Whole Exome Sequencing Analysis

Patient name	:	**Master. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**XX**	**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 was born to non-consanguineous parents. He is diagnosed with Autism Spectrum Disorder. He has a history of delayed cry at birth and ?Birth asphyxia. He is presented with speech delay and intellectual disability. There is a family history of congenital deafness and mutism for his father's two maternal uncle's. Proband, Master XXX has been evaluated for pathogenic variations.

Results

Likely compound heterozygous variants were identified in *TUBGCP2* gene

List of significant variants identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
TUBGCP2 (-)	Exon 7	c.889C>T **(p.Arg297Cys)**	Heterozygous	Pachygyria, microcephaly, developmental delay, and dysmorphic facies, with or without seizures **(OMIM#618737)**	**Likely pathogenic**	Autosomal Recessive
	Intron 9	c.1361-2A>C **(3' splice site)**	Heterozygous		**Likely pathogenic**	

*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 *TUBGCP2* gene

Variant 1: *TUBGCP2*: c.889C>T

Variant summary: A heterozygous missense variation in exon 7 of the *TUBGCP2* gene (chr10:g.133293174G>A, NM_006659.4, Depth: 163x) that results in the amino acid substitution of Cysteine for Arginine at codon 297 (p.Arg297Cys) was detected.

Population frequency: This variant has a minor allele frequency of 0.009% in gnomAD database and has not been reported in 1000 genomes database.

Clinical and Literature evidence: This variant has been previously classified as pathogenic variant in ClinVar database [3]. This variant has been previously reported in patient affected with developmental delay in compound heterozygous state with another variant (c.2025-2A>G) in exon 14 [4].

***In silico* predictions:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2(HumDiv), LRT and MutationTaster2. 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 is 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: *TUBGCP2*: c.1361-2A>C

Variant summary: A heterozygous 3' splice site variation in intron 9 of the *TUBGCP2* gene (chr10:g.133289022T>G, NM_006659.4, Depth: 84x) that affects the invariant AG acceptor splice site of exon 10 (c.1361-2A>C) was detected.

Population frequency: This variant has not been reported in 1000 genomes database and gnomAD database.

***In silico* predictions:** The in-silico predictions of the variant are deleterious by CADD and disrupted by GeneSplicer, MaxEntScan and NNSplice. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

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 is 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: Pachygyria, microcephaly, developmental delay, and dysmorphic facies, with or without seizures (OMIM#618737) is caused by homozygous or compound heterozygous mutation in the *TUBGCP2 gene* (OMIM*617817). 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 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	Literature
HBA1 (-)	Exon 1	c.43T>C **(p.Trp15Arg)**	Heterozygous	Thalassemias, alpha- **(OMIM#604131)**	**Likely Pathogenic**		Clinvar-439103 Pubmed ID - 31304855, 15008259
AIPL1 (-)	Exon 5	c.773G>C **(p.Arg258Pro)**	Heterozygous	Cone-rod dystrophy/ Leber congenital amaurosis 4/ Retinitis pigmentosa, juvenile **(OMIM#604393)**	**Likely Pathogenic**	Autosomal Dominant, Autosomal Recessive	Clinvar-916626 Pubmed ID- 24426771

Recommendations

- The *HBA1* 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	10.63 Gb
Data ≥ Q30	94.38 %

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/VCV000691861.18
4. Mitani T, et al. Bi-allelic Pathogenic Variants in *TUBGCP2* Cause Microcephaly and Lissencephaly Spectrum Disorders. Am J Hum Genet. 2019 Nov 7;105(5):1005-1015. doi: 10.1016/j.ajhg.2019.09.017. Epub 2019 Oct 17. PMID: 31630790; PMCID: PMC6848995.
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.

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