Blueprint Genetics

DIAGNOSTIC FINDING

Cardiomyopathy Panel Plus

PATIENT INFORMATION

NAME	DOB	SSN	AGE	GENDER	ORDER ID
Test, test1	1965-Apr-07	07041965-xxxx	52	Male	54320

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL
Anna Smith	Test hospital

SUMMARY OF CLINICAL HISTORY

Patient is a 52-year-old male with hypertrophic obstructive cardiomyopathy (HOCM). The patient has a maternal uncle who deceased at young age, cause of death is unclear. No genetic testing regarding cardiomyopathy has previously been done in the family.

SEQUENCE ANALYSIS RESULTS

GENE	NOMENCLATURE	INHERITANCE	ZYGOSITY	CLASSIFICATION
SHOC2	c.517A>G, p.(Met173Val)	AD	HET	LIKELY PATHOGENIC

DEL/DUP (CNV) ANALYSIS RESULTS

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

CONCLUSION

We classify the identified *SHOC2* c.517A>G, p.(Met173Val) as likely pathogenic and probable cause for patient's disease, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, identification of the variant as de novo in 2 individuals with the same phenotype, and other rasopathy associated variant at the same codon). However, additional information is still needed to confirm the pathogenicity of the variant, which could allow independent risk stratification based on this mutation. Genetic counseling and family member testing is recommended. Disease caused by *SHOC2* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting the mutation. A proband with autosomal dominant Noonan-like syndrome may have the disorder as a result of a de novo mutation. BpG offers mutation testing for the family if requested.

ORDER INFORMATION

ORDER ID	PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID
54320	DNA	N/A	N/A

ORDER DATE	SAMPLE RECEIVED	RESULTS REPORTED	UPDATED REPORT
Dec 08, 2017	Dec 08, 2017	Jan 17, 2018	N/A

For a full report archive and change history, please login to Nucleus at nucleus.blueprintgenetics.com.

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CAP Number: 9257331

CLINICAL HISTORY

Patient is a 52-year-old male with hypertrophic obstructive cardiomyopathy (HOCM). The patient has a maternal uncle who deceased at young age, cause of death is unclear. No genetic testing regarding cardiomyopathy has previously been done in the family.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Cardiomyopathy Panel identified a heterozygous missense variant c.517A>G, p.(Met173Val) in *SHOC2.* It has not been observed in large reference population cohorts of 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. It is predicted benign by *in silic*o tools PolyPhen and SIFT but deleterious by MutationTaster. Methionine (Met) is completely conserved at this position in mammals as well as evolutionary more distant species suggesting that this position may not tolerate variation.

The *SHOC2* c.517A>G, p.(Met173Val) variant has been reported as a *de novo* variant in association with a RASopathy (PMID: 22670144). This variant has been also observed as apparently de novo in clinical testing (ClinVar 373090). A different *SHOC2* variant, (c.519G>A, p.(Met173Ile), has recently been reported by Hannig et al. in two patients with the mild Noonan-like RASopathy (PMID: 25137548). Functional studies showed impaired ability of the Shoc2 M173I mutant to interact with PP1C.

SHOC2

SHOC2 gene on chromosome 10q25.2 encodes a protein consisting almost entirely of leucine-rich repeats, a domain implicated in protein-protein interactions. The *SHOC2* protein functions as a scaffold linking RAS to downstream signal transducers in the RAS/ERK MAP kinase signaling cascade. In addition to canonical transcript (NM_007373.3) consisting 582 amino acids within 9 exons, *SHOC2* has one other isoform with RefSeq ID. Heterozygous variants in *SHOC2* associate to Noonan syndrome-like disorder with loose anagen hair (OMIM #607721). There are only two *SHOC2* variants classified as pathogenic or likely pathogenic in ClinVar without conflicts (Jan 2018). One per 1,200 individuals in gnomAD reference population carries a unique *SHOC2* missense variant (not present in anybody else in this cohort) and one per 123,000 individuals carry a high-quality truncating *SHOC2* variant. Loss of function variants in *SHOC2* have pLI value of 0.99 in ExAC indicating that they are extremely intolerant.

Dysregulation of the RAS-MAPK signaling pathway has been recognized as the molecular cause underlying a group of clinically related developmental disorders with features including reduced growth, facial dysmorphism, cardiac defects, ectodermal anomalies, variable cognitive deficits and susceptibility to certain malignancies. Nowadays many authors group these disorders under the name of Neuro-Cardio-Facio-Cutaneous syndromes (NCFCS), RAS-MAPK syndromes, or rasopathies. These Mendelian traits are caused by mutations in genes encoding RAS proteins (*KRAS* and *HRAS*), downstream transducers (*RAF1, BRAF, MEK1* and *MEK2*), or pathway regulators (*PTPN11, SOS1, NF1* and *SPRED1*). RAS-MAPK signaling pathway mutations currently explain about 60% of Noonan syndrome cases.

A recurrent pathogenic missense variant c.4A>G, p.(Ser2Gly) in *SHOC2* has been found in a subgroup with features of Noonan syndrome but also growth hormone deficiency, distinctive hyperactive behavior that improves with age in most; hair anomalies including easily pluckable, sparse, thin slow-growing hair (loose anagen hair), darkly pigmented skin with eczema or ichthyosis, hypernasal voice, mitral valve dysplasia, septal defects and structural brain anomalies and myelofibrosis (PMID: 19684605, 23918763). The variant was shown to be *de novo* in the 15 families for whom parental DNA was available (PMID: 19684605). There is only one other *SHOC2* variant reported in literature; c.519G>A, p.(Met173Ile) (PMID: 25137548). We have observed *SHOC2* c.806A>G, p.(Gln269Arg) as de novo in a patient with rasopathy (BpG unpublished observations).

Mutation nomenclature is based on GenBank accession NM_007373.3 (*SHOC2*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

We classify the identified *SHOC2* c.517A>G, p.(Met173Val) as likely pathogenic and probable cause for patient's disease, considering the current evidence of the variant (established association between the gene and patient's phenotype, rarity in control populations, identification of the variant as de novo in 2 individuals with the same phenotype, and other rasopathy associated variant at the same codon). However, additional information is still needed to confirm the pathogenicity of the variant, which could allow independent risk stratification based on this mutation. Genetic counseling and family member testing is recommended. Disease caused by *SHOC2* mutations is inherited in an autosomal dominant manner, thus each child of an affected individual has a 50% chance of inheriting the mutation. A proband with autosomal dominant Noonan-like syndrome may have the disorder as a result of a de novo mutation. BpG offers mutation testing for the family if requested.

CONFIRMATION

The *SHOC2* c.517A>G, p.(Met173Val) was confirmed by bi-directional Sanger sequencing.

On Jan 17, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Kati Kämpjärvi, Ph.D.
Geneticist

Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer

VARIANT TABLE: GENETIC ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	ZYGOSITY	CLASSIFICATION
SHOC2	NM_007373.3	c.517A>G, p.(Met173Val)	missense	HET	Likely pathogenic
	POS	**GNOMAD AC/AN**	**POLYPHEN**	**SIFT**	**MUTTASTER**
	10:112724633	0/0	benign	tolerated	disease causing
	INHERITANCE	**PHENOTYPE**	**COMMENTS**		
	AD	Noonan-like syndrome with loose anagen hair	N/A		

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT ≥ 15X
Cardiomyopathy Panel	134	2463	480813	479755	148	99.7

TARGET REGION AND GENE LIST

Blueprint Genetics Cardiomyopathy Panel (version 2, Jun 26, 2017) consists of sequence analysis of genes associated with arrhythmogenic right ventricular cardiomyopathy (ARVC), cardiomyopathy NAS, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), left ventricular non-compaction cardiomyopathy (LVNC), Noonan syndrome and RCM: *A2ML1, AARS2, ABCC9, ACAD9, ACADVL, ACTA1, ACTC1, ACTN2, AGK*, AGL, ALPK3, ANKRD1, ANO5, APOA1, BAG3, BRAF*, CAPN3, CASQ2, CAV3, CBL, CHKB, COX15, CPT1A, CPT2, CRYAB, CSRP3, CTNNA3, DAG1, DBH, DES, DMD, DNAJC19, DSC2, DSG2, DSP, DYSF, EMD, ETFA, ETFB, ETFDH, EYA4, FBXO32, FHL1*, FKRP, FKTN, FLNC*, FOXRED1, FXN*, GAA, GATAD1, GBE1, GFM1, GLA, GLB1, GMPPB, GNE, GUSB*, HCN4, HFE, HRAS, ISPD, JPH2, JUP, KRAS*, LAMA2, LAMP2, LARGE, LDB3, LMNA, LZTR1, MAP2K1, MAP2K2, MTO1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOT, MYPN, NEXN, NF1*, NRAS, NSUN2, PKP2*, PLEC, PLEKHM2, PLN, PNPLA2, POMGNT1, POMT1, POMT2, PRDM16, PRKAG2, PTPN11, RAF1, RASA2, RBM20, RIT1, RRAS, RYR2, SCN5A, SCNN1B, SCNN1G, SCO2, SDHA*, SELENON, SGCA, SGCB, SGCD, SGCG, SHOC2, SLC22A5, SLC25A4, SLC25A20, SMCHD1, SOS1, SPRED1, TAZ, TCAP, TGFB3, TMEM43, TMEM70, TNNC1, TNNI3, TNNT2, TPM1, TRIM32, TSFM, TTN*, TTR, VCL, VCP* and *XK*. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations** located outside these coding regions. This test covers the majority of arrhythmogenic right ventricular cardiomyopathy (ARVC), cardiomyopathy NAS, dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), left ventricular non-compaction cardiomyopathy (LVNC), Noonan syndrome and RCM mutations known to date and it should be used to detect single nucleotide substitutions and small insertions and deletions (INDELs). In addition, the Cardiomyopathy Panel includes NGS derived Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

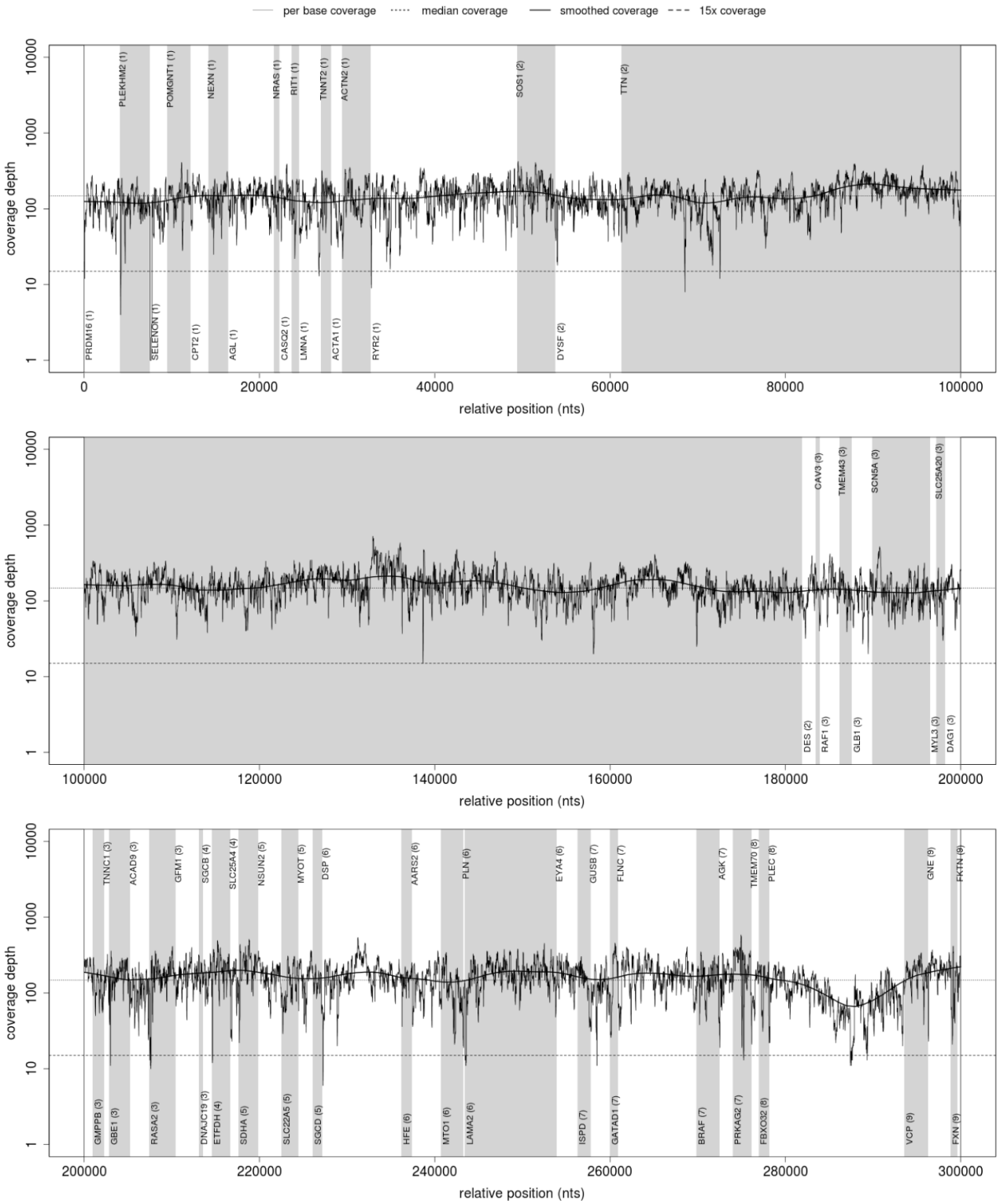
* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions (link to duplicated regions): http://blueprintgenetics.com/pseudogene/. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

** NM_000152.3(GAA):c.-32-13T>G, NM_000152.3(GAA):c.-32-3C>A, NM_000152.3(GAA):c.1076-22T>G, NM_000158.3(GBE1):c.2053-3358_2053-3350delGTGTGGTGGinsTGTTTTTTACATGACAGGT, NM_000169.2(GLA):c.640-801G>A, NM_000256.3(MYBPC3):c.1224-19G>A, NM_000256.3(MYBPC3):c.2309-26A>G, NM_001042492.2(NF1):c.4110+1802delA, NM_001042492.2(NF1):c.5812+332A>G, NM_003239.2(TGFB3):c.*495C>T, NM_003239.2(TGFB3):c.-30G>A, NM_003494.3(DYSF):c.3443-33A>G, NM_003494.3(DYSF):c.4886+1249G>T, NM_004006.2(DMD):c.31+36947G>A, NM_004006.2(DMD):c.6614+3310G>T, NM_004006.2(DMD):c.8217+18052A>G, NM_004006.2(DMD):c.9225-647A>G, NM_004006.2(DMD):c.9225-648A>G, NM_004006.2(DMD):c.961-5831C>T and NM_170707.3(LMNA):c.513+45T>G

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

For a detailed gene table, please login to Nucleus at nucleus.blueprintgenetics.com.

SEQUENCING COVERAGE PLOT



The print file shows the first three rows of the coverage plot. To see the full plot, please login to Nucleus at nucleus.blueprintgenetics.com.

SUMMARY OF METHODS

NOVASEQ (SEQUENCE ANALYSIS)

Laboratory process. Total genomic DNA was extracted from the biological sample using a spin column 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 and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.

Data analysis. Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.

Confirmation. Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.

Reporting. Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

Accreditation. 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. Analysis of patient samples has been carried out with an in vitro diagnostic device, which complies with the essential requirements set out in Annex I of the directive 98/79/EC.

DEL/DUP (CNV) ANALYSIS

Data analysis. Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the NovaSeq data analysis 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).

Interpretation. The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.

Confirmation. Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).

Reporting. Reporting was done using an HGNC-approved gene nomenclature.

Notes. This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

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GLOSSARY OF USED ABBREVIATIONS

POS = genomic position of the variant in the format of chromosome:position

ID = rsID in dbSNP

Transcript = GenBank accession for reference sequence used for variant nomenclature

Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

ExAC AC/AN = allele count/allele number in the Exome Aggregation Consortium Database

AD = autosomal dominant

AR = autosomal recessive

OMIM = Online Mendelian Inheritance in Man®

ExAC = Exome Aggregation Consortium Database (>60,000 unrelated individuals)

HET = heterozygous

HOM = homozygous

Del/Dup = Deletion and Duplication

CNV = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.