Clinical 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 is married consanguineous to Mr. Paul Pandi and has a bad obstetric history. She has a history of two mid-trimester abortions due to autosomal recessive polycystic kidney disease. Her third pregnancy was terminated due to anomaly scan at 24 weeks 5 days GA indicative of single vertical pocket of amniotic fluid measures 2.2 cm (oligohydramnios), both kidneys are enlarged and echogenic measuring 3.8cm & 3.9c with tiny cysts in the cortex, cortico medullary differentiation is lost, no pelvocalyceal or ureteric dilatation observed, renal arterial and venous flow could be seen on color flow mapping, fetal bladder is very small and filled with only minimal urine, thin rim of pericardial effusion is observed, no distinct polydactyly or occipital encephalocele to suggest possibility of meckel gruber syndrome. Findings suggestive of autosomal recessive type of polycystic renal disease. The Product of Conception has been evaluated for pathogenic variations.

Results

Variant of uncertain significance identified in *PKHD1* gene

List of uncertain significant variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
PKHD1 (-)	Exon 53	c.8440G>A **(p.Gly2814Ser)**	Homozygous	Polycystic kidney disease 4, with or without hepatic disease **(OMIM# 263200)**	**Uncertain significance**	Autosomal Recessive

List of additional uncertain significant variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
PKD1 (-)	Exon 9	c.1758A>C **(p.Glu586Asp)**	Heterozygous	Polycystic kidney disease 1 **(OMIM#173900)**	**Uncertain significance**	Autosomal Dominant
GATA5 (-)	Exon 4	c.713G>A **(p.Arg238His)**	Homozygous	Congenital heart defects, multiple types, 5 **(OMIM#617912)**	**Uncertain significance**	Autosomal Dominant, Autosomal Recessive
POLG (-)	Exon 21	c.3383G>A **(p.Arg1128His)**	Heterozygous	Mitochondrial DNA depletion syndrome 4A (Alpers type) **(OMIM#203700)** Mitochondrial DNA depletion syndrome 4B (MNGIE type) **(OMIM#613662)** Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE) **(OMIM#607459)** Progressive external ophthalmoplegia, autosomal recessive 1 **(OMIM#258450)**	**Uncertain significance**	Autosomal Recessive
				Progressive external ophthalmoplegia, autosomal dominant 1 **(OMIM#157640)**		Autosomal Dominant

SLC3A1 (+)	Exon 6	c.1102A>G **(p.Met368Val)**	Heterozygous	Cystinuria **(OMIM#220100)**	**Uncertain significance**	Autosomal Dominant, Autosomal Recessive

List of significant carriers variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
LAMC3 (+)	Exon 20	c.3470_3471delCA **(Thr1157ArgfsTer74)**	Heterozygous	Cortical malformations, occipital **(OMIM#614115)**	**Likely Pathogenic**	Autosomal Recessive
ADAMTS18 (-)	Exon 22	c.3440C>G **(p.Ser1147Ter)**	Heterozygous	Microcornea, myopic chorioretinal atrophy, and telecanthus **(OMIM#615458)**	**Likely Pathogenic**	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

***PKHD1*: c.8440G>A**

Variant summary: A homozygous missense variation in exon 53 of the *PKHD1* gene (chr6:g.51791236C>T, NM_138694.4, Depth: 182x) that results in the amino acid substitution of Serine for Glycine at codon 2814(p.Gly2814Ser) was detected.

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

Literature evidence: This gene has been previously reported in a patient affected with Autosomal recessive polycystic kidney disease in homozygous state [3].

***In-silico* prediction:** 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: Polycystic kidney disease 4, with or without hepatic disease (OMIM#263200) is caused by homozygous or compound heterozygous mutation in the *PKHD1* gene (OMIM*606702). This

disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. **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.**

***PKD1*: c.1758A>C**

Variant summary: A heterozygous missense variation in exon 9 of the *PKD1* gene (chr16:g.2116083T>G, NM_001009944.3, Depth: 125x) that results in the amino acid substitution of Aspartic acid for Glutamic acid at codon 586 (p.Glu586Asp) was detected.

Population frequency: This variant has minor allele frequency of 0.0145% in gnomAD database and has not been reported in 1000 genomes database.

Clinical and Literature evidence: This variant has been classified as uncertain significance in ClinVar database [4]. This variant has been previously reported in a patient affected with Chronic Kidney Disease (CKD) and Autosomal Dominant Polycystic Kidney Disease in heterozygous state [5].

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by SIFT.

OMIM phenotype: Polycystic kidney disease 1 (OMIM#173900) is caused by heterozygous mutation in the *PKD1* gene (OMIM*601313). This disease follows autosomal dominant pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. **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.**

***GATA5*: c.713G>A**

Variant summary: A homozygous missense variation in exon 4 of the *GATA5* gene (chr20:g.62466538C>T, NM_080473.5, Depth: 217x) that results in the amino acid substitution of Histidine for Arginine at codon 238 (p.Arg238His) was detected.

Population frequency: This variant has minor allele frequency of 0.0033% in gnomAD database and has not been reported in 1000 genomes database.

Clinical evidence: This variant has been classified as uncertain significance in ClinVar database [6].

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv), LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Congenital heart defects, multiple types, 5 (OMIM#617912) is caused by heterozygous mutation in the *GATA5* gene (OMIM*611496). This disease follows both autosomal dominant and autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. **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.**

***POLG*: c.3383G>A**

Variant summary: A heterozygous missense variation in exon 21 of the *POLG* gene (chr15:g.89318640C>T, NM_002693.3, Depth: 304x) that results in the amino acid substitution of Histidine for Arginine at codon 1128 (p.Arg1128His) was detected.

Population frequency: This variant has minor allele frequency of 0.0026% in gnomAD database and has not been reported in 1000 genomes database.

Clinical and Literature evidence: This variant has been classified as uncertain significance in ClinVar database [6]. This variant has been previously reported in a patient affected with Mitochondrial Diseases in compound heterozygous state [7].

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv), LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Mitochondrial DNA depletion syndrome 4A (Alpers type) (OMIM#203700), Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE) (OMIM#607459) and Progressive external ophthalmoplegia, autosomal recessive 1 (OMIM#258450) are caused by homozygous or compound heterozygous mutation in the *POLG* gene (OMIM* 174763). Mitochondrial DNA depletion syndrome 4B (MNGIE type) (OMIM#613662) is caused by compound heterozygous mutation in the *POLG* gene (OMIM* 174763). These diseases follow autosomal recessive pattern of inheritane [2]. Progressive external ophthalmoplegia, autosomal dominant 1 (OMIM#157640) is caused by mutation in the *POLG* gene (OMIM* 174763). This disease follows autosomal dominant pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance.

SLC3A1: c.1102A>G

Variant summary: A heterozygous missense variation in exon 6 of the *SLC3A1* gene (chr2:g.44301093A>G, NM_000341.4, Depth: 251x) that results in the amino acid substitution of Valine for Methionine at codon 368 (p.Met368Val) 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, PolyPhen-2 (HumDiv), and LRT. The reference codon is conserved across mammals in PhyloP tool.

OMIM phenotype: Cystinuria (OMIM#220100) can be caused by mutation in the *SLC3A1* gene (OMIM*104614). This disease follows both autosomal dominant and autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a variant of uncertain significance. 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.

LAMC3: c.3470_3471delCA

Variant summary: A heterozygous two base pair deletion in exon 20 of the *LAMC3* gene (chr9:g.131073295delCA, NM_006059.4, Depth: 269x) that results in a frameshift and premature truncation of the protein 74 amino acids downstream to codon 1157 (p.Thr1157ArgfsTer74) was detected. 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* prediction:** The *in-silico* predictions of the variant are deleterious by MutationTaster2. The reference codon is conserved across mammals in PhyloP tool.

OMIM phenotype: Cortical malformations, occipital (OMIM#614115) is caused by homozygous or compound heterozygous mutation in the *LAMC3* gene (OMIM*604349). This disease follows autosomal recessive pattern of inheritance [2].

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

***ADAMTS18*: c.3440C>A**

Variant summary: A heterozygous stop gained variation in exon 22 of the *ADAMTS18* gene (chr16:g.77289374G>C, NM_199355.4, Depth: 125x) that results in the premature truncation of the protein at codon 1147 (p.Ser1147Ter) was detected. This variant is a stop gained variant which occurs in an exon of *ADAMTS18* upstream of 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* prediction:** 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: Microcornea, myopic chorioretinal atrophy, and telecanthus (OMIM#615458) is caused by homozygous mutation in the *ADAMTS18* gene (OMIM*607512). This disease follows autosomal recessive pattern of inheritance [2].

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

MCC Status

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

Recommendations

- 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

DNA extracted from the POC, was used to perform targeted gene capture using a custom 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	:	9.86 Gb
Data ≥ Q30	:	94.34%

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 [9].
- This test does not determine the sex of the fetus in adherence to the PCPNDT act.

References

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3. Serra G, et al. Autosomal recessive polycystic kidney disease: case report of a newborn with rare *PKHD1* mutation, rapid renal enlargement and early fatal outcome. Ital J Pediatr. 2020 Oct 15;46(1):154. doi: 10.1186/s13052-020-00922-4. PMID: 33059727; PMCID: PMC7560064.
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9. 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	Maternal genotype	Fetal genotype	No. of shared alleles	% MCC	Remarks
1	D3S1358	15, 17	15, 17	2	-	Non informative
2	TH01	9	6, 9	1	-	Non informative
3	D21S11	29, 31.2	28, 31.2	1	-	Informative
4	D18S51	14	14	1	-	Non informative
5	Penta E	7, 16	16	1	-	Informative
6	D5S818	11, 12	11, 12	2	-	Non informative
7	D13S317	11	8, 11	1	-	Non informative
8	D7S820	11	9, 11	1	-	Non informative
9	D16S539	11	10, 11	1	-	Non informative
10	CSF1PO	11	11, 12	1	-	Non informative
11	Penta D	9, 12	10, 12	1	-	Informative
12	Amelogenin	X	Not revealed	-	-	-
13	vWA	15, 17	15, 19	1	-	Informative
14	D8S1179	13, 14	13, 14	2	-	Non informative
15	TPOX	8, 11	11	1	-	Informative
16	FGA	20, 24	23, 24	1	-	Informative
The average percentage of maternal cell contamination in the fetal sample is 0%						
