Name	: Mr.RAM BAHADUR SHRESTHA	Centre Details	:KATHMANDU PATHLAB AND DIAGNOSTIC
Age	: 33 Yrs Sex: Male	Accession.ID	:OQG2507090740
Collection Date	: 08/Jul/2025 12:00AM	Referred By	:DR.ANJAN SHRESTHA
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Bone marrow failure syndrome gene panel

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Mr. *Ram Bahadur Shrestha*, presented with clinical indication of pancytopenia. His bone marrow biopsy showed hypocellular bone marrow, consistent with aplastic anemia. His CT KUB showed left distal ureteric calculus with mild hydroureteronephrosis, mild right hydronephrosis, focal cortical thinning in bilateral kidneys with irregular outline and likely sequale of previous infection. Mr. *Ram Bahadur Shrestha* has been evaluated for pathogenic gene variants.

RESULTS

NO PATHOGENIC OR LIKELY PATHOGENIC VARIANTS CAUSATIVE OF THE REPORTED PHENOTYPE WERE DETECTED

VARIANT INTERPRETATION AND CLINICAL CORRELATION

No significant variant(s) for the given clinical indications that warrants to be reported was detected.

ADDITIONAL INFORMATION

- No significant SNV(s)/INDELS or CNV(s) that warrants to be reported were detected. All the genes covered in this assay have been screened for the given clinical indications. To view the coverage of all genes NGS test methodology details of this assay are given in the appendix.
- With regard to ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (PMID: 35802134; ACMG SF v3.1), we report significant pathogenic and/ or likely pathogenic variants in the recommended genes for the recommended phenotypes, only if informed consent is given by the patient.

RECOMMENDATIONS

- Genetic counselling is advised.
- The sensitivity of NGS assay to detect copy number variants (CNV) is 70-75%. We recommend discussing alternative testing methodology options with Laboratory Tech Support as required. In case clinician is suspecting CNV as an important genetic etiology, alternate tests like microarray/ MLPA or qPCR may be considered after discussing with the Laboratory.

Dr. Vinay Bhatia
Ph.D.
Head- Molecular Biology and Genomics

Dr. Shivali Ahlawat
MD. D.N.B (Path)
Lab Director-National Reference Lab
HMC RG-No.17038

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APPENDIX

TEST METHODOLOGY

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions and clinically relevant in the genome is performed. Variants identified in the exonic regions and splice-site are generally actionable compared to variants 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 depth of >80-100X on Illumina sequencing platform. We follow the GATK best practices framework for identification of germline variants in the sample using Sentieon [Sentieon]. The sequences obtained are aligned to human reference genome (GRCh38) using BWA aligner [Sentieon, PMID:20080505] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels [Sentieon]. Sentieon haplotype caller is then used to identify variants in the sample. The germline variants identified in the sample is deeply annotated using VariMAT pipeline. Gene annotation of the variants is performed using VEP program [PMID: 20562413] against the Ensembl release 104 human gene model [PMID: 34791404]. In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth method [PMID: 22942019]. This algorithm detects CNVs based on comparison of the read-depths in the sample of interest with the matched aggregate reference dataset.

Clinically relevant mutations in both coding and non-coding regions are annotated using published variants in literature and a set of diseases databases : ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar [PMID: 26582918, 18842627, 28349240, 21520333, 19344873, 20106818]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genome, TOPMed (Freeze_8), Genome Asia, and our internal Indian population database (MedVarDb v4.0) [PMID: 26432245, 32461613, 11125122, 26292667, 33568819, 31802016]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretation and reporting.

Average sequencing depth (x)	Average on-target sequencing depth (x)	Percentage target base pairs covered		
		0x	≥5x	≥20x
199	102.37	0.32	99.26	97.18

Total data generated (Gb)	5.81
Total reads aligned (%)	99.96
Reads that passed alignment (%)	89.21
Data ≥Q30 (%)	98.22

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[$]The classification of the variants is done based on American College of Medical Genetics as described below [PMID:25741868].

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Pathogenic	A disease causing variant in a gene which can explain the patient's 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 (MANE Select), 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 (v104), [SIFT version - 5.2.2; PolyPhen - 2.2.2; LRT version (November, 2009); CADD (v1.6); Splice AI; dbNSFPv4.2] and MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).

Diseases databases used for annotation includes ClinVar (updated on 17042023), OMIM (updated on 01092023), HGMD (v2023.1), LOVD (Nov-18), DECIPHER (population CNV) and SwissVar.

LIMITATIONS

- Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Accurate interpretation of test results may require knowing the true biological relationships in a family. Failing to accurately state the biological relationships in {my/my child's} family may result in incorrect interpretation of results, incorrect diagnoses, and/or inconclusive test results.
- Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- Specific events like copy number variants, translocations, repeat expansions and chromosomal rearrangements may not be reliably detected with targeted sequencing. Variants in untranslated region, promoters and intronic variants are not assessed using this method.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to: mislabeled samples, inaccurate reporting of clinical/medical information, rare technical errors

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or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).

- The variant population allele frequencies and in silico predictions for GRCh38 version of the Human genome is obtained after lifting over the coordinates from hg19 genome build. The existing population allele frequencies (1000Genome, gnomAD-Exome) are currently available for hg19 genome version only. This might result in some discrepancies in variant annotation due to the complex changes in some regions of the genome.
- It is assumed that the clinician ordering a genetic test is fully aware of these limitations and Laboratory shall not be responsible in case any inappropriate panel/test methodology is selected.

DISCLAIMERS

- 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 Laboratory cannot be held responsible for this. Please feel free to contact Labs in the future to determine if there have been any changes in the classification of any variants. Re-analysis of variants in previously issued reports in light of new evidence is not routinely performed but may be available upon request.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variants (CNV) is 70-75%. The CNVs detected have to be confirmed by 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 variant(s) have not been validated/confirmed by Sanger sequencing.
- Incidental or secondary findings (if any) that meet the ACMG guidelines [PMID: 27854360] 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 recommends 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 and to communicate 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 Laboratory. In case where any test provided by Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by Laboratory in advance, Laboratory shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by Laboratory in advance.

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- Variants of uncertain significance (VUS) which are mentioned in the report need to be further correlated with the clinical phenotype, reports of other investigations, segregation analysis in the parents or affected/unaffected family members. Laboratory shall not be responsible for the inappropriate interpretation/ communication/ clinical actions/ reproductive decisions based on the VUS reported. The classification of VUS may change as the clinical phenotype evolves or more information is available in the scientific literature/ annotated databases.

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END OF REPORT

Disclaimer:

1. The report is validated by third party lab
2. Test results are dependent on the quality of the sample received by the lab
3. Tests are performed as per schedule given in the test listing and in any unforeseen circumstances, report delivery may be delayed
4. Test results may show interlaboratory variations
5. All dispute and claims are subjected to local jurisdiction only. Clinical correlation advised
6. Test results are not valid for medico legal purposes
7. For all queries, feedback, suggestions, and complaints, please contact customer care support +0124 665 0000

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and Genomics

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