Whole Exome Sequencing Analysis

Patient name	:	**Fetus of XXX**	**PIN**	:	**XXX**
Gestational Age	:	**18 Weeks 2 Days**	**Sample number**	:	**XXX**
Referring Clinician	:	**Dr. YYY**	**Sample collection date**	:	**28.03.2025**
Hospital/Clinic	:	**Dr. YYY**	**Sample received date**	:	**29.03.2025**
Specimen	:	**Amniotic fluid**	**Report date**	:	**08.05.2025**

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

Clinical history

Mrs. XXX and Mr. PPP are a non-consanguineous couple. They were presented with their on-going pregnancy of ~21 weeks GA and the TIFFA scan at 20-21 weeks GA s/o length of all long bones in low normal range (Humerus length corresponds to 6th centile and femur length corresponds to 8th centile), no bowing/ fractures observed, shape and echogenicity of all long bones is normal. Double marker screening was indicative of increased risk for down syndrome (1:100). Fluorescence In Situ Hybridization (FISH) Analysis in amniotic fluid (YYY, 2025) revealed a normal chromosome complement for chromosomes 13, 18, 21 and sex chromosomes. Conventional karyotyping in the amniotic fluid (done at AND241H0002285, 2025) was indicative of 46, U (sex chromosomes are not disclosed as indicated by the letter U). Female partner's sister's grand-daughter has been diagnosed with down syndrome. The fetus of Mrs. XXX has been evaluated for pathogenic variations.

Results

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

List of uncertain significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
#TNFRSF11A (+)	Exon 9	c.826T>C **(p.Phe276Leu)**	Heterozygous	{Paget disease of bone 2, early-onset} **(OMIM#602080)** Osteolysis, familial expansile	**Uncertain Significance (PM2)**	Autosomal Dominant

				(OMIM#174810)		
				Osteopetrosis, autosomal recessive 7 **(OMIM#612301)**		Autosomal Recessive

Parental testing is recommended, and classification of the variant(s) may change based on segregation analysis.

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

#A missense variant at the same nucleotide position in *TNFRSF11A* gene (c.826T>G; p.Phe276Val) has been classified as uncertain significance in ClinVar database [3].

*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).

Recommendations

- **Reproductive decisions based on variants of uncertain significance are not recommended.**
- **A heterozygous deletion of size (~1.4mb) on chromosome 17 [chr17:g.(14159947_14191989)_(15574183_15593336)del] encompassing multiple genes suggestive of a copy number variant was detected. The specificity of NGS based assays to detect large deletion is low and an alternate method of testing like MLPA/Microarray/PCR is recommended to confirm the same. Clinical correlation is strongly recommended.**
- 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 recommended.

Methodology: Whole Exome Sequencing Analysis

DNA extracted from amniotic fluid 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	:	8.50 Gb
Data ≥ Q30	:	94.92%

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].
- 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. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002170000.3
4. 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, 16	16, 17	1	-	Informative
2	TH01	9, 9.3	7, 9.3	1	-	Informative
3	D21S11	28, 30	29, 30	1	-	Informative
4	D18S51	15	14, 15	1	-	Non informative
5	Penta E	5, 12	5, 15	1	-	Informative
6	D5S818	13	11, 13	1	-	Non informative
7	D13S317	11, 12	10, 12	1	-	Informative
8	D7S820	9	9, 10	1	-	Non informative
9	D16S539	8, 9	9, 12	1	-	Informative
10	CSF1PO	11, 12	11, 12	2	-	Non informative
11	Penta D	10, 11	11	1	-	Informative
12	Amelogenin	X	Not revealed	-	-	-
13	vWA	15, 16	15, 16	2	-	Non informative
14	D8S1179	11, 13	11, 14	1	-	Informative
15	TPOX	9, 11	11	1	-	Informative
16	FGA	19, 26	19, 24	1	-	Informative
The average percentage of maternal cell contamination in the fetal sample is 0%						
