Blueprint Genetics

Sample report as of July 7, 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***

Mitochondrial Genome Test Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient has a heteroplasmic (39%) *MT-TL1* m.3243A>G variant, which is pathogenic.

PRIMARY MITOCHONDRIAL FINDINGS: SEQUENCE ALTERATIONS

GENE	POS	AF	LEVEL	GENOTYPE	TRANSCRIPT	NOMENCLATURE	CLASSIFICATION
MT-TL1	MT:3243	0.394	39%	HETEROPLASMIC	NC_012920.1	m.3243A>G	**Pathogenic**

PHENOTYPE
Cyclic vomiting syndrome,
Cytochrome c oxidase deficiency,
Diabetes-deafness syndrome,
Mitochondrial myopathy,
encephalopathy,
lactic acidosis,
and stroke-like episodes,
Myoclonic epilepsy with ragged red fibers,
SIDS,
susceptibility to

CONSEQUENCE
non_coding_transcript_exon_variant

INHERITANCE
Mitochondrial

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	4748	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Mitochondrial Genome Test Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *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* and *MT-TY*.

STATEMENT

CLINICAL HISTORY

Patient in their 20s with short stature. Hearing loss was noted before the age of 10 years and has slowly progressed over a number of years progressing to requiring a cochlear implant in the early 20s. The patient had a diagnosis of diabetes made at the age of 18 years. More recently echocardiography was suggestive of mild hypertrophic cardiomyopathy and there was reduced global longitudinal strain suggestive of mild infiltrative disease. These abnormalities persisted at a second echo some months later and the patient has just undergone cardiac biopsy, the results of which were non-specific but there were moderate cardiomyopathic changes with anisonucleosis, myocytolysis and some interstitial fibrosis. There was no myocyte disarray or evidence of iron or amyloid deposition. The patient also had a raised lactate at the time of biopsy. The patient's mother and maternal grandmother have been affected with hearing loss and diabetes.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Mitochondrial Genome Test identified a heteroplasmic variant *MT-TL1* m.3243A>G.

MT-TL1 m.3243A>G

The allele frequency of *MT-TL1*m.3243A>G is 0.29% in the patient population and 0.04% in the healthy population in the HmtVar database, which contains a dataset of >49,304 complete human mitochondrial genomes from GenBank, of which 44,058 from healthy and 5,246 from diseased individuals (PMID: 30371888; HmtVar). Five individuals with m.3243A>G heteroplasmy levels ranging from 10% to 20% and one at 46% have been reported in the gnomAD v3.1 control database. The gnomAD v3.1 data set contains 76,156 whole genomes, of which 56,434 samples passed mitochondrial-specific filters. The variant is present as heteroplasmic in 49 individuals and as homoplasmic in 2 individuals in the HelixMTdb (includes 195,983 unrelated adult individuals sequenced at Helix, population is not enriched for for mitochondrial disorders, or other clinical phenotypes). Nucleotide A at this position is 97.5% conserved in mammals (Mitomaster). This variant has been predicted as pathogenic by MitoTIP (MitoMap).

The m.3243A>G variant (rs199474657) disrupts the mitochondrial tRNA for leucine (UUR) (tRNA-Leu) and is the most common pathogenic variant in the mitochondrial genome. It accounts for up to 11.5% of individuals with mitochondrial disease (PMID: 24375076) and approximately 80% of individuals with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS; NBK1233). The m.3243A>G variant is associated with a wide spectrum of clinical manifestations including Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS), Mitochondrial Myopathy, Leigh syndrome, Chronic Progressive External Ophthalmoplegia (CPEO), Maternally Inherited Deafness and Diabetes (MIDD), hypertrophic cardiomyopathy (HCM), kidney dysfunction, migraine, bowel dysmotility, muscle stiffness, and diabetes (PMID: 24375076, 22403016; NBK1233).

In a cohort of 126 carriers of m.3243A>G, the most frequent features reported were hearing loss (58%), diabetes (41%), stroke-like episodes (41%), muscular weakness (40%), and generalized seizures (37%). About a third of the patients also presented exercise intolerance, heart disease, ptosis/ophthalmoparesis, and/or migraine. Cognitive involvement was reported in 25% of carriers, and 20% had ataxia (PMID: 24375076). Retinopathy was detected in 10% of the patients. In contrast, a separate study of 29 m.3243A>G carriers, identified retinal abnormalities in 86% of the individuals following a 4-grade classification: grade 1 retinal dystrophy (21%) with fine pigment abnormalities; grade 2 abnormalities (38%) with yellowish or mildly pigmented subretinal deposits in the early stage and that encompassed the entire macula and often encircled the optic disc in the advanced stage; grade 3 disease (21%) with profound chorioretinal atrophy present outside the fovea; and grade 4 disease (7%) with atrophy of the fovea and marked loss of visual acuity (PMID: 23806424). The grade of mitochondrial retinal dystrophy correlated significantly with both age and visual acuity, whereas no correlation was observed with heteroplasmy level.

In clinical validation of the Blueprint Genetics mtDNA test, we found the m.3243A>G variant in 26 out of 7000 (0.37%) samples tested for mtDNA due to highly variable clinical indications. The tested DNA was isolated in most cases from either blood or saliva. The median heteroplasmy level was 11.3%. The median heteroplasmy level was 12.7% (range 1.9%-69.7%) in 16 patients without alternative molecular diagnosis and whose phenotype fits potentially to this variant. Conversely, the median heteroplasmy level was significantly lower (3.0%; range 1.6%-19.6%) in 10 patients who had an alternative molecular

diagnosis or whose phenotype did not match with this mtDNA variant. Heteroplasmy level was higher than 15% in six patients: two of them had MELAS/MELAS-like syndrome, two had isolated ophthalmologic phenotype whereas the phenotype in two patients did not match at all with mtDNA disease.

MT-TL1

MT-TL1 is an RNA gene encoding mitochondrial tRNA for leucine 1 (UUA/G) and involved in the tRNA aminoacylation pathway (MIM *590050, *MT-TL1* GeneCards). Pathogenic variants in the *MT-TL1* gene are associated with Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes (MELAS) syndrome (MIM #540000; PMID: 30236074), Maternally Inherited Deafness and Diabetes (MIDD) (MIM #520000), myoclonus epilepsy and ragged-red fibers (MERRF; NIH-MERFF, PMID: 7628497), and MERFF/MELAS overlap syndrome (PMID: 20610441). Less frequently, the pathogenic *MT-TL1* m.3243A>G variant has been reported in individuals with Mitochondrial complex IV deficiency (MIM *220110), Kearns-Sayre syndrome (KSS; MIM *530000) (PMID: 29430542, 9619647), progressive external ophthalmoplegia (PEO) (PMID: 28695364), and cardiomyopathy (PMID: 33113037, 30128910).

MELAS syndrome is one of the most common mitochondrial disorders characterized by mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes affecting many of the body's systems, particularly the nervous system and muscles (MIM #540000). Clinical manifestations include seizures, recurrent headaches, loss of appetite and recurrent vomiting, cerebral infarcts, atherosclerotic lesions, and altered vasculature (GeneReviews NBK1233, PMID: 31641105). Stroke-like episodes with temporary muscle weakness on one side of the body (hemiparesis) may also occur and lead to altered consciousness, vision and hearing loss, loss of motor skills, and intellectual disability. Another distinguishing feature for MELAS syndrome is a lactic acid accumulation that leads to abdominal pain, fatigue, muscle weakness, and difficulty breathing. Less commonly observed manifestations include dementia, and/or a diminished ability to communicate by speech, writing, and/or signs, episodes of confusion, and hallucinations often due to a preceding fever and/or headache. The onset of manifestations occurs before the age of 20 years in approximately 75% of cases. MELAS is a heterogeneous disorder and can be caused by variants in *MT-TL1* (MIM *590050) and several other mitochondrial genes (GeneReviews NBK1233). Approximately 80% of the MELAS cases are due to heteroplasmic *MT-TL1* m.3243A>G variant, and up to 15% due to *MT-TL1* m.3271T>C and *MT-TL1* m.3252A>G (PMID: 2102678, 1932147, 8111377).

Maternally inherited diabetes-deafness syndrome (MIDD; MIM *520000) is a mitochondrial disorder characterized by the onset of sensorineural hearing loss and diabetes in adulthood. Macular pattern dystrophy is present in most cases (PMID: 11329229). Some patients may have additional features observed in mitochondrial disorders, including pigmentary retinopathy, ptosis, cardiomyopathy, myopathy, renal problems, and neuropsychiatric symptoms (MIM *520000, PMID: 11329229). Diabetes may present either as non-insulin-dependent, with secondary requirement for insulin therapy in almost half of patients, or as insulin-dependent from the onset (PMID: 15223991, 11329229, 9105898).

MERRF (myoclonic epilepsy associated with ragged red fibers) syndrome is an extremely rare mitochondrially inherited neuromuscular disorder that begins in childhood and affects the nervous system, skeletal muscle as well as other body systems. The distinguishing feature in MERRF is myoclonus, consisting of sudden, brief, jerking spasms that can affect the arms and legs or the entire body. In addition, individuals with MERRF syndrome may have muscle weakness, ataxia, seizures, and dementia. Short stature, optic atrophy, hearing loss, cardiomyopathy, and abnormal sensation from nerve damage are also common symptoms (MERFF - NORD, GeneReviews NBK1520). MERRF is a heterogeneous disorder and can be caused by variants in several genes, including heteroplasmic m.3256C>T variant *MT-TL1* (MIM *590050; GeneReviews NBK1520).

Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA: GenBank Sequence NC_012920.1

CONCLUSION

MT-TL1 m.3243A>G is classified as pathogenic, based on currently available evidence supporting its disease-causing role. The heteroplasmy level of this mitochondrial variant was 39% in the tested DNA sample. In the presence of heteroplasmy, the proportion of variant to wild-type mtDNA is important in disease expression, with higher levels of variant mtDNA often associated with more severe clinical symptoms (PMID: 28415858). Disease caused by pathogenic mitochondrial variants are maternally inherited or occur *de novo*. A male does not transmit mitochondria to his offspring. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

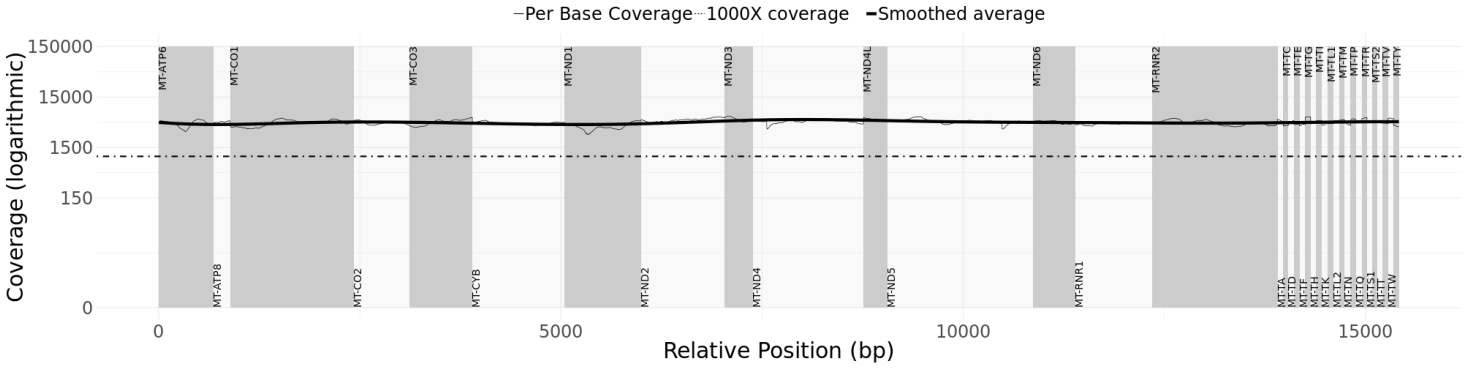
(This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results.)

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.



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 at Blueprint Genetics. 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.These steps were performed at Blueprint Genetics.
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. Bioinformatics and quality control processes were performed by Blueprint Genetics.
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. The interpretation was performed at Blueprint Genetics.
Variant classification: Our variant classification follows the 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. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com
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.The confirmation of sequence alterations was performed at Blueprint Genetics.
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.The confirmation of copy number variants was performed at Blueprint Genetics.
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).

PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director:

CLIA: 99D2092375

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.