administration.

PERFORMING SITES:

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- BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: JUHA KOSKENVUO, MD, PhD, CLIA: 99D2092375

REVIEWING DIRECTOR:

JENNIFER SCHLEIT, PHD, FACMG, Laboratory Director

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
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
MT = Mitochondria
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.