Whole Exome Sequencing Analysis & Whole Mitochondrial Genome Sequencing

Patient name	:	**XX**	**PIN**	:	**XXXX**
Gender/ Age	:	**Female/ 8 years**	**Sample number**	:	**XXXX**
Referring Clinician	:	**XXXX**	**Sample collection date**	:	**25-06- 2024**
Specimen	:	**Peripheral Blood**	**Sample receipt date**	:	**25-06-2024**
			Report date	:	**22-04-2025**

Clinical history

Proband, XX was born to non-consanguineous parents. She is a term baby with chief complaints of delayed walking, can't stand up from sitting or squatting position, can't climb stairs, stumbles while walking and abnormal gait. Her EMG study shows evidence of myogenic pattern in the biceps muscles as well as chronic myogenic/neurogenic pattern in the tibilais muscle. Her MLPA analysis for spinal muscular atrophy (Done at Anderson diagnostics, AND24730003475) revealed homozygous deletion of exon 7 and 8 of *SMN1* gene. She has elevated CPK (259 U/L) and ESR (27mm/hr) levels. Proband, XX has been evaluated for pathogenic variations.

Whole Exome Sequencing Analysis

Results

No pathogenic or likely pathogenic variant causative of the reported phenotype was detected

*Genetic test results are based on the recommendation of American college of Medical Genetics [1].
No other variant that warrants to be reported for the given clinical indication was identified.

Whole Mitochondrial Genome Sequencing

Pathogenic variant causative of the reported phenotype was detected

List of significant variant identified related to the phenotype:

Gene	Variant*	Allele Status	Disease	Classification*
MT-TL1 (+)	m.3243A>G	Heteroplasmic (**Reference base depth: 47.86%; Alternate base depth: 52.14%**)	Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes	**Pathogenic**

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No other variant that warrants to be reported for the given phenotype has been identified.

The mitochondrial genome was completely covered.

Interpretation

***MT-TL1*: m.3243A>G**

Variant summary: A heteroplasmic missense variation in the *MT-TL1* gene (m.3243A>G; Depth: 4524x) was detected.

Clinical and Literature evidence: This variant has been classified as likely pathogenic in ClinVar database [3]. This variant has previously been reported in heteroplasmic state, in patients affected with MELAS. The mutation m.3243A>G results in multisystem failure, where 70% of the mutant mtDNA impairs the mitochondrial unfolded protein response [4]. This variant has previously been reported inpatient with proximal muscle weakness in the lower extremities and moderate weakness in the upper extremities [5].

OMIM phenotype: Mutations in the *MT-TL1* gene have been reported to be associated with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes [2,6].

Variant classification: Based on the evidence, **this *MT-TL1* variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms.**

Inspite of a partial clinical match, the variant has been classified as pathogenic based on current literature evidence. The results have to be carefully correlated with the clinical findings of the patient.

Recommendations

- Sequencing the variant in the mother and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is advised.

Methodology: Whole Exome Sequencing Analysis

DNA extracted from the blood was used to perform whole exome using whole exome capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using GenoLab M sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAscope has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (v3.1,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Methodology: Whole Mitochondrial Genome Sequencing

DNA extracted from blood was used to amplify the mitochondrial genome by LA-PCR followed by GenoLab M DNA library preparation. The libraries were sequenced to mean >100X coverage on GenoLab M sequencing platform. The sequences obtained were aligned to revised Cambridge mitochondrial reference genome (rCRS) analyzed using sentieon to identify variants relevant to the clinical indication. We follow the Varseq best practices framework for identification of variants in the sample. Clinically relevant mutations were annotated using published variants in literature and Mito Map database. Only non-synonymous variants found in the sample were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Sequence data attributes

Total reads generated	11.66 Gb
Data ≥ Q30	88.45 %

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed

Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [7].

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. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000009589.63
4. Aras S, et al. Mitochondrial Nuclear Retrograde Regulator 1 (MNRR1) rescues the cellular phenotype of MELAS by inducing homeostatic mechanisms. Proc Natl Acad Sci U S A. 2020 Dec 15;117(50):32056-32065. doi: 10.1073/pnas.2005877117. Epub 2020 Nov 30. Erratum in: Proc Natl

Acad Sci U S A. 2021 Feb 16;118(7):e2100262118. doi: 10.1073/pnas.2100262118. PMID: 33257573; PMCID: PMC7749287.

5. Kawakami Y, et al. Mitochondrial myopathy with progressive decrease in mitochondrial tRNA(Leu)(UUR) mutant genomes. Ann Neurol. 1994 Mar;35(3):370-3. doi: 10.1002/ana.410350322. PMID: 8122892.
6. https://www.mitomap.org/MITOMAP
7. Miller, David T., et al. "ACMG SF v3. 1 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)." Genetics in Medicine 24.7 (2022): 1407-1414.

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