Clinical Exome Sequencing Analysis & Whole Mitochondrial Genome Sequencing

Patient name	:	**Miss. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Female/ 13 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, Miss. XXX presented with new onset scholastic difficulties, fall in grades, cognitive decline, slow speech, decreased blink rate, recent memory impaired, vacuous smile. Miss. XXX is suspected to be affected with Inborn errors of metabolism/Mitochrondrial disorders/Leukodystrophies. Miss. XXX has been evaluated for pathogenic variations.

Clinical Exome Sequencing Analysis

Results

Variant of Uncertain significance identified in *ARSA* gene

List of uncertain significance variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
ARSA (-)	Exon 8	c.1294G>T **(p. Asp432Tyr)**	Homozygous	Metachromatic leukodystrophy **(OMIM#250100)**	**Uncertain Significance**	Autosomal Recessive

List of additional carrier variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
HBB (-)	Exon 1	c.79G>A **(p. Glu27Lys)**	Heterozygous	Thalassemia, beta **(OMIM#613985)**	**Likely pathogenic**	Autosomal Recessive
IDUA (+)	Exon 7	c.895G>T **(p. Glu299Ter)**	Heterozygous	Mucopolysaccharidosis Ih **(OMIM#607014)**	**Likely pathogenic**	Autosomal Recessive
				Mucopolysaccharidosis Ih/s **(OMIM#607015)**		
				Mucopolysaccharidosis Is **(OMIM#607016)**		
OTOGL (+)	Exon 25	c.2724C>A **(p. Cys908Ter)**	Heterozygous	Deafness, autosomal recessive 84B **(OMIM#614944)**	**Likely pathogenic**	Autosomal Recessive
SERPINB8 (+)	Exon 5	c.537C>A **(p. Tyr179Ter)**	Heterozygous	Peeling skin syndrome 5 **(OMIM#617115)**	**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

***ARSA:* c.1294G>T**

Variant summary: A homozygous missense variation in exon 8 of the *ARSA* gene (chr22: g.50625381C>A, NM_000487.6, Depth: 192x) that results in the amino acid substitution of Tyrosine for Aspartic acid at codon 432 (p. Asp432Tyr) 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 damaging by SIFT, PolyPhen-2 (HumDiv), LRT and MutationTaster2.

OMIM phenotype: Metachromatic leukodystrophy (OMIM#250100) is caused by homozygous or compound heterozygous mutation in the *ARSA* gene (OMIM*607574). This disease follows autosomal recessive pattern of inheritance [2].

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

***HBB:* c.79G>A**

Variant summary: A heterozygous missense variation in exon 1 of the *HBB* gene (chr11: g.5226943C>T, NM_000518.5, Depth: 215x) that results in the amino acid substitution of Lysine for Glutamic acid at codon 27 (p. Glu27Lys) was detected.

Population frequency: This variant has minor allele frequency of 0.042% in gnomAD database and has minor allele frequency of 0.2796% in 1000 genomes databases.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [3]. This HbE variation in heterozygous and homozygous condition results in benign disorders. However, when it occurs in combination with β thalassaemia alleles it results in HbE/β0 Thalassemia with severe clinical manifestations [4,5].

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by SIFT, PolyPhen-2 (HumDiv) and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Thalassemia, beta (OMIM#613985) can be caused by homozygous or compound heterozygous mutation in the *HBB* gene (OMIM*141900). This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a likely pathogenic variant.

***IDUA:* c.895G>T**

Variant summary: A heterozygous stop gained variation in exon 7 of the *IDUA* gene (chr4:g.1002084G>T, NM_000203.5, Depth: 204x) that results in the premature truncation of the protein at codon 299 (p. Glu299Ter) was detected. This variant is a stop gained variant which occurs in an exon of *IDUA* upstream of 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 0.0007% in gnomAD database and has not been reported 1000 genomes databases.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [6]. This variant has been previously reported in Indian patients affected with Mucopolysaccharidosis Ih/s in compound heterozygous state [7].

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

OMIM phenotype: Mucopolysaccharidosis Ih (OMIM#607014), Mucopolysaccharidosis Ih/s (OMIM#607015) and Mucopolysaccharidosis Is (OMIM#607016) are caused by homozygous or compound heterozygous mutation in the *IDUA* gene (OMIM*252800) These diseases follow autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a likely pathogenic variant.

***OTOGL:* c.2724C>A**

Variant summary: A heterozygous stop gained variation in exon 25 of the *OTOGL* gene (chr12:g.80278210C>A, NM_001378609.3, Depth: 167x) that results in the premature truncation of the protein at codon 908 (p. Cys908Ter) was detected. This variant is a stop gained variant which occurs in an exon of *OTOGL* upstream of 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.

***In-silico* prediction:** The *in-silico* predictions of the variant are deleterious by CADD.

OMIM phenotype: Deafness, autosomal recessive 84B (OMIM#614944) is caused by homozygous or compound heterozygous mutation in the *OTOGL* gene (OMIM*614925). This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a likely pathogenic variant.

***SERPINB8:* c.537C>A**

Variant summary: A heterozygous stop gained variation in exon 5 of the *SERPINB8* gene (chr18:g.63983691C>A, NM_002640.4, Depth: 139x) that results in the premature truncation of the protein at codon 179 (p. Tyr179Ter) was detected. This variant is a stop gained variant which occurs in an exon of *SERPINB8* upstream of 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 0.0007% in gnomAD database and has not been reported 1000 genomes databases.

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

OMIM phenotype: Peeling skin syndrome 5 (OMIM#617115) is caused by homozygous mutation in the *SERPINB8* gene (OMIM*601697). This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a likely pathogenic variant.

Whole Mitochondrial Genome Sequencing

No pathogenic or likely pathogenic variant associated with the given phenotype was detected

*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 phenotype has been identified.

Recommendations

- The *HBB* gene has pseudogene in the human genome. Validation of the variant 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: Clinical Exome Sequencing Analysis and Whole Mitochondrial Genome Sequencing

DNA extracted from the blood was used to perform targeted gene capture using a custom 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.

The sequences obtained were aligned to revised Cambridge mitochondrial reference genome (rCRS) analyzed using sentieon to identify variants relevant to the clinical indication. We follow the Varseq best practices framework for identification of variants in the sample. Clinically relevant mutations were annotated using published variants in literature and Mito Map database. Only non-synonymous variants found in the sample 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	8.82 Gb
Data ≥ Q30	93.98%

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 [8].

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.
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8. 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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