Whole Exome Sequencing Analysis

Patient name	:	**Baby. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Male/ 1 Year**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Specimen	:	**Peripheral Blood**	**Sample receipt date**	:	**XX**
			Report date	:	**XX**

Clinical history

Proband, Baby. XXX was first born male child to non-consanguineous endogamous parents. He is a term baby with chief complaint of skull deformity with mildly hypertrophic scalp scar and his developmental milestones were uneventful. He is diagnosed with craniosynostosis premature fusion of metopic and bilateral coronal sutures. He underwent bilateral frontal and parietal cranial vault remodelling, orbital advancement, bilateral frost tarsorrhaphy surgery at 5 months of his age. His 64 SLICE - MDCT Facial bone 3D indicative of premature coronal suture and normal sagittal, lambdoid suture. His parents had a history of abortion and his mother was diagnosed with hyperthyroidism. Proband is suspected to be affected with infantile neuroaxonal dystrophy, craniosynostosis syndrome, frontal bossing, retinitis pigmentosa and biocoronal craniosynostosis. Proband, Baby. XXX has been evaluated for pathogenic variations.

Results

Variant of uncertain significance was identified in *SMAD6* gene

List of uncertain significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
SMAD6 (+)	Exon 1	c.465_471delCGGGCGG **(p.Gly156ValfsTer23)**	Heterozygous	{Craniosynostosis 7, susceptibility to} **(OMIM#617439)** {Radioulnar synostosis, nonsyndromic} **(OMIM#179300)** Aortic valve disease 2 **(OMIM#614823)**	**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

***SMAD6*: c.465_471delCGGGCGG**

Variant summary: A heterozygous seven base pair deletion in exon 1 of the *SMAD6* gene (chr15:g.66703713delCGGGCGG, NM_005585.5, Depth: 83x) that results in a frameshift and premature truncation of the protein 23 amino acids downstream to codon 156 (p.Gly156ValfsTer23) was detected. This variant is a frameshift variant which occurs in an exon of *SMAD6* 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 minor allele frequency of 0.0013% in gnomAD database and has not been reported in 1000 genomes databases.

Clinical and Literature evidence: This variant has been previously classified as uncertain significance in ClinVar database [3]. A frameshift variant in the *SMAD6* gene (P152fs) has been previously reported in a patient affected with complex craniosynostosis with another frameshift variant in the *TCF12* gene (E548fs) [4].

***In-silico* prediction:** The *in-silico* predictions of the variant are deleterious by MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: {Craniosynostosis 7, susceptibility to} (OMIM#617439) is conferred by heterozygous mutation in the *SMAD6* gene (OMIM*602931). Craniosynostosis is a primary abnormality of skull growth involving premature fusion of the cranial sutures such that the growth velocity of the skull often cannot match that of the developing brain. This produces skull deformity and, in some cases, raises intracranial pressure, which must be treated promptly to avoid permanent neurodevelopmental disability. {Radioulnar synostosis, nonsyndromic} (OMIM#179300) is caused by sex chromosome aneuploidy or by heterozygous variants in the *SMAD6* gene (OMIM*602931), Aortic valve disease 2 (OMIM#614823) is caused by heterozygous mutation in the SMAD6 gene (OMIM*602931). These diseases follow autosomal dominant pattern of inheritance [2].

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

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 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	10.58 Gb
Data ≥ Q30	97.31%

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/VCV000638816.14
4. Timberlake, Andrew T., et al. "Co-occurrence of frameshift mutations in *SMAD6* and *TCF12* in a child with complex craniosynostosis." Human Genome Variation 5.1 (2018): 14.
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:

Sivasankar.S, Ph.D
Molecular Biologist

Muthukumaran. S, Ph.D
Clinical Bioinformatician

Sachin. D.Honguntikar, Ph.D,
Molecular Geneticist

Dr. G. Suriyakumar
Director