Whole Exome Sequencing Analysis

Patient name	**:**	**XXX**	**PIN**	**:**	**XX**
Gender/ Age	**:**	**Male / 4 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 Arpit Raja is born to non-consanguineous parents. He is presented with complaints of global developmental delay, abnormal gait, diplegic gait and learning difficulties. He started waking after 2 years of age. MRI brain revealed no significant diagnostic abnormality. No evidence of acute infarct or intracranial SOL seen. MRI whole spine screening is unremarkable. Proband Master Arpit Raja has been evaluated for pathogenic variations.

Results

Likely compound heterozygous variants were identified in *GEMIN5* gene

List of variants identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
GEMIN5 (-)	Intron 12	c.1674-1G>A **(3' Splice site)**	Heterozygous	Neurodevelopmental disorder with cerebellar atrophy and motor dysfunction **(OMIM#619333)**	**Likely pathogenic**	Autosomal Recessive
	Exon 24	c.3581A>T **(p.Asn1194Ile)**			**Uncertain Significance**	

*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

Likely compound heterozygous variants were identified in *GEMIN5* gene

Variant 1: *GEMIN5*: c.1674-1G>A

Variant summary: A heterozygous 3' splice site variation in intron 12 of the *GEMIN5* gene (chr5:g.154917180C>T, NM_015465.5, Depth: 81x) that affects the invariant AG acceptor splice site of exon 13 (c.1674-1G>A) 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 deleterious by CADD and disrupting in GeneSplicer, MaxEntScan, NNSplice and SpliceAI. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

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

Variant 2: *GEMIN5*: c.3581A>T

Variant summary: A heterozygous missense variation in exon 24 of the *GEMIN5* gene (chr5:g.154896108T>A, NM_015465.5, Depth: 75x) that results in the amino acid substitution of Isoleucine for Asparagine at codon 1194 (p.Asn1194Ile) was detected.

Population frequency: This variant has minor allele frequency of 0.0007% in gnomAD database and has not been reported in 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.

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

OMIM phenotype: Neurodevelopmental disorder with cerebellar atrophy and motor dysfunction (OMIM#619333) is caused by homozygous or compound heterozygous mutation in the *GEMIN5* gene (OMIM*607005). Neurodevelopmental disorder with cerebellar atrophy and motor dysfunction (NEDCAM) is an autosomal recessive disorder characterized by global developmental delay with prominent motor abnormalities, mainly axial hypotonia, gait ataxia, and appendicular spasticity. Affected

individuals have cognitive impairment and speech delay; brain imaging shows cerebellar atrophy. The severity is variable. This disease follows autosomal recessive pattern of inheritance [2].

The variants are reported to be in likely compound heterozygous state. These likely compound heterozygous variants are strongly recommended to confirm the cis or trans status by parental segregation analysis.

Additional Variant(s)

The additional variants identified which are significant but may not be related to patient's phenotype. Phenotype – genotype correlation is recommended.

List of additional variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
GRIA4 (+)	Exon 10	c.1194G>C **(p.Leu398Phe)**	Heterozygous	Neurodevelopmental disorder with or without seizures and gait abnormalities **(OMIM#617864)**	**Uncertain Significance**	Autosomal Dominant

Recommendations

- Sanger sequencing is 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 recommended.

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 (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	9.62 Gb
Data ≥ Q30	89.04 %

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

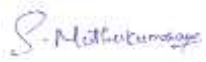
- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations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

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