Whole Exome Sequencing Analysis

Patient name	:	**Miss. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Female / 7 years**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Hospital/Clinic	:	**XX**	**Sample receipt date**	:	**XX**
Specimen	:	**Peripheral Blood**	**Report date**	:	**XX**

Clinical history

Proband, Miss XXX was born to non- consanguineous parents. She was born FTLSCS with birth weight of 3.50kg. She was presented with complaints of delayed milestones. Her CBC in 2019 was indicative of normocytic smear with leucocytosis. Her MRI brain in 2020 was indicative of thickened elongated superior cerebellar peduncles with vermian hypoplasia ? Joubert syndrome. Proband's mother have had a history of two missed abortions and one pregnancy was terminated due to renal/congenital anomalies. Proband mother is currently 12 weeks 4 days pregnant. Proband, Miss XXX has been suspected to be affected with ?Joubert syndrome and has been evaluated for pathogenic variations.

Results

Likely compound heterozygous variants was identified in *AHI1* gene

List of significant variants identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
AHI1[#] (-)	Exon 10	c.1197T>A **(p. Tyr399Ter)**	Heterozygous	Joubert syndrome 3 **(OMIM#608629)**	**Likely Pathogenic**	Autosomal Recessive
	Exon 11	c.1414C>T **(p. Arg472Trp)**	Heterozygous		**Uncertain Significance**	

#Sanger sequencing is recommended for *AHI1* to rule out the 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.

Likely pathogenic variant in *AHI1* gene (c.1197T>A; p. Tyr399Ter) identified in the sample of Miss. XXX in likely compound heterozygous has been identified in her father Mr. YYY in heterozygous state.

Uncertain significant variant in *AHI1* gene (c.1414C>T; p. Arg472Trp) identified in the sample of Miss. XXX in likely compound heterozygous state has been identified in her mother Mrs. ZZZ in heterozygous state.

Additional variants:

Likely pathogenic carrier variant in *CYP11B1* gene (c.427C>T; p. Arg143Trp) identified in the sample of her father YYY and her mother ZZZ in heterozygous state associated with Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency (AR) has not been identified in the sample of Miss. XXX.

Likely pathogenic carrier variant in *GLB1* gene (c.785G>A; p. Gly262Glu) identified in the sample of her father YYY in heterozygous state associated with GM1-gangliosidosis, type I / GM1-gangliosidosis, type II, GM1-gangliosidosis, type III and Mucopolysaccharidosis type IVB (Morquio) (AR) has been identified in the sample of Miss. XXX in heterozygous state.

Likely pathogenic carrier variant in *ZNF142* gene (c.4868delC; p. Pro1623ArgfsTer17) identified in the sample of her father YYY in heterozygous state associated with Neurodevelopmental disorder with impaired speech and hyperkinetic movements (AR) has been identified in the sample of Miss. XXX in heterozygous state.

Likely pathogenic carrier variant in *PAPSS2* gene (c.1000C>T; p. Arg334Ter) identified in the sample of her mother Mrs. ZZZ in heterozygous state associated with Brachyolmia 4 with mild epiphyseal and metaphyseal changes (AR) has been identified in the sample of Miss. XXX in heterozygous state.

Likely pathogenic carrier variant in *MARVELD2* gene (c.1183-1G>A; 3' splice site) identified in the sample of her mother Mrs. ZZZ in heterozygous state associated with Deafness, autosomal recessive 49 (AR) has not been identified in the sample of Miss. XXX.

Likely pathogenic carrier variant in *CHRNE* gene (c.1327delG; p. Glu443LysfsTer64) identified in the sample of her mother Mrs. ZZZ in heterozygous state associated with Myasthenic syndrome, congenital, 4B, fast channel and Myasthenic syndrome, congenital, 4C, associated with acetylcholine receptor deficiency (AR) and Myasthenic syndrome, congenital, 4A, slow channel (AR, AD) has not been identified in the sample of Miss. XXX.

Interpretation

Likely compound heterozygous variants was identified in *AHI1* gene

Variant 1: *AHI1*: c.1197T>A

Variant summary: A heterozygous stop gained variation in exon 10 of the *AHI1* gene (chr6:g.135455881A>T, NM_001134831.2, Depth: 57x) that results in the premature truncation of the protein at codon 399 (p.Tyr399Ter) was detected. This variant is a frameshift variant which occurs in an exon of *AHI1* 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* prediction:** The *in-silico* predictions of the variant are deleterious by CADD. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

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

This variant has been identified in her father, YYY in heterozygous state.

Variant 2: *AHI1*: c.1414C>T

(chr6:g.135453367G>A, NM_001134831.2, Depth: 51x) that results in the amino acid substitution of Tryptophan for Arginine at codon 472 (p.Arg472Trp) was detected.

Population frequency: This variant has minor allele frequency of 0.0013% gnomAD database and has not been reported in 1000 genomes database.

Clinical evidence: This variant has been classified as uncertain significance in ClinVar database [3].

***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 and GERP++ tools.

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

These *AHI1* variations are considered to be likely compound heterozygous variants and must be carefully correlated with the clinical symptoms.

OMIM phenotype: Joubert syndrome 3 (OMIM#608629) is caused by homozygous mutation in the *AHI1* gene (OMIM*608894). This disease follows autosomal recessive pattern of inheritance [2].
This variant has been identified in her mother, Mrs. ZZZ in a heterozygous state.

Recommendations

- **Sanger sequencing is recommended for *AHI1* to rule out the false positives.**
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is advised.

Methodology

DNA extracted from the blood 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 (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	9.43 Gb
Data ≥ Q30	97.47%

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

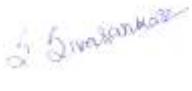
- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations 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 [4].

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/VCV000939721.4

4. 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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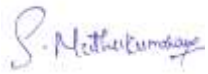

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