Blueprint Genetics

Sample report as of June 14th, 2022. 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***

Metabolic Myopathy and Rhabdomyolysis Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		28		

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *CPT2* c.338C>T, p.(Ser113Leu), which is classified as pathogenic.

Del/Dup (CNV) analysis

The patient is heterozygous for a deletion *CPT2* c.341-2621_1121del, which encompasses part of exon 4 of *CPT2*. This alteration is classified as likely pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CPT2	NM_000098.2	c.338C>T, p.(Ser113Leu)	HET	missense_variant, splice_region_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	1:53668099	C/T		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	393/282834	probably damaging	deleterious	disease causing	Carnitine palmitoyltransferase II deficiency	

PRIMARY FINDINGS: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
CPT2	DELETION	1	HET	CPT2:Partial gene	UCSC	**Likely pathogenic**
	OMIM	**PHENOTYPE**			**COMMENT**	
		Carnitine palmitoyltransferase II deficiency			-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Metabolic Myopathy and Rhabdomyolysis Panel	90	1440	253270	253040	221	99.91

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	6185	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Metabolic Myopathy and Rhabdomyolysis Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ACAD9, ACADL, ACADM, ACADVL, ADCK3, AGL, AHCY, ALDOA, AMPD1, ANO5, ATP2A1, B3GALNT2#, B4GAT1, C10ORF2, CAPN3, CASQ1, CAV3, CHKB, COQ2, CPT2, CTDP1, DAG1, DGUOK, DMD, DNAJB6, DPM1, DPM2, DYSF, EMD, ENO3, ETFA, ETFB, ETFDH, FDX1L, FHL1*, FKRP, FKTN, FLAD1, GAA, GBE1, GMPPB, GYG1, GYS1, HADHA, HADHB, ISCU, LAMA2, LAMP2, LARGE, LDHA, LPIN1, 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, MYH3, OPA1, OPA3, PDSS2, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKB, PNPLA2, POLG, POLG2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, PYGM, RBCK1, RRM2B, RYR1, SCN4A, SGCA, SGCB, SGCD, SGCG, SIL1, SLC22A5, SLC25A20, STAC3, SUCLA2, SUCLG1, TANGO2, TCAP, TK2#, TNPO3, TRIM32 and TYMP.* The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: TK2 (NM_001271934:3).

#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 a number sign (#).

STATEMENT

CLINICAL HISTORY

Patient is a 28-year-old individual with a second episode of exercise-induced rhabdomyolysis with acute tubular necrosis. There is no family history of similar disease.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Metabolic Myopathy and Rhabdomyolysis Panel identified a heterozygous missense variant *CPT2* c.338C>T, p.(Ser113Leu). Del/Dup (CNV) analysis identified a heterozygous deletion c.341-2621_1121del encompassing part of exon 4 of *CPT2*. Due to the large genomic distance between these variants, NGS-based methods cannot determine whether they occur on the same (in *cis*) or different (in *trans*) parental alleles.

CPT2 c.338C>T, p.(Ser113Leu)

There are 385 individuals heterozygous and 4 individuals homozygous 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. All in silico tools utilized predict this variant to be damaging to protein structure and function. The p.(Ser113Leu) variant is the most commonly occurring disease causing variant in carnitine palmitoyltransferase II deficiency (CPT2), accounting for approximately 60% of mutant alleles in European patients with the adult myopathic form of CPT2 deficiency (CPT2 - GeneReviews, ClinVar ID: 8953). In a series of 32 affected individuals, 14 were homozygous for this common allele and 17 were compound heterozygous for this common pathogenic variant and a second pathogenic variant (PMID: 12707442).

Deletion *CPT2* c.341-2621_1121del

CPT2 c.341-2621_1121del is a gross deletion which involves part of exon 4 of *CPT2*. This deletion encompasses the genomic region 1:53673066-53676467 and is 3402 base pairs in size. There are no individuals with this deletion listed in the ExAC control dataset (n>46,000 exomes) nor in the gnomAD population database of structural variation (>10,000 genomes) (gnomAD SVs v2.1, ExAC data available in the gnomAD browser). 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. However, loss of *CPT2* function is an established disease mechanism, and other truncating variants in the gene have been described in patients with phenotypes consistent with *CPT2*-related disease (HGMD).

CPT2

CPT2 gene (OMIM *600650) encodes a nuclear protein, carnitine palmitoyltransferase 2, which is transported to the mitochondrial inner membrane. It releases fatty acids from acylcarnitines for beta-oxidation. It has two isoforms with RefSeq IDs. Carnitine palmitoyltransferase II deficiency (CPT II, MIM #600649, 608836, 255110) is an autosomal recessive mitochondrial disorder of long-chain fatty-acid oxidation. The three clinical presentations described are: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form (which is usually mild and can manifest from infancy to adulthood (GeneReviews). Heterozygotes have a biochemically intermediate phenotype (with markedly reduced enzyme activity) but generally do not display symptoms. (GeneReviews). While the former two are severe multisystemic diseases characterized by liver failure with hypoketotic hypoglycemia, cardiomyopathy, seizures, and early death, the last one is characterized by exercise-induced muscle pain and weakness, sometimes associated with myoglobinuria. Cardiac manifestations may include dilated cardiomyopathy, left ventricular hypertrophy and susceptibility to different arrhythmias. In 2017, Pereyra et al. CPT2 deficient (Cpt2M-/-) mice developed cardiac hypertrophy and systolic dysfunction, evidenced by a 5-fold greater heart mass, 60-90% reduction in blood ejection fraction relative to control mice, and eventual lethality in the absence of cardiac fibrosis (PMID 28916721). The myopathic form of CPT II deficiency is the most common disorder of lipid metabolism affecting skeletal muscle and the most frequent cause of hereditary myoglobinuria. Males are more likely to be affected than females. The age of onset in

myopathic form has variable onset from 1st to 6th decade (CPT2 - GeneReviews). Most individuals with the myopathic form of CPT II deficiency have normal serum CK concentration (<80 U/L) between attacks; however, permanent elevation of serum CK concentration (≤313 U/L) is observed in approximately 10% of affected individuals (PMID 12707442). The mild adult form, detected in more than 200 families, is characterized by episodes of rhabdomyolysis triggered by prolonged exercise (ClinVar ID8953). Abnormal results in either CPT enzyme activity assay or molecular genetics analysis can be confirmed with the other assay. There are over 50 *CPT2* variants classified as pathogenic or likely pathogenic in ClinVar (Sept 2019): 35 frameshift, 11 nonsense, 7 missense, and one splice site variant. HGMD Professional (November 2019) lists 100 variants in *CPT2* under the Disease Mutation (DM) category of which 62% are missense, 22% frameshift, 7% nonsense and 5% splicing variants, and 2% are gross deletions or complex rearrangements. Truncating loss of function *CPT2* variants usually cause a severe form of the disease, even though the clinical picture of the disease may vary from person to person (CPT2 - GeneReviews).

Mutation nomenclature is based on GenBank accession NM_000098.2 (*CPT2*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

CPT2 c.338C>T, p.(Ser113Leu) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Deletion *CPT2* c.341-2621_1121del, affecting part of exon 4, is classified as likely pathogenic, based on the established association between the gene and the patient’s phenotype, the variant's rarity in control populations, and variant type (gross deletion). Disease caused by *CPT2* variants is inherited in an autosomal recessive manner. Testing of parental/offspring samples is needed to determine whether the variants occur in cis (on the same allele) or in trans (on different alleles). Compound heterozygosity of the variants (in trans) would explain the patient’s clinical presentation. If both of these variants are parentally inherited, any siblings of the patient will have a 25% chance of being compound heterozygous and thus affected, a 50% chance of being an unaffected carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

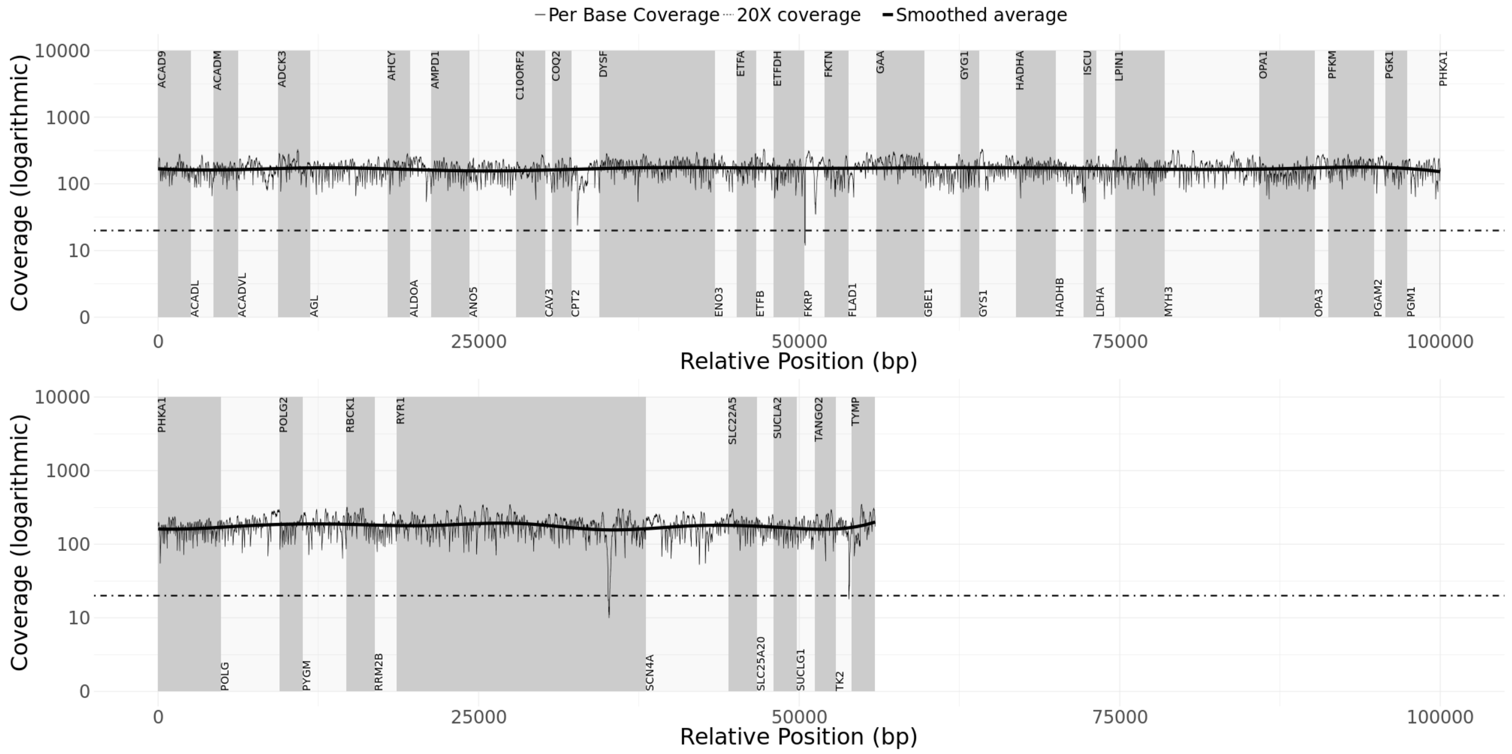
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

Signature

Name

Title

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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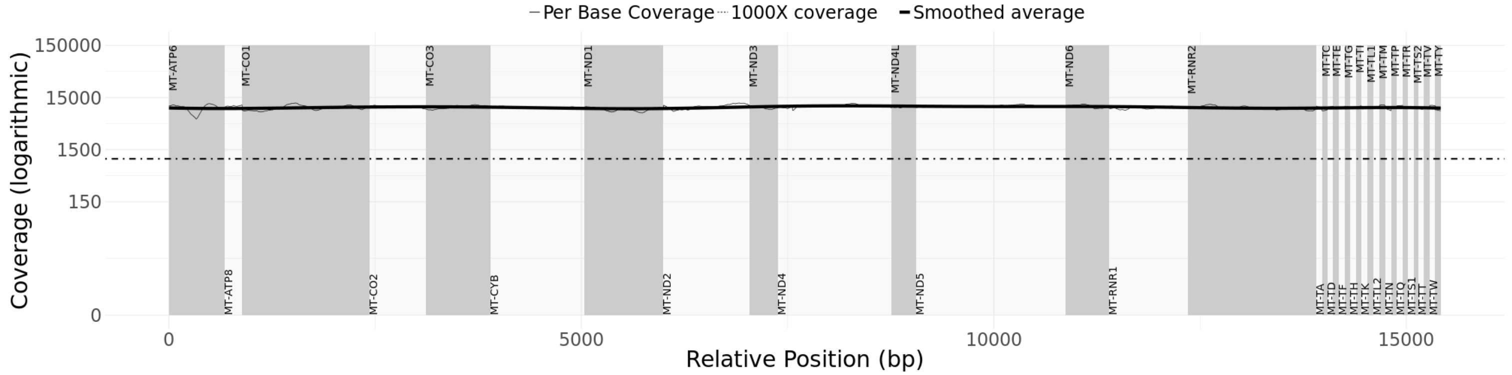

APPENDIX 5: SUMMARY OF THE TEST

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_000016.4(ACADM):c.388-19T>A
NM_000016.4(ACADM):c.600-18G>A
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_020247.4(ADCK3):c.*72dupG
NM_000028.2(AGL):c.4260-12A>G
NM_003494.3(DYSF):c.3443-33A>G
NM_003494.3(DYSF):c.4410+13T>G
NM_003494.3(DYSF):c.4886+1249G>T
NM_003494.3(DYSF):c.5668-824C>T
NM_003494.3(DYSF):c.*107T>A
NM_004453.2(ETFDH):c.-75A>G
NM_004453.2(ETFDH):c.176-636C>G
NM_024301.4(FKRP):c.-272G>A
NM_006731.2(FKTN):c.648-1243G>T
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_000158.3(GBE1):c.2053-3358_2053-3350delGTGTGGTGGinsTGTTTTTTACATGACAGGT
NM_004130.3(GYG1):c.481+3276C>G
NM_000183.2(HADHB):c.442+614A>G
NM_000183.2(HADHB):c.442+663A>G
NM_213595.2(ISCU):c.418+382G>C
NM_130837.2(OPA1):c.449-34dupA
NM_130837.2(OPA1):c.2179-40G>C
NM_001166686.1(PFKM):c.1626-64A>G
NM_000291.3(PGK1):c.1214-25T>G
NM_001172818.1(PGM1):c.1199-222G>T
NM_005609.2(PYGM):c.661-601G>A
NM_005609.2(PYGM):c.425-26A>G
NM_000540.2(RYR1):c.8692+131G>A
NM_000540.2(RYR1):c.14647-1449A>G
NM_003060.3(SLC22A5):c.394-16T>A
NM_003060.3(SLC22A5):c.825-52G>A

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
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.