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Julio C. Delgado, MD, MS, Director of Laboratories



Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

## Patient: Patient, Example

| | |
|---|---|
| **DOB** | 5/11/1989 |
| **Gender:** | Female |
| **Patient Identifiers:** | 01234567890ABCD, 012345 |
| **Visit Number (FIN):** | 01234567890ABCD |
| **Collection Date:** | 00/00/0000 00:00 |

## Childhood-Onset Epilepsy Panel, Sequencing and Deletion/Duplication

ARUP test code 2007545

Ordering Physician Name

TEST, TEST

Performed by: GeneDx
207 Perry Parkway
Gaithersburg, MD 20877

Anne Maddalena, Ph.D.,FACMG,

Ordering Physician Phone Number

8002422787

Performed by: GeneDx
207 Perry Parkway
Gaithersburg, MD 20877

Anne Maddalena, Ph.D.,FACMG,

Childhood-Onset Epilepsy Panel

**POSITIVE** *

Date Test(s) Started: 12/11/2018 17:19:00
Test(s) Requested: Childhood Epilepsy Panel / Sequencing and Deletion/Duplication Analysis
Test Indications: Not Provided.
Result: POSITIVE

| Gene | Coding DNA | Variant | Zygosity | Classification |
|---|---|---|---|---|
| CACNA1A | c.4177 G>A | p.Val1393Met (V1393M) | Heterozygous | Pathogenic Variant |

UNCERTAIN CLINICAL SIGNIFICANCE

| Gene | Coding DNA | Variant | Zygosity | Classification |
|---|---|---|---|---|
| POLG | c.2369 G>A | p.Arg790His (R790H) | Heterozygous | Variant of Uncertain Significance |

No other reportable variants were detected by sequencing and deletion/duplication analysis of the genes included on this panel. See the attached table for a list of genes included in the panel.
Interpretation: This individual is heterozygous for a published pathogenic variant in the CACNA1A gene. This gene is associated with an autosomal dominant disorder. This result is consistent with the diagnosis of a CACNA1Arelated disorder.
This individual is also heterozygous for a single variant of uncertain significance in the POLG gene. No second pathogenic variant was identified by sequencing and deletion/duplication analysis of POLG. Pathogenic variants in the POLG gene causing infantile or childhood-onset symptoms typically have an

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autosomal recessive inheritance pattern; therefore, the clinical significance of a single POLG variant is uncertain.
CACNA1A Summary: The CACNA1A gene encodes the alpha-1a subunit of the P/Q-type calcium channel Cav2.1, which is the vital voltage sensor that modulates channel activity and has roles in synaptic transmission, gene expression, and other intracellular processes (Pietrobon, 2010; Pietrobon, 2013). Pathogenic variants in CACNA1A cause the autosomal dominant disorders episodic ataxia type 2 (EA2), familial hemiplegic migraine (FHM), and spinocerebellar ataxia type 6 (SCA6) (Terwindt et al, 1998; Pietrobon, 2010; Pietrobon, 2013). Individuals with EA2 typically present with cerebellar symptoms such as ataxia or nystagmus, but the initial symptoms may also include epileptic encephalopathy, generalized or febrile seizures, developmental delay, intellectual disability, or autism spectrum disorders (Damaj et al., 2015). De novo CACNA1A variants have been reported in association with more severe presentations including cerebellar atrophy in childhood, Lennox-Gastaut syndrome, and early-onset epileptic encephalopathy (Ohba et al., 2013; Allen et al., 2013; Epi4K Consortium, 2016). Low-level mosaicism has been reported in an unaffected parent (Epi4k Consortium, 2016). CACNA1A-related disorders demonstrate significant clinical variability and reduced penetrance (Pietrobon, 2013).
CACNA1A p.V1393M: p.Val1393Met (V1393M) (GTG>ATG): c.4177 G>A in exon 26 of the CACNA1A gene (NM_001127221.1)
The V1393M missense variant in the CACNA1A gene has been reported previously as a de novo variant in an individual who also has a de novo variant in another gene; however, additional information regarding the phenotype was not provided (Posey et al., 2017). It has been previously identified as a confirmed de novo variant in other patients with epilepsy undergoing testing at GeneDx. The V1393M variant is not observed in large population cohorts (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; Exome Variant Server). It is a conservative amino acid substitution that occurs at a conserved position predicted to be within transmembrane segment S5 in the third homologous domain of the protein. In silico analysis predicts this variant is probably damaging to the protein structure/function. Therefore, the V1393M variant is considered to be a pathogenic variant, and its presence is consistent with the diagnosis of a CACNA1A-related disorder in this individual.
POLG Summary: Pathogenic variants in the POLG gene cause mitochondrial DNA (mtDNA) depletion and mitochondrial dysfunction resulting in an overlapping spectrum of mitochondrial diseases. Classic POLG-related disorders include progressive external ophthalmoplegia (PEO), sensory ataxic neuropathy dysarthria and ophthalmoparesis (SANDO), Alpers syndrome, and mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE). However, the spectrum of clinical presentations is broad, and many patients do not fit the diagnostic criteria for a specific defined syndrome (Chinnery and Zeviani, 2008). Symptoms range from early-onset intractable epilepsy with severe encephalopathy and liver failure to late-onset isolated external ophthalmoplegia (Cohen et al., 2014; Chinnery and Zeviani, 2008). Generalized and focal seizures, status epilepticus, and epilepsia partialis continua are common in individuals with pathogenic variants in POLG, and valproic acid and sodium divalproate should be avoided since they may precipitate and/or accelerate liver disease (Cohen et al., 2014; Milone and Massie, 2010). Other clinical features may include ataxia, myopathy, muscle pain, cardiomyopathy, cardiac conduction defects, depression, hearing loss, diffuse degeneration of cerebral gray matter, hepatic cirrhosis, diffuse leukoencephalopathy, hypotonia, Parkinsonism, and premature ovarian failure (Cohen et al., 2014; Horvath et al., 2006). POLG-related disorders can be inherited in an autosomal recessive or an autosomal dominant manner and may lead to different clinical presentations, even within the same family (Cohen et al., 2014). POLG-related disorders associated with autosomal dominant inheritance typically result in adult-onset symptoms that include PEO, while autosomal recessive inheritance is associated with childhood-onset symptoms. Heterozygous

carriers of autosomal recessive pathogenic variants are typically asymptomatic, although some carriers have been reported to develop clinical manifestations of a POLG disorder (Cohen et al., 2014).
POLG p.R790H: p.Arg790His (R790H) (CGT>CAT): c.2369 G>A in exon 14 of the POLG gene (NM_002693.2)
A variant of uncertain significance has been identified in the POLG gene. The R790H variant has been reported previously in a patient with Alper's syndrome in whom a second POLG pathogenic variant was not identified (Tang et al., 2011). The R790H variant is observed in 6/16508 (0.04%) alleles from individuals of South Asian background, including one homozygous individual (Lek et al., 2016; 1000 Genomes Consortium et al., 2015; Exome Variant Server). The R790H variant is a conservative amino acid substitution, which is not likely to impact secondary protein structure as these residues share similar properties. However, this substitution occurs at a position that is conserved in mammals, and in silico analysis predicts this variant is probably damaging to the protein structure/function. Therefore, based on the currently available information, it is unclear whether this variant is a pathogenic variant or a rare benign variant.
POLG-related disorders can be inherited in an autosomal recessive or an autosomal dominant manner, although POLG-related disorders causing epilepsy typically have an autosomal recessive inheritance pattern (Cohen et al., 2010). A second pathogenic variant, as expected for a recessive disorder, was not detected by sequencing or deletion/duplication analysis of POLG; however, the possibility that this patient harbors a second POLG pathogenic variant that is undetectable by this test cannot be excluded. The finding of a single variant of uncertain clinical significance makes the molecular diagnosis inconclusive, and clinical findings should also be considered in the diagnosis of this patient.
Recommendation: 1. Targeted testing for the V1393M pathogenic variant in the CACNA1A gene is available to the parents of this individual for an additional charge to determine if the pathogenic variant was inherited or arose de novo. If desired, molecular prenatal diagnosis is available to at-risk family members to address the recurrence risk.
2. Genetic counseling is recommended to discuss the implications of this test report, specifically including the risk of recurrence for this family, clinical variability associated with CACNA1A pathogenic variants, and testing options for other at-risk family members.
Resources: GenomeConnect is an NIH initiative created to enable individuals and families with the same genetic variant or medical history to connect and share de-identified information. If you are interested in participating, please visit www.genomeconnect.org.
Methods: Genomic DNA from the submitted specimen was enriched for the complete coding regions and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). The enriched targets were simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads were assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data were analyzed to identify sequence variants and most deletions and duplications involving coding exons For the CHRNA7 and MAGI2 gene(s), deletion/duplication analysis, but not sequencing was performed since only large deletions have been reported in these genes. For the FOXG1 and SLC6A8 gene(s), sequencing but not deletion/duplication analysis, was performed. Alternative sequencing or copy number detection methods were used to analyze regions with inadequate sequence or copy number data by Next generation sequencing (NGS). Reported clinically significant variants were confirmed by an appropriate method. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines,

respectively. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.
Report electronically signed by: Dianalee McKnight PhD, FACMG
References: Pietrobon (2010) Pflugers Archiv: European Journal Of Physiology 460 (2): 375-93 (PMID: 20204399); Pietrobon (2013) Biochimica Et Biophysica Acta 1828 (7): 1655-65 (PMID: 23165010); Terwindt et al. (1998) European Journal Of Human Genetics: Ejhg 6 (4): 297-307 (PMID: 9781035); Damaj et al. (2015) Eur. J. Hum. Genet. 23 (11): 1505-12 (PMID: 25735478); Ohba et al. (2013) Neurogenetics 14 (3-4): 225-32 (PMID: 24091540); Allen et al., (2013) Nature 501 (7466): 217-221 (PMID: 23934111); Epi4k Consortium (2016) Am. J. Hum. Genet. 99 (2): 287-98 (PMID: 27476654); Posey et al. (2017) N. Engl. J. Med. 376 (1): 21-31 (PMID: 27959697); Lek et al. (2016) Nature 536 (7616): 285-91 (PMID: 27535533); 1000 Genomes Project Consortium, (2015) Nature 526 (7571): 68-74 (PMID: 26432245); Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http: //evs.gs.washington.edu/EVS/) [Accessed 2017]; Stenson et al (2014), The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet 133: 1-9 (PMID: 24077912); Chinnery et al. (2008) Neuromuscular Disorders : Nmd 18 (3): 259-67 (PMID: 18160290); Cohen BH, Chinnery PF, Copeland WC. POLG-Related Disorders. 2010 Mar 16 [Updated 2014 Dec 18]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. Available from: https: //www.ncbi.nlm.nih.gov/books/NBK26471/; Milone and Massie (2010) The Neurologist 16 (2): 84-91 (PMID: 20220442); Horvath et al. (2006) Brain : A Journal Of Neurology 129 (Pt 7): 1674-84 (PMID: 16621917); Tang et al. (2011) Journal Of Medical Genetics 48 (10): 669-81 (PMID: 21880868).
Limitations: Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 500bp in size, or insertions of 10bp to 500 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negative results may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.
Disclaimer: This test was performed at GeneDx, 207 Perry Parkway, Gaithersburg, MD 20877. Laboratory data interpretation was performed at either GeneDx or BioReference Laboratories, 481 Edward H. Ross Drive, Elmwood Park, NJ 07407.
Performed by: GeneDx
207 Perry Parkway
Gaithersburg, MD 20877

Anne Maddalena, Ph.D.,FACMG,

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|---|---|
| EER Childhood-Onset Epilepsy Panel | See Note<br>Access ARUP Enhanced Report using either link below:<br>-Direct access:<br>-Enter Username, Password: https://erpt.aruplab.com<br>Username:<br>Password: |

VERIFIED/REPORTED DATES

| Procedure | Accession | Collected | Received | Verified/Reported |
|---|---|---|---|---|
| Ordering Physician Name | 18-345-105223 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |
| Ordering Physician Phone Number | 18-345-105223 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |
| Childhood-Onset Epilepsy Panel | 18-345-105223 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |
| EER Childhood-Onset Epilepsy Panel | 18-345-105223 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |

END OF CHART

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