Whole Exome Sequencing Analysis

Patient name	**: Fetus of XX**	**PIN**	**: XXXXXXX**
Gender/ Age	**: 22 Weeks 4 Days**	**Sample number**	**: XXXX**
Hospital/Clinic	**: XXXXXX**	**Sample collection date**	**: XXX**
Specimen	**: Amniotic Fluid**	**Sample receipt date**	**: XXX**
		Report date	**: 08.03.2025**

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

Clinical history

Mrs. XX has an ongoing pregnancy. Anomaly scan findings are suggestive of dilated cardiomyopathy with gross tricuspid regurgitation and prolonged myocardial performance. Index is 0.63+ prolonged. Fetus of Mrs. XX has been evaluated for pathogenic variations

Results

Variant of uncertain significance related to the given phenotype was detected

List of copy number variant identified:

Gene	Region	Variant	Allele status	Disease	Classification*	Inheritance pattern
MYBPC3 (-)	Exons 12 to 18	c.(1090+1_10911)_(1790+1_1791-1)del Exonic deletion	Homozygous	Dilated Cardiomyopathy 1MM; Left ventricular noncompaction-10 **(OMIM#615396)**	**Uncertain Significance**	Autosomal Dominant
				Cardiomyopathy, hypertrophic, 4 **(OMIM#115197)**		Autosomal Dominant, 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

CNV deletion [chr11:g.(47341245_47341990)_(47343625_47346206)del]

CNV summary: On *in silico* CNV analysis, a contiguous homozygous deletion of size [~3.77 Kb], on chromosome 11 spanning genomic location chr11:g.47341245_47341990_47343625_47346206 del comprising exons 12 to 18 of the *MYBPC3* gene, suggestive of a copy number variant was detected. (Refer Appendix II).

CNV score: *In-silico* analysis identified CNV score of 0.00, are likely to be suggestive of a homozygous deletion of this region. The coverage and depth of these regions are sufficiently targeted in this assay.

OMIM phenotype: Dilated cardiomyopathy-1MM (CMD1MM)/Left ventricular noncompaction-10(LVNC10) (OMIM#615396) are caused by heterozygous mutation in the *MYBPC3* gene (OMIM*(600958).These diseases follow autosomal dominant pattern of inheritance. Cardiomyopathy, hypertrophic, 4 (OMIM#115197) is caused by heterozygous, homozygous, or compound heterozygous mutation in the *MYBPC3* gene (OMIM*(600958).These disorders are characterized by dilated cardiomyopathy, heart failure, progressive and sometimes fatal ventricular flutter, non-sustained, and left ventricular noncompaction at apex and/or midventricular wall. These diseases follows both autosomal dominant and autosomal recessive pattern of inheritance. [2].

Variant classification: Due to lack of literature evidence, this variant is classified as a variant of uncertain significance and has to be carefully correlated with the clinical symptoms.

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)

The additional variants identified may not be related to patient's phenotype. Phenotype – genotype correlation is recommended.

List of additional variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern	Literature
RNASEH2C (-)	Exon 2	c.205C>T **(p.Arg69Trp)**	Homozygous	Aicardi-Goutieres syndrome-3 **(OMIM#610329)**	**Likely Pathogenic**	Autosomal Recessive	PubMed 31529068 34302356 ClinVar 1260
SLC39A4 (-)	Exon 6	c.1102_1109dup **(p.Ala371GlnfsTer13)**	Homozygous	Acrodermatitis enteropathica **(OMIM#201100)**	**Likely Pathogenic**	Autosomal Recessive	-
TTN (-)	Exon 214	c.40135G>A **(p. Ala13379Thr)**	Heterozygous	Dilated cardiomyopathy - 1G **(OMIM#604145)**	**Uncertain Significance**	Autosomal Dominant	-

Recommendations

- Reproductive decisions based on variants of uncertain significance are not recommended.
- The *RNASEH2C* gene has pseudogenes in the human genome. Validation of the variant by an alternate technique is recommended to rule out false positives.
- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Sensitivity of NGS to identify large deletion and duplication is low. It is recommended to do SNP Chromosomal microarray in order to confirm this CNV finding.
- Genetic counselling is recommended.

Methodology: Whole Exome Sequencing Analysis

DNA extracted from the Amniotic Fluid, was used to perform whole exome using a whole exome capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using Illumina 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 haplotype caller has been used to call the variants. Gene annotation of the variants is performed using Variant Effect predictor program against the Ensembl release 99 human gene model. Small Indels and copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM, GWAS, HGMD (v2020.2) and SwissVar. Common variants are filtered based on allele frequency in 1000 Genome Phase 3, gnomAD (v3.1 & 2.1.1), EVS, dbSNP (v151), 1000 Japanese Genome database. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Splicing effect has been elucidated with MaxEntScan, GeneSplicer, NNSplice. 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	:	12.44 Gb
Total reads aligned (%)	:	99.93%
Data ≥ Q30	:	98.63%

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 are not assessed using this assay.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variations (CNV) is 70-75%. The CNVs detected must be confirmed by 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. 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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Appendix I

Estimation of maternal cell contamination %

S. No.	Marker	Fetal genotype	Maternal genotype	No. of shared alleles	MCC	Remarks
1	D3S1358	17	17, 18	1	-	Informative
2	TH01	6, 9	6, 9	2	-	Non informative
3	D21S11	32.2	32.2	1	-	Informative
4	D18S51	14, 17	14, 15	1	-	Informative
5	Penta E	16, 17	13, 16	1	-	Informative
6	D5S818	10	10, 13	1	-	Informative
7	D13S317	11, 13	11, 12	1	-	Informative
8	D7S820	8	8, 12	1	-	Informative
9	D16S539	11, 12	11	1	-	Non informative
10	CSF1PO	10, 11	10, 11	2	-	Non informative
11	Penta D	11	11, 13	1	-	Informative
12	Amelogenin	Not revealed	X	-	-	-
13	vWA	19	13, 19	1	-	Informative
14	D8S1179	9, 10	9, 14	1	-	Informative
15	TPOX	8, 11	11	1	-	Non informative
16	FGA	22	22, 24	1	-	Informative
The average percentage of maternal cell contamination in the fetal sample is 0%						

Appendix II

The list genes encompassing the copy number variation and their phenotypes based on OMIM Morbid map have been listed below.

CNV	GENES	PHNOTYPES	INHERITANCE	HI/PLi/TS
CNV#1	*MYBPC3*	Cardiomyopathy, dilated, 1MM (OMIM#615396) Cardiomyopathy, hypertrophic, 4 (OMIM#115197), Left ventricular noncompaction 10 (OMIM#615396)	Autosomal Dominant	3.00/0.00/9,562,578.00