Whole Exome Sequencing Analysis & Whole Mitochondrial Genome Sequencing

Patient name	:	**Mr. 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, Mr. XXX is affected with end-stage renal disease and is kidney transplant recipient. His transplanted kidney is showing signs of TMA. He is suspected to be affected with complement-mediated HUS and has been evaluated for pathogenic variations.

Whole Exome Sequencing Analysis

Results

Copy Number Variation of Uncertain Significance related to the given phenotype was detected

List of uncertain significant Copy Number Variant identified related to the phenotype:

Chromosome	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
Chromosome 1	chr1:g.(?_196774887)_(196831999_?)del	Deletion	Homozygous	Susceptibility to atypical hemolytic uremic syndrome **(OMIM#235400)**	**Uncertain significance**	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

CNV deletion [chr1:g.(?_196774887)_(196831999_?)del]

CNV summary: On *in silico* CNV analysis, a contiguous homozygous deletion of size ~57.1120 KB], on chr1:g.(?_196774887)_(196831999_?)del encompassing *CFHR1* and *CFHR3* genes is observed. (Refer Appendix I).

CNV score: *In-silico* analysis identified CNV score of 0.03, which indicates homozygous deletion of these segments. The coverage and depth of these regions are sufficiently targeted in this assay.

Literature evidence: Homozygous deletions involving *CFHR3* and *CFHR1* genes are known to be associated with atypical hemolytic uremic syndrome [3].

OMIM phenotype: Susceptibility to atypical hemolytic uremic syndrome (OMIM#235400) is caused by mutations in *CFHR1* (OMIM#134371) and *CFHR3* (OMIM#605336) genes. Typical hemolytic uremic syndrome is characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia associated with distorted erythrocytes ('burr cells'). These diseases follow both autosomal dominant and autosomal recessive pattern of inheritance. Clinical correlation is recommended [2].

Variant classification: Due to lack of adequate literature evidence, this deletion is classified as copy number variant of uncertain significance and has to be carefully correlated with the clinical symptoms.

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

- Sequencing the variant 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: Whole Exome Sequencing Analysis and Whole Mitochondrial Genome Sequencing

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 haplotype caller 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	12.36 Gb
Data ≥ Q30	96.63

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

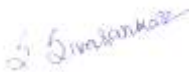
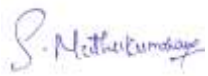
- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations 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 [4].

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. Zipfel PF, et al. Deletion of complement factor H-related genes *CFHR1* and *CFHR3* is associated with atypical hemolytic uremic syndrome. PLoS Genet. 2007 Mar 16;3(3):e41. doi: 10.1371/journal.pgen.0030041. Epub 2007 Feb 1. PMID: 17367211; PMCID: PMC1828695.

4. 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

Appendix I

The list genes encompassing the copy number variation and their phenotypes based on OMIM Morbid map have been listed below

CNV#	Genes	Phenotypes	Inheritance	HI/PLi/TS
Cnv#1	*CFHR1*	Hemolytic uremic syndrome, atypical, susceptibility to (OMIM#235400)	Autosomal dominant; Autosomal recessive	
	CFHR3	Hemolytic uremic syndrome, atypical, susceptibility to (OMIM#235400)	Autosomal dominant; Autosomal recessive	