Molecular Genetics and Metabolism 116 (2015) 139–145

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Recurrent *ACADVL* molecular findings in individuals with a positive newborn screen for very long chain acyl-coA dehydrogenase (VLCAD) deficiency in the United States

Marcus J. Miller [a], Lindsay C. Burrage [a], James B. Gibson [b], Meghan E. Strenk [c], Edward J. Lose [d], David P. Bick [e], Sarah H. Elsea [a], V. Reid Sutton [a], Qin Sun [a], Brett H. Graham [a], William J. Craigen [a], Victor Wei Zhang [a], Lee-Jun C. Wong [a,*]

[a] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77021, United States
[b] Section of Clinical Genetics and Metabolism, 'Specially for Children, Austin, TX 78723, United States
[c] Children's Mercy Hospital, Kansas City, MO 64108, United States
[d] Department of Genetics, University of Alabama Birmingham, Birmingham, AL 35294, United States
[e] Section of Genetics, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, United States

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form 26 August 2015
Accepted 26 August 2015
Available online 2 September 2015

Keywords:
VLCAD
Inborn error of metabolism
Fatty acid oxidation
Newborn screening
ACADVL
VLCADD
NBS
V283A
V243A

ABSTRACT

Very long chain acyl-coA dehydrogenase deficiency (VLCADD) is an autosomal recessive inborn error of fatty acid oxidation detected by newborn screening (NBS). Follow-up molecular analyses are often required to clarify VLCADD-suggestive NBS results, but to date the outcome of these studies are not well described for the general screen-positive population. In the following study, we report the molecular findings for 693 unrelated patients that sequentially received Sanger sequence analysis of *ACADVL* as a result of a positive NBS for VLCADD. Highlighting the variable molecular underpinnings of this disorder, we identified 94 different pathogenic *ACADVL* variants (40 novel), as well as 134 variants of unknown clinical significance (VUSs). Evidence for the pathogenicity of a subset of recurrent VUSs was provided using multiple in silico analyses. Surprisingly, the most frequent finding in our cohort was carrier status, 57% all individuals had a single pathogenic variant or VUS. This result was further supported by follow-up array and/or acylcarnitine analysis that failed to provide evidence of a second pathogenic allele. Notably, exon-targeted array analysis of 131 individuals screen positive for VLCADD failed to identify copy number changes in ACADVL thus suggesting this test has a low yield in the setting of NBS follow-up. While no genotype was common, the c.848T>C (p.V283A) pathogenic variant was clearly the most frequent; at least one copy was found in ~10% of all individuals with a positive NBS. Clinical and biochemical data for seven unrelated patients homozygous for the p.V283A allele suggests that it results in a mild phenotype that responds well to standard treatment, but hypoglycemia can occur. Collectively, our data illustrate the molecular heterogeneity of VLCADD and provide novel insight into the outcomes of NBS for this disorder.

1. Introduction

ACADVL encodes for very long chain acyl CoA dehydrogenase (VLCAD), a mitochondrial enzyme that catalyzes the initial rate-limiting step of β-oxidation of long chain fatty acids [1,2]. Patients with autosomal recessive VLCAD deficiency (VLCADD; OMIM #201475) accumulate high plasma levels of long chain acylcarnitine conjugates, especially the tetradecenoyl (C14:1) acylcarnitine, and can exhibit a wide range of clinical outcomes including (i) a severe neonatal onset disease associated with cardiomyopathy and a high mortality rate, (ii) an infantile onset form usually presenting with non-ketotic hypoglycemia and hepatic dysfunction, and (iii) an adult onset myopathic form characterized by exercise induced muscle weakness/pain and rhadomyolysis [3–5]. *ACADVL* null alleles are associated with a severe early onset phenotype whereas missense or in frame deletion alleles are often, but not always, associated with a milder later onset form of VLCADD [6].

VLCADD is detected by newborn screening (NBS) laboratories in the United States on the basis of blood spot acylcarnitine levels (typically C14:1 acylcarnitine or a ratio involving this compound) [7,8]. Large multicenter US NBS studies predicated on standard tandem mass spectrometry based approaches to acylcarnitine analysis have reported positive predictive values for VLCADD of 20–30% [8,9]. Positive NBS results can occur for many reasons including (i) truly affected early onset forms of the disorder requiring immediate clinical attention, (ii) later onset forms of the disorder that may not manifest symptoms until adulthood, (iii) unaffected carrier status [9,10], (iv) maternal effect [11], (v) false positives, possibly resulting from fasting, diet, or other factors not

* Corresponding author.
E-mail address: ljwong@bcm.edu (L.-J.C. Wong).

http://dx.doi.org/10.1016/j.ymgme.2015.08.011
1096-7192/

related to VLCADD. There have also been multiple reports of confirmed affected individuals that screen positive for VLCADD but then appear asymptomatic by follow-up quantitative plasma acylcarnitine analysis [10,12–14].

Given this complexity, diagnostic decisions can be challenging and advisory panels have advocated for additional testing to clarify positive NBS results such as enzymatic studies and/or molecular analysis of *ACADVL* (*https://www.acmg.net/StaticContent/ACT/C14.pdf*; [15]). Numerous groups have published case reports describing follow-up testing results from clinically interesting screen positive individuals, but there are limited data describing the specific molecular findings in a large unselected cohort of individuals screen positive for VLCADD [5,9,11,16].

We describe our experiences as a reference laboratory that has completed molecular follow-up analysis for hundreds of VLCADD NBS screen positive individuals from testing facilities across the US. Results from this analysis provide a novel perspective on NBS outcomes that can be used to further advance an evidence-based approach to NBS follow-up for VLCADD.

2. Materials and methods

2.1. Specimen collection

The 1080 specimens reported in this study represent all patients for whom Sanger sequence analysis of *ACADVL* was performed at the Baylor College of Medicine (Houston, Texas) from 06/2007 to 12/2014 (Fig. 1). Samples were collected as whole blood in EDTA containing tubes and shipped at ambient temperature to the Baylor College of Medicine. Approximately 50% of samples came from the state of Texas, with nearly all of the remainder collected in 1 of 40 different states; the following were represented by 20 or more unique patients: PA, CA, IA, CT, WI, MA, MO, AL, and OH. Each specimen was submitted with a requisition form intended to gather additional patient information including ethnicity, indication for study, and relevant family history. All procedures were approved by the Baylor College of Medicine Institutional Review Board with a waiver of informed consent.

2.2. Sanger sequencing

DNA was extracted from EDTA preserved whole blood using a commercially available DNA isolation kit (Gentra Systems Inc., Minneapolis, MN) according to the manufacturer's protocol. The coding regions of the *ACADVL* gene (NM_000018.2), as well as proximal intronic sequences, were PCR amplified and then sequenced in the forward and reverse directions using automated fluorescent dideoxy sequencing methods. Nucleotide 1 corresponds to the A of the start codon ATG (NM_000018.2). Variants detected in exons and in introns within up to 20 bp of the exon/intron boundaries were studied.

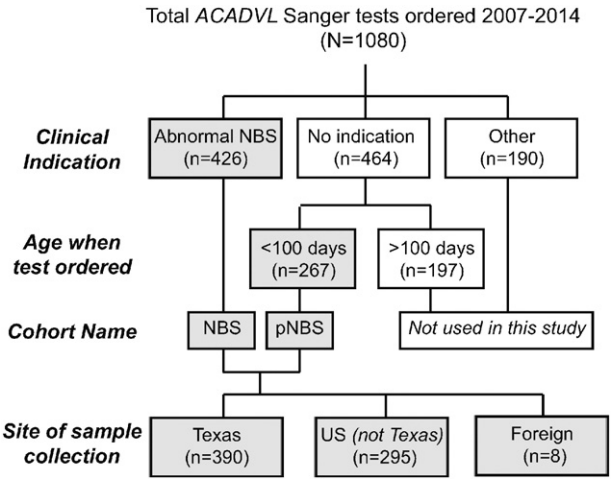

Fig. 1. The flowchart describes the samples sent to our laboratory for Sanger sequence analysis of *ACADVL* over the course of seven years. Gray boxes indicate samples analyzed in this study. pNBS = presumptive newborn screen positive.

2.3. Array CGH

A custom-designed oligonucleotide 180 K exon-targeted CGH array (MitoMet v3) was used to assess for copy number changes involving *ACADVL*. The average probe density was greater than four probes per exon, with 1-kb spacing in the intronic regions. The targeted region of the aCGH contained probes for the coding exons and 50 bp of the flanking intronic regions. The criteria for a potential CNV call in the aCGH are at least two contiguous probes with a $\log_2$ ratio >0.3 for duplication and <−0.3 for deletion in regions of interest. Findings were reported according to human genome build hg19.

2.4. Biochemical analyses

Plasma acylcarnitine analysis described in Fig. 4 was completed as described previously [17]. NBS, enzymatic testing, and quantitative acylcarnitine analysis described in Table 3 and Fig. 5 were completed by outside CLIA certified clinical testing laboratories on a fee for service basis. Enzymatic testing was completed using peripheral leukocytes or fibroblasts.

2.5. Data analysis

Classification of variants was completed by a team of Fellow of the American College of Medical Genetics credentialed molecular geneticists following ACMG standards and guidelines [18]. Novel pathogenic variants were classified as base pair changes that resulted in a premature stop codon or that occurred within the canonical splice donor or acceptor region of *ACADVL* but that were not listed in the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php). Variant enrichment analysis was completed using a Fisher's exact test and the allele frequencies reported in the ExAC Database. Expected allele numbers were calculated by multiplying the ExAC allele frequency by the number of alleles in our cohort of NBS and likely NBS patients (n = 1386). To determine if a variant had been previously detected, we searched the Baylor College of Medicine exome database (accessed 11/14/2014), the ExAC database (accessed 2/17/2015), and the exome variant server (accessed 2/17/2015) with each database comprised of exome data from ~5000, 60,542, and ~6500 individuals, respectively, (Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) and Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/). Normal reference ranges for quantitative acylcarnitine analysis were calculated using data from all patients receiving testing in our biochemical genetics laboratory over the course of 10 years. Samples used for reference range calculations were collected within the first 100 days of life from patients that did not have a biochemical genetics diagnosis (n = 2438). The upper limit of normal was defined as the 95th percentile of this reference population.

2.6. In silico predictions

Variant pathogenicity predictions were completed using PolyPhen-2 (version2.2.2) and SIFT (Ensembl 63) [19,20]. The structure of human VLCAD was examined using a previously generated 1.45 Å resolution crystal structure (PDB# 2UXW). PyMOL (DeLano Scientific, CA) was used to visualize the protein structure and model amino acid changes in the three-dimensional structure.