Whole Exome Sequencing Analysis

Patient name	:	**Master. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Male/ 2 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, Master XXX is second born male child to non-consanguineous parents. He presented with delayed milestones, abnormal posturing, seizures, and facial dysmorphisms - bilateral cataract, frontal bossing, patchy hair on the head, short neck, and low set ears. He is currently 2 years of age. Proband's elder brother presented with delayed milestones and facial dysmorphisms. Proband's maternal and paternal grandparents are consanguineous, and his maternal uncle succumbed at 8 months of age and was suspected to have delayed milestones. His mother is having an ongoing pregnancy. Proband, Master XXX has been evaluated for pathogenic variations.

Results

Likely pathogenic variant identified in *KIAA0586* gene

List of significant variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
KIAA0586 (+)	Exon 3	c.326delA **(p.Asn109ThrfsTer22)**	Homozygous	Joubert syndrome 23 **(OMIM#616490)** Short-rib thoracic dysplasia 14 with polydactyly **(OMIM#616546)**	**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

***KIAA0586*: c.326delA**

Variant summary: A homozygous single base pair deletion in exon 3 of the *KIAA0586* gene (chr14:g.58430702delA, NM_001329943.3, Depth: 94x) that results in a frameshift and premature truncation of the protein 22 amino acids downstream to codon 109 (p.Asn109ThrfsTer22) was detected. This variant is a frameshift variant which occurs in an exon of *KIAA0586* 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.

OMIM phenotype: Joubert syndrome 23 (OMIM#616490) is caused by homozygous or compound heterozygous mutation in the *KIAA0586* gene (OMIM*610178). Short-rib thoracic dysplasia 14 with polydactyly (OMIM#616546) is caused by homozygous mutation in the *KIAA0586* gene (OMIM*610178). Joubert syndrome-23 is an autosomal recessive neurodevelopmental disorder characterized by delayed development, abnormal eye movements, and abnormal breathing pattern associated with a characteristic hindbrain malformation apparent on brain imaging and known as the 'molar tooth sign.' Compared to other forms of Joubert syndrome, the phenotype is relatively mild, and other organ systems are generally not affected. Short-rib thoracic dysplasia (SRTD) with or without polydactyly refers to a group of autosomal recessive skeletal ciliopathies that are characterized by a constricted thoracic cage, short ribs, shortened tubular bones, and a 'trident' appearance of the acetabular roof. SRTD encompasses Ellis-van Creveld syndrome (EVC) and the disorders previously designated as Jeune syndrome or asphyxiating thoracic dystrophy (ATD), short rib-polydactyly syndrome (SRPS), and Mainzer-Saldino syndrome (MZSDS). Polydactyly is variably present, and there is phenotypic overlap in the various forms of SRTDs, which differ by visceral malformation and metaphyseal appearance. Nonskeletal involvement can include cleft lip/palate as well as anomalies of major organs such as the brain, eye, heart, kidneys, liver, pancreas, intestines, and genitalia. Some forms of SRTD are lethal in the neonatal period due to respiratory insufficiency secondary to a severely restricted thoracic cage, whereas others are compatible with life. These diseases follow autosomal recessive pattern of inheritance [2].

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

Additional Variant(s)

The additional variants identified may or 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
WRN (+)	Intron 2	c.97-1G>A **(3' splice site)**	Heterozygous	Werner syndrome **(OMIM#277700)**	**Likely pathogenic**	Autosomal Recessive
HBB (-)	Intron 1	c.92+5G>C **(5' splice site)**	Heterozygous	Thalassemia, beta **(OMIM# 13985)**	**Likely pathogenic**	Autosomal Recessive
ODAD1 (-)	Exon 6	c.448C>T **(p.Arg150Ter)**	Heterozygous	Ciliary dyskinesia, primary, 20 **(OMIM#615067)**	**Likely pathogenic**	Autosomal Recessive
ZMYM3 (-)	Exon 2	c.356A>G **(p.Gln119Arg)**	Hemizygous	Intellectual developmental disorder, X-linked 112 **(OMIM#301111)**	**Uncertain significance**	X – Linked Recessive

Recommendations

- The *HBB* and *WRN* gene has pseudogene in the human genome. Validation of the variant(s) by Sanger sequencing is strongly 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.
- 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	14.65 Gb
Data ≥ Q30	93.65 %

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].

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.

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