PerkinElmer
For the Better

Whole Exome Sequencing

CLIENT	SPECIMEN	PATIENT
Client Name:	Specimen Type:	Patient's Name:
Hospital/Institution:	Collection Date:	Date of Birth:
Mailing Address:	Receive Date:	Gender:
Toll Free Phone Number:	Report Date:	Patient's PKI ID:
		Accession ID:
		Cross Reference

Test Performed: Whole Exome Sequencing, Proband Only

TEST RESULT SUMMARY

Pathogenic variants detected. One copy of a c.1750C>T (p.Q584X) pathogenic variant and one copy of a c.2872C>T (p.R958X) pathogenic variant were detected in the *AP3B2* gene in this individual. Clinical and biochemical correlation is required.

Diagnostic findings related to phenotype:

Gene/ OMIM	DNA Change[a]	Protein Change	Exon	Disease	Inheritance	Zygosity	Classification
AP3B2/ 602166	c.1750C>T (g.83335601; coverage 204; allele fraction 47.1/52.9)	p.Q584X	15	Early infantile epileptic encephalopathy 48	Autosomal Recessive	Heterozygous	Pathogenic
	c.2872C>T (g.83330664; coverage 86; allele fraction 51.2/48.8)	p.R958X	24	Early infantile epileptic encephalopathy 48	Autosomal Recessive	Heterozygous	Pathogenic

[a]Positions relative to the reference sequences listed below.

VARIANT INTERPRETATION

A sample from this individual was referred to our laboratory for focused exome sequencing. Information provided to us indicates that this individual has a suspected diagnosis of early infantile epileptic encephalopathy, an EEG suggestive of evolving modified hypsarrhythmia, and a normal MRI.

***AP3B2* c.1750C>T (p.Q584X) – Pathogenic.** The c.1750C>T (p.Q584X) nonsense variant results in the substitution of the glutamine codon at amino acid position 584 with a termination codon. This variant has not been reported in individuals with disease to our knowledge, but is of a type expected to cause disease. The c.1750C>T (p.Q584X) *AP3B2* variant is classified as pathogenic. Clinical and biochemical correlation is required.

***AP3B2* c.2872C>T (p.R958X) – Pathogenic.** The c.2872C>T (p.R958X) nonsense variant results in the substitution of the arginine codon at amino acid position 958 with a termination codon. This variant has been reported in a single individual with early-onset epileptic encephalopathy and optic atrophy[1] and is of a type expected to cause disease. The c.2872C>T (p.R958X) *AP3B2* variant is classified as pathogenic. Clinical and biochemical correlation is required.

1. Assoum et al. Am J Hum Genet. 2016 Dec 1;99(6):1368-1376. PMID: 27889060.

Recommendations: The detection of two pathogenic variants in the *AP3B2* gene is consistent with a diagnosis of early infantile epileptic encephalopathy; however, these results must be interpreted in the context of this individual's clinical and biochemical profile.

Sequence analysis cannot determine if multiple variants are on the same (in *cis*) or opposite (in *trans*) copies of a gene. Targeted testing of the parents of this individual to determine if the c.1750C>T (p.Q584X) *AP3B2* pathogenic variant and the c.2872C>T (p.R958X) *AP3B2* pathogenic variant are on opposite chromosomes in this individual is available.

Targeted testing is available for family members at risk to carry the pathogenic variants identified in this individual.

Genetic counseling is recommended. Identified data will be stored at PerkinElmer Genetics. For more information, please contact the laboratory at 1-866-463-6436.

Diagnostic findings not related to phenotype: No pathogenic variants in genes that are unrelated to the patient's phenotype were detected in this individual.

Carrier status in genes related to disease: No pathogenic variants in genes related to disease were detected in this individual.

Carrier status in genes on ACMG recommended secondary list: No pathogenic variants in genes related on the ACMG recommended secondary list were detected in this individual.

Pharmacogenetic variants:

The following pharmacogenetic variants were detected:

Gene	Allele haplotype	Drugs	Enzyme Activity	Predicted Metabolizer Status
CYP2C9	CYP2C9*3/CYP2C9*3	Warfarin	Reduced	Poor Metabolizer

Variants: A list of variants identified in this individual by whole exome sequencing is available upon request. Raw sequencing data is also available upon request.

Note: Variants are evaluated by their reported frequency.[1-5] Variants that have a population frequency greater than expected given the prevalence of the disease in the general population are considered to be benign variants. Silent variants are not reported unless known to be pathogenic or other evidence suggests potential disruption of splicing.

These interpretations may change over time as more information about the exome and this individual's clinical phenotype becomes available. Only variants in genes associated with the phenotype observed in this individual, or thought to be clinically relevant for the proband, are reported here. These include pathogenic or likely pathogenic variants in genes described in the ACMG Recommendations for Reporting of Incidental Findings in Exome and Genome Sequencing, when requested by the patient.[6] These results must be interpreted in the context of this individual's clinical and biochemical profile. Genetic counseling is recommended.

METHODS AND LIMITATIONS

Exome Data Quality Statistics:

Exome coverage	92.9%
Coding regions covered	>98%
No. of variants identified and analyzed	27,664
No. of exons evaluated from genes associated with disease	62,668
No. of genes associated with disease with 100% coverage	3048
Low coverage exons (<8X coverage)	0.8% (list available upon request)

Patient Name: Accession Number: Cross Reference:

Medical Exome Sequencing:

The Medical Exome is performed on genomic DNA using the Agilent V5PlusV2 targeted sequence capture method to enrich for the exome. These targeted regions are then sequenced using the Illumina NovaSeq sequencing system with 100 basepair (bp) paired-end reads at a mean coverage of 100X in the target region. This sequencing provides >97% coverage of the 22,000 genes in the exome at >20x. This includes 100% coverage (>20X) of all exons of 3000 disease-associated genes. The target region includes the exon and 10bp of flanking intronic sequence. The DNA sequence is mapped to and analyzed in comparison with the published human genome build GRCh Build 37 (hg19) reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes are assessed for the depth of coverage and data quality threshold values. Variants with 8X or more coverage are analyzed. PKIG has developed an Exome bioinformatics analysis pipeline to compare sequence changes in the individual being tested to the reference sequence. High-quality single nucleotide variants (SNVs), which pass PKIG's quality filters are not confirmed by Sanger sequencing. Reportable SNVs that do not pass the quality filters are confirmed using bidirectional Sanger sequence analysis. Possible diagnostic errors include sample mix-ups, genetic variants that interfere with analysis, and other sources. Allele Fraction denoted as Reference allele/Alternate allele.

Reference Sequences for Genes Reported: *AP3B2* (NM_004644.4)

References:

1. NCBI dbSNP: www.ncbi.nih.gov/dbSNP
2. Exome Variant Server: evs.gs.washington.edu/EVS/EVS
3. 1000 Genomes: browser.1000genomes.org/
4. Exome Aggregation Consortium (ExAC): exac.broadinst28825itute.org/
5. genome Aggregation Database (gnomAD): gnomad.broadinstitute.org/
6. Kalia et al. Genet Med. 2017 Feb;19(2):249-255. (PMID: 27854360)

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Pursuant to the requirements of CLIA ’88, this test was developed and its performance validated by PerkinElmer Genetics. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.