Excellence In Health Care

DEP6RĪMENĪ OI MOĮE‡UĮ6R CENEĪI‡S 6ND CENOMI‡S

Patient Name	Master. Snehasish Panda	Ref. Doctor	-

Clinical History:

Master. Snehasish Panda presented with steroid-resistant nephrotic syndrome and referred for clinical exome sequencing to rule out the genetic etiology of his condition.

Results: Heterozygous Variant of Uncertain Significance (VUS) was identified in *KANK2* gene.

Variants in genes known to be associated with the provided phenotype

Gene and Transcript	Exon	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
KANK2 NM_001136191.3	10	c.2152G>A, p.Asp718Asn chr19:11173040C>T	Heterozygous	Variant of Uncertain Significance (VUS)	Nephrotic syndrome, type 16 (OMIM# 617783)	Autosomal Recessive

The second heterozygous variant is not identified in the *KANK2* gene for an autosomal recessive disorder due to sensitivity of NGS testing. Hence, detection of the second heterozygous variant by alternate testing method is suggested.

Variant Interpretation:

The exome data analysis identified a heterozygous missense variant **c.2152G>A, p.Asp718Asn (chr19:11173040C>T)** in ***KANK2*** gene. The observed variant has a minor allele frequency of 0.004% in gnomAD database. The *In silico* predictions by DANN and MutationTaster found the mutation to have deleterious effect. The p.Asp718Asn variant was reported in ClinVar [2150260]. No functional studies ascertained the pathogenic role of this variant yet. Therefore, the observed variant was classified as a **Variant of Uncertain Significance (VUS).**

Genotype-Phenotype Correlation:

In 3 patients from 2 unrelated families with NPHS16, Gee *et al*. (2015) identified homozygous missense mutations in the *KANK2* gene (S181G and S684F). The mutation in 2 affected sibs in a consanguineous family of Arab descent (family A982) was found by a combination of homozygosity mapping and whole-exome sequencing. The mutation in the unrelated patient (patient A1751-21) was found by direct sequencing of the *KANK2* gene in over 1,100 patients with nephrotic syndrome; this was the only patient found to have a KANK2 mutation in that cohort.

Clinical Features:

Nephrotic syndrome, type 16 is an autosomal recessive disorder characterized by proteinuria, hematuria, renal biopsy shows minimal change disease and onset between 2 and 3 years of age.

References:

1. Gee, H. Y., et al. KANK deficiency leads to podocyte dysfunction and nephrotic syndrome. J. Clin. Invest. 125: 2375-2384, 2015. [PubMed: 25961457].

Carrier status in Genes as per ACMG guidelines:

No pathogenic or likely pathogenic variants were detected. (PMCID: **PMC8488021**).

Secondary findings as per ACMG guidelines:

No pathogenic variants were detected in the ACMG recommended secondary gene list in this individual (Miller DT *et al.,* Genet Med. 2022 Jul;24(7):1407-1414. PMID: 35802134).

Additional findings:

Gene and Transcript	Exon	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
CPT2 NM_000098.3	1	c.136C>T, p.Gln46* chr1:53197079C>T	Heterozygous	Carrier of Likely Pathogenic (LP)	Stress-induced myopathic form of carnitine palmitoyltransferase II deficiency (OMIM# 255110)	Autosomal Dominant/ Autosomal Recessive

Recommendations:

- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- Genetic counselling and clinical correlation for accurate interpretation of test results are recommended.
- If the above result does not correlate completely with the patient phenotype, additional testing is advised based on the clinician's discretion.
- Based on genetic testing on the parents and other family members, the significance/ classification of the variant(s) may alter.

Test Information

The total genomic DNA was extracted from the biological sample using the column-based method and DNA quality and quantity were assessed using electrophoretic and Qubit methods. The QC-qualified genomic DNAwas randomly fragmented and ligating sequencing adapters were added to both ends of DNA fragments. Sequencing libraries were size-selected using beads to optimal template size and amplified by polymerase chain reaction. The regions of interest (exons and flanking intronic targets) are targeted by a hybridization-based target capture method. Sequencing libraries that passed the quality control were sequenced on the MGI platform using paired-end chemistry. Reads were assembled and are aligned to reference sequences based on NCBI Ref Seq transcripts and human genome build GRCh38. Data was filtered and analyzed to identify variants of interest related to patients' clinical phenotype.

Tools and databases used for data analysis:

We followed the Genome Analysis Toolkit (GATK) best practices framework for the identification of variants in the sample. The sequences obtained were subjected to quality assessment and pre-processing. The pre-processed sequences were aligned with human reference genome sequence (assembly GRch38) by Burrows-Wheeler Aligner and post-alignment processing like read duplicate removal and base quality score recalibration (BQSR) was carried out by using GATK (v4.2.5.0). Variant calling was done by using the GATK Haplotype Caller. Each called variant is annotated using different clinical and population databases. Common variants were filtered out based on minor

allele frequency (MAF) in 1000Genome Phase 3[5], gnomAD (v4.1), ExAC [3], and dbSNP (v156). Non-synonymous variants effect is calculated using multiple in-silico algorithms. Only non-synonymous and splice site variants with clinical relevance were selected using published literature and a set of disease databases -ClinVar, OMIM, GWAS, and SwissVar. The classification of the variant is done based on American College of Medical Genetics guidelines.

QC METRICS

Total data generated	6.13Gb
Total reads aligned reads (%)	99.22%
Data ≥Q30(%)	94.23%
Total data which passed mapping quality cut-off	5.05Gb

Variant or Mutation	The change(s) in a gene. This could be disease-causing (pathogenic) or non-disease-causing (benign).
Pathogenic	A disease-causing mutation in a gene has been identified, which may explain or correlate with the patient's symptoms. This usually denotes the confirmation of a suspected condition for which testing was requested.
Likely Pathogenic	A variant that is very likely to play a role in the development of disease, but currently scientific evidence is inadequate, additional evidence in the future may declare the pathogenicity of this variant.
Variant of Uncertain Significance	A variant has been identified, but existing scientific information makes it impossible to define as pathogenic (disease-causing) or benign (non-disease-causing) and further required functional studies. The clinician may recommend additional tests for the patient or family members. It is likely that their relevance will only be determined over time, depending on the availability of scientific information.

Test Limitations:

A negative or normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate/incomplete information may lead to misinterpretation of the results.

Test Attributes:

- It is presumed that the specimen used to perform the test belongs to the patient specified above, such verification having been carried out at the collection level of the sample.
- The current results are based on analysis of coding regions (exons) as well as certain intron padding regions on the patient's genomic DNA with respect to patient phenotype as defined in the target regions. However, due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants of insufficient coverage, as well as those variants that currently do not correlate with the provided phenotype may not be analysed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- The test methodology currently does not detect large deletions/duplications, triplet repeat expansions, and epigenetic changes. The test also does not include an analysis of predictors for multifactorial, polygenic, and/or complex diseases. Novel synonymous changes as well as intronic mutations (excludingthoseaffecting invariant splice nucleotides) are not routinely reported.

- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity & specificity for variant detection, analysis, and interpretation due to the inability of the data tools to unambiguously determine the origin of the sequence data. The mutations have not been validated by Sanger sequencing unless specified.
- Regions other than the targeted are not covered and hence cannot be reported.
- Phenotype variability may be due to modifying genetic/non-genetic factors and is not a part of the current analysis.
- This test has not been validated by the FDA, NABL, or CAP, and it has been determined by the accrediting bodies that such validation is not required atthis time.
- In some instances, the classification and interpretation of variants (VUS) may change as new scientific information comes to light. We recommend a re-analysis of this report yearly. Please contact the laboratory in case a re-analysis of the report is desired. It is the lab's policy to perform re-analysis once on a complimentary basis. However, this re-analysis is performed only when requested.

References:

1. Clarke L, et al. The International Genome Sample Resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. Nucleic Acids Research. 2016 Sep 15;45(D1): D854–9.
2. Jiaxin Wu and Rui Jiang., Prediction of Deleterious Nonsynonymous Single-Nucleotide Polymorphism for Human Diseases; The Scientific World Journal, Volume 2013, Article ID 675851, 10 pages.
3. Karczewski KJ, et al. The ExAC browser: displaying reference data information from over 60,000 exomes. Nucleic Acids Research. 2016 Nov 28;45(D1): D840–5.
4. Karczewski, K.J. et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581, 434–443 (2020).
5. Landrum M. J. et al., ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res., 44(D1): D862-8, 2015. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015.
6. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics [Internet]. 2010 Jan 15;26(5):589-95.
7. McKenna A, et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res, 20:1297-303.
8. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
9. McLaren W. et al., Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, 26(16):2069-70, 2010.
10. Miller DT, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2022 Jul;24(7):1407-1414. doi: 10.1016/j.gim.2022.04.006. Epub 2022 Jun 17. PMID: 35802134.
11. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003 Jul 1;31(13):3812-4. doi: 10.1093/nar/gkg509. PMID: 12824425; PMCID: PMC168916.
12. 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, Genet Med., 17(5):405-24, 2015.
13. Sherry ST. dbSNP: the NCBI database of genetic variation. Nucleic Acids Research. 2001 Jan 1;29(1):308–11.
14. Welter D. et al., The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res., 42: D1001-1006, 2014.

--------- End of the Report ---------

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