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PROBAND WHOLE GENOME SEQUENCING

PATIENT		SPECIMEN/TEST		PHYSICIAN
Patient:	Jane Doe	Sample/Data:	Blood/FASTQ	Dr. Gene
Date of Birth:	01/01/2020	Collection Date:	03/25/2024	Genetics Clinic
Gender:	Female	Receiving Date:	03/26/2024	
ID:	LP9999999	Test Order Date:	03/26/2024	
Case ID:	200000000	Report Date:	05/01/2024	
Family ID:	123456789	Test code:	1000	

CLINICAL SUMMARY

The patient is a four-year-old female with a history of Leber Hereditary Optic Neuropathy. We were requested to perform whole genome sequencing on a blood sample from the patient (LP9999999).

RESULT SUMMARY

Significance of the Findings	Gene	Variant Count
High Impact	ND1	1 (P)
Possibly High Impact	--	--
Uncertain Impact	--	--
Medically Actionable Incidental	--	--
Carrier Status	--	--

COMMENTS & RECOMMENDATIONS

A homoplasmic pathogenic variant, m.3460G>A (p.A52T), in the ND1 gene was detected. The m.3460G>A (p.A52T) variant has been previously reported (ClinVar Variation ID 9722). Defects in the ND1 gene are associated with Leber Hereditary Optic Neuropathy (LHON; MIM# 535000). Clinical correlations and genetic counseling are recommended.

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RESULT DETAILS

High Impact — Gene: MT-ND1

Position	Variant	Zygosity	Inheritance	Comments	Population Freq*	Variant Sig.
ChrM:3460 G>A	ENST00000361390 exon1 c.G154A p.A52T	Hom 1.0	Not assessed	ACMG: PS3+PS4+PM6+PP1+PP3 PMID:8680405, 1732158, 1928099, 1734726, 7901141, 1674640, 1550131, 8213820, 1444915, 8270249, 8024249, 1959619, 8401538, 8071952, 8496715, 8195807, 7853025, 7770132, 7611298, 7635294, 7603534, 7924787, 7821467, 7710535, 7629530, 7977345, 7735876, 7760326, 7599218, 9012411, 8659512, 8571959, 10976107, 9302261, 9412783, 9561832, 9150158, 9852675, 10426140, 10520236, 8755941, 10426138, 10939569, 12205655, 11074292, 15638829, 12446713, 16083845, 15033723, 16523671, etc.	0.05/0.0007 39645	P

*Frequencies in GenBank and MitoDB

INTERPRETATIONS:

Variant:
A homoplasmic pathogenic variant, m.3460G>A (p.A52T), in the ND1 gene was detected. The m.3460G>A (p.A52T) variant has been previously reported in affected individuals from more than 50 families (PMID: 1928099, 1674640, 7629530, 1734726, 1550131, 8496715, 8024249, 8556281, 8571959, 12205655, 11906302, 12807863, 12518276, 16738010, 17122117, 18216301, 18562849, 20232220, 21887510, 25338955, 25053773, 28314831, 30053855, 30591017; PS4_Strong). The m.3460G>A (p.A52T) variant segregated with disease in multiple affected members in multiple families, and several healthy family members had lower to undetectable levels of the variant (PMID: 1734726, 8571959, 11906302; PP1_Moderate). There is one reported de novo occurrence to our knowledge (PMID: 12518276; PM6_Supporting). This variant is present in the healthy population, consistent with reduced penetrance of this variant. The computational predictor APOGEE gives a consensus rating of pathogenic with a score of 0.86, which predicts a damaging effect on gene function (PP3_Supporting). Extensive cybrid studies support the functional impact of this variant, as do E. coli and mouse studies (PMID: 35383288, 22079202, 15720387, 15883259, 15342361, 10976107; PS3_Moderate). In summary, evidence suggests that the m.3460G>A in ND1 is pathogenic for LHON.

Gene:
Subunit 1 is one of 7 mitochondrial DNA (mtDNA) encoded subunits (MTND1, MTND2, MTND3, MTND4, MTND4L, MTND5, MTND6) included among the approximately 41 polypeptides of respiratory Complex I (NADH:ubiquinone oxidoreductase). Complex I is the first step in the electron transport chain of mitochondrial oxidative phosphorylation (OXPHOS) and is located within the mitochondrial inner membrane. It accepts electrons from NADH and transfers them, through a series of electron carriers, to ubiquinone (Coenzyme Q10). The internal electron carriers of complex I include flavin mononucleotide (FMN) and 6 iron-sulfur clusters designated N-1a, N-1b, N-2, N-3, N-4, and N-5.

Disease:
Defects in the ND1 gene are associated with Leber Hereditary Optic Neuropathy (LHON; MIM# 535000). LHON patients present with midlife, acute or subacute, painless, central vision loss leading to central scotoma.

METHODOLOGY & LIMITATIONS

Short-read whole genome sequencing (WGS) was performed using genomic DNA. The DNA was fragmented and ligated to unique index adapters using an Illumina library preparation. No library amplification (PCR) was performed. The library was assessed for quality and sequenced using an Illumina NovaSeq 6000 instrument (150-bp paired end sequence reads) to a mean coverage of 30X. The output data were converted from BCL file to FASTQ format and aligned to human reference genome GRCh38. Variant calling and annotation were performed using AiLife's AilisNGS platform. The variants were interpreted using modified ACMG/AMP guidelines[1]. Medically actionable and carrier findings are reported according to ACMG recommendations (see Supplemental Tables)[2-3].

Regions of the human genome exist that are difficult to resolve with current technology. This test was designed to detect single nucleotide variants (SNVs) and small insertions/deletions (indels). The test is limited in its ability to detect copy number variants (CNVs), short tandem repeat expansions (STRs), complex structural variation (SV), and mosaicism. The sensitivity of the test varies across variant types, with highest sensitivity for SNVs and indels (>95%).

Diagnostic yield for whole genome sequencing tests in rare disease is reported to be 25-60%, depending on the indication for testing and extent of prior clinical testing[4-6]. Therefore, a negative test result does not mean that the patient does not have a genetic disease. The interpretations set forth in this report are based on current knowledge in the field and may change over time. Variants identified may not be reported as causative due to limited evidence to support a gene-disease association. Variants may also be reclassified as new evidence becomes available. As such, periodic reanalysis of the patient's data should be considered (additional fees apply). Results should be interpreted in the context of the patient's medical and family history. Genetic counseling is recommended to discuss the implications of this report.

This test was developed and its performance characteristics determined by The Jackson Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test may be used for clinical purposes and should not be regarded as purely investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88) as qualified to perform high complexity clinical testing. The Jackson Laboratory makes no promises or guarantees that a healthcare provider, insurer or other third-party payor, whether private or governmental, will reimburse a patient for the cost of this test.

References:

[1]Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753.

[2]Miller DT, et al. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2022 Jul;24(7):1407-1414. doi: 10.1016/j.gim.2022.04.006. Epub 2022 Jun 17. PMID: 35802134.

[3]Edwards JG, et al. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol. 2015 Mar;125(3):653-662. doi: 10.1097/AOG.0000000000000666. PMID: 25730230.

[4]Pagnamenta AT, et al. Structural and non-coding variants increase the diagnostic yield of clinical whole genome sequencing for rare diseases. Genome Med. 2023 Nov 9;15(1):94. doi: 10.1186/s13073-023-01240-0. PMID: 37946251; PMCID: PMC10636885.

[5]Lee HF, Chi CS, Tsai CR. Diagnostic yield and treatment impact of whole-genome sequencing in paediatric neurological disorders. Dev Med Child Neurol. 2021 Aug;63(8):934-938. doi: 10.1111/dmcn.14722. Epub 2020 Nov 26. PMID: 33244750.

[6]van der Sanden BPGH, et al The performance of genome sequencing as a first-tier test for neurodevelopmental disorders. Eur J Hum Genet. 2023 Jan;31(1):81-88. doi: 10.1038/s41431-022-01185-9. Epub 2022 Sep 16. PMID: 36114283; PMCID: PMC9822884.

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Supplementary Table 1. ACMG SF v3.2 list for reporting of secondary findings*

ACTA2	ACTC1	ACVRL1	APC	APOB	ATP7B	BAG3	BMPR1A	BRCA1	BRCA2
BTD	CACNA1S	CALM1	CALM2	CALM3	CASQ2	COL3A1	DES	DSC2	DSG2
DSP	ENG	FBN1	FLNC	GAA	GLA	HFE	HNF1A	KCNH2	KCNQ1
LDLR	LMNA	MAX	MEN1	MLH1	MSH2	MSH6	MUTYH	MYBPC3	MYH11
MYH7	MYL2	MYL3	NF2	OTC	PALB2	PCSK9	PKP2	PMS2	PRKAG2
PTEN	RB1	RBM20	RET	RPE65	RYR1	RYR2	SCN5A	SDHAF2	SDHB
SDHC	SDHD	SMAD3	SMAD4	STK11	TGFBR1	TGFBR2	TMEM127	TMEM43	TNNC1
TNNI3	TNNT2	TP53	TPM1	TRDN	TSC1	TSC2	TTN	TTR	VHL
WT1									

* D.T. Miller et al. Genet Med (2023). https://doi.org/10.1016/j.gim.2023.100866

Supplementary Table 2. Genes and Diseases for Carrier Status Reporting**

Diseases	Gene
Cystic Fibrosis	CFTR
Beta Hemoglobinopathies (Beta-thalassemia and Sickle Cell)	HBB
Niemann-Pick Disease	NPC1, SMPD1
Gaucher Disease	GBA
Tay-Sachs Disease	HEXA
Maple Syrup Urine Disease	DBT, BCKDHA, BCKDHB
Glycogen Storage Disease: Type Ia	G6PC
Usher Syndrome	PCDH15, CLRN1, USH2A, CDH23
Bloom Syndrome	BLM
Fanconi Anemia	FANCC
Familial Dysautonomia	ELP1 (IKBKAP)
Familial Hyperinsulinism	ABCC8
Canavan Disease	ASPA
Mucolipidosis IV	MCOLN1
Joubert Syndrome	TMEM216

** Edwards et al. Obstet Gynecol. 2015 Mar;125(3):653