Blueprint Genetics

Congenital Structural Heart Disease Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
Unknown

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**
DNA

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *KMT2D* c.10342C>T, p.(Pro3448Ser), which is a variant of uncertain significance (VUS).

Del/Dup (CNV) analysis

Negative for explaining the patient's phenotype.

ADDITIONAL FINDINGS

The patient is heterozygous for *PKD1L1* c.5553-1G>A, which is likely pathogenic.

Please see APPENDIX 2: Additional Findings for further details

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
KMT2D	NM_003482.3	c.10342C>T, p.(Pro3448Ser)	HET	missense_variant	AD	**Variant of uncertain significance**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	12:49428608	G/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	1/249254	possibly damaging	deleterious	disease causing	Kabuki syndrome	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Congenital Structural Heart Disease Panel	114	1948	427191	426750	260	99.9

TARGET REGION AND GENE LIST

The Blueprint Genetics Congenital Structural Heart Disease Panel (version 4, Oct 19, 2019) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ABL1, ACTA2, ACTB*, ACTC1, ACTG1*, ACVR1, ACVR2B, ADAMTS10, ADAMTS17, AFF4, AMMECR1, ARHGAP31, ARID1A, ARID1B, B3GAT3*,#, BCOR, BMPR2, BRAF*, C12ORF57, CBL, CDK13, CDK9, CFAP53, CHD4, CHD7, CHRM2, CREBBP, CRELD1, CTC1, DHCR7, DLL4, DOCK6, EFTUD2, EHMT1, EIF2AK4, ELN, ENG, EOGT, EP300, EVC, EVC2, FLNA, FOXC1, FOXF1, FOXH1, FOXP1, GATA4*, GATA5, GATA6, GDF1, GJA1*, GJA5, GPC3, HAND1, HAND2, HDAC8, HNRNPK*, HOXA1, HRAS, JAG1, KDM6A, KMT2D, KRAS*, KYNU, LEFTY2*, MED12, MED13L, MEIS2, MMP21, MYCN, MYO18B, MYRF, NAA15, NF1*, NIPBL, NKX2-5, NKX2-6, NODAL, NONO, NOTCH1, NOTCH2*, NR2F2, NSD1, PITX2, PKD1L1, PPP1CB, PRDM6, PRKD1, PTPN11, PUF60, RAB23, RAF1, RBM10, RERE*, RIT1, SALL4, SMARCB1, SMC1A, SMC3, SOS1, SOS2, STAG2, STRA6, TAB2, TBX1, TBX20*, TBX5, TFAP2B, TGDS, TLL1, TMEM94, ZEB2*, ZFPM2 and ZIC3. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: B3GAT3 (NM_001288722:5). 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) 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

This tested prenatal sample is the DNA extracted from the amniocytes of a 35-year-old female. The fetus has a complex congenital heart defect: pulmonary artery and pulmonary valve stenosis; tricuspid atresia; ventricular septal defect; and ductal and aortic arch anomalies. Maternal cell contamination testing has been carried out by the customer. The results of the analysis show no signs of maternal contamination.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Congenital Structural Heart Disease Panel identified a heterozygous missense variant *KMT2D* c.10342C>T, p.(Pro3448Ser).

KMT2D c.10342C>T, p.(Pro3448Ser)

There is 1 individual heterozygous for this variant 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. Moreover, there is another missense variant, p.(Pro3448Thr) at this position in the gnomAD. Both of the carriers of these variants existing in the reference population are heterozygous that suggest that these variants may be tolerated. However, all *in silico* tools utilized predict this variant to be damaging to protein structure and function. The affected amino acid is moderately conserved in mammals as well as in evolutionarily more distant species, which suggests that this position may not tolerate variation. There is a moderate physicochemical difference between proline and serine, therefore this is considered a non-conservative substitution (Grantham score = 74). To the best of our knowledge, this variant has not been described in the medical literature or reported in disease-related variation databases such as ClinVar or HGMD.

KMT2D

KMT2D (MIM *602113) gene encodes a histone methyltransferase that methylates the Lys-4 position of histone H3. The protein is part of a large protein complex called ASCOM, which has been shown to be a transcriptional regulator of the beta-globin and estrogen receptor genes. Heterozygous pathogenic variants in this gene have been shown to cause Kabuki syndrome (KS; MIM #147920). KS is a multiple congenital anomaly syndrome characterized by typical facial features, skeletal anomalies, mild to moderate intellectual disability and postnatal growth deficiency (ORPHA:2322). Individuals with KS often (70%) have congenital heart defects, coarctation of aorta being the most common, frequent ear infections, hearing loss and early puberty (GeneReviews NBK62111). The diagnosis of KS is established in a proband of any age with a history of infantile hypotonia, developmental delay, and/or intellectual disability AND one or both of the following 1) Typical dysmorphic features (long palpebral fissures with eversion of the lateral third of the lower eyelid, and ≥2 of the following: arched and broad eyebrows with the lateral third displaying notching or sparseness; short columella with depressed nasal tip; large, prominent, or cupped ears; persistent fingertip pads) or 2) a heterozygous pathogenic variant in *KMT2D* or a heterozygous or hemizygous pathogenic variant in *KDM6A.* KS was initially described in Japan, but has now been observed in all ethnic groups. Prevalence of KS is estimated to be 1:32,000. The inheritance pattern of KS is autosomal dominant. Majority of the KS cases are associated with mutations in the *KMT2D* gene. Deletions in the *KDM6A* (MIM *300128) gene have also been reported in few cases. In a study of 347 patients with clinical diagnosis of KS, 208 mutations were identified in the *KMT2D* gene (60%) and 12 in the *KDM6A* gene (3%) while 37% of the cases could not be molecularly confirmed (PMID: 27302555).

The proportion of KS caused by *de novo* variants is unknown but it is likely to be high based on clinical experience. Human Gene Mutation Database (HGMD Professional 2019.4) reports >600 disease-causing variants in the *KMT2D* gene. These include small deletions, insertions and insertion-deletions (44%) most of those causing frameshift, nonsense variants (31%), missense variants (15%), splice site variants (9%) gross deletions (1%), gross insertions (0.7%) and more complex rearrangements (0.3%). Vast majority of Kabuki associated variants cause loss of gene function. Patients with whole-gene deletion of *KMT2D* or pathogenic

truncating variants that occur in the first half of the gene may have more severe intellectual disability (PMID: 28295206).

Mutation nomenclature is based on GenBank accession NM_003482.3 (*KMT2D*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

KMT2D c.10342C>T, p.(Pro3448Ser) is classified as a variant of uncertain significance (VUS), as there is insufficient evidence to evaluate its clinical relevance. This variant should not be used for clinical decision-making or risk evaluation in family members. Management of the patient and family should be based on clinical evaluation and judgment. Genetic counselling is recommended.

The identified *KMT2D* c.10342C>T, p.(Pro3448Ser) variant is not eligible for the VUS Clarification Service at this time as family member testing is not sufficient to result in reclassification to likely pathogenic (please refer to our variant classification schemes on our website for additional information). The BpG VUS Clarification Service is offered when testing additional family members is likely to result in reclassification of the variant to likely pathogenic. Testing of the VUS in family members is available as part of our Familial Variant Testing service.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On Jun 9, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Nelli Paasikoski, MSc

Geneticist

Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

APPENDIX 2: ADDITIONAL FINDINGS

This table includes variants that:

1. are not thought to be the likely cause for, or sufficient to cause the patient's phenotype
    a. a single variant (pathogenic, likely pathogenic or variant of uncertain significance) in a gene that causes an autosomal recessive or X-linked recessive disorder
2. are findings potentially relevant to the patient's medical care
    a. risk variants identified in genes included on the panel
    b. potentially disease-causing variants for an autosomal dominant disorder not related to patient's current phenotype
3. indicate carrier status for pathogenic or likely pathogenic variants in a gene that causes an autosomal recessive or X-linked disorder not suspected in the patient

ADDITIONAL FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKD1L1	NM_138295.3	c.5553-1G>A	HET	splice_acceptor_variant	AR	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	7:47879261	C/T		
					PHENOTYPE	
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	Heterotaxy, visceral, 8, autosomal	
	0/0	N/A	N/A	disease causing		

NOTES REGARDING ADDITIONAL FINDINGS

The patient is heterozygous for a likely pathogenic variant *PKD1L1* c.5553-1G>A. Disease caused by *PKD1L1* variants is inherited in an autosomal recessive manner, and no second rare or potentially disease-causing variant in *PKD1L1* was detected in the patient. Therefore, this heterozygous variant is not expected to be related to the patient's clinical presentation. However, as the patient is a carrier of a likely pathogenic variant for recessive disease, genetic counselling is recommended.

PKD1L1

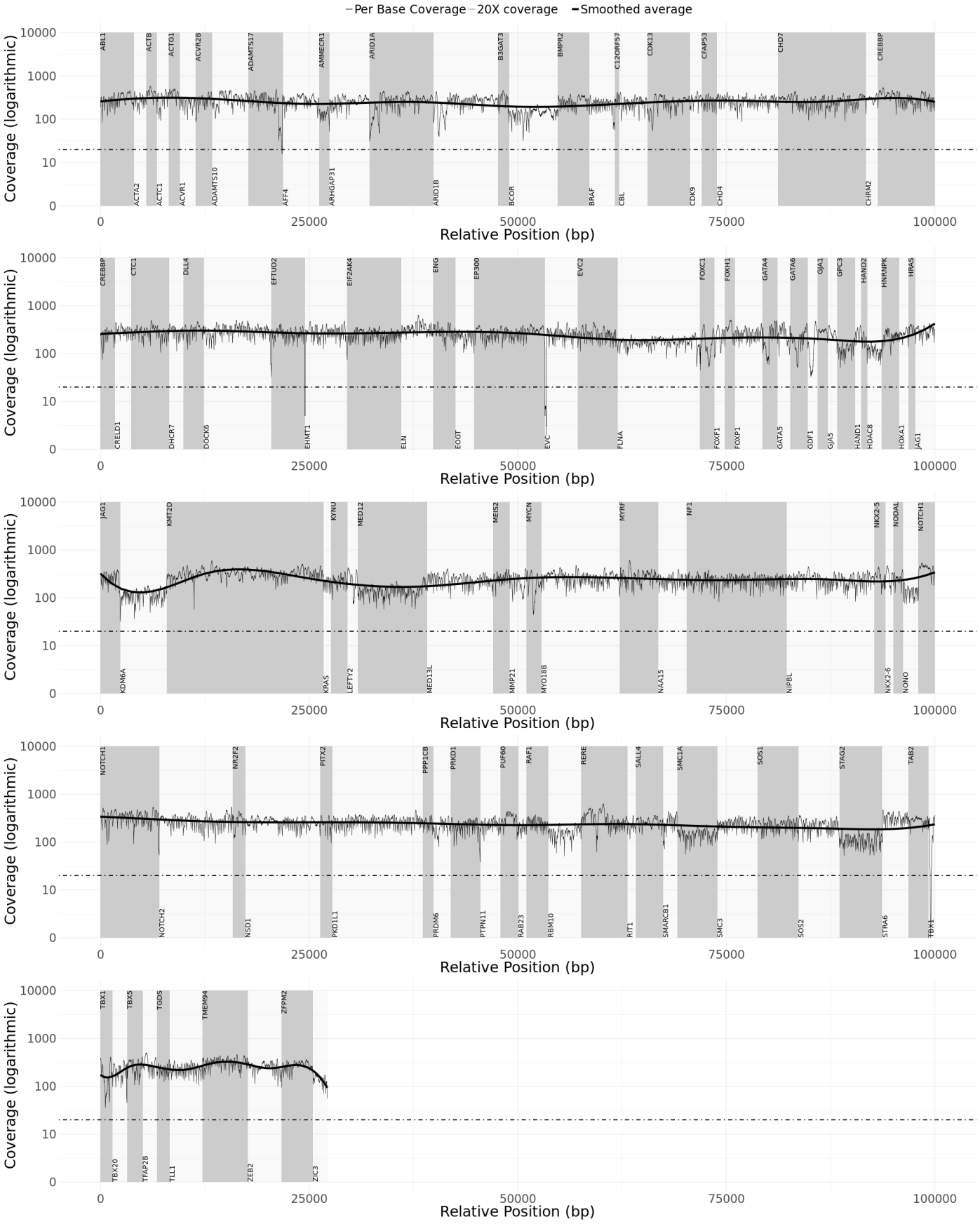
PKD1L1 on chromosome 7p12.3 encodes a Polycystic kidney disease protein 1-like 1 (polycystin 1-like 1), a member of the polycystin protein family (OMIM #609721). The PKD1L1 protein contains 11 transmembrane domains, 2 Ig-like PKD domains, a small REJ domain, a GPS domain, an LH2/PLAT domain, and a coiled-coil domain (Uniprot Q8TDX9). In embryo primitive node, the PKD1L1 protein is involved in sensation and response to nodal flow, which is required for the establishment of left-right asymmetry, by inhibiting NODAL signaling to the left lateral plate mesoderm (PMID 21307093, 27272319). The encoded protein acts by interacting with PKD2 to form a calcium-permeant ciliary channel (PMID 27272319). It is also important for regulation of sonic hedgehog/SHH signaling and GLI2 transcription (PMID 24336288, 21307093). The importance of the gene for left-right determination has been shown in the *Pkd1l1* mouse model (PMID 21307093). In Pkd1l1-/- mice, the absence of Pkd1l1 results in reduced viability during development or early postnatal. The surviving mice showed situs inversus but were otherwise normal (PMID 20080492). Kamura et al. reported a pkd1l1 nonsense mutation in medaka fish and noticed an altered asymmetrical expression of gene involved in left-right patterning and concluded that PKD1L1-PKD2 complex is involved in left-right patterning (PMID 21307098).

Homozygous or compound heterozygous pathogenic variants in *PKD1L1*, are reported to cause autosomal visceral heterotaxy type 8 (HTX8), a rare sub-type of HTX, characterized by situs inversus (SI) of visceral organs, associated with complex congenital heart defects, which is inherited in an autosomal recessive manner (OMIM #617205, PMID 31026592, 27616478). The typical congenital cardiac manifestations include R or L atrial isomerism, double outlet R ventricle (DORV) with malposed great arteries,

ventricular size discordance (VSD), left ventricular outflow obstruction, pulmonary stenosis, left ventricular hypoplasia, left-sided obstructive lesions. Both types of cardiac positions including dextrocardia and levocardia have been observed (PMID 31026592, 27616478, 28991257). Moreover, L-bronchial isomerism is also commonly reported. Most of the patients exhibit abdominal heterotaxic features such as R-sided stomach, L-sided liver; small, midline gall bladder associated with biliary tract abnormalities; polysplenia, and intestinal atresia.

Vetrini et al. reported two HTX8 patients with homozygous splice site mutation (c.6473+2_6473+3delTG) and a patient with SIT and congenital heart disease with a missense mutation (p.(Cys1691Ser)) in *PKD1L1* (PMID 27616478). In addition, two fetuses of non-consanguineous couple, were reported to be compound heterozygous for (p.Gln1600AspfsTer4) and (p.Arg1347Ter) variants, that were associated with HTX8, with some unique heart anomalies such as antenatal fetal bradyarrhythmia with complete heart block, bilateral superior vena cava (SVC), R-sided aorta, co-arctation of the aorta (CoA), and large atrioventricular septal defect (AVSD); extracardiac defects such as bi-lobed lungs, a central liver, intestinal malrotation, duodenal atresia. Facial dysmorphisms in one of the fetus included low-set, posteriorly rotated ears (PMID 31026592). A patient with situs inversus, CHD, and hematological abnormalities were reported to have a homozygous nonsense mutation (p.(Arg2269*)) in PKD1L1 (PMID 30791085). Few cases with bilateral atresia splenic syndrome carrying pathogenic missense or nonsense variants have also been reported (PMID 30664273). Currently there are 16 variants in the HGMD database (August 2019), five nonsense, nine missense, a splice site and a deletion mutation. The Clinvar database lists only one pathogenic deletion in *PKD1L1*. The pLI of *PKD1L1* is 1 suggesting that heterozygous loss of function is not tolerated. The overall prevalence of heterotaxy /situs ambiguus spectrum is estimated to be around 1/10,000 individuals (PMID 25099286). For patients with suggestive clinical findings, the diagnosis is based on molecular genetic testing. The treatment can be symptomatic. However, a female patient with HTX8 was subjected to the placement of left ventricle to pulmonary artery conduit and ventricular septal defect closure. She has a paroxysmal atrial flutter and a dual-chamber pacemaker (PMID 27616478).

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.



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. 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.
Bioinformatics and quality control: Base called raw sequencing data were 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 were 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 were adjusted to account for the effects of varying guanine and cytosine content.
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.
Variant classification: Our variant classification follows the Blueprint Genetics 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.
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.
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.

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.

Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. 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 (excluding mtDNA testing and digital PCR confirmation).

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_005159.4(ACTC1):c.*1784T>C
NM_001204.6(BMPR2):c.-947_-946delGCinsAT
NM_001204.6(BMPR2):c.-347C>T
NM_001204.6(BMPR2):c.-279C>A
NM_001204.6(BMPR2):c.-92C>A
NM_001204.6(BMPR2):c.968-12T>G
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_004380.2(CREBBP):c.4281-11C>G
NM_024757.4(EHMT1):c.2382+1697T>G
NM_001278939.1(ELN):c.2272+20C>G
NM_001114753.2(ENG):c.1742-22T>C
NM_001114753.2(ENG):c.361-11T>A
NM_001114753.2(ENG):c.-58G>A
NM_001114753.2(ENG):c.-127C>T
NM_001114753.2(ENG):c.-142A>T
NM_001429.3(EP300):c.1879-12A>G
NM_153717.2(EVC):c.940-150T>G
NM_001110556.1(FLNA):c.6023-27_6023-16delTGACTGACAGCC
NM_001453.2(FOXC1):c.-429C>G
NM_002052.3(GATA4):c.-989C>T
NM_002052.3(GATA4):c.-902G>T
chr8:g.11561399-11561399

NM_002052.3(GATA4):c.910-55T>C
NM_002052.3(GATA4):c.997+103G>T
NM_002052.3(GATA4):c.998-26G>A
NM_080473.4(GATA5):c.-201A>G
chr20:g.61051462-61051462
NM_005257.4(GATA6):c.-530A>T
NM_005257.4(GATA6):c.-409C>G
NM_002140.3(HNRNPK):c.214-35A>G
NM_000214.2(JAG1):c.1349-12T>G
NM_003482.3(KMT2D):c.10356-12G>A
NM_001042492.2(NF1):c.-273A>C
NM_001042492.2(NF1):c.-272G>A
NM_001042492.2(NF1):c.60+9031_60+9035delAAGTT
NM_001042492.2(NF1):c.61-7486G>T
NM_001042492.2(NF1):c.288+2025T>G
NM_001042492.2(NF1):c.587-14T>A
NM_001042492.2(NF1):c.587-12T>A
NM_001042492.2(NF1):c.888+651T>A
NM_001042492.2(NF1):c.888+744A>G
NM_001042492.2(NF1):c.888+789A>G
NM_001042492.2(NF1):c.889-12T>A
NM_001042492.2(NF1):c.1260+1604A>G
NM_001042492.2(NF1):c.1261-19G>A
NM_001042492.2(NF1):c.1392+754T>G
NM_001042492.2(NF1):c.1393-592A>G
NM_001042492.2(NF1):c.1527+1159C>T
NM_001042492.2(NF1):c.1642-449A>G
NM_001128147.2(NF1):c.*481A>G
NM_001042492.2(NF1):c.2002-14C>G
NM_001042492.2(NF1):c.2252-11T>G
NM_001042492.2(NF1):c.2410-18C>G
NM_001042492.2(NF1):c.2410-16A>G
NM_001042492.2(NF1):c.2410-15A>G
NM_001042492.2(NF1):c.2410-12T>G
NM_001042492.2(NF1):c.2851-14_2851-13insA
NM_001042492.2(NF1):c.2991-11T>G
NM_001042492.2(NF1):c.3198-314G>A
NM_001042492.2(NF1):c.3974+260T>G
NM_001042492.2(NF1):c.4110+945A>G
NM_001042492.2(NF1):c.4173+278A>G
NM_001042492.2(NF1):c.4578-20_4578-18delAAG
NM_001042492.2(NF1):c.4578-14T>G
NM_001042492.2(NF1):c.5269-38A>G
NM_001042492.2(NF1):c.5610-456G>T
NM_001042492.2(NF1):c.5812+332A>G
NM_001042492.2(NF1):c.5813-279A>G
NM_001042492.2(NF1):c.6428-11T>G
NM_001042492.2(NF1):c.6642+18A>G
NM_001042492.2(NF1):c.7190-12T>A
NM_001042492.2(NF1):c.7190-11_7190-10insGTTT
NM_001042492.2(NF1):c.7971-321C>G

NM_001042492.2(NF1):c.7971-17C>G
NM_001042492.2(NF1):c.8113+25A>T
NM_133433.3(NIPBL):c.-321_-320delCCinsA
NM_133433.3(NIPBL):c.-94C>T
NM_133433.3(NIPBL):c.-79-2A>G
NM_133433.3(NIPBL):c.5329-15A>G
NM_133433.3(NIPBL):c.5710-13_5710-12delCTinsAA
chr5:g.172662741-172662741
chr5:g.172672291-172672291
chr5:g.172672303-172672303
NM_001145155.1(NR2F2):c.-60C>T
NM_000325.5(PITX2):c.*520_*522delTAT
NM_000325.5(PITX2):c.412-11A>G
NM_153426.2(PITX2):c.-1214_-1213delAT
NM_002834.3(PTPN11):c.934-59T>A
NM_003073.3(SMARCB1):c.93+559A>G
NM_003073.3(SMARCB1):c.1119-12C>G
NM_003073.3(SMARCB1):c.*70C>T
NM_003073.3(SMARCB1):c.*82C>T
NM_080647.1(TBX1):c.-777C>T
NM_080647.1(TBX1):c.-620A>C
NM_001077653.2(TBX20):c.-549G>A
NM_000192.3(TBX5):c.*88822C>A
NM_014795.3(ZEB2):c.-69-1G>A
NM_014795.3(ZEB2):c.-69-2A>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.