Name:	**Dummy**	Case ID:	**XXX**
Age:	**19 Years**	Sample Type:	**Blood**
Sex:	**Male**	Sample receipt date:	**11/03/2024**
Referring Clinician:	**Dr. XXX**	Reporting date:	**11/04/2024**
Test Requested:	**Whole Genome Mitochondrial NGS analysis**		

CLINICAL HISTORY:

Dummy, a 19-year-old male came for the genetic evaluation for LHON.

Clinician's Suspicion: ?LHON (Leber hereditary optic neuropathy).

VARIANTS RELEVANT TO INDICATION FOR TESTING

A *likely pathogenic* variant in the *MT-ND4* gene was identified in this individual. No other variants of relevance to the indication were identified. Please see below for more detailed variant information.

Gene & Transcript	Variant	Zygosity	Location	Disorder	Inheritance	ACMG Classification
MT-ND4	c.1019G>A p.Arg340Hisfs*121	Homoplasmy	Exon 1	Leber hereditary optic neuropathy [OMIM ID: 535000]	Mitochondrial Inheritance	Likely pathogenic PM2, PM1, PP5, & PM5

DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)

***MT-ND4* Chr. MT:11778 – Likely pathogenic:**

The missense variant *MT-ND4*(*MT-ND4*):c.1019G>A (p.Arg340Hisfs*121) causes a change at the same amino acid residue as a previously established pathogenic variant. This variant was found in ClinVar (Variant 9708) with a classification of Pathogenic and a review status of 3 stars, reviewed by an expert panel. The p.Arg340Hisfs*121 variant is not reported in any individuals in gnomAD All. The p.Arg340Hisfs*121 variant is not reported in any individuals in 1kG All. There is a small physicochemical difference between arginine and histidine, which is not likely to impact secondary protein structure as these residues share similar properties. 2 variants within 6 amino acid positions of the variant p.Arg340Hisfs*121 have been shown to be pathogenic, while none have been shown to be benign. For these reasons, this variant has been classified as **Likely Pathogenic.**

Leber hereditary optic neuropathy [OMIM ID: 535000]:

Leber optic atrophy, also known as Leber hereditary optic neuropathy (LHON), can be caused by mutation in multiple genes encoded by the mitochondrial genome (mtDNA). Leber hereditary optic neuropathy (LHON) typically presents in young adults as bilateral painless subacute visual failure. The peak age of onset in LHON is in the second and third decades of life, with 95% of those who lose their vision doing so before age 50 years. Very rarely, individuals first manifest LHON in the seventh and eighth decades of life. Males are four to five times more likely to be affected than females, but neither sex nor mutational status significantly influences the timing and severity of the initial visual loss (GeneReviews).

.0001 LEBER OPTIC ATROPHY -MTND4, LHON11778A: The allele changes the highly conserved arginine at amino acid 340 to a histidine (R340H). This allele accounts for over 50% of Leber hereditary optic neuropathy (LHON; 535000) cases among Caucasians and over 90% of the cases in Asians. The mutation has not been observed in random population controls, may be either homoplasmic or heteroplasmic within families, and has been shown to have arisen multiple times on different mtDNA haplotypes in association with the disease (Wallace et al., 1988; Singh et al., 1989). In families harboring this mutation, approximately 33 to 60% of the maternal relatives are affected and of these, about 80% are males. Visual recovery is seen in only 4% of cases. Chinnery

et al. (2001) analyzed 17 independent pedigrees that harbored the 11778G-A mutation. They made the following observations: (1) The frequency of blindness in males was related to the mutation load in the individual's blood. (2) Mothers with 80% or less mutant mtDNA in blood were less likely to have clinically affected sons than mothers with 100% mutant mtDNA in their blood. (3) Within individual lineages, changes in mutation load from one generation to the next were largely determined by random genetic drift. Phasukkijwatana et al. (2006) examined 30 unrelated pedigrees of Thai or Chinese origin with LHON and the 11778G-A mutation. Compared to Caucasian and Japanese populations with the same mutation, the pedigrees in the study showed a lower male-to-female ratio (2.6:1) of affected persons and a higher prevalence of blood heteroplasmy (37% of the pedigrees contained at least 1 heteroplasmic 11778G-A individual). The estimated overall penetrance was 37% for males and 13% for females (OMIM).

RECOMMENDATIONS

Based on the clinical features and the observed genetic findings the following have been recommended:

1. Please note that the genetic information obtained from the patient's genomic DNA was analyzed for regions of the mtDNA, and mutations in regions other than these regions have not been assessed.
2. Genetic counseling is recommended for the patient to discuss the potential clinical implications of this result.
3. **Clinical/ Genotype-phenotype correlation is strongly recommended.**
4. **Sanger validation of identified variant(s) in the proband and segregation analysis in the parents, affected and unaffected family members and close relatives is recommended.**
5. If the above results do not correlate completely with patient phenotype, additional testing is advised based on clinician's recommendation.

METHODOLOGY

DNA extraction is done by QIAamp Blood mini kit. Mitogenome is amplified in two amplicons using long range PCR kit. Amplified Amplicons were pooled in equimolar concentration and then the whole genome library was prepared by QIASeq FX DNA Library Kit. Mito-WGS library was sequenced on Novaseq 6000 to generate with 2X150 bp chemistry to generate 0.5 GB of data. The sequence reads were pre-processed to remove adapter contamination and low-quality bases using the fastp tool. The high-quality trimmed reads were mapped on rCRS mitochondrial sequence using BWA-MEM. Duplicated reads were removed using Mark Duplicate followed by a variant recalibration module incorporated in the GATK tool. Variant calling was performed using GATK mutect2 mitochondrial mode in the setting of ploidy 1. Mitochondrial haplogroup assignment and contamination check was done using haplocheck. Mitochondrial variant annotation was carried out using Varseq & further checked using web server MSeqDR mvTool.

GENES STUDIED

MT-ND1, MT-ND2, MT-ND3, MT-ND4L, MT-ND4, MT-ND5, MT-ND6, MT-CYB, MT-CO1, MT-CO2, MT-CO3, MT-ATP6,MTATP8, MT-RNR2, MT-RNR1, MT-RNR2, MT-TA, MT-TR, MT-TN, MT-TD, MT-TC, MT-TE, MT-TQ, MT-TG, MT-TH, MT-TI, MT-TL1, MT-TL2, MT- TK, MT-TM, MT-TF, MT-TP, MT-TS1, MT-TS2, MT-TT, MT-TW, MT-TY, MT-TV.

IMPORTANCE

1. These test results should be interpreted by the referring clinician only in conjunction with the patient's clinical history, other test results and any previous analysis of appropriate family members.
2. Only phenotype-related Pathogenic and Likely Pathogenic variations reported in the Mito Map database as well as literatures are reported. Haplogroups are not analyzed. A list of variants other than the above is available on request.
3. The classification and interpretation of all the variants in this assay reflects the current state of scientific understanding at the time this report was issued. In some instances, the classification and interpretation of such variants may change as new scientific information comes to light.

LIMITATIONS

1. This report is for research purposes only, not for use in clinical diagnostic or therapeutic applications.
2. This test has not been validated by the FDA, NABL or CAP, and it has been determined by the accrediting bodies that such validation is not required at this time.
3. The analysis is based on the clinical summary provided by the clinician.
4. DNA studies do not constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it is not a diagnosis itself.
5. The significance/classification of the variant(s) may change based on the genetic testing in the parents and other family members.
6. This test was developed, and its performance characteristics were determined by Redcliffe Life Sciences. It has not been cleared or approved by the FDA.
7. The classification of variants of unknown significance can change over time and Redcliffe Life Sciences cannot be held responsible for this. Please contact Redcliffe Life Sciences later to inquire about any changes.
8. Intronic variants are not assessed using this method.
9. Large deletions of more than 10 bp or copy number variations/chromosomal rearrangements cannot be assessed using this method.
10. Certain genes may not be covered completely, and few variants could be missed. Variants not detected by the assay that was performed may impact the phenotype.
11. The variants have not been validated by Sanger sequencing.
12. Incidental or secondary findings (if any) that meet the ACMG guidelines can also be given upon request.
13. Variants that have coverage below ≤ 20 are not taken into consideration.

---------- End Of Report ----------

Govind M Suresh
Senior Genome Analyst

Dr. Aruna Priya
Lead – Genetic Counselor & Reporting

Dr. Himani Pandey
Lab Head - Clinical Genomics
Post-Doc. Fellowship
(Medical Genetics) SGPGIMS

Dr. Shivani Mishra
DM (Medical Genetics, SGPGIMS)
MD(Obgyn), MBBS
Former Associate Professor,
KMC Hospital Manipal (MAHE)
Consultant Clinical Geneticist

Conditions for Reporting

1. It is presumed that the specimen belongs to the patient named or identified, such verification being carried out at the point of generation of said specimen.
2. A test might not be performed due to following reasons:
   a. Specimen quantity not sufficient (Inadequate collection/spillage during transit).
   b. Specimen quality not acceptable (Hemolysis/clotted/lipemic).
   c. Incorrect sample type.
   d. Test canceled either on request of patient or doctor.
3. In any of the above cases a fresh specimen will be required for testing and reporting.
4. The results of the tests may vary from lab to lab, time to time for the same patient.
5. The reported results are dependent on individual assay methods, equipment, method sensitivity, specificity and quality of the specimen received.
6. Partial representation of the report is not allowed.
7. The reported tests are for the notification of the referring doctor, only to assist him/her in the diagnosis and management of the patient.
8. Report with status "Preliminary" means one or more tests are yet to be reported.
9. This report is not valid for Medico Legal Purpose.
10. Applicable Jurisdiction will be of "Delhi" for any dispute/claim concerning the test(s) & results of the test(s).

Disclaimer: Method given in report are only indicative and can be changed depending upon type of machine and kit available at time of testing. Not all tests at all locations are under NABL scope. Availability of tests under NABL scope varies from lab to lab.

Terms and Conditions of Reporting

1. The presented findings in the Reports are intended solely for informational and interpretational purposes by the referring physician or other qualified medical professionals possessing a comprehensive understanding of reporting units, reference ranges, and technological limitations. The laboratory shall not be held liable for any interpretation or misinterpretation of the results, nor for any consequential or incidental damages arising from such interpretation.

2. It is to be presumed that the tests performed pertain to the specimen/sample attributed to the Customer's name or identification. It is presumed that the verification particulars have been cleared out by the customer or his/her representation at the point of generation of said specimen / sample. It is hereby clarified that the reports furnished are restricted solely to the given specimen only.

3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

4. This report shall not be deemed valid or admissible for any medico-legal purposes.

5. The Customers assume full responsibility for apprising the Company of any factors that may impact the test finding. These factors, among others, includes dietary intake, alcohol, or medication / drug(s) consumption, or fasting. This list of factors is only representative and not exhaustive.