Whole Exome Sequencing Analysis

Patient name	:	**Baby. 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, Baby XXX is third born female child to non- consanguineous parents. She is presented with chief complaints of global developmental delay, neuroregression, hypotonia, ?seizures, blue sclera, nystagmus and microcephaly. Her fundus examination in 2023 revealed cherry red spots. Her MRI brain with whole spine screening in 2023 indicative of bilateral near symmetrical altered signal intensity areas with restricted diffusion in supra as well as infratentorial white matter and deep grey matter brain parenchyma. Her EEG in 2023 indicative of normal findings. Her Echocardiography in 2023 indicative of normal study. Proband, Baby XXX has been evaluated for pathogenic variations.

Results

Likely pathogenic variant was identified in *SURF1* gene

List of significant variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
SURF1 (-)	Exon 8	c.804delG **(p. Asn269ThrfsTer8)**	Homozygous	Charcot-Marie-Tooth disease, type 4K **(OMIM#616684)** Mitochondrial complex IV deficiency, nuclear type 1 **(OMIM#220110)**	**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

***SURF1*: c.804delG**

Variant summary: A homozygous single base pair deletion in exon 8 of the *SURF1* gene (chr9:g.133352090delC, NM_003172.4, Depth: 115x) that results in a frameshift and premature truncation of the protein 8 amino acids downstream to codon 269 (p. Asn269ThrfsTer8) was detected.

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

***In-silico* prediction:** The *in-silico* predictions of the variant is damaging by MutationTaster2. TThe reference codon is conserved across mammals in PhyloP tool.

OMIM phenotype: Charcot-Marie-Tooth disease, type 4K (OMIM#616684) and Mitochondrial complex IV deficiency, nuclear type 1 (OMIM#220110) are caused by homozygous or compound heterozygous mutation in the *SURF1* gene (OMIM*185620). Charcot-Marie-Tooth disease type 4K is an autosomal recessive demyelinating peripheral neuropathy characterized by onset in the first decade of distal muscle weakness and atrophy associated with impaired distal sensation. Both upper and lower limbs are affected. Affected individuals may also have nystagmus and late-onset cerebellar ataxia. Mitochondrial complex IV deficiency nuclear type 1 (MC4DN1) is an autosomal recessive metabolic disorder characterized by rapidly progressive neurodegeneration and encephalopathy with loss of motor and cognitive skills between about 5 and 18 months of age after normal early development. Affected individuals show hypotonia, failure to thrive, loss of the ability to sit or walk, poor communication, and poor eye contact. Other features may include oculomotor abnormalities, including slow saccades, strabismus, ophthalmoplegia, and nystagmus, as well as deafness, apneic episodes, ataxia, tremor, and brisk tendon reflexes. Brain imaging shows bilateral symmetric lesions in the basal ganglia, consistent with a clinical diagnosis of Leigh syndrome. 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.**

Appendix Table

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
MFSD8 (-)	Exon 9	c.894T>G **(p. Tyr298Ter)**	Heterozygous	Ceroid lipofuscinosis, neuronal, 7 **(OMIM#610951)** Macular dystrophy with central cone involvement **(OMIM#616170)**	**Likely Pathogenic**	Autosomal Recessive
APOB (-)	Exon 26	c c.10579C>T **(p. Arg3527Trp)**	Homozygous	Hypercholesterolemia, familial, 2 **(OMIM#144010)**	**Likely Pathogenic**	Autosomal Dominant
				Hypobetalipoproteinemia **(OMIM#615558)**		Autosomal Recessive
CHST14 (+)	Exon 1	c.196G>A **(p. Ala66Thr)**	Homozygous	Ehlers-Danlos syndrome, musculocontractural type 1 **(OMIM#601776)**	**Uncertain significance**	Autosomal Recessive

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	9.46 Gb
Data ≥ Q30	91.05%

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:

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