MyWES

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Scientific Director: George Nasioulas PhD

SAMPLE INFORMATION

Name :	-	**Date Received :**	-
Medical ID :	-	**Date of Report :**	-
Date of Birth :	-	**Req. Physician :**	-
Location :	-	**Barcode :**	-
Material :	WHOLE PERIPHERAL BLOOD	**Reason of referal:**	e.g. epileptic encephalopathy

Whole Exome analysis (WES) by Next Generation Sequencing

Results associated with the reason of referral

PATHOGENIC VARIANT IDENTIFIED

Gene	Variant	Clinical Significance	Zygosity
STXBP1	NM_001032221.6:c.1652G>A, p.(Arg551His)	Pathogenic variant	Heterozygous

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Variants Details

***STXBP1*, Exon 18, NM_001032221.6:c.1652G>A, p.(Arg551His)** ClinGen HPO ClinVar

This is a single nucleotide variant that results in the substitution of arginine by histidine at codon 551 of the STXBP1 protein (p.Arg551His). The arginine is located in a domain of the protein that is known to be functionally important and there is a small physicochemical difference between arginine and histidine (Grantham score: 29). The variant has been reported in population databases (rs796053374) without a defined allele frequency and is listed in ClinVar (Variation ID: 566474). This variant has been reported as de novo in multiple unrelated individuals with STXBP1-related developmental and epileptic encephalopathy (PMID: 35655584, 35007884, 26865513, 23409955). In silico prediction tools assessing the functional impact of missense variants suggest that this change may affect protein structure and function. The variant affects the arginine residue at position 551 (p.Arg551), and other variants disrupting the same residue have been described as pathogenic (PMID: 23409955, 26865513, 27069701). Based on the ACMG/AMP guidelines (PMID: 25741868), this variant is classified as pathogenic.

The *STXBP1* gene, located on chromosome 9q34.11, encodes the syntaxin-binding protein 1, which plays a critical role in neurotransmitter release by regulating the function of syntaxin, a component of the SNARE complex involved in synaptic vesicle exocytosis. Pathogenic or likely pathogenic variants in STXBP1 are associated with early infantile epileptic encephalopathy type 4 (EIEE4), also known as Ohtahara syndrome. The mode of inheritance is autosomal dominant, and most variants occur de novo.The estimated prevalence of STXBP1-related developmental and epileptic encephalopathy (STXBP1-RD) is approximately 1 in 30,000 individuals. Recent studies have identified STXBP1 as one of the five most frequently implicated genes in developmental and epileptic encephalopathies.Clinically, STXBP1 encephalopathy is characterized by early-onset developmental delay, intellectual disability or cognitive impairment, and epilepsy. The median age of seizure onset is six weeks (range: 1 day to 13 years). Reported seizure types include infantile spasms, generalized tonic-clonic, clonic or tonic seizures, myoclonic, atonic, absence, and focal seizures. Additional neurological findings include abnormalities of muscle tone, movement disorders (particularly ataxia and dystonia), behavioral disturbances, and autistic features. Feeding difficulties are also common and may require specialized nutritional support. Management typically involves anti-seizure medications (ASMs) for seizure control; however, approximately 25% of patients show inadequate response to pharmacologic therapy (PMID: 18469812, 38898886, 39456768, 32643187).

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Methodology

Genomic DNA was extracted from the sample under investigation. The DNA was analyzed using a target enrichment method (exome capture) covering all coding exons and flanking intronic regions of the human genome (21,285 genes), utilizing the Twist Human Core Exome EF Multiplex Complete kit (Twist Bioscience). Sequencing of the enriched targets was performed on the MGI DNBSEQ-T7 platform. Bioinformatic analysis and variant interpretation were carried out using the Breakthrough Genomics bioinformatics platform VG PLUS ver3.0.8, aligned to the reference genome GRCh37/hg19.

The mean coverage depth was 107x, with 99.9% of target regions sequenced at a depth ≥20x. Large genomic rearrangements (CNVs) were evaluated in silico using validated algorithms of the Breakthrough Genomics bioinformatics platform VG PLUS ver3.0.8.

Based on the available clinical data and the reported phenotype, a phenotype-driven analysis approach was followed.

-Genes were prioritized according to the OMIM and Human Phenotype Ontology (HPO) databases, selecting those associated with the patient's phenotype.

- Variant classification was performed according to the ACMG/AMP guidelines (PMID: 25741868).

-Variants predicted to have a deleterious impact (frameshift, nonsense, missense, or splice-site changes), as well as potential de novo variants, were evaluated. Only variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) relevant to the phenotype were reported.

- All clinically significant variants were confirmed by Sanger sequencing, when technically feasible.

RECOMMENDATIONS

1)Genetic counseling is recommended to discuss the implications of this test and to interpret the results in the context of the patient's overall clinical evaluation and family history.

2) Reinterpretation of genome sequencing data is recommended on an annual basis and may be requested by the referring clinician and one should be cautious about that variant classification and/or interpretation may change over time if more information becomes available and identification of new variants associated with disease phenotype during the re-assessment.

3) Targeted testing of the identified pathogenic variant in the VCP gene is recommended in the extended family members if deemed necessary, for identifying those at risk for the clinical condition or reproductive planning. Consult with the referring physician to discuss about risk assessment and disease management measures.

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*Note:

- Every molecular test has an internal 0,5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.
-Unless otherwise stated, the target regions enriched in this assay include all coding exons and 15 base pairs of the flanking intronic sequences on either side. Therefore, this method does not detect variants located deep within introns, in regulatory regions (enhancers or promoters), or in non-coding RNAs.

-The applied methodology achieves >99% sensitivity and specificity for the detection of single-nucleotide variants (SNVs) and small insertions/deletions (INDELs) as well as >90% sensitivity for the detection of large genomic rearrangements (CNVs) using validated computational algorithms.
- CNV calls generated from sequencing coverage data should be interpreted with caution and confirmed by an independent method. In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected.
- This methodology does not detect structural alterations such as translocations, balanced rearrangements, or nucleotide repeat expansions in genes associated with these disorders. In addition, it cannot detect low-level mosaicism (coverage <25%).
-Next generation sequencing technologies and our bioinformatics analysis significantly reduce the contribution of pseudogene sequences or other highly-homologous sequences, these may still occasionally interfere with the technical ability of the assay to identify pathogenic variant alleles in both sequencing and deletion/duplication analyses.
-The analysis also includes the mitochondrial genome (mtDNA) for the detection of single-nucleotide variants. However, the level of heteroplasmy may vary considerably among different tissues; therefore, a pathogenic variant present in tissues such as muscle or nervous system may not be detectable in peripheral blood, and alternative tissue testing may be required.

GLOSSARY OF USED ABBREVIATIONS:
AD = autosomal dominant
AR = autosomal recessive
HEM = hemizygous
HET = heterozygous
HOM = homozygous
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)

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Details about non-pathogenic variants

Each individual carries a large number of genetic variants, most of which are not associated with an increased risk of disease. Variants that, according to bioinformatic analysis and the ACMG/AMP classification criteria, are considered benign or likely benign are not reported, as they are not known to confer an increased risk of disease and do not alter medical management beyond what is indicated by the patient's family and personal history. Only variants that are relevant to the reported clinical phenotype and that have been classified, according to the ACMG/AMP guidelines (2015) and ClinGen specifications (2021), as pathogenic or likely pathogenic are included in this report. Variants of uncertain clinical significance (VUS) are reported only when found in genes potentially related to the patient's phenotype and predicted by most computational algorithms (e.g., REVEL, MetaLR) to have a damaging effect on protein function. VUS identified in autosomal recessive genes are not reported unless another variant (pathogenic, likely pathogenic, or VUS) is detected in the same gene. Furthermore, variants that are not related to the indication for testing are not reported. Secondary findings are not included unless the patient has opted to receive such information, in accordance with ACMG SF v3.1 (2022) (PMID: 35802134).

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Family tree

Note: **The information shown on the family tree has been provided by the patient and not by medical records.**

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Literature

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