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

Physician: Doctor, Example

## Patient: Patient, Example

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

## Fatty Acid Oxidation Disorders Panel, Sequencing

ARUP test code 3001851

| | |
|---|---|
| Fatty Acid Oxidation Disorders Specimen | Whole Blood |

Fatty Acid Oxidation Disorders Interp

Positive

RESULT
Two pathogenic variants were detected in the ACADVL gene.

PATHOGENIC VARIANT
Gene: ACADVL (NM_000492.3)
Nucleic Acid Change: c.779C>T; Heterozygous
Amino Acid Alteration: p.Thr260Met
Inheritance: Autosomal recessive

PATHOGENIC VARIANT
Gene: ACADVL (NM_000492.3)
Nucleic Acid Change: c.1349G>A; Heterozygous
Amino Acid Alteration: p.Arg450His
Inheritance: Autosomal recessive

INTERPRETATION
Two pathogenic variants, c.779C>T; p.Thr260Met, and c.1349G>A; p.Arg450His, were detected in the ACADVL gene by massively parallel sequencing. This individual is predicted to be affected with very long-chain acyl CoA (VLCAD) deficiency; clinical manifestations are highly variable. Although the identified variants have not previously been reported to occur on the same chromosome, parental testing could confirm they are located on opposite chromosomes.

Please refer to the background information included in this report for a list of the genes analyzed, methodology and limitations of this test.

Evidence for variant classifications:
The ACADVL c.779C>T; p.Thr260Met variant (rs113994168), also known as Thr220Met, has been reported in multiple individuals diagnosed with very long chain acyl-CoA dehydrogenase deficiency, often found in-trans with another pathogenic variant (Andresen 1996, Andresen 1999, Gobin-Limballe 2010, Laforet 2009, Mathur 1999). Additionally, functional characterization of the variant protein in patient fibroblasts demonstrate significantly decreased enzymatic activity (Andresen 1996, Andresen 1999, Hoffman 2012, Laforet 2009). This variant is reported in ClinVar (Variation ID: 21024) and observed in the general population at a low overall frequency of 0.002% (5/246264 alleles) in the Genome Aggregation Database. The threonine at residue 260 is highly conserved, and computational analyses predict that this variant is deleterious (REVEL: 0.875). Based on available information, this variant is

considered pathogenic.

The ACADVL c.1349G>A; p.Arg450His variant (rs118204016), also known as Arg410His for traditional nomenclature, is reported multiple times in the literature in association with VLCAD deficiency and is reported both in the homozygous and compound heterozygous states in affected individuals (Andresen 1999, Fukao 2001, Gobin-Limballe 2010, Kang 2018, Ohashi 2004, Smelt 1998, Zhang 2014). Additionally, functional analyses of the variant protein show decreased expression and enzymatic activity (Fukao 2001, Smelt 1998). This variant is reported in ClinVar (Variation ID: 1634) and found in the general population with a low overall allele frequency of 0.003% (8/277108 alleles) in the Genome Aggregation Database. The arginine at codon 450 is highly conserved, and computational analyses predict that this variant is deleterious (REVEL: 0.920). Based on available information, this variant is considered to be pathogenic.

RECOMMENDATIONS
Genetic consultation is indicated; diagnosis and management should rely on clinical symptoms and biochemical/functional assays. Parental testing may be considered to confirm the chromosomal origin of the identified variants. At-risk family members should be offered testing for the identified pathogenic ACADVL variants (Familial Targeted Sequencing, ARUP test code 3005867). This individual's reproductive partner should be offered genetic testing to determine carrier status.

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations:
NONE

REFERENCES
Andresen B et al. Cloning and characterization of human very-long-chain acyl-CoA dehydrogenase cDNA, chromosomal assignment of the gene and identification in four patients of nine different mutations within the VLCAD gene. Hum Mol Genet. 1996; 5(4):461-72. PMID: 8845838.
Andresen B et al. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. Am J Hum Genet. 1999; 64(2):479-94. PMID: 9973285.
Fukao T et al. Myopathic form of very-long chain acyl-coa dehydrogenase deficiency: evidence for temperature-sensitive mild mutations in both mutant alleles in a Japanese girl. Pediatr Res. 2001 Feb;49(2):227-31. PMID: 11158518.
Gobin-Limballe S et al. Compared effects of missense mutations in Very-Long-Chain Acyl-CoA Dehydrogenase deficiency: Combined analysis by structural, functional and pharmacological approaches. Biochim Biophys Acta. 2010; 1802(5):478-84. PMID: 20060901.
Hoffman L et al. VLCAD enzyme activity determinations in newborns identified by screening: a valuable tool for risk assessment. J Inherit Metab Dis. 2012; 35(2):269-77. PMID: 21932095.
Kang E et al. Clinical and genetic characteristics of patients with fatty acid oxidation disorders identified by newborn screening. BMC Pediatr. 2018 Mar 8;18(1):103. PMID: 29519241.
Laforet P et al. Diagnostic assessment and long-term follow-up of 13 patients with Very Long-Chain Acyl-Coenzyme A dehydrogenase (VLCAD) deficiency. Neuromuscul Disord. 2009; 19(5):324-9. PMID: 19327992.
Mathur A et al. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. Circulation. 1999; 99(10):1337-43. PMID: 10077518.
Ohashi Y et al. A new diagnostic test for VLCAD deficiency using immunohistochemistry. Neurology. 2004 Jun 22;62(12):2209-13.

PMID: 15210884.
Smelt AH et al. Very long chain acyl-coenzyme A dehydrogenase deficiency with adult onset. Ann Neurol. 1998 Apr;43(4):540-4. PMID: 9546340.
Zhang RN et al. Clinical features and mutations in seven Chinese patients with very long chain acyl-CoA dehydrogenase deficiency. World J Pediatr. 2014 May;10(2):119-25. PMID: 24801231.

This result has been reviewed and approved by [redacted]

H=High, L=Low, *=Abnormal, C=Critical

BACKGROUND INFORMATION: Fatty Acid Oxidation Disorders Panel, Sequencing

CHARACTERISTICS: Fatty acid oxidation disorders can present with hypoketotic hypoglycemia, lethargy, episodic emesis, seizures, dicarboxylic aciduria, hepatomegaly, hepatic failure, cardiomyopathy, Reye-like symptoms, skeletal myopathy, myalgia, exercise intolerance, coma, and sudden death. Clinical presentation varies in severity and age of onset.

INCIDENCE: Approximately 1 in 5,000 to 1 in 10,000 births.

CAUSE: Pathogenic germline variants in genes associated with fatty acid oxidation disorders.

INHERITANCE: Mostly autosomal recessive; rarely autosomal dominant or X-linked.

CLINICAL SENSITIVITY: May be as high as 96 percent.

GENES TESTED: ACAD9, ACADM, ACADS, ACADVL, ACAT1, CPT1A, CPT2, ECHS1, ETFA, ETFB, ETFDH, FLAD1, HADH, HADHA, HADHB, HMGCL, HMGCS2, HSD17B10, LPIN1*, MLYCD, SLC22A5, SLC25A20, SLC52A1, SLC52A2, SLC52A3
*One or more exons are not covered by sequencing for the indicated gene; see limitations section below.

METHODOLOGY: Capture of all coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed to fill in regions of low coverage and confirm reported variants as necessary. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity of this test is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions from 1-10 base pairs in size. Variants greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced.

LIMITATIONS: A negative result does not exclude a diagnosis of a fatty acid oxidation disorder. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. Regulatory region variants and deep intronic variants will not be identified.
Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay may not detect low-level mosaic or somatic variants associated with disease. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding transcripts were not analyzed.

The following regions are not sequenced due to technical limitations of the assay:
LPIN1(NM_001349200) exon 13
LPIN1(NM_001349201) exon 12

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

| Procedure | Accession | Collected | Received | Verified/Reported |
|---|---|---|---|---|
| Fatty Acid Oxidation Disorders Specimen | 22-301-104620 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |
| Fatty Acid Oxidation Disorders Interp | 22-301-104620 | 00/00/0000 00:00 | 00/00/0000 00:00 | 00/00/0000 00:00 |

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical