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

Physician: Doctor, Example

Patient: Patient, Example

DOB	12/31/1752
Sex:	Male
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	01/01/2017 12:34

Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (ACADVL) Sequencing and Deletion/Duplication

ARUP test code 3004419

VLCAD Specimen	Whole Blood
VLCAD Interp	Positive

RESULT
Two pathogenic variants on opposite chromosomes were detected in the ACADVL gene.

PATHOGENIC VARIANT
Gene: ACADVL (NM_000018.4)
Nucleic Acid Change: c.848T>C; heterozygous
Amino Acid Alteration: p.Val283Ala
Inheritance: Autosomal recessive

PATHOGENIC VARIANT
Gene: ACADVL (NM_000018.4)
Nucleic Acid Change: c.869dupG; heterozygous
Amino Acid Alteration: p.Ile291HisfsTer7
Inheritance: Autosomal recessive

INTERPRETATION
Two pathogenic variants, c.848T>C; p.Val283Ala and c.869dup; p.Ile291HisfsTer7 were detected in the ACADVL gene by massively parallel sequencing. This individual is predicted to be affected with very long-chain acyl-CoA dehydrogenase (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. The presence of two pathogenic variants, one in each copy of the ACADVL gene, is causative for VLCAD deficiency.

No additional pathogenic variants were identified in ACADVL by massively parallel sequencing or deletion/duplication analysis. Please refer to the background information included in this report for limitations of this test.

Evidence for variant classifications:
The ACADVL c.848T>C; p.Val283Ala variant (rs113994167), also known as Val243Ala, is reported by the literature in individuals affected with VLCAD deficiency (Andresen, 1996) and accounts for 20% of all pathogenic alleles in VLCAD individuals identified by newborn screening (Leslie, 2009). It is reported as pathogenic by multiple laboratories in ClinVar (Variation ID: 21025) and functional studies demonstrate that this variant reduces the amount of ACADVL protein produced, which causes a decrease in enzymatic activity (Andresen, 1999; Goetzman, 2007; Hoffmann, 2012). Based on available information, this variant is

considered to be pathogenic.

The ACADVL c.869dupG; p.Ile291HisfsTer7 variant (rs886044671) has been reported in two patients affected with VLCAD deficiency (Miller, 2015). This variant is also reported in ClinVar (Variation ID: 291163) and is only observed on three allele in the Genome Aggregation Database, indicating it is not a common polymorphism. This variant causes a frameshift by inserting a single nucleotide and is predicted to result in a truncated protein or mRNA subject to nonsense-mediated decay. Based on available information, this variant is considered to be pathogenic.

RECOMMENDATIONS
Genetic and dietary consultations are strongly recommended, including a discussion of medical screening and management. 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 variants (Familial Mutation, Targeted Sequencing; ARUP test code 2001961). This individual's future reproductive partner should be offered ACADVL 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.

REFERENCES
Andresen BS, 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 BS, et al. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. Am J Hum Genet. Feb;64(2):479-94. PMID: 9973285

Goetzman ES, et al. Expression and characterization of mutations in human very long-chain acyl-CoA dehydrogenase using a prokaryotic system. Mol Genet Metab. 2007;91(2):138-47. PMID: 17374501

Hoffmann 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

Leslie ND, et al. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews (Internet). University of Washington, Seattle; 1993-2017. May 2009 (Updated Sep 2014). Available from: https://www.ncbi.nlm.nih.gov/books/NBK6816/.

Miller MJ, et al. Recurrent ACADVL molecular findings in individuals with a positive newborn screen for very long chain acyl-coA dehydrogenase (VLCAD) deficiency in the United States. Mol Genet Metab. 2015;116(3):139-45. PMID: 26385305

BACKGROUND INFORMATION: Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (ACADVL) Sequencing and Deletion/Duplication

CHARACTERISTICS: VLCAD deficiency is a long-chain fatty acid oxidation disorder associated with three phenotypes that vary in age of onset and severity. Clinical symptoms may include cardiomyopathy, pericardial effusion, hypotonia, hepatomegaly, hypoketotic hypoglycemia, skeletal myopathy, exercise intolerance, and rhabdomyolysis induced by exercise.

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

EPIDEMIOLOGY: Approximately 1 in 40,000

CAUSE: Pathogenic germline variants in the ACADVL gene

INHERITANCE: Autosomal recessive

CLINICAL SENSITIVITY: 95-97 percent

GENE TESTED: ACADVL (NM_000018)

METHODOLOGY: Probe hybridization-based capture of all coding exons and exon-intron junctions of the ACADVL gene, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and confirm reported variants. A proprietary bioinformatic algorithm was used to detect large (single exon-level or larger) deletions or duplications in the indicated genes. Large deletions/duplications were confirmed using an orthogonal exon-level microarray. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY/SPECIFICITY: The analytical sensitivity is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions (indels) from 1-10 base pairs in size. Indels greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced. Deletions of 2 exons or larger are detected with sensitivity greater than 97 percent; single exon deletions are detected with 62 percent sensitivity. Duplications of 3 exons or larger are detected at greater than 83 percent sensitivity. Specificity is greater than 99.9 percent for all variant classes.

LIMITATIONS: A negative result does not exclude a diagnosis of VLCAD deficiency. This test only detects variants within the coding regions and intron-exon boundaries of the ACADVL gene. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. Regulatory region variants and deep intronic variants will not be identified. Precise breakpoints for large deletions or duplications are not determined in this assay and single exon deletions/duplications may not be detected based on the breakpoints of the rearrangement. The actual breakpoints for the deletion or duplication may extend beyond or be within the exon(s) reported. This test is not intended to detect duplications of 2 or fewer exons in size, though these may be identified. Single exon deletions are reported but called at a lower sensitivity. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, repetitive, or homologous regions. This test is not intended to detect low-level mosaic or somatic variants, gene conversion events, complex inversions, translocations, mitochondrial DNA (mtDNA) mutations, or repeat expansions. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Non-coding transcripts were not analyzed.

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.

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

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
VLCAD Specimen	22-054-104358	2/23/2022 11:02:00 AM	2/23/2022 11:03:05 AM	2/23/2022 12:19:00 PM
VLCAD Interp	22-054-104358	2/23/2022 11:02:00 AM	2/23/2022 11:03:05 AM	2/23/2022 12:19:00 PM

END OF CHART

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