Clinical Exome Sequencing Analysis

Patient name	**: Baby. XXX**	**PIN**	**: XX**
Gender/ Age	**: Female/ 2 Years**	**Sample number**	**: XX**
Hospital/Clinic	**: XX**	**Sample collection date**	**: XX**
Specimen	**: Peripheral Blood**	**Sample receipt date**	**: XX**
		Report date	**: XX**

Clinical history

Proband, Baby XXX was first born female to nonconsanguineous parents. She was presented with chief complaints of oligohydramnios, IUGR with Cephalopelvic disproportion, strabismus and fine motor delay. Her MRI brain findings was indicative of hypoplasia of cerebellar superior vermis and abnormal decussation with stretching of superior cerebellar peduncle leading to molar tooth appearance of midbrain, bullet shaped appearance of fourth ventricle, mild thinning of corpus callosum seen with dilatation and splaying of lateral ventricles, reduction in volume of superior temporal and inferior frontal gyrus, thinning of both optic nerve and optic chiasm, left CP angle and para pontine arachnoid cyst measuring 1.80*1.10cms, congenital hypoplasia of pons seen. There is a family history of paternal cousin brother affected with strabismus. Baby XXX is suspected to be affected with Joubert's syndrome and has been evaluated for pathogenic variations.

Results

Likely pathogenic variant identified in *NFIX* gene

List of significant variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
NFIX (+)	Exon 2	c.520G>T **(p.Glu174Ter)**	Heterozygous	Malan syndrome **(OMIM#614753)** Marshall-Smith syndrome **(OMIM#602535)**	**Likely pathogenic**	Autosomal Dominant

List of uncertain significant variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
USP7 (-)	Exon 30	c.3160G>T **(p.Asp1054Tyr)**	Heterozygous	Hao-Fountain syndrome **(OMIM#616863)**	**Uncertain Significance**	Autosomal Dominant

*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

***NFIX*:c.520G>T**

Variant summary: A heterozygous stop gained variation in exon 2 of the *NFIX* gene (chr19:g.13025513G>T, NM_001365902.3, Depth: 276x) that results in the premature truncation of the protein at codon 174 (p.Glu174Ter) was detected. This variant is a stop gained variant which occurs in an exon of *NFIX* 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.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [3]. This variant has been previously reported in a patient affected with Malan syndrome in heterozygous state as a de novo variant [4].

***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: Malan syndrome (OMIM#614753) and Marshall-Smith syndrome (OMIM#602535) are caused by heterozygous mutation in the *NFIX* gene (OMIM*164005). These diseases follow autosomal dominant 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.**

***USP7*:c.3160G>T**

Variant summary: A heterozygous missense variation in exon 30 of the *USP7* gene (chr16:g.8894592C>A, NM_003470.3, Depth: 213x) that results in the amino acid substitution of Tyrosine for Aspartic acid at codon 1054 (p.Asp1054Tyr) was detected.

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 damaging by SIFT, PolyPhen-2 (HumDiv) and LRT. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Hao-Fountain syndrome (OMIM#616863) is caused by heterozygous mutation in the *USP7* gene (OMIM*602519). This disease follows autosomal dominant pattern of inheritance [2].

Variant classification: Based on the evidence, this variant has been 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.**

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 blood, 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 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 (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	11.43 Gb
Data ≥ Q30	94.63%

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, 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 [5].

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/VCV000191097.4
4. Priolo M, et al. Further delineation of Malan syndrome. Hum Mutat. 2018 Sep;39(9):1226-1237. doi: 10.1002/humu.23563. Epub 2018 Jun 25. PMID: 29897170; PMCID: PMC6175110.
5. 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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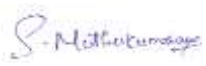

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