Whole Exome Sequencing Analysis

Patient name	:	**Fetus of YYY**	**PIN**	:	**...**
Gestational Age	:	**24 Weeks 1 Day**	**Sample number**	:	**...**
Referring Clinician	:	**Dr. ...**	**Sample collection date**	:	**05.04.2025**
Hospital/Clinic	:	**...**	**Sample received date**	:	**06.04.2025**
Specimen	:	**Amniotic fluid**	**Report date**	:	**05.05.2025**

(This test confers to the PCPNDT act and does not determine the sex of the fetus)

Clinical history

Mrs. YYY is of third-degree consanguineous marriage. Her first-born male child presented with seizures and global developmental delay and succumbed during the infancy period. Her second-born female child presented with seizures and global developmental delay and succumbed during the neonatal period. She has an ongoing pregnancy of ~24 weeks GA. USG at 18 weeks 5 days GA revealed single umbilical artery. The fetus of YYY has been evaluated for pathogenic variations.

Results

Variants of uncertain significance related to given phenotype were detected

List of uncertain significant variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
CPS1 (+)	Exon 2	c.203C>A **(p.Ala68Asp)**	Homozygous	Carbamoylphosphate synthetase I deficiency **(OMIM#237300)**	**Uncertain significance** (PM2, PP3)	Autosomal Recessive
LMBRD1 (-)	Exon 6	c.517G>C **(p.Glu173Gln)**	Homozygous	Methylmalonic aciduria and homocystinuria, cblF type **(OMIM#277380)**	**Uncertain significance** (PM2)	Autosomal Recessive

Reproductive decisions based on variants of uncertain significance are not recommended.

The variant (c.203C>A; p.Ala68Asp) in *CPS1* gene has been detected in Father - PPP (…) and Mother - YYY (…) in heterozygous state.

The variant (c.517G>C; p.Glu173Gln) in *LMBRD1* gene has been detected in Father – PPP (…) and Mother YYY (…) in heterozygous state.

*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

***CPS1:* c.203C>A**

Variant summary: A homozygous missense variation in exon 2 of the *CPS1* gene (chr2:g.210573374C>A, NM_001875.5, Depth: 120x) that results in the amino acid substitution of Aspartic acid for Alanine at codon 68 (p.Ala68Asp) 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, LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Carbamoylphosphate synthetase I deficiency **(**OMIM#237300) is caused by homozygous or compound heterozygous mutation in the *CPS1* gene (OMIM*608307). Carbamoyl phosphate synthetase I deficiency is an inborn error of metabolism of the urea cycle which causes hyperammonemia. There are 2 main forms: a lethal neonatal type and a less severe, delayed-onset type. Urea cycle disorders are characterized by the triad of hyperammonemia, encephalopathy, and respiratory alkalosis. Five disorders involving different defects in the biosynthesis of the enzymes of the urea cycle have been described: ornithine transcarbamylase deficiency, carbamyl phosphate synthetase deficiency, argininosuccinate synthetase deficiency, or citrullinemia, argininosuccinate lyase deficiency and arginase deficiency. This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance.
Clinical correlation is strongly recommended.
The variant has been detected in Father - PPP and Mother – YYY in heterozygous state.

***LMBRD1:* c.517G>C**

Variant summary: A homozygous missense variation in exon 6 of the *LMBRD1* gene (chr6:g.69741834C>G, NM_018368.4, Depth: 46x) that results in the amino acid substitution of Glutamine for Glutamic acid at codon 173 (p.Glu173Gln) 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 LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Methylmalonic aciduria and homocystinuria of the cblF type **(**OMIM#277380) is caused by homozygous or compound heterozygous mutation in the *LMBRD1* gene (OMIM*612625). Combined methylmalonic aciduria (MMA) and homocystinuria is a genetically heterogeneous disorder of cobalamin metabolism. The defect causes decreased levels of the coenzymes adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), which results in decreased activity of the respective enzymes methylmalonyl-CoA mutase and methyltetrahydrofolate:homocysteine methyltransferase, also known as methionine synthase. This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance. **Clinical correlation is strongly recommended.**

The variant has been detected in Father - PPP and Mother – YYY in heterozygous state.

MCC Status

- The sample has been screened for maternal cell contamination and it is found to be negative for MCC (Refer Appendix: I).

Additional Variant(s): Segregation of the variant(s) in the family members

Gene and Region	Inheritance	Fetus YYY	YYY (Mother)	PPP (Father)	Variant Classification
CPS1 (c.203C>A; p.Ala68Asp)	AR	Homozygous	Heterozygous	Heterozygous	Uncertain Significance
LMBRD1 (c.517G>C); p.Glu173Gln)	AR	Homozygous	Heterozygous	Heterozygous	Uncertain Significance

Recommendations

- **Reproductive decisions based on variants of uncertain significance are not recommended**.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is recommended.

Methodology: Whole Exome Sequencing Analysis

DNA extracted from amniotic fluid was used to perform whole exome using whole exome capture kit. The libraries were sequenced to mean depth of >80-100X on Illumina sequencing platform. We follow the GATK best practices framework for identification of germline variants in the sample using Sentieon [Sentieon]. The sequences obtained are aligned to human reference genome (GRCh38) using BWA aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels [Sentieon]. Sentieon haplotype caller is then used to identify variants in the sample. The germline variants identified in the sample is deeply annotated using Inhouse pipeline. Gene annotation of the variants is performed using VEP program against the Ensembl release 99 human gene model. In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth method. This algorithm detects CNVs based on comparison of the read-depths in the sample of interest with the matched aggregate reference dataset.

Clinically relevant mutations in both coding and non-coding regions are annotated using published variants in literature and a set of diseases databases: ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v4.1.0, v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genome, TOPMed (Freeze_8), Genome Asia, and our internal Indian population database. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretation and reporting.

Sequence data attributes

Total reads generated	:	8.76 Gb
Total reads aligned (%)	:	99.99 %
Data ≥ Q30	:	98.32 %

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 [3].
- This test does not determine the sex of the fetus in adherence to the PCPNDT act.

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 American 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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Appendix I

Estimation of maternal cell contamination %

No	Marker	Maternal genotype	Fetal genotype	No. of shared alleles	MCC	Remarks
1	D8S1179	16	8, 16	1	-	Non informative
2	D21S11	32.2, 33.2	32.2	1	-	Informative
3	D7S820	8, 10	8, 11	1	-	Informative
4	CSF1PO	11	11, 12	1	-	Non informative
5	D3S1358	16	15, 16	1	-	Non informative
6	TH01	7, 9	7,9	2	-	Non informative
7	D13S317	9	9,12	1	-	Non informative
8	D16S539	10, 13	10, 11	1	-	Informative
9	D2S1338	22, 23	22, 23	2	-	Non informative
10	D19S433	12.2, 14.2	12.2, 13	1	-	Informative
11	vWA	15,18	15,17	1	-	Informative
12	TPOX	8, 11	8, 11	2	-	Non informative
13	D18S51	13, 18	13, 16	1	-	Informative
14	D5S818	12	12	1	-	Non informative
15	FGA	22, 27	27	1	-	Informative
The average percentage of maternal cell contamination in the fetal sample is 0%						
