

# Comprehensive Skeletal Dysplasias and Disorders Panel Plus

## REFERRING HEALTHCARE PROFESSIONAL

NAME                      HOSPITAL

## PATIENT

NAME                      DOB                      AGE                      GENDER                      ORDER ID

6

PRIMARY SAMPLE TYPE                      SAMPLE COLLECTION DATE                      CUSTOMER SAMPLE ID

## SUMMARY OF RESULTS

### PRIMARY FINDINGS

The patient is heterozygous for *TRPV4* c.2396C>T, p.(Pro799Leu), which is classified as pathogenic.

### Del/Dup (CNV) analysis

Negative for explaining the patient’s phenotype.

### PRIMARY FINDINGS: SEQUENCE ALTERATIONS

| GENE         | TRANSCRIPT   | NOMENCLATURE             | GENOTYPE     | CONSEQUENCE      | INHERITANCE   | CLASSIFICATION    |
|--------------|--------------|--------------------------|--------------|------------------|---|-------------------|
| <b>TRPV4</b> | NM_021625.4  | c.2396C>T, p.(Pro799Leu) | HET          | missense_variant | AD  | <b>Pathogenic</b> |
|              | ID           | ASSEMBLY                 | POS          | REF/ALT          |   |                   |
|              |              | GRCh37/hg19              | 12:110222183 | G/A              |   |                   |
|              | gnomAD AC/AN | POLYPHEN                 | SIFT         | MUTTASTER        | <b>PHENOTYPE</b><br>Brachyolmia (autosomal dominant type),<br>Charcot-Marie-Tooth disease,<br>Familial Digital arthropathy with brachydactyly,<br>Hereditary motor and sensory neuropathy,<br>Metatropic dysplasia,<br>Parastremmatic dwarfism,<br>Spinal muscular atrophy,<br>Spondyloepiphyseal dysplasia Maroteaux type,<br>Spondylometaphyseal dysplasia Kozlowski type |                   |
|              | 0/0          | possibly damaging        | deleterious  | disease causing  |   |                   |

## SEQUENCING PERFORMANCE METRICS

| PANEL   | GENES | EXONS / REGIONS | BASES  | BASES > 20X | MEDIAN COVERAGE | PERCENT > 20X |
|---|-------|-----------------|--------|-------------|-----------------|---------------|
| Comprehensive Skeletal Dysplasias and Disorders Panel | 252   | 4053            | 816901 | 812241      | 199             | 99.43         |

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## TARGET REGION AND GENE LIST

The Blueprint Genetics Comprehensive Skeletal Dysplasias and Disorders Panel (version 4, Oct 19, 2019) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ACAN#, ACP5, ACVR1, ADAMTS10, ADAMTS17, ADAMTSL2\*,#, AGPS, AIFM1, AKT1, ALPL, ALX3, ALX4, AMER1, ANKH, ANKRD11\*, ANO5, ARHGAP31, ARSB, ARSE\*, ATP6V0A2, B3GALT6#, B3GAT3\*,#, B4GALT7, BGN, BHLHA9, BMP1, BMP2, BMPER, BMPR1B, CA2, CANT1, CASR, CDC45, CDC6, CDKN1C, CDT1, CHST14, CHST3, CHSY1, CKAP2L, CLCN5, CLCN7, COL10A1, COL11A1, COL11A2, COL1A1, COL1A2, COL27A1, COL2A1, COL9A1, COL9A2, COL9A3, COMP, CREBBP, CRLF1, CRTAP, CSPP1, CTSK, CUL7, CYP27B1, DDR2, DHCR24, DLL3, DLL4, DLX3, DMP1, DOCK6, DVL1, DYM, DYNC2H1, EBP, EFNB1, EFTUD2, EIF2AK3, ENAM, ENPP1, EOGT, EP300, ESCO2, EVC, EVC2, EXT1, EXT2, EXTL3, EZH2, FAM111A, FAM20A, FAM20C, FAM83H, FANCB, FANCC, FBN1, FBN2, FGF23, FGFR1, FGFR2, FGFR3, FKBP10, FLNA, FLNB, GALNT3, GDF5, GJA1\*, GLI3, GNAS, GNPAT, GPC6, HDAC8, HOXA13#, HOXD13, HSPG2, IDS\*, IDUA, IFT122\*, IFT140, IFT172, IFT43, IFT80, IHH, IMPAD1, INPPL1, KAT6B, KIF22, KIF7, KMT2A, LBR, LEMD3, LIFR, LMNA, LMX1B, LONP1, LRP4, LRP5\*, LTBP2, LTBP3, MAFB, MATN3, MESP2, MGP, MMP13, MMP2, MMP9, MSX2\*, MYCN, MYO18B, NANS, NEK1, NF1\*, NFIX, NIPBL, NKX3-2, NOG, NOTCH2\*, NPR2, NSD1, NSDHL, OBSL1, ORC1, ORC4, ORC6, OSTM1, P3H1, PAPSS2, PCNT, PCYT1A, PDE4D, PEX7, PGM3, PHEX, PIK3CA\*, PISD, PLOD2, PLS3, POLR1C#, POLR1D, POR, PPIB, PRKAR1A, PTDSS1, PTH1R, PTHLH, PTPN11, PYCR1, RAB33B, RAD21\*, RBPJ\*, RECQL4, RMRP, RNU4ATAC, ROR2, RUNX2, SBDS\*, SERPINF1, SERPINH1, SETBP1, SF3B4, SH3BP2, SH3PXD2B, SHOX\*,#, SKI, SLC26A2, SLC29A3, SLC34A3, SLC35D1, SLC39A13, SLCO2A1, SMAD3, SMAD4, SMARCA1, SMC1A, SMC3, SNX10, SOST, SOX9, SP7, STAMBP, TBX15, TBX3, TBX4, TBX6, TCF12, TCIRG1, TCOF1, TCTN3, TGFB1, TGFB2, TGFB3, TGFB3R1, TGFB3R2, TMEM38B, TNFRSF11A, TNFRSF11B, TNFSF11, TP63, TRAPPC2\*, TRIP11\*, TRPS1, TRPV4, TTC21B, TWIST1, TYROBP, VDR, VIPAS39, WDR19, WDR34, WDR35, WDR60, WISP3, WNT1, WNT5A and XYLT1. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: ADAMTSL2 (NM\_014694:11-19), B3GAT3 (NM\_001288722:5), POLR1C (NM\_001318876:9) and SHOX (NM\_006883:6). This panel targets protein coding exons, exon-intron boundaries ( $\pm$  20 bps) and selected noncoding, 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 (#).

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## STATEMENT

### CLINICAL HISTORY

Patient is a 6-year-old child with suspected spondyloepiphyseal chondrodysplasia or pseudoachondroplasia.

### CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Comprehensive Skeletal Dysplasias and Disorders Panel identified a heterozygous missense variant *TRPV4* c.2396C>T, p.(Pro799Leu).

#### ***TRPV4* c.2396C>T, p.(Pro799Leu)**

This variant is absent in the Genome Aggregation Database control population cohorts ([gnomAD](#), n>120,000 exomes and >15,000 genomes). The affected amino acid is highly conserved in mammals as well as in evolutionarily more distant species, which suggests that this position does not tolerate variation. All in silico tools utilized predict this variant to be damaging to protein structure and function. Pro799 is the most commonly mutated codon in *TRPV4*-related metatropic dysplasia (MD) with four different amino acid substitutions reported (PMID: [21658220](#), [20577006](#)). The p.(Pro799Leu) variant has been reported in multiple patients with MD, including also patients with a de novo mutation (PMID: [19232556](#), [20425821](#), [20577006](#), [21658220](#), [31808622](#)). The p.(Pro799Leu) variant has been also reported in patients with Spondylo-epiphyseal dysplasia (SED), Maroteaux type (PMID: [20503319](#)). The variant has been detected by several other laboratories in the context of clinical testing and submitted to ClinVar (variation ID [4998](#)). Experimental studies have demonstrated that the Pro799Leu change results in constitutive activation of the *TRPV4* protein channel (PMID: [20425821](#), [21573172](#), [26170305](#)).

#### ***TRPV4***

The *TRPV4* (MIN \*[605427](#)) gene encodes a member of the OSM9-like transient receptor potential channel (OTRPC) subfamily in the transient receptor potential (TRP) superfamily of ion channels. The encoded protein is a Ca<sup>2+</sup>-permeable, nonselective cation channel that is thought to be involved in the regulation of systemic osmotic pressure. Pathogenic variants in the *TRPV4* gene are the cause of autosomal dominant *TRPV4*-associated disorders (GeneReviews [NBK201366](#)) that are grouped into neuromuscular disorders and skeletal dysplasia. Neuromuscular disorders from mildest to most severe include: Charcot-Marie-Tooth disease type 2C (CMT2C, also known as Hereditary motor and sensory neuropathy, type IIc, MIM #[606071](#)), Scapuloperoneal spinal muscular atrophy (SPSMA, MIM #[181405](#)) and congenital distal spinal muscular atrophy (CDSMA, MIM #[600175](#)). The neuromuscular disorders are characterized by a progressive peripheral neuropathy with variable combinations of laryngeal dysfunction (i.e. vocal fold paresis), respiratory dysfunction, and joint contractures. The skeletal dysplasia associated with *TRPV4* include: Familial digital arthropathy-brachydactyly (mildest) (MIM #[606835](#)), Autosomal dominant brachyolmia (MIM #[113500](#)), SED, Maroteaux type (MIM #[184095](#)) Spondylometaphyseal dysplasia, Kozlowski type (intermediate) (SMDK, MIM #[184252](#)), Parastremmatic dysplasia (MIM #[168400](#)) and Metatropic dysplasia (most severe) (MIM #[156530](#)). The skeletal dysplasia are characterized by brachydactyly. Patients with intermediate and severe skeletal dysplasia have short stature that varies from mild to severe with progressive spinal deformity and involvement of the long bones and pelvis. In the mildest of the *TRPV4*-associated disorders life span is normal, while in the most severe it is shortened. In general, specific sets of *TRPV4* pathogenic variants have been associated with either neuromuscular disorders or skeletal dysplasia. However, making precise genotype-phenotype correlations is challenging due to considerable overlap between the phenotypes. In the surveillance of *TRPV4*-related bone dysplasias, it is important to obtain flexion/extension cervical spine films before school age or general anesthesia to determine if there is atlanto-axial instability secondary to odontoid hypoplasia. Scoliosis requires yearly evaluation.

More than 80 mutations have been reported in association with *TRPV4*-associated disorders, the vast majority being missense variants (90%) (HGMD Professional 2020.1). Two *TRPV4* variants causing a frameshift (1-bp deletion and insertion) have been reported in association with intellectual disability and autism spectrum disorder (PMID: [25473036](#), [25621899](#)). ClinVar reports more than 50 pathogenic or likely pathogenic missense variants in *TRPV4* detected in clinical testing (May 2020). Pro799 and Arg594 are the most commonly mutated codons in the *TRPV4* skeletal dysplasias; Pro799 in exon 15 is a hot codon for metatropic dysplasia (MD) mutations with four different amino acid substitutions reported, while Arg594 in exon 11 is a hotspot for SMDK mutations (PMID: [21658220](#), [20577006](#)).

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Mutation nomenclature is based on GenBank accession NM\_021625.4 (*TRPV4*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

## CONCLUSION

*TRPV4* c.2396C>T, p.(Pro799Leu) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *TRPV4* variants is inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. *TRPV4*-related disease may be caused by a de novo variant. Genetic counseling and family member testing are recommended.

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| STEP               | DATE |
|--------------------|------|
| Order date         |      |
| Sample received    |      |
| Sample in analysis |      |
| Reported           |      |

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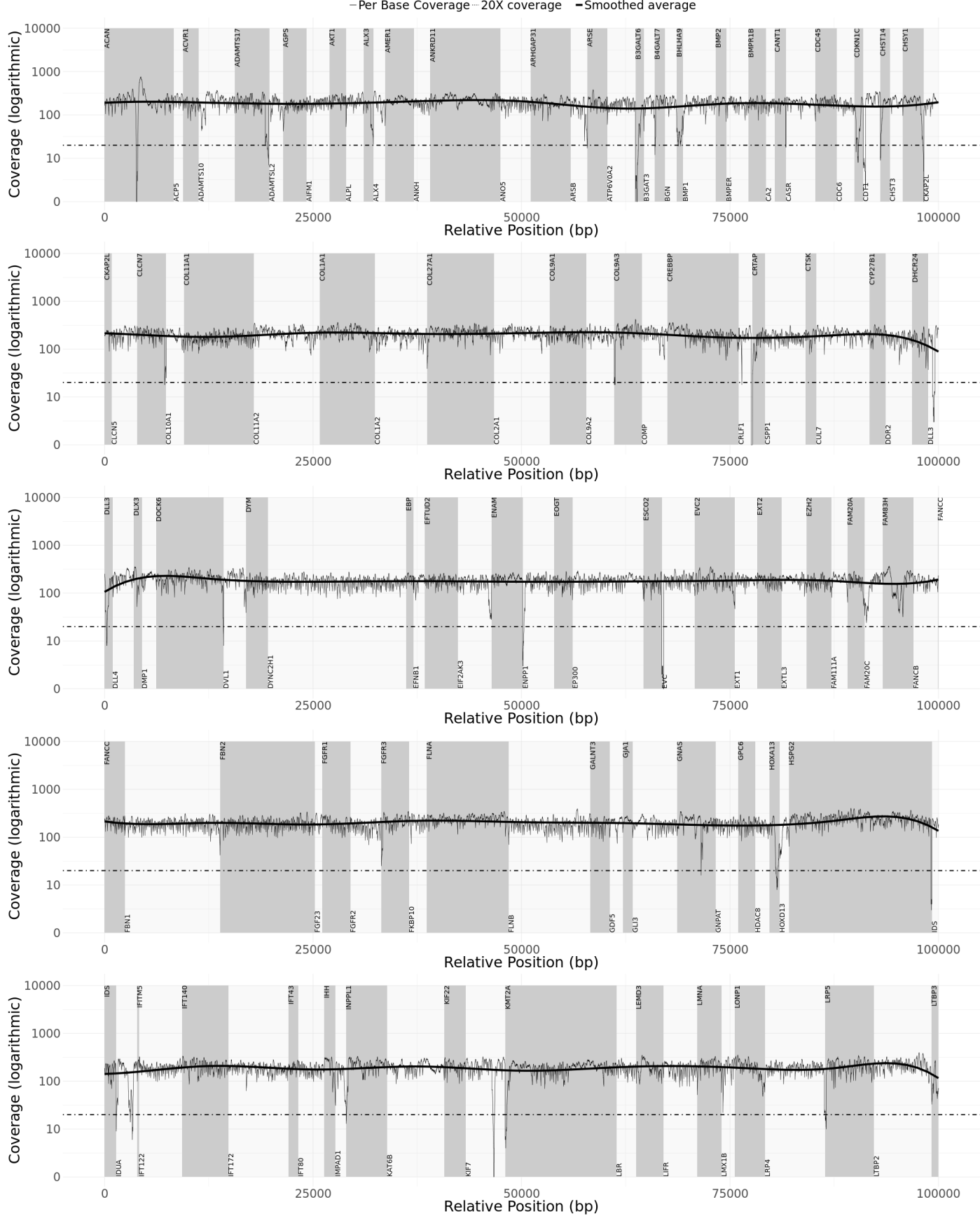
On November 1, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

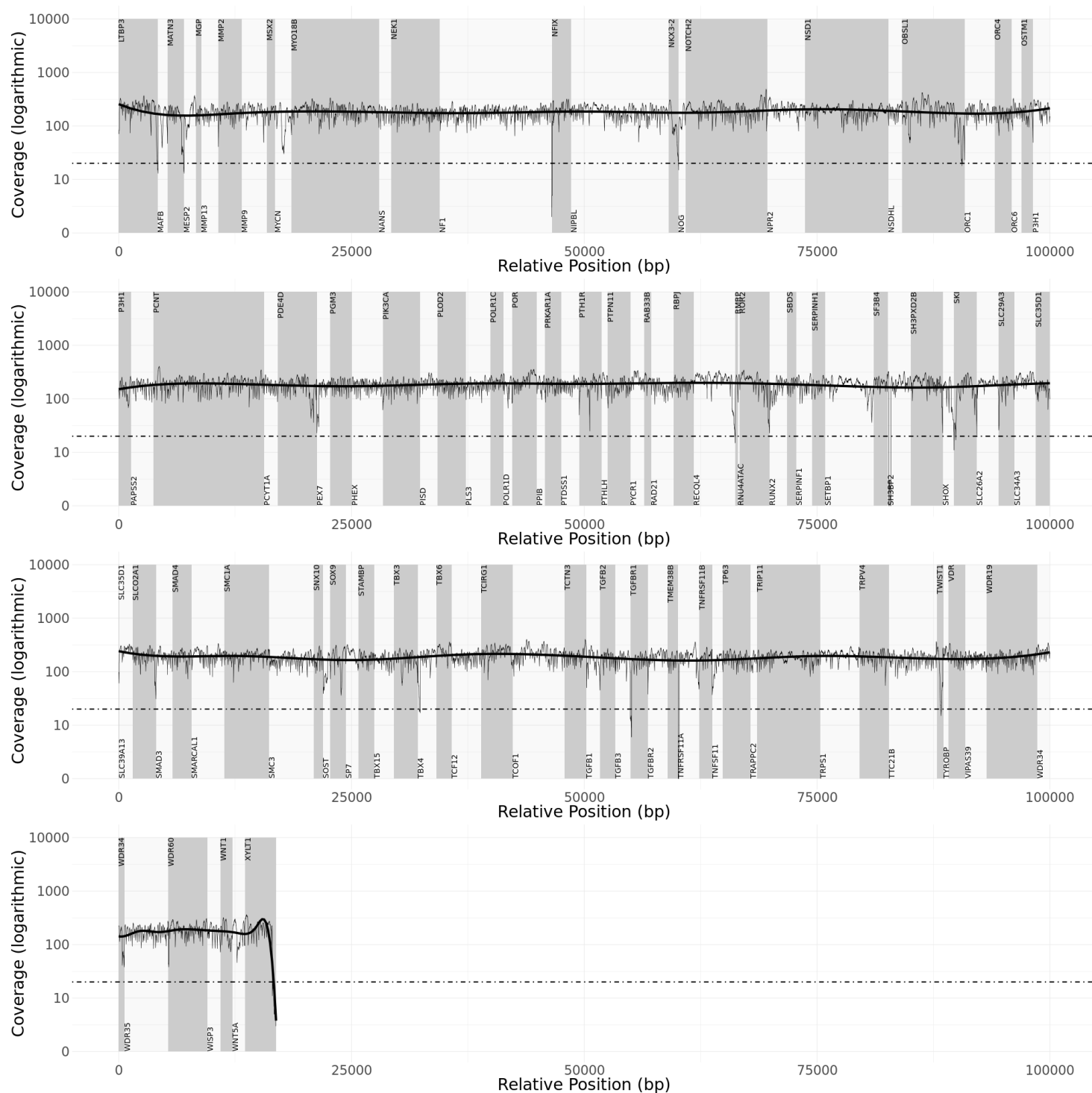


Liisa Pelttari, PhD  
Geneticist



Juha Koskenvuo, MD, PhD  
Lab Director, Chief Medical Officer





## 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 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.

**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

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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, noncoding variants deeper than  $\pm 20$  base pairs from exon-intron boundary unless otherwise indicated (please see the list of noncoding 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).

#### NONCODING VARIANTS COVERED BY THE PANEL:

NM\_004208.3(AIFM1):c.697-44T>G  
NM\_004208.3(AIFM1):c.-123G>C  
NM\_000478.4(ALPL):c.-195C>T  
NM\_000478.4(ALPL):c.793-30\_793-11delGGCATGTGCTGACACAGCCC  
NM\_054027.4(ANKH):c.-11C>T  
NM\_001199.3(BMP1):c.\*241T>C  
NM\_001203.2(BMPR1B):c.-113+2T>G  
NM\_138793.3(CANT1):c.-342+1G>A  
NM\_001178065.1(CASR):c.1378-19A>C  
NM\_000076.2(CDKN1C):c.\*5+20G>T  
NM\_001287.5(CLCN7):c.916+57A>T  
NM\_001287.5(CLCN7):c.739-18G>A  
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\_000088.3(COL1A1):c.2668-11T>G  
NM\_000088.3(COL1A1):c.2451+94G>T  
NM\_000088.3(COL1A1):c.2451+77C>T  
NM\_000088.3(COL1A1):c.2343+31T>A  
NM\_000088.3(COL1A1):c.1354-12G>A  
NM\_000088.3(COL1A1):c.1003-43\_1003-32delTGCCATCTCTTC  
NM\_000088.3(COL1A1):c.958-18\_958-15delTTCC  
NM\_000088.3(COL1A1):c.904-14G>A  
NM\_000088.3(COL1A1):c.904-15T>A  
NM\_000089.3(COL1A2):c.70+717A>G  
NM\_000089.3(COL1A2):c.226-22\_226-11delTTTTTTTTTTTT  
NM\_001844.4(COL2A1):c.1527+135G>A  
NM\_004380.2(CREBBP):c.4281-11C>G  
NM\_006371.4(CRTAP):c.472-1021C>G  
NM\_000396.3(CTSK):c.244-29A>G  
NM\_001168370.1(CUL7):c.3897+29G>A



NM\_001080463.1(DYNC2H1):c.2819-14A>G  
NM\_001080463.1(DYNC2H1):c.6478-16G>A  
NM\_004429.4(EFNB1):c.-411C>G  
NM\_004429.4(EFNB1):c.-95T>C/G  
NM\_004429.4(EFNB1):c.-95T>C  
NM\_004429.4(EFNB1):c.-95T>G  
NM\_001429.3(EP300):c.1879-12A>G  
NM\_001017420.2(ESCO2):c.1354-18G>A  
NM\_153717.2(EVC):c.940-150T>G  
NM\_000136.2(FANCC):c.-78-2A>G  
NM\_000136.2(FANCC):c.-79+1G>A  
NM\_000138.4(FBN1):c.8051+375G>T  
NM\_000138.4(FBN1):c.6872-14A>G  
NM\_000138.4(FBN1):c.6872-961A>G  
NM\_000138.4(FBN1):c.5672-87A>G  
NM\_000138.4(FBN1):c.5672-88A>G  
NM\_000138.4(FBN1):c.4211-32\_4211-13delGAAGAGTAACGTGTGTTTCT  
NM\_000138.4(FBN1):c.2678-15C>A  
NM\_000138.4(FBN1):c.1589-14A>G  
NM\_000138.4(FBN1):c.863-26C>T  
NM\_001999.3(FBN2):c.3974-24A>C  
NM\_001999.3(FBN2):c.3974-26T>G  
NM\_001999.3(FBN2):c.3725-15A>G  
chr10:g.123099960-123099960  
NM\_001110556.1(FLNA):c.6023-27\_6023-16delTGACTGACAGCC  
NM\_080425.2(GNAS):c.2242-11A>G  
NM\_005529.5(HSPG2):c.1654+15G>A  
NM\_005529.5(HSPG2):c.574+481C>T  
NM\_000202.5(IDS):c.1181-15C>A  
NM\_006123.4(IDS):c.\*57A>G  
NM\_000202.5(IDS):c.709-657G>A  
NM\_001025295.2(IFITM5):c.-14C>T  
NM\_052985.3(IFT122):c.2005-13T>A  
NM\_014714.3(IFT140):c.2577+25G>A  
NM\_170707.3(LMNA):c.513+45T>G  
NM\_170707.3(LMNA):c.937-11C>G  
NM\_170707.3(LMNA):c.1608+14G>A  
NM\_170707.3(LMNA):c.1609-12T>G  
NM\_001174146.1(LMX1B):c.140-37\_140-21delGGCGCTGACGGCCGGGC  
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  
NM\_015922.2(NSDHL):c.\*129C>T  
NM\_000288.3(PEX7):c.-45C>T  
NM\_000444.4(PHEX):c.349+11149A>T  
NM\_000444.4(PHEX):c.849+1268G>T  
NM\_000444.4(PHEX):c.1701-16T>A  
NM\_000444.4(PHEX):c.1768+177\_1768+180dupGTAA  
NM\_000444.4(PHEX):c.\*231A>G  
NM\_005032.5(PLS3):c.74-24T>A  
NM\_000941.2(POR):c.-5+4A>G  
NM\_002734.4(PRKAR1A):c.-97G>A  
NM\_002734.4(PRKAR1A):c.-7G>A  
NM\_002734.4(PRKAR1A):c.-7+1G>A  
NM\_002734.4(PRKAR1A):c.550-17T>A  
NM\_002734.4(PRKAR1A):c.709-7\_709-2delTTTTTA  
NM\_000316.2(PTH1R):c.544-25\_544-23delCTG  
NM\_000316.2(PTH1R):c.1049+29C>T  
NM\_002834.3(PTPN11):c.934-59T>A  
chr9:g.35658026-35658026  
chr9:g.35658026-35658026  
chr9:g.35658026-35658026  
chr9:g.35658026-35658026  
chr9:g.35658027-35658027  
chr9:g.35658027-35658027

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chr9:g.35658027-35658027  
chr9:g.35658027-35658027  
chr9:g.35658027-35658027  
chr9:g.35658028-35658028  
chr9:g.35658028-35658028  
chr9:g.35658029-35658029  
chr9:g.35658029-35658029  
chr9:g.35658032-35658032  
NM\_002615.5(SERPINF1):c.-9+2dupT  
NM\_002615.5(SERPINF1):c.439+34C>T  
NM\_002615.5(SERPINF1):c.440-40\_440-38delTCG  
NM\_002615.5(SERPINF1):c.787-617G>A  
NM\_000451.3(SHOX):c.-645\_-644insGTT  
NM\_000451.3(SHOX):c.-645\_-644insGTT  
NM\_000451.3(SHOX):c.-432-3C>A  
NM\_000451.3(SHOX):c.-65C>A  
NM\_000112.3(SLC26A2):c.-26+2T>C  
NM\_018344.5(SLC29A3):c.\*413G>A  
NM\_000346.3(SOX9):c.-185G>A  
NM\_006463.4(STAMPB):c.1005+358A>G  
chr12:g.115122148-115122148  
NM\_004608.3(TBX6):c.\*21C>T  
NM\_207036.1(TCF12):c.1468-20T>A  
NM\_006019.3(TCIRG1):c.-5+1G>C/T  
NM\_006019.3(TCIRG1):c.-5+1G>C  
NM\_006019.3(TCIRG1):c.-5+1G>T  
NM\_006019.3(TCIRG1):c.1887+132T>C  
NM\_006019.3(TCIRG1):c.1887+142T>A  
NM\_006019.3(TCIRG1):c.1887+146G>A  
NM\_006019.3(TCIRG1):c.1887+149C>T  
NM\_003239.2(TGFB3):c.\*495C>T  
NM\_003239.2(TGFB3):c.-30G>A  
NM\_001024847.2(TGFBR2):c.-59C>T  
NM\_014112.2(TRPS1):c.2824-23T>G  
NM\_000474.3(TWIST1):c.-255G>A  
NM\_000474.3(TWIST1):c.-263C>A  
NM\_001006657.1(WDR35):c.1434-684G>T  
NM\_001006657.1(WDR35):c.143-18T>A  
NM\_198239.1(WISP3):c.103-763G>T  
NM\_198239.1(WISP3):c.643+27C>G

## 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, eg, 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

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**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.

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