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DNA TEST REPORT - MEDGENOME LABS

Full Name / Ref No:	[redacted]	Order ID/Sample ID:	**1114565/8816592**
Gender:	Male	Sample Type:	Blood
Date of Birth / Age:	14 years	Date of Sample Collection:	17th November 2024
Referring Clinician:	[redacted]	Date of Sample Receipt:	20th November 2024
		Date of Order Booking:	20th November 2024
		Date of Report:	6th January 2025
Test Requested:	**[SBZ_A] Whole Exome Sequencing (BPL Subsidised test)**		

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Master [redacted] born of a consanguineous marriage, presented with clinical indications of short stature, stage 5 chronic kidney disease, hypertensive crisis, retinitis pigmentosa, nystagmus, hematuria and anemia. Master [redacted] is suspected to be affected with nephronophthisis and has been evaluated for pathogenic variations.

RESULTS

PATHOGENIC VARIANT CAUSATIVE OF THE REPORTED PHENOTYPE WAS DETECTED

SNV(s)/INDELS

Gene# (Transcript)	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification$
***IQCB1* (-)** (ENST00000310864.11)	Intron 6	c.488-1G>A **(3' Splice site)**	Homozygous	Senior-Loken syndrome 5 (OMIM#609254)	Autosomal recessive	Pathogenic **(PVS1,PM2,PP5)**

COPY NUMBER VARIANTS CNV(s)

No significant CNVs for the given clinical indications that warrants to be reported was detected.

VARIANT INTERPRETATION AND CLINICAL CORRELATION

Variant description: A homozygous 3' splice site variant in intron 6 of the ***IQCB1*** gene (**chr3:g.121807444C>T; Depth: 80x**) that affects the invariant AG acceptor splice site upstream of exon 7 (**c.488-1G>A**; **ENST00000310864.11**) was detected (Table). The observed variant has previously been reported in patients affected with Senior Loken syndrome [PMID: 18076122; ClinVar; VCV000812120.3]. This variant has not been reported in the 1000 genomes and gnomAD (v3.1) databases and has a minor allele frequency of 0.001%, 0.001% and 0.006% in the gnomAD (v2.1), topmed and our internal databases respectively. The *in-silico* prediction# of the variant is damaging by MutationTaster2. The reference base is conserved across species.

OMIM phenotype: Senior-Loken syndrome 5 (OMIM#609254) is caused by homozygous or compound heterozygous mutations in the *IQCB1* gene (OMIM*609237). Senior-Loken syndrome is an autosomal recessive disorder with the main features of nephronophthisis (NPHP) and leber congenital amaurosis (LCA) [PMID: 18842627].

Based on the above evidence[$], ***this IQCB1 variation is classified as a pathogenic variant and has to be carefully correlated with the clinical symptoms.***

The significance/classification of the variant may change based on the genetic testing in parents and other family members.

ADDITIONAL INFORMATION

- No other 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 Click here. NGS test methodology details of this assay are given in the appendix.
- [$]Genetic test results are reported based on the recommendations of American College of Medical Genetics and Genomics (ACMG) [PMID: 25741868, 31690835, 32906214].
- 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.
- Please write an email to genetic.counseling@medgenome.com in case you need assistance for genetic counselling. For any further technical queries please write an email to techsupport@medgenome.com

RECOMMENDATIONS

- **The *IQCB1* gene has a pseudogene in the human genome. Validation of the variant by an alternate technique 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).
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.
- The sensitivity of NGS assay to detect copy number variants (CNV) is 70-75%. We recommend discussing alternative testing methodology options with MedGenome Tech Support (techsupport@medgenome.com) 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 MedGenome TechSupport team.

[redacted] Ph.D
Sr. Manager -
Variant Interpretation

[redacted] Ph.D
Director - Clinical Bioinformatics

[redacted] DNB(Medical Genetics), MD(Pediatrics),DCh
Consultant- Senior Clinical Geneticist

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APPENDIX

TEST METHODOLOGY

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions and clinically relevant in th genome is performed. Variants identified in the exonic regions and splice-site are generally actionable compared t variants that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect varian 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 wer sequenced to mean depth of >80-100X on Illumina sequencing platform. We follow the GATK best practices framewo for identification of germline variants in the sample using Sentieon [Sentieon]. The sequences obtained are aligned t human reference genome (GRCh38) using BWA aligner [Sentieon, PMID:20080505] and analyzed using Sentieon fo removing duplicates, recalibration and re-alignment of indels [Sentieon]. Sentieon haplotype caller is then used to identi variants in the sample. The germline variants identified in the sample is deeply annotated using VariMAT pipeline. Ger annotation of the variants is performed using VEP program [PMID: 20562413] against the Ensembl release 104 huma gene model [PMID: 34791404]. In addition to SNVs and small Indels, copy number variants (CNVs) are detected fro targeted sequence data using the ExomeDepth method [PMID: 22942019]. This algorithm detects CNVs based o 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 literatu and a set of diseases databases : ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar [PMI 26582918, 18842627, 28349240, 21520333, 19344873, 20106818]. Common variants are filtered based on alle frequency in 1000Genome Phase 3, gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genom TOPMed (Freeze_8), Genome Asia, and our internal Indian population database (MedVarDb v4.0) [PMID: 2643224 32461613, 11125122, 26292667, 33568819, 31802016]. Non-synonymous variants effect is calculated using multip algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretatio and reporting.

Average sequencing depth (x)	Average on-target sequencing depth (x)	Percentage target base pairs covered		
		0x	≥5x	≥20x
214	85.05	0.17	99.64	98.56

Total data generated (Gb)	8.02
Total reads aligned (%)	99.94
Reads that passed alignment (%)	86.13
Data ≥Q30 (%)	97.19

$The classification of the variants is done based on American College of Medical Genetics as described belo [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 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 MedGenome 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 MedGenome cannot be held responsible for this. Please feel free to contact MedGenome Labs (techsupport@medgenome.com)

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. MedGenome 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. MedGenome 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. MedGenome 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 MedGenome. In case where any test provided by MedGenome fails for unforeseeable or unknown reasons that cannot be influenced by MedGenome in advance, MedGenome shall not be responsible for the incomplete, potentially misleading or even wrong result of any testing if such could not be recognized by MedGenome in advance.
- 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. MedGenome 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.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by MedGenome.

END OF REPORT