Whole Exome Sequencing Analysis & Whole Mitochondrial Genome Sequencing

Patient name	:	**Baby of. XX**	**PIN**	:	**XXXX**
Gender/ Age	:	**Female / 103 Days**	**Sample number**	:	**XXXX**
Clinic/Hospital	:	**XXXX**	**Sample collection date**	:	**11-03-2025**
Specimen	:	**Peripheral Blood**	**Sample received date**	:	**12-03-2025**
			Report date	:	**22-04-2025**

Clinical history

Baby of XX is presented with chief complaints of seizure, poor metabolism, occult blood in stool, urine analysis indicative of calcium carbonate crystals, urine albumin was positive. Her blood investigations was indicative of low levels of calcium (7.63mg/dl), potassium (2.47 m.mol/liter) and haemoglobin (9.4 gm/dl) levels. Her total protein and albumin levels are low. Proband. Baby of XX has been evaluated for pathogenic variations.

Additional Indication: Baby of XX is suspected to be affected by congenital nephrotic syndrome or Steroid resistant nephrotic syndrome.

Whole Exome Sequencing Analysis

Results

Variant of uncertain significance related to given phenotype was detected

List of uncertain significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
LAMB2 (-)	Exon 5	c.545G>A **(p.Cys182Tyr)**	Homozygous	Nephrotic syndrome, type 5, with or without ocular abnormalities **(OMIM#614199)** Pierson syndrome **(OMIM#609049)**	**Uncertain significance** (PM2, PP3)	Autosomal recessive

*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.

Interpretation

***LAMB2:* c.545G>A**

Variant summary: A homozygous missense variation in exon 5 of the *LAMB2* gene (chr3:g.49131638C>T, NM_002292.4, Depth: 140x) that results in the amino acid substitution of Tyrosine for Cysteine at codon 182 (p.Cys182Tyr) was detected.

Population frequency: This variant has not been reported in gnomAD database and 1000 genomes database.

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv), LRT and deleterious by CADD and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Nephrotic syndrome, type 5, with or without ocular abnormalities (OMIM#614199) and Pierson syndrome (OMIM#609049) are caused by homozygous or compound heterozygous mutation in the *LAMB2* gene (OMIM*150325). Nephrotic syndrome type 5 (NPHS5) is an autosomal recessive disorder characterized by very early onset of progressive renal failure manifest as proteinuria with consecutive edema starting in utero or within the first 3 months of life. A subset of patients may develop mild ocular anomalies, such as myopia, nystagmus, and strabismus. Pierson syndrome (PIERS) is an autosomal recessive disorder comprising congenital nephrotic syndrome with diffuse mesangial sclerosis and distinct ocular abnormalities, including microcoria and hypoplasia of the ciliary and pupillary muscles, as well as

other anomalies. Many patients die early, and those who survive tend to show neurodevelopmental delay and visual loss. These diseases follow autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

CNV Findings

No significant Copy Number Variations (CNV) related to phenotype was detected.

Whole Mitochondrial Genome Sequencing

No pathogenic or likely pathogenic variant associated with the given 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 phenotype has been identified.

Recommendations

- Sequencing the variant(s) in the parents 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

DNA extracted from the blood was used to perform whole exome and whole mitochondrial using Exome + Mitochondrial capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using SURFSeq 5000 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

(v4.1.0,3.1,2.1.1), 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.

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.

Sequence data attributes

Total reads generated	13.68 Gb
Data ≥ Q30	97.11%

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 and Promoter region variants are not assessed using this assay.
- This assay has a sensitivity of 70-75% in detecting large deletions/duplications of more than 10 base pairs or copy number variations (CNV). However, it is important to note that any CNVs detected must be confirmed using an alternate method.
- 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 [3].

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. 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 Americans College of Medical Genetics and Genomics (ACMG)." Genetics in Medicine 24.7 (2022): 1407-1414.

This report has been reviewed and approved by:

			
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