Name:	**Anushka Yadav**	Case ID:	**MD059902**
Age:	**09 years**	Sample Type:	**Blood**
Sex:	**Female**	Sample collection date:	**09/11/2023**
Referring Clinician:	**Dr. Abhishek Abhinay**	Sample collection time:	**NA**
Test Requested:	**Clinical Exome Sequencing (CES)**	Reporting date:	**12/12/2023**

CLINICAL INFORMATION/HISTORY

Anushka Yadav, a 09-year-old female child, presented to the clinician with the chief complaints of whitish urinary discharge, CECT abdomen shows dense calcification involving corticomedullary function of bilateral kidney (medullary nephrocalcinosis grade-III), bilateral pelvicalyceal system (L>R) and distal renal tubular acidosis.
Clinical suspicion for Medullary nephrocalcinosis grade-III.

RESULT SUMMARY

No Pathogenic, Likely Pathogenic, or Variants of Uncertain Significance related to the reported phenotype were found

VARIANTS RELEVANT TO INDICATION FOR TESTING

No variants were found in the sequence data which may be associated with the clinical history of the patient.

Gene & Transcript	Variant	Zygosity	Location	Disorder	Inheritance	ACMG Classification
No significant variant related to phenotype detected.						

FINDINGS UNRELATED TO PHENOTYPE

This section provides information on variants identified which are unrelated to the provided phenotype.

ACMG SECONDARY FINDINGS

No clinically relevant variants associated with the ACMG recommended secondary list of genes were found in the sequence data.

INCIDENTAL FINDINGS

No variants were detected as incidental findings in the sequenced data which may not be associated with the diagnostic indication for which the sequencing test was performed.

CARRIER STATUS IN THE GENES RELATED TO DISEASE

The following Pathogenic or Likely Pathogenic variants were detected.

Gene & Transcript	Variant	Zygosity	Location	Disorder	Inheritance	ACMG Classification
HHAT NM_018194.6	c.10C>T p.Arg4Ter	Heterozygous	Exon 2	Nivelon-Nivelon-Mabille syndrome [OMIM ID: 600092]	Autosomal Recessive	Likely pathogenic PM2 & PVS1

DETAILED VARIANT INFORMATION (CARRIER STATUS)

***HHAT* Chr. 1:210522329 – Likely pathogenic:**

The stop gained NM_018194.6(*HHAT*):c.10C>T (p.Arg4Ter) has not been reported previously on a disease database like ClinVar, to our knowledge. The p.Arg4Ter variant is observed in 4/251,258 (0.0016%) alleles from individuals of gnomAD All background. The p.Arg4Ter variant is not reported in any individuals in 1kG All. This variant is predicted to cause loss of normal protein function through protein truncation. This variant is a stop gained variant which occurs in an exon of *HHAT* upstream of where nonsense mediated decay is predicted to occur. There is another pathogenic loss of function variant 374 residues downstream of this variant, indicating that the region is critical to protein function. The p.Arg4Ter variant is a loss of function variant in the gene *HHAT*, which is intolerant of Loss of Function variants, as indicated by the presence of existing pathogenic loss of function variant NP_060664.2:p.W378*. For these reasons, this variant has been classified as **Likely Pathogenic**.

Nivelon-Nivelon-Mabille syndrome [OMIM ID: 600092]:

Nivelon-Nivelon-Mabille syndrome (NNMS) is caused by homozygous mutation in the *HHAT* gene (605743) on chromosome 1q32. Nivelon-Nivelon-Mabille syndrome (NNMS) is characterized by progressive microcephaly, vermis hypoplasia, and skeletal dysplasia. Variable features include infantile-onset seizures, dwarfism, generalized chondrodysplasia, and micromelia.

RECOMMENDATIONS

Based on the clinical features and the observed genetic findings the following have been recommended:

1. Genetic counseling is recommended to discuss the potential clinical implications of this result.
2. Clinical/ Genotype-phenotype correlation is strongly recommended.
3. Sanger evaluation of the identified variant in the proband and segregation analysis in the parents and close relatives is recommended.
4. If the above results do not correlate completely with patient phenotype, additional testing is advised based on clinician's recommendation.
5. Whole exome sequencing (WES) can be offered to look for other single gene disorders for the provided clinical indication.

REPORTED VARIANTS STATISTICS:

Gene/Transcript	Variant	Depth	Allelic Depth	Alternate Allele Fraction	dbSNP rsID
HHAT NM_018194.6	c.10C>T	82X	42X	0.51	rs779025178

DATA STATISTICS

Total data generated (Gb)	**4.76 GB**

Reads that passed alignment (%)	**99.49%**
Data > Q30 (%)	**92.78%**

METHODOLOGY

Sequencing of the protein coding regions of approximately 30Mb of the human exome (targeting approximately 99% of regions in CCDS and RefSeq) was performed using Illumina NovaSeq platform at a mean depth of 80-100X and % of bases covered at 20X depth >90% in the target region. The individual's DNA was extracted and fragmented, with fragments from the coding regions of the selected gene panel targeted for amplification and sequencing. Reads from the sequence output were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner (BWA). Duplicate reads identification and removal, base quality recalibration and re-alignment of reads based on indels were done using inbuilt DRAGEN bio-IT pipeline. Variants to the reference were called using the Genomic Analysis Tool Kit (GATK). The variants were annotated and filtered using the **Golden Helix VarSeq** and **Varsome** analysis workflow implementing the ACMG guidelines for interpretation of sequence variants. This includes comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes Project Consortium's publication of 2,500 genomes, the NCBI ClinVar database of clinical assertions on variant's pathogenicity and multiple lines of computational evidence on conservation and functional impact. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in **HGMD**, in ClinVar are considered. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM information). All potential modes of inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and causality. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

VARIANT ASSESSMENT PROCESS

The following databases and in-silico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCBI RefSeq Genes, ExAC Gene Constraints, VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using the to HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar.

LIMITATIONS

It should be noted that this test is limited to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meet a level of evidence threshold for cause or contribution to disease. Certain classes of genomic variants are also not covered using the NGS testing technology, including triplet repeat expansions, copy number alterations, translocations and gene fusions or other complex structural rearrangements. More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.

VARIANT CLASSIFICATION BASED ON ACMG RECOMMENDATIONS

Genetic test results are reported based on the recommendations of American College of Medical Genetics (ACMG) as described below [1]

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.

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 which is difficult to classify either as pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence.

ACMG Criteria for classifying Variants

Very Strong (PVS1)	
PVS1	Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease.
Strong (PS)	
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
PS2	De novo variant (both maternity and paternity confirmed) in a patient with the disease and no family history.
PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.
Moderate (PM)	
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
PM2	Absent from controls (or at extremely low frequency if recessive) in reputed databases.
PM3	Variant (one of the compound heterozygous), is segregated with a pathogenic variant with known phase after testing of parents.
PM4	An in-frame deletions/insertions in non-repeat regions or stop-loss can alter the protein length.
PM5	A novel missense change at the same amino acid residue where a pathogenic missense variant has already been determined.
PM6	De novo, without testing in the family.
Supporting (PP)	
PP1	A variant in known gene for a disease which is co-segregating in multiple affected family members
PP2	Missense variants are a common mechanism of disease in a gene which has low benign missense variants.
PP3	A deleterious effect of the variant is predicted by multiple lines of computational evidence (conservation, evolutionary, splicing impact, etc.)
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.
PP5	Reputable source recently reported the variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

DISCLAIMER

In accordance with the Pre-Conception and Pre-Natal Diagnostic Testing (PCPNDT) Act, 2003- Govt. of India; Lab does not disclose the gender of the fetus.

- Interpretation of variants in this report is performed to the best knowledge of the laboratory based on the information available at the time of reporting. The classification of variants can change over time and the laboratory cannot be held responsible for this. Re-analysis of variants in previously issued reports in light of new evidence is not routinely performed, but may be available upon request.
- Negative results do not completely exclude the risk/carrier status for these disorders tested (residual risk)
- The sensitivity of this assay to detect large deletions/duplications of more than 10bp or copy number variations (CNV) is 70-75%. The CNVs detected have to be confirmed by an alternate method.
- Due to inherent technology limitations of the assay, not all bases of the exome can be covered by this test. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that pathogenic variants are present in one or more of the genes analyzed, but have not been detected. The variants not detected by the assay that was performed may impact the phenotype.
- 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.
- The mutations have not been validated/confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines [2] can be given upon request.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. Laboratory under no circumstances will be liable for any delay beyond aforementioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommend any cure in any manner. Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to the laboratory. In case where any test provided by the laboratory fails for unforeseeable or unknown reasons that cannot be influenced by the laboratory in advance, the laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by the laboratory in advance.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by laboratory.

REFERENCES

1. Hamosh, A., Scott, A. F., Amberger, J. S., Bocchini, C. A., & McKusick, V. A. (2005). Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Research, 33(Database Issue), D514–D517. http://doi.org/10.1093/nar/gki033, https://www.omim.org/
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Conditions for Reporting

1. It is presumed that specimen belongs to the patient named or identified, such verification being carried out at the point of generation of said specimen.
2. A test might not be performed due to following reasons:
   a. Specimen Quantity not sufficient (Inadequate collection/spillage during transit).
   b. Specimen Quality not acceptable (Hemolysis/clotted/lipemic).
   c. Incorrect sample type.
   d. Test canceled either on request of the patient or doctor
3. In any of the above case a fresh specimen will be required for testing and reporting.
4. The results of the tests may vary from lab to lab; time to time for the same patient.
5. The reported results are dependent on individual assay methods, equipment, method sensitivity, specificity and quality of the specimen received.
6. Partial representation of the report is not allowed.
7. The reported tests are for the notification of the referring doctor, only to assist him/her in the diagnosis and management of the patient.
8. Report with status "Preliminary" means one or more test are yet to be reported.
9. This report is not valid for Medico Legal Purpose.
10. Applicable Jurisdiction will be of "Delhi" for any dispute/claim concerning the test(s) & results of the test(s).

SANGER SEQUENCING ANALYSIS

Patient ID	8434851	**Gender**	Female	**Location**	Varanasi
Patient Name	Anushka Yadav	**Clinician Name**	Dr. Abhishek Abhinay	**Sample Collected**	28-05-2024
DOB	NA	**GA/LMP Date**	NA	**Sample Received**	29-05-2024
Age	09 Years	**Hospital Name**	BHU Hospital	**Report Released**	12-06-2024

Test Requested:- Sanger Sequencing **Sample Type:- Blood** **Sample Quality:- Acceptable**

RESULT

Fig No.	Sample Name	Gene Name	Variant Reported in the Index Patient	Variant Status	Inheritance
1.	**Anushka Yadav**	HHAT	**c.10C>T (p.Arg4Ter)**	Heterozygous	Autosomal Recessive

TEST INFORMATION

This assay tests for the confirmation of variant in the Anushka Yadav which has been detected in HHAT gene in her CES. Analysis is performed only for variant at c.10C>T (p.Arg4Ter) in HHAT gene.

RECOMMENDATION

Please correlate clinically and genetic counselling is recommended.

TECHNOLOGY

Targeted sequencing and mutation analysis was performed by Polymerase Chain Reaction (PCR) followed by automated DNA sequencing of the amplicon using BigDye ABI Genetic Analyzer 3500XL platform. The raw data obtained is subsequently analyzed for the nucleotide variants.

DISCLAIMER

This test is designed to detect mutations in the above-mentioned regions only. Sequences surrounding the regions of interest are analysed but not reported.In rare cases because of allele dropout, heterozygosity may be reported as homozygosity.This assay is unable to differentiate between cis and trans mutations. Though oligos are designed specifically to parent gene using bioinformatics tool, Interference of pseudogene sequence cannot be ruled out completely. Any change in primer binding site can result and interfere with the results and allele dropout cannot be ruled out using this experiment.

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ANNEXURE

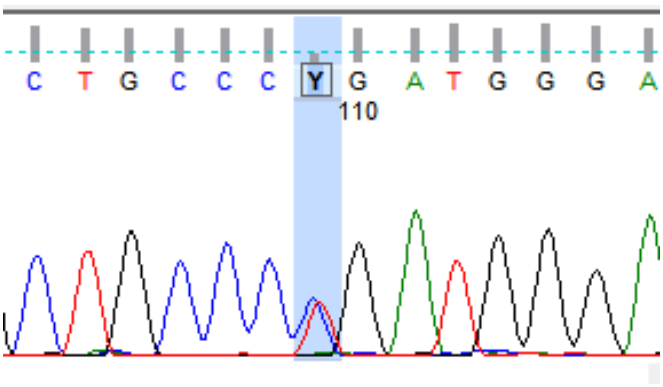

Fig 1: Sanger sequencing data (electropherogram) for the provided sample showing nucleotide change at c.10C>T (p.Arg4Ter) in HHAT gene.

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2. It is to be presumed that the tests performed pertain to the specimen/sample attributed to the Customer's name or identification. It is presumed that the verification particulars have been cleared out by the customer or his/her representation at the point of generation of said specimen / sample. It is hereby clarified that the reports furnished are restricted solely to the given specimen only.

3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

4. This report shall not be deemed valid or admissible for any medico-legal purposes.

5. The Customers assume full responsibility for apprising the Company of any factors that may impact the test finding. These factors, among others, includes dietary intake, alcohol, or medication / drug(s) consumption, or fasting. This list of factors is only representative and not exhaustive.