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DEPARTMENT OF MEDICAL GENETICS
NEXT GENERATION SEQUENCING TEST REPORT

Hospital No.	MH001145657	**Referral Hospital**	Manipal Hospital Kanakpura Road & MNSH
Patient Name	Ms Radhika Subramani	**Sample Collection Date**	01-07-2026
Age / Gender	22 years / Female	**Test Initiation Date**	01-07-2026
Test Requested	Whole Exome with Mitochondrial Sequencing	**Report Date**	25-07-2026
Referred by	Dr Deepesh V / Dr. Mitesh Shetty	**Specimen**	Peripheral blood

CLINICAL DIAGNOSIS / FEATURES / HISTORY

Ms Radhika Subramani is presented with childhood-onset steroid-resistant nephrotic syndrome diagnosed at 10 years of age, persistent proteinuria despite immunosuppressive therapy, progressive renal dysfunction and chronic kidney disease. Previous renal biopsy demonstrated mesangial proliferative glomerulonephritis. Her sample has been evaluated to investigate an underlying genetic etiology of hereditary nephrotic syndrome and progressive kidney disease.

RESULT

No disease-causing pathogenic or likely pathogenic variant or No significant single nucleotide variation (SNV) or copy number variation (CNV) related to the phenotypes was detected in this sample

MITOCHONDRIAL RESULTS

No disease-causing pathogenic or likely pathogenic variant or no other significant variant related to the phenotype was detected in this sample

MITOCHONDRIAL GENES

MT-TF, MT-RNR1, MT-TV, MT-RNR2, MT-TL1, MT-ND1, MT-TI, MT-TQ, MT-TM, MT-ND2, MT-TW, MT-TA, MT-TN, MT-TC, MT-TY, MT-CO1, MT-TS1, MT-TD, MT-CO2, MT-TK, MT-ATP8, MT-ATP6, MT-CO3, MT-TG, MT-ND3, MT-TR, MT-ND4L, MT-ND4, MT-TH, MT-TS2, MT-TL2, MT-ND5, MT-ND6, MT-TE, MT-CYB, MT-TT and MT-TP

0RECOMMENDATIONS

- Genetic Counseling

METHODOLOGY

Sequencing of the protein coding regions approximately 30Mb of the human Exome (targeting approximately 99% of regions in CCDS and RefSeq) and complete mitochondrial genome sequencing was performed using MGI next generation sequencing (NGS) systems at a mean depth of 80-100X with percentage of bases covered at 20X depth >90% in the target region and the depth for mitochondrial genome at 1000-2000X. In some cases, due to the complexity of the sequence, not all variants in the flanking regions are able to be analyzed. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. GATK best practice framework was followed for variant identification. Duplicate reads identification and removal, Base quality recalibration and re-alignment of reads based on indels were done using DRAGEN BIO IT platform (2). Quality checks (QC) will be performed on all VCF files to exclude variants where sequencing is of poor quality. Additional QC metrics includes total homozygous and heterozygous calls (SNVs and indels),

proportion of variant calls that were common, number of variants falling into different annotated consequence categories, numberof extreme heterozygotes (alternate allele proportion 0.8). Oneseq 1MB backbone has been used to pick the CNV above 1MB in size with the limitation of 70-75% detection rate. Variant annotations will be done using published databases like OMIM, GWAS, GNOMAD, 1000Genome etc (3-6). Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, Mutation Assessor and LRT. Non-synonymous and splice site variants will be used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported. Geneyx (version 5.12) was used for variant annotation, analysis and reporting (7).

LIMITATIONS AND DISCLAIMER

Any preparation and processing of a sample from patient material provided to the department of medical genetics for the requested genetic testing is performed on the highest and most current scientific and analytical standards. Currently available data indicates that technical error rate for analysis involving DNA tests is anywhere between 1-3%. In order to minimize this inherent limitation of targeted gene panel sequencing, a high coverage depth (>100X) is ensured and >90% of all amplicons are covered at least by 20X. Intronic variants are not assessed using this method. Test results are always interpreted in the context of clinical findings, family history and other laboratory data. Rare polymorphisms may lead to false negative or positive results. This does not imply that reported variants can always explain all symptoms of the patient. Copy number variations or large deletion/duplications may not be detected by NGS. Incidental or secondary findings (if any) that meet the ACMG guidelines can also be given upon request. Additionally, it is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the impact of variants on those genes. Not all variants detected may be listed in the report. Inclusion of variants is dependent upon provided clinical information, its significance and currently available literature. Incidental or secondary findings (if any) that meet the ACMG guidelines can also be given upon request. The classification of variants of unknown significance can change over time.

DR.MITESH SHETTY
HOD & Consultant Medical Geneticist

REFERENCES:

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