Whole Exome Sequencing Analysis

Patient name	:	**Fetus of ZZZ**	**PIN**	:	**XXX**
Gestational Age	:	**16 Weeks 4 Days**	**Sample number**	:	**XXX**
Referring Clinician	:	**Dr. XXX**	**Sample collection date**	:	**06.03.2025**
Hospital/Clinic	:	**XXX**	**Sample received date**	:	**10.03.2025**
Specimen	:	**Amniotic fluid cultured cells**	**Report date**	:	**02.05.2025**

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

Clinical history

Mrs. ZZZ is married consanguineous to Mr. YYY Their first-born female child presented with chief complaints of delayed milestones, mild mental retardation, hypotonia and after 1 year of age child developed motor regression, convulsions following an episode of fever and URTI and succumbed to illness at 1.5 years. Molecular diagnosis for Neimann-Pick Disease A/B (Done elsewhere, 2022) revealed a compound heterozygous variant of uncertain significance in the index child c.1772G>A; p.Arg591His and c. 1529C>T; p.Ser510Phe in the *SMPD1* gene associated with Neimann-Pick Disease. Carrier screening by whole exome sequencing in both partners revealed YYY (2024) is found to be heterozygous carrier for variant of uncertain significance c.1772G>A; p.Arg591His in *SMPD1* gene and Mrs. ZZZ (… 2024) is found to be carrier of variant of uncertain significance c.1529C>T; p.Ser510Phe in *SMPD1* gene associated with Niemann-Pick disease, type A and type B. Mr. YYY is found to be heterozygous carrier for likely pathogenic variant in c.737G>A; p.Arg246Gln in *SRD5A2* gene associated with Pseudovaginal perineoscrotal hypospadias. Mrs. ZZZ is found to be heterozygous carrier for likely pathogenic variants: c.709G>C; p. Glu237Gln in *PIGT* gene associated with Multiple congenital anomalies-hypotonia-seizures syndrome 3, c.720C>A; p.Cys240Ter in *FOXE3* gene associated with Anterior segment dysgenesis 2, multiple subtypes, c.4092+1G>A in *PKD1L1* gene associated with Heterotaxy, visceral, 8, autosomal, c.20905+1G>A in *OBSCN* gene associated with {Rhabdomyolysis, susceptibility to, 1}. Additionally both partners heterozygous carriers for variants of uncertain significance: c.5C>T; p.Thr2Met in *HPD* gene associated with Tyrosinemia, type III, c.2428G>A; p.Glu810Lys in *MSH6* gene associated with Mismatch repair cancer syndrome 3, c.509G>C; p.Arg170Pro in *PEX13* gene associated with Peroxisome biogenesis disorder 11A (Zellweger), Peroxisome biogenesis disorder 11B. USG at 13 weeks 1 day GA indicative of normal findings and double marker screening showed low risk for trisomy 13,18 and 21. She is currently 16 weeks 4 days pregnant. The fetus of ZZZ has been evaluated for pathogenic variations.

Results

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

Previously reported (c.1772G>A; p.Arg591His) variant in *SMPD1* gene in Father - YYY in heterozygous state and sibling (done elsewhere) in compound heterozygous state has been detected in the Fetus of ZZZ in heterozygous state in this assay.

Previously reported variant (c.1529C>T; p.Ser510Phe) in *SMPD1* gene in Mother – ZZZ in heterozygous state and sibling (done elsewhere) in compound heterozygous state has not been detected in the Fetus of ZZZ.

*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.

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 of ZZZ	Mrs. ZZZ (Mother)	Mr.YYY (Father)	Variant Classification
SMPD1 (c.1772G>A; p.Arg591His)	AR	Heterozygous	Absent	Heterozygous	Uncertain Significance
SMPD1 (c.1529C>T; p.Ser510Phe)	AR	Absent	Heterozygous	Absent	Uncertain Significance
SRD5A2 (c.737G>A; p.Arg246Gln)	AR	Heterozygous	Absent	Heterozygous	Likely pathogenic
PIGT (c.709G>C; p.Glu237Gln)	AR	Heterozygous	Heterozygous	Absent	Likely pathogenic

FOXE3 (c.720C>A; p.Cys240Ter)	AR	Absent	Heterozygous	Absent	**Likely pathogenic**
PKD1L1 (c.4092+1G>A; 5' Splice site)	AR	Absent	Heterozygous	Absent	**Likely pathogenic**
OBSCN (c.20905+1G>A; 5' Splice site)	AR	Heterozygous	Heterozygous	Absent	**Likely pathogenic**
HPD (c.5C>T; p.Thr2Met)	AR	Homozygous	Heterozygous	Heterozygous	**Uncertain Significance**
MSH6 (c.2428G>A; p.Glu810Lys)	AR	Homozygous	Heterozygous	Heterozygous	**Uncertain Significance**
PEX13 (c.509G>C; p.Arg170Pro)	AR	Homozygous	Heterozygous	Heterozygous	**Uncertain Significance**

Recommendations

- **Reproductive decisions based on variants of uncertain significance are not recommended**.
- The *SRD5A2* gene has pseudogene in the human genome.
- **Alternative test is strongly recommended to rule out the deletion/duplication**.
- Genetic counselling is recommended.

Methodology: Whole Exome Sequencing Analysis

DNA extracted from the Amniotic fluid cultured cells 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 (v4.1.0, 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

Sequence data attributes

Total reads generated	9.20 Gb
Data ≥ Q30	96.54%

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.

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Appendix I

Estimation of maternal cell contamination %

No	Marker	Maternal genotype	Fetal genotype	No. of shared alleles	MCC	Remarks
1	D3S1358	15	15	1	-	Non informative
2	TH01	7, 9	9	1	-	Informative
3	D21S11	29, 31	28, 31	1	-	Informative
4	D18S51	14, 19	15, 19	1	-	Informative
5	Penta E	12, 16	11, 16	1	-	Informative
6	D5S818	9, 10	9, 12	1	-	Informative
7	D13S317	9, 10	10, 11	1	-	Informative
8	D7S820	8, 10	8, 11	1	-	Informative
9	D16S539	10, 12	10, 12	2	-	Non informative
10	CSF1PO	10, 13	13	1	-	Informative
11	Penta D	10, 13	9, 13	1	-	Informative
12	Amelogenin	X	Not revealed	-	-	-
13	vWA	16, 18	16, 18	2	-	Non informative
14	D8S1179	15, 16	13, 16	1	-	Informative
15	TPOX	8, 10	9, 10	1	-	Informative
16	FGA	23, 25	20, 23	1		Informative
The average percentage of maternal cell contamination in the fetal sample is 0%						
