OtoGenex™
GENOMIC DRIVEN OTOLOGY

Sample report as of # July 1st, 202 2. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in otegenix online portal. Accreditation and certification information available at otogenix.com

REFERRING HEALTHCARE PROFESSIONAL

NAME **Hospital**

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		4		
PRIMARY SAMPLE TYPE		SAMPLE COLLECTION DATE		CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for GJB2 c.109G>A, p.(Val37Ile), which is pathogenic.
The patient is heterozygous for GJB2 c.1A>T, p.(Met1?), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GJB2	NM_004004.6	c.109G>A, p.(Val37Ile)	HET	missense_variant	AD,AR	Pathogenic
	ID	ASSEMBLY	POS	REF/ALT		
	rs72474224	GRCh37/hg19	13:20763612	C/T		
	gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE	
	2132/282164	probably damaging	tolerated	disease causing	Bart-Pumphrey syndrome, Deafness, Hystrix-like ichthyosis with deafness, Keratitis-icthyosis-deafness syndrome, Keratoderma, palmoplantar, with deafness, Vohwinkel syndrome	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GJB2	NM_004004.6	c.1A>T, p.(Met1?)	HET	start_lost	AD,AR	Pathogenic
	ID	ASSEMBLY	POS	REF/ALT		
		GRCh37/hg19	13:20763720	T/A		

gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE
0/0	possibly damaging	deleterious	disease causing	Bart-Pumphrey syndrome, Deafness, Hystrix-like ichthyosis with deafness, Keratitis-icthyosis-deafness syndrome, Keratoderma, palmoplantar, with deafness, Vohwinkel syndrome

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Resonate Program Panel	251	4135	839683	837905	523	99.79

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	30171	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Resonate Program Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABHD12*, *ABHD5*, *ACOX1*, *ACTB**, *ACTG1**, *ADCY1*, *ADGRV1*, *AIFM1*, *ALMS1**, *AMMECR1*, *ANKH*, *ANLN*, *ARSG*, *ATP2B2*, *ATP6V0A4*, *ATP6V1B1*, *ATP6V1B2*, *BCS1L*, *BDP1**, *BSND*, *BTD*, *C10ORF2*, *CABP2*, *CACNA1D*, *CATSPER2**, *CCDC50*, *CD151*, *CD164*, *CDC14A*, *CDC42**, *CDH23*, *CDK9*, *CDKN1C*, *CEACAM16*, *CEP250*, *CEP78*, *CHD7*, *CHSY1*, *CIB2*, *CISD2**, *CLDN14*, *CLIC5*, *CLPP*, *CLRN1*, *COCH*, *COL11A1*, *COL11A2*, *COL2A1*, *COL4A3*, *COL4A4*, *COL4A5*, *COL4A6*, *COL9A1*, *COL9A2*, *COL9A3*, *CRYM*, *DCAF17*, *DCDC2*, *DFNA5*, *DFNB31*, *DFNB59*, *DIABLO*, *DIAPH1*, *DIAPH3*, *DLX5*, *DMXL2*, *DNMT1*, *DSPP*, *EDN3*, *EDNRA*, *EDNRB*, *EFTUD2*, *EIF3F*, *ELMOD3*, *EPS8*, *EPS8L2*, *ESPN**, *ESRRB*, *EYA1*, *EYA4*, *FAM136A**, *FAM65B*, *FDXR*, *FGF3*, *FGFR2*, *FGFR3*, *FITM2*, *FOXC1*, *FOXI1*, *GATA3*, *GDF6*, *GIPC3*, *GJA1**, *GJB2*, *GJB3*, *GJB6*, *GPSM2*, *GREB1L*, *GRHL2*, *GRXCR1*, *GRXCR2*, *HARS*, *HARS2*, *HGF*, *HOMER2*, *HOXA2*, *HOXB1*, *HSD17B4*, *ILDR1*, *KARS*, *KCNE1*, *KCNJ10*, *KCNQ1*, *KCNQ4*, *KIT*, *KITLG*, *KMT2D*, *LARS2*, *LHFPL5*, *LHX3*, *LMX1A*, *LOXHD1*, *LRP2*, *LRTOMT*, *MAN2B1*, *MANBA*, *MARVELD2*, *MASP1*, *MCM2*, *MET*, *MGP*, *MIR96*, *MITF*, *MPZL2*, *MSRB3*, *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*, *MYH14*, *MYH9*, *MYO15A*, *MYO3A*, *MYO6*, *MYO7A*, *NARS2*, *NDP*, *NDRG1*, *NEFL*, *NF2*, *NLRP3*, *NOG*, *NR2F1*, *OPA1*, *OSBPL2*, *OTOA# **, *OTOF*, *OTOG*, *OTOGL*, *P2RX2*, *PAX1*, *PAX3*, *PCDH15*, *PCGF2*, *PDE1C*, *PDZD7*[#], *PEX1*, *PEX11B*, *PEX12*, *PEX13*, *PEX14*, *PEX2*, *PEX26*, *PEX5*, *PEX6*, *PEX7*, *PHYH*, *PISD*, *PMP22*, *PNPT1**, *POLR1C*# , *POLR1D*, *POU3F4*, *POU4F3*, *PRPS1**, *PTPRQ*, *RAI1*, *RDX**, *REST*, *RMND1**, *ROR1*, *RPS6KA3*, *S1PR2*, *SALL1**, *SALL4*, *SEMA3E*, *SERAC1*, *SERPINB6*, *SH3TC2*, *SIX1*, *SIX2*, *SIX5*, *SLC17A8*, *SLC19A2*, *SLC22A4*, *SLC26A4*, *SLC26A5*, *SLC29A3*, *SLC33A1**, *SLC44A4*, *SLC4A11*, *SLC52A2*, *SLC52A3*, *SLITRK6*, *SMAD4*, *SMPX*, *SNAI2*, *SOX10*, *SPATA5*, *STAG2*, *STRC*# **, *SUCLA2*, *SUCLG1*, *SYNE4*, *SYT2**, *TBC1D24*, *TBL1X*, *TBX1*, *TCOF1*, *TECTA*, *TFAP2A*, *TIMM8A**, *TJP2*, *TMC1*, *TMEM126A*, *TMEM132E*, *TMIE*, *TMPRSS3*, *TNC*, *TPRN*# , *TRIOBP*, *TRMU*, *TRRAP*, *TSHZ1*, *TSPEAR*, *TUBB4B*, *TYR**, *UBR1*, *USH1C*, *USH1G*, *USH2A*, *VCAN*, *WBP2*, *WFS1* and *XYLT2*.
The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: OTOA (NM_144672:22-27), PDZD7 (NM_024895:10), POLR1C (NM_001318876:9) and STRC (NM_153700:1-18).
*Some, or all, of the gene is duplicated in the genome. Read more:

#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 4-year-old child with history of bilateral SNHL and chronic otitis media. The patient first underwent an ABR in 12/2017 after failing the newborn hearing screen twice, which showed slight to mild hearing loss in the right ear and mild hearing loss in the left ear at 8000 Hz. The patient underwent BMT in 5/2018 and 1/2020 for continued chronic OMs. Post-op audiogram on 2/10/2020 showed improved hearing within normal limits from 500-4000 Hz and mild hearing loss at 8000 Hz. Audiogram at the last ENT appointment on 9/30/2021 showed a mild to moderately severe mixed right-sided hearing loss from 500 to 6000 Hz rising to normal hearing at 8000 Hz. There is also a mild conductive hearing loss from 3000 to 6000 Hz in the left ear. CT/MR imaging: not done. No affected family members.

ICD-10-CM diagnosis codes: H91.90 (unspecified hearing loss, unspecified ear), H90.3 (sensorineural hearing loss, bilateral), F80.9 (developmental disorder of speech and language, unspecified), H65.493 (other chronic nonsuppurative otitis media, bilateral).

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Resonate Program Panel identified a heterozygous missense variant *GJB2* c.109G>A, p.(Val37Ile) and a heterozygous start lost variant *GJB2* c.1A>T, p.(Met1?). The NGS data indicates that these variants are on different parental chromosomes (*in trans*) in this patient.

GJB2 c.109G>A, p.(Val37Ile)

There are 1934 individuals heterozygous and 99 individuals homozygous 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 *GJB2* c.109G>A, p.(Val37Ile) variant, located in exon 2, has been observed with a particularly high allele frequency in East Asian population (gnomAD: MAF 8.3%). Most *in silico* tools utilized predict this variant to be damaging to protein structure and function. Despite of its high population frequency, *GJB2* c.109G>A p.(Val37Ile) is a well-known disease-causing variant with wide consensus on the pathogenicity. It has been identified in patients with autosomal recessive hearing loss in several studies (e.g. PMIDs 10633133, 22106692, 12121355, 24654934, 23873582, 19043807, 26061099, 17036313, 22574200, 27153395, 17935238, 10830906). The variant has also been detected by other laboratories in the context of clinical testing and submitted to ClinVar (variation ID 17023). The variant induces an inability to formation of homotypic gap junction channels, but is associated only with mild hearing loss (GeneReviews NBK1272). Homozygous knock-in mouse model of c.109G>A in *Gjb2* was reported to have progressive mild hearing loss, more pronounced at higher sound frequencies (PMID 27623246, 31160754). A comprehensive study (in 2016) of over 1000 patients with hearing loss found this variant in a homozygous state in 13 patients and in a compound heterozygous state in 14 patients (PMID 26969326). Majority of these patients had mild-to-moderate hearing loss, but three patients (two compound heterozygous and one homozygous) were regarded as having severe-to-profound hearing loss. However, mostly because of its population frequency, this variant has been previously classified as a benign polymorphism (PMID 12792423, 9529365) or as a hypomorphic allele (PMID 24645897). However, a comprehensive publication evaluating the data using American College of Medical Genetics and Genomics (ACMG) Guidelines-based variant classification scheme concluded that this variant is likely pathogenic and overrode the preliminary classification because of the extensive supporting literature (PMID 27153395). The ClinGen Hearing Loss Variant Curation Expert Panel recently classified the *GJB2* c.109G>A, p.(Val37Ile) variant as pathogenic based on ACMG Guidelines modified for hearing loss (PMID 30311386, 31160754; FDA-approved Database, ClinVar variation ID 17023). It was concluded that the *GJB2* c.109G>A, p.(Val37Ile) variant is one of the most common pathogenic variants in hearing loss, and is considered as a low penetrant variant based on the ClinGen Expert Panel's judgement (PMID 30311386, 31160754).

GJB2 c.1A>T, p.(Met1?)

This variant is absent 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 disrupts the canonical start codon of transcript NM_004004.6. This is predicted to lead to a failure of protein translation, or to result in an abnormal protein due to the use of an alternative start site. The next in-frame methionine is located at codon 34. The variant has been submitted to ClinVar by another clinical testing laboratory (variation ID 550716).

Different variants affecting the start codon, *GJB2* c.3G>T, p.(Met1?) and *GJB2* c.1A>G,p.(Met1?), have been described in patients with non-syndromic hearing loss (HGMD). The *GJB2* c.3G>T, p.(Met1?) variant was described together with a pathogenic *GJB2* variant, c.235del, p.(Leu79Cysfs*3), in one patient with non-syndromic hearing loss (PMID: 29605365); and *GJB2* c.1A>G, p.(Met1?) has been reported as disease-causing for autosomal recessive non-syndromic deafness in different studies, in which the variant was identified in homozygous or compound heterozygous state with another pathogenic *GJB2* variant (ClinVar variation ID 44729; e.g PMID: 9482292, 10218527, 16380907, 17666888, 18983339, 18941476). An *in vitro* functional study provided some evidence that the *GJB2* c.1A>G, p.(Met1?) variant might result in complete absence of protein (PMID: 12189493).

GJB2

The *GJB2* gene (MIM *121011) encodes a protein called gap junction beta 2 (widely known as connexin 26, CX26), which is a member of the gap junction protein family. The gap junctions are characterized as regionally specialized structures on plasma membranes of contacting adherent cells. These structures consist of cell-to-cell channels that facilitate the transfer of ions and small molecules between the cytoplasm of adjacent cells. The gap junction proteins are different in different tissues. The *GJB2* gene contains only 2 exons and exon 1 is untranslated (PMID 9358053). High levels of GJB2 protein expression have been observed in the cochlea (PMID 9139825) and both GJB2 and GJB6 (also known as CX30) are expressed in the same inner-ear structures and have been suggested to play a role in endolymph potassium recycling (PMID 10980526).

Pathogenic variants in *GJB2* cause both autosomal recessive (DFNB1A, MIM #220290) and autosomal dominant (DFNA3A, MIM #601544) forms of sensorineural non-syndromic hearing loss and deafness. Deafness is the most frequent form of sensorial deficit with between 1/1,000 and 1/700 children being born with profound or severe hearing loss (ORPHA87884). Of early-onset hearing loss cases, 60–80% are of genetic origin, and in 85% of cases, deafness is transmitted as an autosomal recessive trait. Approximately 50% of that is attributable to the *DFNB1* locus that harbors both *GJB2* and *GJB6*. The carrier rate in the general population for a recessive deafness-causing *GJB2* variant is approximately 1 in 33 (GeneReviews NBK1434). Only a small percentage of prelingual deafness is syndromic or non-syndromic autosomal dominant. Recessive non-syndromic hearing loss caused by biallelic *GJB2* variants has typically prelingual onset, and is often stable and severe-to-profound in degree, while dominant hearing loss caused by heterozygous *GJB2* variants is characterized by pre- or postlingual, mild-to-profound progressive high-frequency sensorineural hearing impairment (GeneReviews NBK1434, NBK1536). All disease-causing variants associated with autosomal dominant hearing loss in HGMD (Professional 2019.4) are missense variants. Also, a digenic pattern of inheritance of prelingual deafness at the *DFNB1* locus has been suggested (Deafness, digenic *GJB2*/*GJB6*, MIM #220290). Two large deletions, known as del(*GJB6*-D13S1830) and del(*GJB6*-D13S1854), are relatively common in some populations and several cases have been reported to have a pathogenic *GJB2* variant on one allele and a gross deletion of *GJB6* on the other allele (PMID 11807148, 11896458, 15994881, 15150777). However, these *GJB6* deletions likely involve a regulatory element of *GJB2* (PMID 19723508, 21738759, 14571368, 16380907). Thus, even though *GJB2/GJB6*-related hearing loss has causal variants in two genes, the inheritance pattern reflects autosomal recessive inheritance and the combination of a disease-causing variant in *GJB2* and a deletion in *GJB6* in *trans* can be considered as a compound heterozygous state (PMID 23695287).

In a comprehensive clinical genetic testing with targeted genomic enrichment and massively parallel sequencing on 1119 sequentially accrued patients, causative variants in *GJB2* were found in 95/440 (21.6%) of the patients diagnosed with non-syndromic hearing loss (PMID 26969326). Ninety-four out of all 95 cases showed recessive inheritance, while only 1/95 showed dominant inheritance model. The *GJB2* c.35del variant (previously also known as c.35delG and c.30delG) was particularly prevalent with 53% of patients having it in a homozygous or compound heterozygous state. Also, different missense variants are common. In a large study of 1531 patients with identified *GJB2* variants, the degree of hearing impairment associated with biallelic truncating variants was significantly more severe than the hearing impairment associated with biallelic nontruncating variants (PMID 16380907).

In addition to recessive and dominant non-syndromic hearing loss, pathogenic variants in *GJB2* cause various disorders with dominant inheritance model. These include Bart-Pumphrey syndrome (MIM #149200), hystrix-like ichthyosis with deafness syndrome (HID, MIM #602540), keratitis-ichthyosis-deafness (KIDAD, MIM #148210), palmoplantar keratoderma with

deafness (MIM #148350), and Vohwinkel syndrome (MIM #121011). Many of these diseases are characterized by hearing loss or deafness in addition to other varying clinical characteristics. Of note, ClinGen Hearing Loss Working Group has found no difference in molecular mechanism (dominant-negative mechanism of disease) and inheritance pattern for autosomal dominant non-syndromic hearing loss, keratitis-ichthyosis-deafness, and palmoplantar keratoderma with deafness. The expert group suggests that it is possible that these phenotypes exist on a spectrum of disease (The classification for "Definitive" gene-disease validity for AD disorders caused by *GJB2* has been approved by the ClinGen Hearing Loss Working Group on 6/26/2018).

There are currently 363 variants in *GJB2* listed as disease-causing (DM) in the HGMD Professional variant database (version 2021.4). The majority (approximately 62.5%) are missense alterations, 125 (34%) are small deletions/insertions, indels, splicing, and nonsense variants. In addition, there are 5 start-loss and regulatory variants, and 8 gross deletions/insertions.

Mutation nomenclature is based on GenBank accession NM_004004.6 (*GJB2*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

GJB2 c.109G>A, p.(Val37Ile) and *GJB2* c.1A>T, p.(Met1?) are both classified as pathogenic, based on currently available evidence supporting their disease-causing roles. It should be noted that the *GJB2* c.109G>A, p.(Val37Ile) missense variant is associated mainly with mild-to-moderate hearing loss and reduced penetrance. Disease caused by these variants is expected to be inherited in an autosomal recessive manner. Next-generation sequencing data strongly suggests that these variants are in trans (on different alleles) in this patient, which explains the patient's clinical presentation. Parental testing is recommended to confirm that these variants are parentally inherited, in which case any siblings of the patient will have a 25% chance of being compound heterozygous for these variants and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

Name

Title

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:

- BLUEPRINT GENETICS,INC, 2505 3RD AVE, SUITE 204, SEATTLE, WA 98121 Laboratory Director: PHD, FACMG, CLIA: 50D2140410
- QUEST DIAGNOSTICS NICHOLS INSTITUTE, 33608 ORTEGA HIGHWAY, SAN JUAN CAPISTRANO, CA 92690 Laboratory Director: MD, PHD, MBA, CLIA: 05D0643352
- BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: MD, PhD, CLIA: 99D2092375

REVIEWING DIRECTOR:

Laboratory Director

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_002241.4(*KCNJ10*):c.-1+1G>T, 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.486-14G>A, NM_206933.2(*USH2A*):c.-259G>T, NM_017433.4(*MYO3A*):c.1777-12G>A, NM_001142763.1(*PCDH15*):c.-29+1G>C, NM_018344.5(*SLC29A3*):c.*413G>A, chr10:g.123099960-123099960,

chr11:g.2484803-2484803, NM_000076.2(*CDKN1C*):c.*5+20G>T, NM_000260.3(*MYO7A*):c.-48A>G, NM_000260.3(*MYO7A*):c.3109-21G>A, NM_000260.3(*MYO7A*):c.5327-14T>G, NM_000260.3(*MYO7A*):c.5327-11A>G, NM_000260.3(*MYO7A*):c.5857-27_5857-26insTTGAG, NM_000372.4(*TYR*):c.1037-18T>G, NM_001844.4(*COL2A1*):c.1527+135G>A, NM_003482.3(*KMT2D*):c.10356-12G>A, NM_004004.5(*GJB2*):c.-22-2A>C, NM_004004.5(*GJB2*):c.-23+2T>A, NM_004004.5(*GJB2*):c.-23+1G>A, NM_004004.5(*GJB2*):c.-23G>T, NM_004004.5(*GJB2*):c.-259C>T, NM_004004.5(*GJB2*):c.-260C>T, NM_001042517.1(*DIAPH3*):c.-172G>A, NM_001042517.1(*DIAPH3*):c.-173C>T, NM_174916.2(*UBR1*):c.1911+14C>G, NM_174916.2(*UBR1*):c.1094-12A>G, NM_174916.2(*UBR1*):c.1094-13A>G, NM_174916.2(*UBR1*):c.529-13G>A, NM_000304.3(*PMP22*):c.79-13T>A, NM_025000.3(*DCAF17*):c.322-14delC, NM_004328.4(*BCS1L*):c.-147A>G, NM_004328.4(*BCS1L*):c.-50+155T>A, NM_181459.3(*PAX3*):c.958+28A>T, NM_000092.4(*COL4A4*):c.4334-23A>G, NM_000091.4(*COL4A3*):c.2224-11C>T, NM_000091.4(*COL4A3*):c.4028-27A>G, NM_000091.4(*COL4A3*):c.4462+457C>G, NM_000091.4(*COL4A3*):c.4463-18dupA, NM_001174090.1(*SLC4A11*):c.1077+26_1077+44delCGGCAGGGTCGGCGGGGGC, NM_000114.2(*EDN3*):c.-125G>A, NM_000114.2(*EDN3*):c.-19C>A, NM_080647.1(*TBX1*):c.-777C>T, NM_080647.1(*TBX1*):c.-620A>C, NM_000268.3(*NF2*):c.516+232G>A, NM_006941.3(*SOX10*):c.-84-2A>T, NM_006941.3(*SOX10*):c.-31954C>T, NM_006941.3(*SOX10*):c.-32520C>G, NM_000060.2(*BTD*):c.310-15delT, NM_000060.2(*BTD*):c.*159G>A, NM_001195794.1(*CLRN1*):c.254-649T>G, NM_130837.2(*OPA1*):c.449-34dupA, NM_130837.2(*OPA1*):c.2179-40G>C, NM_006005.3(*WFS1*):c.-43G>T, NM_001080476.2(*GRXCR1*):c.627+19A>T, NM_005612.4(*REST*):c.983-2247C>G, NM_054027.4(*ANKH*):c.-11C>T, NM_001199291.1(*HSD17B4*):c.1285-11C>G, NM_024577.3(*SH3TC2*):c.2873-14T>A, NM_024577.3(*SH3TC2*):c.386-15G>A, NM_001453.2(*FOXC1*):c.-429C>G, NM_000287.3(*PEX6*):c.2301-15C>G, NM_000287.3(*PEX6*):c.2300+28G>A, NM_004999.3(*MYO6*):c.2417-1758T>G, NM_004100.4(*EYA4*):c.1282-12T>A, NM_004100.4(*EYA4*):c.1341-19T>A, NM_000288.3(*PEX7*):c.-45C>T, NM_032861.3(*SERAC1*):c.92-165C>T, NM_032861.3(*SERAC1*):c.92-239G>C, NM_004403.2(*DFNA5*):c.991-15_991-13delTTC, NM_000601.4(*HGF*):c.482+1991_482+2000delGATGATGAAA, NM_000601.4(*HGF*):c.482+1986_482+1988delTGA, NM_000441.1(*SLC26A4*):c.-103T>C, NM_000441.1(*SLC26A4*):c.-60A>G, NM_000441.1(*SLC26A4*):c.-4+1G>C, NM_000441.1(*SLC26A4*):c.-4+5G>A, NM_000441.1(*SLC26A4*):c.918+45_918+47delCAA, NM_000441.1(*SLC26A4*):c.1150-35_1150-28delTTTGTAGG, NM_000441.1(*SLC26A4*):c.1264-12T>A, NM_000441.1(*SLC26A4*):c.1438-7dupT, NM_000441.1(*SLC26A4*):c.1708-27_1708-11delTAAGTAACTTGACATTT, NM_000441.1(*SLC26A4*):c.2090-52_2090-49delCAAA, NM_003068.4(*SNAI2*):c.-149_-148delCGinsTA, NM_017780.3(*CHD7*):c.2836-15C>G, NM_017780.3(*CHD7*):c.5051-15T>A, NM_017780.3(*CHD7*):c.5405-18C>A, NM_017780.3(*CHD7*):c.5405-17G>A, NM_017780.3(*CHD7*):c.5405-13G>A, NM_000503.4(*EYA1*):c.1051-12T>G, NM_000503.4(*EYA1*):c.640-15G>A, NM_024915.3(*GRHL2*):c.20+133delA, NM_024915.3(*GRHL2*):c.20+257delT, NM_024915.3(*GRHL2*):c.20+544G>T, NM_024531.4(*SLC52A2*):c.-110-1G>A, NM_138691.2(*TMC1*):c.362+18A>G, NM_004586.2(*RPS6KA3*):c.1228-279T>G, NM_004586.2(*RPS6KA3*):c.326-11A>G, 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_000266.3(*NDP*):c.-343A>G, NM_000266.3(*NDP*):c.-391_-380delCTCTCTCTCCCTinsGTCTCTC, NM_000266.3(*NDP*):c.-396_-383delTCCCTCTCTCTCTC, NM_004085.3(*TIMM8A*):c.133-23A>C, NM_033380.2(*COL4A5*):c.385-719G>A, NM_033380.2(*COL4A5*):c.466-12G>A, NM_033380.2(*COL4A5*):c.609+875G>T, NM_033380.2(*COL4A5*):c.646-12_646-11delTT, NM_033380.2(*COL4A5*):c.1423+57dupC, NM_033380.2(*COL4A5*):c.1424-20T>A, NM_033380.2(*COL4A5*):c.1948+894C>G, NM_033380.2(*COL4A5*):c.2042-18A>G, NM_033380.2(*COL4A5*):c.2245-40A>G, NM_033380.2(*COL4A5*):c.2245-14T>A, NM_033380.2(*COL4A5*):c.2395+2750A>G, NM_033380.2(*COL4A5*):c.3374-11C>A, NM_033380.2(*COL4A5*):c.4529-2300T>G, NM_033380.2(*COL4A5*):c.4529-345A>G, NM_033380.2(*COL4A5*):c.4821+121T>C, NM_033380.2(*COL4A5*):c.4822-152dupT, NM_033380.2(*COL4A5*):c.4822-151_4822-150insT, NM_004208.3(*AIFM1*):c.697-44T>G, NM_004208.3(*AIFM1*):c.-123G>C

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.