Suman

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MEDGENOME

DNA TEST REPORT – MEDGENOME LABORATORIES

Full Name / Ref No:	SUMAN MALLICK	**Order ID/Sample ID:**	**42175/130279**
Gender:	Male	Sample Type:	Blood
Date of Birth / Age:	21 years	Date of Sample Collection:	15th December 2017
Referring Clinician:	Dr. Sana Islam, Institute of Child Health, Kolkata	Date of Sample Receipt:	16th December 2017
		Date of Order Booking:	18th December 2017
		Date of Report:	16th January 2018, 6.30 PM
Test Requested:	**Clinical Exome**		

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Mr. *Suman Mallick,* born of a non-consanguineous marriage, presented with clinical indications of mild developmental delay, speech issues, coarse facies, hepatosplenomegaly, left inguinal hernia (operated), coronary heart disease (operated at 18 years for thickened aortic valve) and presence of diverticula in urinary bladder. Two of his younger brothers are similarly affected and have been diagnosed with mucopolysaccharidosis II (Hunter syndrome). Mr. *Suman Mallick* is suspected to be affected with mucopolysaccharidosis II (Hunter syndrome) or mucopolysaccharidosis IV (Maroteaux–Lamy syndrome) and has been evaluated for pathogenic gene variations.

RESULTS

PATHOGENIC VARIANT CAUSATIVE OF THE REPORTED PHENOTYPE WAS IDENTIFIED

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
***IDS* (-)** (ENST00000340855)	Exon 9	c.1345_1349del **(p.Glu449SerfsTer6)**	Hemizygous	Mucopolysaccharidosis II	X-Linked recessive	Pathogenic

ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED

No other variant that warrants to be reported was detected. Variations with high minor allele frequencies which are likely to be benign will be given upon request.

The coverage of mucopolysaccharidosis genes is given in appendix 1.

VARIANT INTERPRETATION AND CLINICAL CORRELATION

Variant description: A hemizygous 5 base pair deletion in exon 9 of the ***IDS*** gene **(chrX:148564581_148564585delTCCTC; Depth: 395x)** that results in a frameshift and premature truncation of the protein 6 amino acids downstream to codon 449 **(p.Glu449SerfsTer6; ENST00000340855)** was detected (Table). Another frameshift variant (c.1349_1364delATCCGTACCTCCC TGG), in the nearby region and affecting protein similarly, has previously been reported in a patient affected with mucopolysaccharidosis II [23]. The observed variant has not been reported in the 1000 genomes, ExAC and our internal databases. The *in silico* prediction# of the variant is damaging by MutationTaster2. The reference region is conserved across species.

OMIM phenotype: Mucopolysaccharidosis II (OMIM#309900) is caused by mutation in the *IDS* gene (OMIM*300823). Mucopolysaccharidosis II is a rare X-linked recessive disorder caused by deficiency of the lysosomal enzyme iduronate sulfatase, leading to progressive accumulation of glycosaminoglucans in nearly all cell types, tissues, and organs. Patients with MPS II excrete excessive amounts of chondroitin sulfate B (dermatan sulfate) and heparitin sulfate (heparan sulfate) in the urine [10].

Based on the above evidence, ***this IDS variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms.***

RECOMMENDATIONS

The *IDS* gene has a pseudogene in the human genome. Validation of the variant(s) by 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.

Genetic counselling is advised for interpretation on the consequences of the variant(s).

TEST METHODOLOGY

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions of the genome/genes is performed. Mutations identified in the exonic regions are generally actionable compared to variations that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.

DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80-100X coverage on Illumina sequencing platform. The sequences obtained are aligned to human reference genome (GRCh37/hg19) using BWA program [2, 3] and analyzed using Picard and GATK version 3.6 [4, 5] to identify variants relevant to the clinical indication. We follow the GATK best practices framework for identification of variants in the sample. Gene annotation of the variants is performed using VEP program [6] against the Ensembl release 87 human gene model [7]. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases – ClinVar, OMIM, GWAS, HGMD and SwissVar [8-15]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, ExAC, EVS, dbSNP147, 1000 Japanese Genome and our internal Indian population database [16-20]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, Mutation Assessor and LRT. Only non-synonymous and splice site variants found in the clinical exome panel consisting of 6763 genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Total data generated (Gb)	15.24
Total reads aligned (%)	99.97
Reads that passed alignment (%)	96.47
Data ≥ Q30 (%)	94.86

***Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:**

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).

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.

‡The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 87gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

#The *in silico* predictions are based on Variant Effect Predictor, Ensembl release 87 (SIFT version - 5.2.2; PolyPhen - 2.2.2); LRT version - November, 2009 release from dbNSFPv3.1 and MutationTaster2 based on build NCBI 37 / Ensembl 69 [21].

For any further technical queries please contact techsupport@medgenome.com.

DISCLAIMER

- The classification of variants of unknown significance can change over time and MedGenome cannot be held responsible for this. Please contact MedGenome at a later date to inquire about any changes.
- Intronic variants are not assessed using this method.
- Large deletions of more than 10 bp or copy number variations /chromosomal rearrangements cannot be assessed using this method.
- Certain genes may not be covered completely and few mutations could be missed. Variants not detected by the assay that was performed may impact the phenotype.
- The mutations have not been validated by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines [22] can also be given upon request.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by MedGenome.

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END OF REPORT

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): 405–24.
2. Li, H, and R Durbin. "Fast and Accurate Long-Read Alignment with Burrows-Wheeler Transform." Bioinformatics (Oxford, England). 26.5 (2010): 589–95.
3. Meyer, LR, *et al.* "The UCSC Genome Browser Database: Extensions and Updates 2013." Nucleic acids research. 41. (2012): n.pag.
4. McKenna, A, *et al.* "The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data." Genome research. 20.9 (2010): 1297–303.
5. Li, H, *et al.* "The Sequence Alignment/map Format and SAMtools." Bioinformatics (Oxford, England). 25.16 (2009): 2078–9.
6. McLaren, W, *et al.* "Deriving the Consequences of Genomic Variants with the Ensembl API and SNP Effect Predictor." Bioinformatics (Oxford, England). 26.16 (2010): 2069–70.
7. ENSEMBL: http://www.ensembl.org
8. Landrum, MJ, *et al.* "ClinVar: Public Archive of Interpretations of Clinically Relevant Variants." Nucleic acids research. 44. (2015): n.pag.
9. Hamosh, A, *et al.* "Online Mendelian Inheritance in Man (OMIM), a Knowledgebase of Human Genes and Genetic Disorders." Nucleic acids research. 33. (2004): n.pag.
10. OMIM: http://www.omim.org
11. GWAS: http://www.ebi.ac.uk/gwas/
12. Welter, D, *et al.* "The NHGRI GWAS Catalog, a Curated Resource of SNP-Trait Associations." Nucleic acids research. 42. (2013): n.pag.
13. Stenson, PD, *et al.* "Human Gene Mutation Database (HGMD): 2003 Update." Human mutation. 21.6 (2003): 577–81.
14. HGMD: http://www.biobase-international.com/product/hgmd
15. Mottaz, A, *et al.* "Easy Retrieval of Single Amino-Acid Polymorphisms and Phenotype Information Using SwissVar." Bioinformatics (Oxford, England). 26.6 (2010): 851–2.
16. The 1000 Genomes Project Consortium. "A Global Reference for Human Genetic Variation." Nature 526.7571 (2015): 68–74. PMC.
17. Lek, M, *et al.* "Analysis of Protein-Coding Genetic Variation in 60,706 Humans." Nature. 536.7616 (2016): 285–91.
18. NHLBI: https://esp.gs.washington.edu/drupal
19. Nagasaki, Masao *et al.* "Rare Variant Discovery by Deep Whole-Genome Sequencing of 1,070 Japanese Individuals." Nature Communications 6 (2015): 8018. PMC. n.pag
20. dbSNP: http://www.ncbi.nlm.nih.gov/SNP/
21. MutationTaster2: http://www.mutationtaster.org/
22. Green, RC, *et al.* "ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing." Genetics in medicine: official journal of the American College of Medical Genetics. 15.7 (2013): 565–74.
23. Brusius-Facchin, AC, *et al.* "Mucopolysaccharidosis type II: identification of 30 novel mutations among Latin American patients." Molecular genetics and metabolism 111.2 (2014): 133-8.

APPENDIX 1: COVERAGE OF MUCOPOLYSACCHARIDOSIS GENES

Gene	Percentage of coding region covered
ARSB	100.00
GALNS	100.00
GLB1	100.00
GNS	100.00
GUSB	100.00
HGSNAT	90.55
HYAL1	100.00

Gene	Percentage of coding region covered
IDS	100.00
IDUA	100.00
NAGLU	100.00
SGSH	100.00
SUMF1	100.00