Blueprint Genetics

*Sample report as of May 13, 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***

Mitochondrial Genome Test Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		45	Female	

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE	CUSTOMER SAMPLE ID

SUMMARY OF RESULTS

TEST RESULTS

Patient has a homoplasmic (100%) *MT-ND4* m.11778G>A, p.(Arg340His) variant, which is classified as pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing or rare copy number variants which could explain the patient's reported phenotype.

PRIMARY MITOCHONDRIAL VARIANT TABLE: SEQUENCE ALTERATIONS

GENE	POS	AF	LEVEL	GENOTYPE	TRANSCRIPT	NOMENCLATURE	CLASSIFICATION
MT-ND4	MT:11778	1	100%	HOMOPLASMIC	NC_012920.1	m.11778G>A	**Pathogenic**

CONSEQUENCE	PHENOTYPE	INHERITANCE
missense_variant	Leber hereditary optic neuropathy, Leber optic atrophy and dystonia, Mitochondrial complex I deficiency	Mitochondrial

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	11247	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Mitochondrial Genome Test (version 1, Nov 29, 2019) 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 is a 45-year-old female with vision loss. She has family history of LHON; Nephew age 23 and 3 maternal cousins diagnosed in childhood.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Mitochondrial Genome Test identified a homoplasmic missense variant *MT-ND4* m.11778G>A, p.(Arg340His).

MT-ND4 m.11778G>A, p.(Arg340His)

The *MT-ND4* m.11778G>A, p.(Arg340His) allele frequency in patient population is 0.02 and in healthy population 0.00035 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). The variant has been seen as homoplasmic in the 1000G population cohort. All *in silico* tools utilized (MutPred, Panther, PhDSNP, SNPsGO, PolyPhen2) predict this variant to be damaging to protein structure and function. The variant has been detected by other laboratories in the context of clinical testing and submitted to ClinVar (ID: 24747).

The *MT-ND4* m.11778G>A, p.(Arg340His) is one of the most common Leber's hereditary optic neuropathy (LHON) variants and together with m.3460G>A, and m.14484T>C variants account for the majority of the LHON cases. The *MT-ND4* m.11778G>A, p.(Arg340His) variant is shown to be responsible for about 70 percent of all LHON cases worldwide, except in Canada (PMID: 28768321). In addition, the m.11778G>A, p.(Arg340His) variant in *MT-ND4* is reported to cause the most severe form of LHON-related visual loss (PMID: 15548492; PMID: 31605306). LHON is one of the most common maternally inherited mitochondrial disorders. (LHON -GeneReviews). Only approximately 50% of males and approximately 10% of females carrying these mutations develop optic neuropathy and blindness (PMID: 28768321). Khan et al. investigated 543 individuals (295 male, 248 female) from 64 unrelated Indian families harbouring the *MT-ND4* m.11778G>A, p.(Arg340His) variant (PMID: 28768321). The overall disease penetrance was 27.1% (146 of 543) and higher in males (37.9%; 112/295) than females (13.7%; 34/248). The mtDNA haplogroup analysis revealed that all affected probands belonged to different mtDNA haplogroups. In addition, Ji et al. identified 175 Chinese families harbouring the *MT-ND4* m.11778G>A variant. All identified individuals were homoplasmic for the *MT-ND4* m.11778G>A, p.(Arg340His) variant (PMID: 19026397). No association between the m.11778G>A, p.(Arg340His) variant and the background mtDNA haplogroup was detected.

MT-ND4

The *MT-ND4* (MIM: *516003) gene encodes subunit 4 of mitochondrial NADH dehydrogenase (also known as Complex I), the major component involved in the first step of the electron transport chain of oxidative phosphorylation. NADH dehydrogenase 4 extracts electrons from NADH, donates them to ubiquinone, and utilizes the energy released to pump protons out from the mitochondrial inner membrane (PMID: 27716073). NADH dehydrogenase 4 may serve as a component of the hydrophobic protein fragment. Homoplasmic or heteroplasmic mtDNA variants in *MT-ND4* gene are known to cause Leber hereditary optic neuropathy (LHON; MIM: #535000; GeneReviews NBK1174; PMID: 31718067) and Leigh syndrome (PMID: 24062162). Heteroplasmic mtDNA variants in *MT-ND4* have also been associated with MELAS-like syndrome (PMID: 12707444, PMID: 30949164) and Leber optic atrophy with hereditary spastic dystonia (MIM: #500001). Diseases caused by *MT-ND4* pathogenic variants are transmitted by maternal inheritance. LHON can also be sporadic with no definitive family history. LHON is a relatively common and rapidly progressing optic neuropathy, which is characterised by painless, progressive bilateral loss of central vision loss, which may lead to blindness, scotomas (central and cecocentral), optic disc atrophy, and a significant reduction in the activity of mitochondrial NADH dehydrogenase subunit 4 (PMID: 28768321). The typical age of onset is during the early adulthood (PMID: 28768321). Males are four to five times more likely than females to be affected (GeneReviews NBK1174). Neurologic abnormalities such as postural tremor, peripheral neuropathy, nonspecific myopathy, and movement disorders have been reported to be more common in individuals with LHON than in the general population. The overall prevalence of LHON is estimated to be 1 in 30,000 (PMID: 31605306). About 90% of people affected by LHON in Asian countries like China, Thailand, and Japan have the *MT-ND4*

m.11778G>A variant. In a study conducted among the Chinese cohorts, other *MT-ND4* variants, such as m.11204T>C, m.11430C>G, m.11213T>G, m.11447G>A, and m.10934G>A were reported with the incidence of 0,312%, 0.078%, 0.078%, 0.078%, and 0.078%, respectively (PMID: 29133631). The penetrance of LHON-causing variants in *MT-ND4* is incomplete, suggesting that additional factors such as sex bias, mtDNA haplogroup, heteroplasmy, nuclear background, and environmental factors contribute to the disease manifestation (GeneReviews NBK1174, PMID: 28768321, PMID: 29133631).

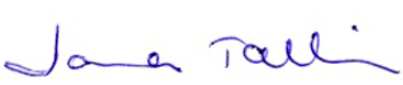
Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA: GenBank sequence NC_012920 gi:251831106.4

CONCLUSION

The *MT-ND4* m.11778G>A, p.(Arg340His) variant is classified as pathogenic based on the current evidence of the variant, including the established association between the *MT-ND4* gene and the patient's phenotype, the variant's rarity in control populations, *in silico* predicted pathogenicity, and identification of the variant in multiple individuals with the same phenotype. This mitochondrial variant was homoplasmic in the tested saliva sample. *MT-ND4* m.11778G>A, p.(Arg340His) occurs typically as homoplasmic and is maternally inherited. Any offspring of the patient are at risk of inheriting the variant and of being affected. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

On May 13, 2020 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



Jonna Tallila, Ph.D.

Senior Geneticist

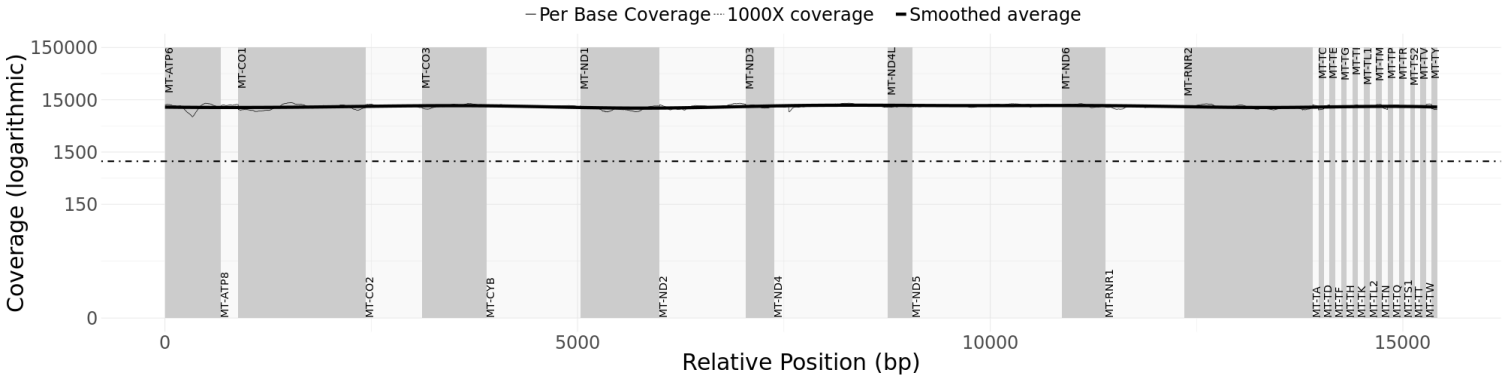
Juha Koskenvuo, MD, Ph.D.

Lab Director, Chief Medical Officer

Jennifer Schleit, Ph.D., FACMG

Senior Geneticist

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.



*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***

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 droplet 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 droplet PCR confirmation).

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.