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Patient Information

Name: Master Karn Pandkule	**Sample ID:** NA
Age/Gender: 2 years/Male	**Patient ID:** 1002320917
Specimen Type: NA	**Collected On:** NA
Referring Physician: Dr. Chaitanya Datar	**Received On:** 09.05.2023
Referring Centre: Sujanan-Pune 1	**Report Released On:** 06.07.2023

WHOLE EXOME SEQUENCING TEST REPORT [DATA REANALYSIS]

Clinical History

Master Karn Pandkule, born of a consanguineous marriage, presented with clinical indications of global developmental delay, macrocephaly, iron-deficiency anemia, hypotonia, tremulousness, ?seizures. On examination- relative macrocephaly, frontal bossing, midface retrusion, upslant to palpebral fissures, short smooth philtrum, open mouth, and baseline hypotonia. MRI Brain- suggestive of leukodystrophy, EEG-Normal. The whole exome sequencing was performed and a heterozygous variant of uncertain significance in the *CSF1R* gene (c.488C>T; p.Thr163Ile) was reported. His mitochondrial genome sequencing test result did not reveal any clinically significant variants. He is suspected to be affected with leukodystrophy or lysosomal storage disorder or Krabbe's and has been re-evaluated for clinically significant variations.

Results

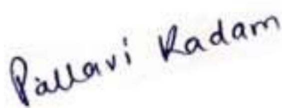
Diagnostic findings related to phenotype:
No significant variant was identified in Master Karn Pandkule.

Diagnostic findings not related to phenotype:
No other significant variants have been detected.

The previously identified CSF1R: c.488C>T, gene variant was also identified during this assay.

Recommendations

1. Clinical correlation and Genetic counseling.
2. Further tests can be considered after a discussion with a clinical geneticist or a genetic counselor.



Pallavi Kadam
Scientific Officer - Genomics

Dr. Madhavi Pusalkar, Ph.D
General Manager: Genomics

PRECAUTIONS: Although all precautions are taken during DNA tests, the currently available data indicate that the technical error rate of all types of DNA analysis is approximately 2%. All clinicians or persons requesting DNA diagnostic tests must be aware of these data before acting upon these results.

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Test Methodology

The sequences obtained are aligned to human reference genome (GRCh37/Hg19) using Sentieon aligner [3, 4] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels [3]. Sentieon haplotype caller has been used to identify variants which are relevant to the clinical indication. Gene annotation of the variants is performed using VEP program [5] against the Ensembl release 99 human gene model [6] In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth (v1.1.10) method [7]. This algorithm detects rare CNVs based on a comparison of the read-depths of the test data with the matched aggregate reference dataset.

Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on 11th May 2020), GWAS, HGMD (v2020.2) and SwissVar [8-12]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v2.1), EVS, dbSNP (v151), 1000 Japanese Genome and our internal Indian population database [13-16]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Only non-synonymous and splice site variants found in the panel genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

ACMG-Rule of Classification (ROC)

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

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

Pathogenic Very Strong (PVS)	
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 segregating with a pathogenic variant with known phase after testing of parents.
PM4	An in-frame deletions/insertions in non-repeat region 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.

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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 reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 87 gene model), 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**.**

The in silico predictions are based on Variant Effect Predictor, Ensembl release 91 (SIFT version - 5.2.2; PolyPhen - 2.2.2); LRT version – December 5, 2019 release from dbNSFPv4.0 and MutationTaster2 based on build NCBI/ Ensembl 66.

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. 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 variations, translocations, repeat expansions and chromosomal rearrangements may not be reliably detected with targeted panel 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).

Disclaimer

- 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. 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 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.

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- 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 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 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.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by laboratory.

References

1. Fast gapped-read alignment with Bowtie 2 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3322381/)
2. McKenna A. et al., The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297-303. doi: 10.1101/gr.107524.110. Epub 2010 Jul 19. PMID: 20644199; PMCID: PMC2928508.
3. VarSeqTM (Version 8.x) [Software]. Bozeman, MT: Golden Helix, Inc. Available from http://www.goldenhelix.com.
4. Landrum M. J. et al., ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res., 44(D1):D862-8, 2015.
5. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998.
6. Welter D. et al., The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res., 42:D1001-1006,2014.
7. Stenson P. D. et al., The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Human Genet., 136(6): 665-677, 201.
8. Mottaz A. et al., Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. Bioinformatics, 26(6): 851-852, 2010.
9. 1000 Genomes Project Consortium et al., A global reference for human genetic variation. Nature, 526(7571): 68-74, 2015..
10. Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/).

*****End of The Report*****

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