Whole Exome Sequencing Analysis

Patient name	:	**POC of XXX**	**PIN**	:	**XX**
Gestational Age	:	**XX**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Hospital/Clinic	:	**XX**	**Sample receipt date**	:	**XX**
Specimen	:	**Product of conception**	**Report date**	:	**XX**

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

Clinical history

Mrs. XXX marriage is consanguineous and has a bad obstetric history. Her first pregnancy resulted in pre-term delivery at 28 weeks GA and succumbed. Her second pregnancy resulted in still birth at 32 weeks GA. Her previous pregnancy USG at 13 weeks indicative of bilateral renal agenesis and Oligohydramnios and terminated at 13 weeks 2 days GA. Chromosomal Microarray revealed increased total homozygosity (ROH) and no significant Copy Number Variations (CNV). The Product of Conception has been evaluated for pathogenic variations.

Results

Likely pathogenic variant was identified in *ITGA8* gene

List of uncertain significant variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
ITGA8 (-)	Exon 8	c.803-2_807dupAGGATAC **(p.Ser270ArgfsTer35)**	Homozygous[#]	Renal hypodysplasia/aplasia 1 **(OMIM#191830)**	**Likely Pathogenic**	Autosomal Recessive

[#]Sanger sequencing is recommended for the *ITGA8* variant to rule out false positives.

*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

***ITGA8:*c.803-2_807dupAGGATAC**

Variant summary: A homozygous seven base pair insertion in exon 8 of the *ITGA8* gene (chr10:g.15671643insATCCTGT, NM_003638.3, Depth: 66x) that results in a frameshift and premature truncation of the protein 35 amino acids downstream to codon 270 (p.Ser270ArgfsTer35) was detected. This variant is a frameshift variant which occurs in an exon of *ITGA8* upstream where nonsense mediated decay is predicted to occur. This variant is predicted to cause loss of normal protein function through protein truncation.

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

***In silico* predictions:** The *in-silico* predictions of the variant are deleterious by CADD. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Renal hypodysplasia/aplasia 1 (OMIM#191830) is caused by homozygous or compound heterozygous mutation in the *ITGA8* gene (OMIM*604063). This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant has been classified as a likely pathogenic variant. 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.

MCC Status

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

Recommendations

- **Sanger sequencing is recommended for the *ITGA8* variant to rule out false positives.**
- Genetic counselling is recommended.

Methodology

DNA extracted from the POC, 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 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 haplotype caller 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 (3.1.2,2.1.1), 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	:	10.51 Gb
Data ≥ Q30	:	92.77%

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. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

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	15, 17	15, 16	2	-	Non informative
2	TH01	9	9	1	-	Non informative
3	D21S11	30, 33.2	30, 31.2	1	-	Informative
4	D18S51	12, 15	12, 19	1	-	Informative
5	Penta E	12, 13	11, 12	1	-	Informative
6	D5S818	11, 12	12	1	-	Non informative
7	D13S317	9, 12	9, 12	2	-	Non informative
8	D7S820	10	10	1	-	Non informative
9	D16S539	9, 11	9, 11	2	-	Non informative
10	CSF1PO	12	12	1	-	Non informative
11	Penta D	10	10	1	-	Non informative
12	Amelogenin	Not revealed	X	-	-	-
13	vWA	17	15, 17	1	-	Informative
14	D8S1179	13, 17	13, 17	2	-	Non informative
15	TPOX	8, 11	8	1	-	Non informative
16	FGA	21, 25	21, 25	2	-	Non informative
The average percentage of maternal cell contamination in the fetal sample is 0%						
