Blueprint Genetics

*Sample report as of Nov 1st, 2020. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications***

# Hypertrophic Cardiomyopathy (HCM) Panel Plus

## REFERRING HEALTHCARE PROFESSIONAL

**NAME** **HOSPITAL**

## PATIENT

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

51

**CUSTOMER SAMPLE ID**

**PRIMARY SAMPLE TYPE** **SAMPLE COLLECTION DATE**

## SUMMARY OF RESULTS

### PRIMARY FINDINGS

The patient is heterozygous for *MYBPC3* c.1224-52G>A, which is pathogenic.

### Del/Dup (CNV) analysis

Negative for explaining the patient's phenotype.

#### PRIMARY FINDINGS: SEQUENCE ALTERATIONS

| GENE | TRANSCRIPT | NOMENCLATURE | GENOTYPE | CONSEQUENCE | INHERITANCE | CLASSIFICATION |
|---|---|---|---|---|---|---|
| MYBPC3 | NM_000256.3 | c.1224-52G>A | HET | intron_variant | AD | **Pathogenic** |

| ID | ASSEMBLY | POS | REF/ALT |
|---|---|---|---|
| | GRCh37/hg19 | 11:47364865 | C/T |

| gnomAD AC/AN | POLYPHEN | SIFT | MUTTASTER | PHENOTYPE |
|---|---|---|---|---|
| 1/31334 | N/A | N/A | N/A | Dilated cardiomyopathy (DCM), Hypertrophic cardiomyopathy (HCM), Left ventricular noncompaction |

#### SEQUENCING PERFORMANCE METRICS

| PANEL | GENES | EXONS / REGIONS | BASES | BASES > 20X | MEDIAN COVERAGE | PERCENT > 20X |
|---|---|---|---|---|---|---|
| Hypertrophic Cardiomyopathy (HCM) Panel | 47 | 718 | 131157 | 131157 | 287 | 100 |

| PANEL | GENES | EXONS / REGIONS | BASES | BASES > 1000X | MEDIAN COVERAGE | PERCENT > 1000X |
|---|---|---|---|---|---|---|
| Mitochondrial genome | 37 | - | 15358 | 15358 | 12622 | 100 |

## TARGET REGION AND GENE LIST

The Blueprint Genetics Hypertrophic Cardiomyopathy (HCM) Panel (version 7, Feb 22, 2020) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: ABCC9, ACAD9, ACADVL, ACTA1, ACTC1, ACTN2, AGK*, AGL, ALPK3, APOA1, BAG3, BRAF*, CBL, COX15, CSRP3, ELAC2, EPG5, FBXL4, FHL1*, FHOD3, FLNC*, FXN*, GAA, GLA, GSK3B, HRAS, JPH2, KLHL24, LAMP2, MIPEP*, 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, MYBPC3, MYH7, MYL2, MYL3, NDUFAF2, PLN, PRKAG2, PTPN11, RAF1, RIT1, SLC25A4, SOS1, TNNC1, TNNI3, TNNT2, TPM1 and TTR.
*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

## STATEMENT

### CLINICAL HISTORY

Patient is a 51-year-old individual with hypertrophic cardiomyopathy (HCM). A cardiac MRI revealed severe mid-cavity and apical hypertrophy (21 mm) with sparing of lateral wall, and an extensive non-ischemic pattern of LGE in hypertrophied segments. The individual has decreased exercise tolerance. Family history: Parent reportedly diagnosed with HCM, died suddenly at the age of 70.

### CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hypertrophic Cardiomyopathy (HCM) Panel identified a heterozygous intron variant *MYBPC3* c.1224-52G>A.

#### *MYBPC3* c.1224-52G>A

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.

The *MYBPC3* c.1224-52G>A variant in intron 13 results in the nucleotide substitution 52 bp from the splice acceptor site of exon 14. Three *in silico* tools included in the Alamut Visual software (SSF, MaxEntScan, and NNSPLICE) predict the variant to create a cryptic acceptor splice site and thus likely lead to abnormal splicing. The variant has been reported 3 individuals affected with hypertrophic cardiomyopathy (HCM). Two of them from the same family. Functional mRNA analysis of the variant has been shown to create a cryptic splice acceptor site and an inclusion of 50 bp of intron 13 leading to a frameshift p.(Gly407fs*30) (PMID 30025578). We have identified the variant in a boy with hypertrophic cardiomyopathy (HCM) and in two adult patients with HCM (BpG unpublished observation). In addition, other intronic variants c.1224-19G>A, c.1224-80G>A affecting splicing in intron 13 have been reported in HCM patients (PMID 18258667, 28797094, 31006259, ClinVar 138326, 693982).

#### *MYBPC3*

*MYBPC3* gene on chromosome 11p11.2 encodes the cardiac isoform of myosin-binding protein C found in the cross-bridge-bearing zone (C region) of sarcomere A band. Heterozygous mutations in *MYBPC3* are associated with hypertrophic cardiomyopathy (HCM; OMIM *115197*), left ventricular non-compaction (LVNC; OMIM *600958*), and occasionally dilated cardiomyopathy (DCM) at so called burned-out phase. This gene has only one RefSeq transcript (NM_000256.3) consisting 1,274 amino acids within 34 exons. There are 546 HCM related *MYBPC3* mutations listed in HGMD, of which 246 (45%) are missense, 68 (12.5%) nonsense, 79 (14.5%) splicing, 98 (18.0%) small deletions, 35 (6.4%) small insertions, and 10 (1.8%) small indels. One per 398 individuals in gnomAD reference population carries a unique *MYBPC3* missense variant (not present in anybody else in this cohort) and one per 1,139 individuals carry a high-quality truncating *MYBPC3* variant.

#### Hypertrophic cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is defined by the presence of left ventricular hypertrophy (LVH) that is not solely explained by abnormal loading conditions such as hypertension or aortic stenosis. In adults, diagnostic criteria for HCM is a diastolic wall thickness ≥15 mm measured by any imaging technique. Lower cut-off value (≥13 mm) for diagnosis is applied for first-degree relatives of patients with unequivocal HCM. (PMID *25173338*). Surprisingly, even less extensive deviation from normal wall thickness is used for diagnostic criteria in children as z-score >2 is considered diagnostic (PMID *10814626*). Clinical manifestations range from asymptomatic LVH to progressive heart failure to sudden cardiac death, and vary from individual to individual even within the same family. Common symptoms include shortness of breath, chest pain, palpitations, and pre-syncope/ syncope. Most often the LVH of HCM becomes apparent during young adulthood, although it may also develop late in

life or at infancy. Prevalence of HCM is estimated to be 1:500 (HCM - *GeneReviews*). Relying on >6,000 HCM patients tested in OGT and Harvard LMM, monogenic molecular genetic cause for HCM is found in one third of patients with the phenotype (PMID *27532257*). The most common causative genes are *MYBPC3* (15.0%), *MYH7* (9.8%), *TNNI3* (1.5%), *TNNT2* (1.2%), *TPM1* (0.7%), *PRKAG2* (0.6%), *GLA* (0.6%), and *LAMP2* (0.5%). The presence of any sarcomere gene mutation in HCM patient associates with a younger age at presentation (38.4 vs 46.0 years, p<0.0005), a family history of HCM (50.6% vs 23.1%, p<0.0005), a family history of SCD (27.0% vs 14.9%, p<0.0005) and greater MLVWT (21.0 vs 19.3 mm, p=0.03). Some of the *MYBPC3* mutations are more severe, with high risk of sudden death (PMID *25740977*). Primary HCM rarely turns into DCM phenotype at so-called burn out phase. The hazard ratio for death is significantly higher in males vs. females (HR 2.0 (95% CI: 1.5 to 2.6) with a truncating *MYBPC3* mutation (PMID *22115648*). Depending on symptoms and disease presentation, treatments may include pharmacological and septal reduction therapies, and devices (pacemakers, ICD). Cardiac transplantation may be considered as last option for a very severe disease. European Society of Cardiology does not recommend competitive sports for cardiomyopathy patients and even family members who are genotype positive yet phenotype negative are advised to avoid competitive sports (PMID *15923204*).

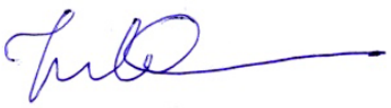
Mutation nomenclature is based on GenBank accession NM_000256.3 (*MYBPC3*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

## CONCLUSION

*MYBPC3* c.1224-52G>A is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *MYBPC3* 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. *MYBPC3*-related disease may be caused by a de novo variant. Genetic counseling and family member testing are recommended.

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

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



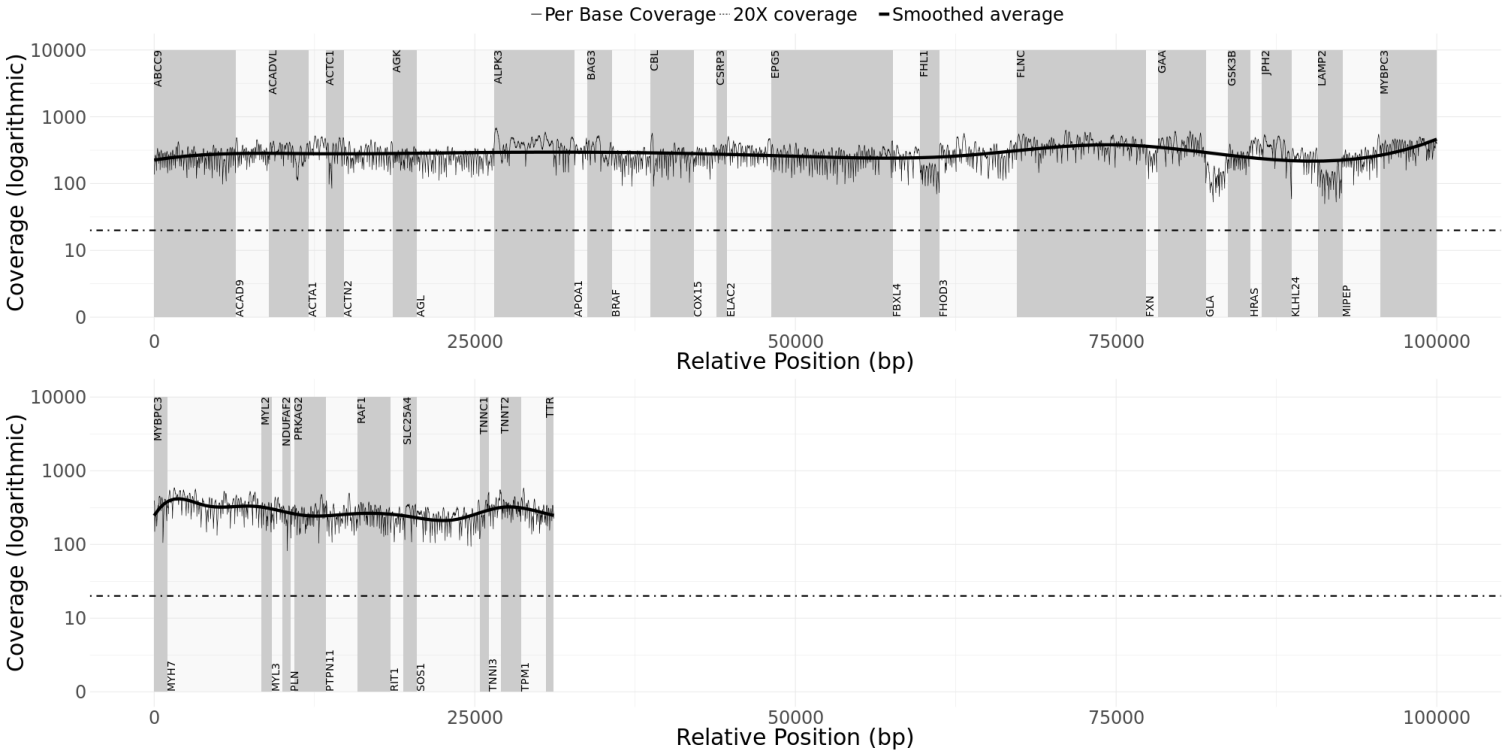
Saija Ahonen, Ph.D.

Geneticist

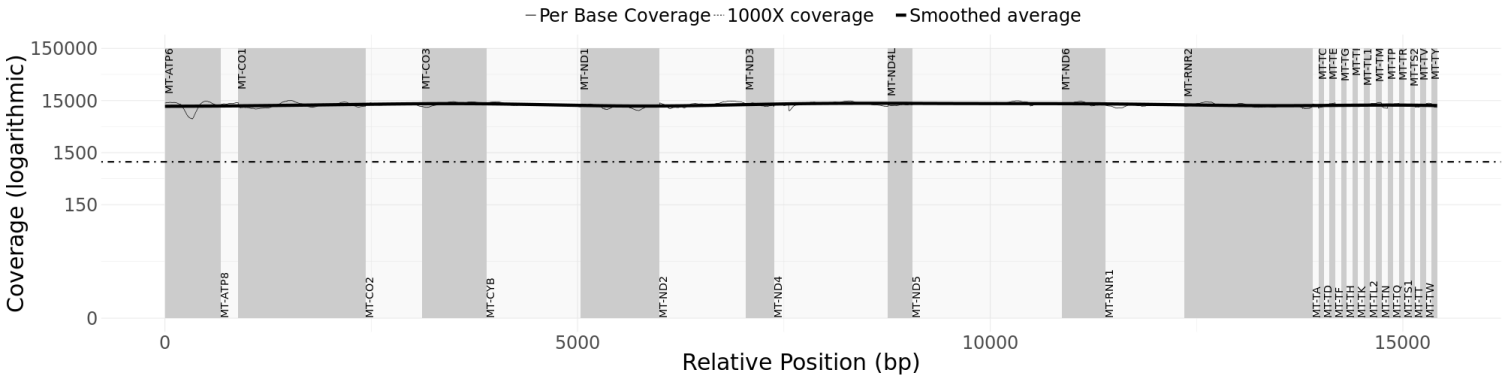
Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

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.



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## 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 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_000018.3(ACADVL):c.-144_-132delCCCAGCATGCCCCinsT
NM_001270447.1(ACADVL):c.822-27C>T
NM_001270447.1(ACADVL):c.822-11T>G
NM_001270447.1(ACADVL):c.1146+15C>T
NM_001270447.1(ACADVL):c.1252-15A>G
NM_001270447.1(ACADVL):c.1747+23C>T
NM_005159.4(ACTC1):c.*1784T>C
NM_000028.2(AGL):c.4260-12A>G
NM_000039.1(APOA1):c.-21+22G>A
NM_000039.1(APOA1):c.-65A>C
NM_000152.3(GAA):c.-32-13T>G
NM_000152.3(GAA):c.-32-13T>A
NM_000152.3(GAA):c.-32-3C>A/G
NM_000152.3(GAA):c.-32-2A>G
NM_000152.3(GAA):c.-32-1G>C
NM_000152.3(GAA):c.-17C>T
NM_000152.3(GAA):c.1076-22T>G
NM_000152.3(GAA):c.2190-345A>G
NM_000152.3(GAA):c.2647-20T>G
NM_000169.2(GLA):c.640-11T>A
NM_000169.2(GLA):c.640-801G>A
NM_000169.2(GLA):c.640-859C>T
NM_000169.2(GLA):c.547+395G>C
NM_000256.3(MYBPC3):c.*26+2T>C
NM_000256.3(MYBPC3):c.3628-12C>G
NM_000256.3(MYBPC3):c.2309-26A>G

NM_000256.3(MYBPC3):c.2149-80G>A
NM_000256.3(MYBPC3):c.1227-13G>A
NM_000256.3(MYBPC3):c.1224-19G>A
NM_000256.3(MYBPC3):c.1224-52G>A
NM_000256.3(MYBPC3):c.1091-575A>C
NM_000256.3(MYBPC3):c.1090+453C>T
NM_000256.3(MYBPC3):c.906-22G>A
NM_000256.3(MYBPC3):c.906-36G>A
NM_002667.4(PLN):c.-271A>G
NM_002667.4(PLN):c.-236C>G
NM_002834.3(PTPN11):c.934-59T>A
NM_001018005.1(TPM1):c.241-12_241-11delCTinsTG

**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.