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DEPARTMENT OF MOLECULAR GENETICS AND GENOMICS

Patient Name	Master Aarav Kumar Sahoo	Ref. Doctor	Self
Age/Gender	11M / MALE		
Test Name	Clinical Exome Sequencing	Reported Date	11/10/2023

Clinical History:

Master Aarav Kumar Sahoo is presented with clinical indications of seizure, recurrent episodes of generalized tonic-clonic seizure (GTCS), status epilepticus with pneumonia and neutrophilic leukocytosis. He is suspected to be with idiopathic epilepsy and has been referred for clinical exome sequencing.

Results: Heterozygous likely pathogenic (LP) variant has been identified in *SCN1A* gene.

Variants in genes known to be associated with the provided phenotype

Gene and Transcript	Exon	Variant Nomenclature	Zygosity	Classification	Disease	Inheritance
SCN1A NM_001165963.4	29	c.5330T>A, p.Val1777Glu chr2:165991945A>T	Heterozygous	Likely Pathogenic (LP)	Generalized epilepsy with febrile seizures plus, type 2 (OMIM# 604403)	Autosomal Dominant

Variant Interpretation:

The exome data analysis identified a heterozygous missense variant **c.5330T>A, p.Val1777Glu (chr2:165991945A>T)** in ***SCN1A*** gene. The observed variant has not been reported in gnomAD database. The In *silico* predictions by Revel, SIFT, DANN and MutationTaster found to have deleterious effect. The variant lies in the hot spot region and a different missense change (c.5330T>C) at same amino acid position has been reported in Clinvar (953613). No functional studies have been done on the pathogenic role of this variant yet. Therefore, the observed variant has been classified as **Likely Pathogenic (LP).**

Genotype-Phenotype Correlation:

Orrico *et al*. (2009) identified 21 mutations, including 14 novel mutations, in the *SCN1A* gene in 22 (14.66%) of 150 Italian pediatric probands with epilepsy. *SCN1A* mutations were found in 21.2% of patients with GEFS+. In 2 unrelated Japanese families with GEFS+2 associated with development of partial epilepsy, Sugawara *et al*. (2001) identified 2 novel mutations in the *SCN1A* gene.

Clinical Features:

Generalized epilepsy with febrile seizures plus, type 2 (GEFSP2) is an autosomal dominant neurologic disorder characterized by the onset of seizures associated with fever in the first months or years of life. Affected individuals continue to have various types of febrile and afebrile seizures later in life, including generalized tonic-clonic seizures (GTCS). Some patients may have offset of seizures in the first or second decades; rare patients may have mildly impaired intellectual development. Mutations in the *SCN1A* gene thus cause a spectrum of seizure disorders,

ranging from early-onset isolated febrile seizures to generalized epilepsy with febrile seizures plus, type 2, which represents a more severe phenotype.

References:

1. Orrico, A., *et al*. Mutational analysis of the *SCN1A, SCN1B* and *GABRG2* genes in 150 Italian patients with idiopathic childhood epilepsies. (Letter) Clin. Genet. 75: 579-581, 2009. [PubMed: 19522081].
2. Sugawara, T., *et al*. Na-v-1.1 mutations cause febrile seizures associated with afebrile partial seizures. Neurology 57: 703-705, 2001. [PubMed: 11524484].

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).

Recommendations:

- Genetic counselling and clinical correlation for accurate interpretation of test results are recommended.
- If the above result does not correlate completely with patient phenotype, additional testing is advised based on clinician's discretion.
- Based on genetic testing in 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 column-based method and DNA quality and quantity were assessed using electrophoretic and Qubit method. 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 hybridization- based target capture method. Sequencing libraries that passed the quality control were sequenced on 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 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) had been carried out by using GATK (v4.2.5.0). Variant calling was done by using the GATK Haplotype Caller. Each called variant annotated using different clinical and population databases. Common variants were filtered out based on minor allele frequency (MAF) in 1000Genome Phase 3[4], gnomAD (v3), ExAC [3], dbSNP (v155). 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 diseases databases -ClinVar, OMIM, GWAS and SwissVar. The classification of the variant is done based on American College of Medical Genetics guidelines.

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QC METRICS

Total data generated	10.38 Gb
Total reads aligned reads (%)	85.01
Data ≥Q30(%)	92.69
Total data which passed mapping quality cut-off	9.30 Gb

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 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 development of disease, but currently scientific evidence is inadequate, additional evidence in 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 sample.
- The current results are based on analysis of coding regions (exons) as well as certain intron padding regions on patient's genomic DNA with respect to patient phenotype as defined in the target regions (link available below). However, due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants of insufficient coverage, as well as those variants which 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 reported variants have not been Sanger confirmed. Sanger confirmation is recommended for the same.
- The test methodology currently does not detect large deletions/duplications, triplet repeat expansions and epigenetic changes. The test also does not include analysis of predictors for multifactorial, polygenic and/or complex diseases. Novel synonymous changes as well as intronic mutations (excludingthose affecting invariant splice nucleotides) are not routinely reported.
- CNV analysis is not included.

- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity & specificity for variant detection, analysis, interpretation due to 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 re- analysis of this report yearly. Please contact laboratory in case 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:

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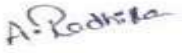

A.Radhika
Co-Head,
Genomics

Dr.B.Chandan
Head,
Bioinformatics

Dr.S.M.Naushad
Chief Scientific Officer