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Outcomes and genotype-phenotype correlations in 52 individuals with VLCAD deficiency diagnosed by NBS and enrolled in the IBEM-IS database

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ABSTRACT

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency can present at various ages from the neonatal period to adulthood, and poses the greatest risk of complications during intercurrent illness or after prolonged fasting. Early diagnosis, treatment, and surveillance can reduce mortality; hence, the disorder is included in the newborn Recommended Uniform Screening Panel (RUSP) in the United States. The Inborn Errors of Metabolism Information System (IBEM-IS) was established in 2007 to collect longitudinal information on individuals with inborn errors of metabolism included in newborn screening (NBS) programs, including VLCAD deficiency. We retrospectively analyzed early outcomes for individuals who were diagnosed with VLCAD deficiency by NBS and describe initial presentations, diagnosis, clinical outcomes and treatment in a cohort of 52 individuals ages 1–18 years. Maternal prenatal symptoms were not reported, and most newborns remained asymptomatic. Cardiomyopathy was uncommon in the cohort, diagnosed in 2/52 cases. Elevations in creatine kinase were a common finding, and usually first occurred during the toddler period (1–3 years of age). Diagnostic evaluations required several testing modalities, most commonly plasma acylcarnitine profiles and molecular testing. Functional testing, including fibroblast acylcarnitine profiling and white blood cell or fibroblast enzyme assay, is a useful diagnostic adjunct if uncharacterized mutations are identified.

Abbreviations: VLCAD, very long chain acyl-CoA dehydrogenase; NBS, newborn screening; MCT, medium chain triglycerides; IBEM-IS, Inborn Errors of Metabolism Information System; RUSP, Recommended Uniform Screening Panel; CK, creatine kinase; HELLP syndrome, hemolysis, elevated liver enzymes, or low platelets syndrome.

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1. Introduction

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is a disorder involving the initial step of fatty acid beta-oxidation in the mitochondrial matrix. The deficient enzyme is encoded by the gene *ACADVL* [1,2]. The prevalence of VLCAD deficiency is estimated at 1:30,000 to 1:100,000 births [3,4]. The disorder can present at various ages from the neonatal period to adulthood, and poses the greatest risk of complications during intercurrent illness or after prolonged fasting. Symptoms can include hypoglycemia, rhabdomyolysis, skeletal muscle weakness, and cardiomyopathy. Multiple genotypic variants have been described in patients with VLCAD deficiency, and some genotype-phenotype correlations have been reported [5–8]. Treatment emphasizes avoidance of fasting and often includes a specialized diet that is low in long chain fats and supplemented with medium chain triglycerides (MCT). Carnitine is sometimes prescribed, but its use is controversial [9]. Additional novel therapeutic agents are currently under investigation [10–14]. Recognizing that morbidity and mortality related to VLCAD deficiency can be reduced with early diagnosis, treatment, and surveillance, this disorder is included in the newborn Recommended Uniform Screening Panel (RUSP) in the United States [15].

While VLCAD deficiency was described over 20 years ago [2], limited information is available regarding long-term outcomes in affected individuals, especially those identified through newborn screening. A recent report of 32 cases indicated that the majority of those identified by NBS were asymptomatic at diagnosis and remained so during seven years of follow up [4]. Those who were diagnosed while symptomatic had complications that included cardiomyopathy, arrhythmias, skeletal myopathy, and hypoglycemia [4]. Significant long-term symptoms have been reported in patients identified both symptomatically and through newborn screening. Improvement in these symptoms with the use of the investigational drug triheptanoin has been reported [10,14], but not with bezafibrate [16].

The Inborn Errors of Metabolism Information System (IBEM-IS) is a robust data collection tool used to collect demographic and longitudinal clinical information from patients with inborn errors of metabolism and their clinicians at metabolic specialty centers in multiple states. In this study, the IBEM-IS was used to retrospectively analyze early outcomes for infants who were diagnosed with VLCAD deficiency following positive NBS. Initial presentations, diagnostic testing, maternal complications, cardiac outcomes and dietary treatment are described in a cohort of 52 individuals who participate in the IBEM-IS. We addressed the following key questions in our data review:

What symptoms do the majority of individuals exhibit at diagnosis or develop later in childhood? The majority of previously published case series for individuals with VLCAD deficiency reports on individuals who are symptomatic. Our cohort presented a unique opportunity to study clinical outcomes in individuals diagnosed early in life through NBS.

Can molecular testing help to resolve pitfalls in diagnosis? We recognize the existence of overlap of biochemical analytes for VLCAD deficiency in affected and unaffected individuals and thus explored the utility of genotyping for diagnostic purposes [17].

Do maternal complications occur during pregnancy? Acute fatty liver in pregnancy and other complications have been described in women carrying fetuses affected with long-chain FAOD [18–21]. We explored whether this was an observed complication for VLCAD deficiency.

Is there variability in the specific treatment modalities used for VLCAD deficiency? Treatment modalities for VLCAD deficiency can be variable and are based on expert opinion [3] but evidence-based treatment protocols are greatly needed. We analyzed treatment approaches for affected patients, including diet, supplements and sick day management.

2. Materials and methods

IBEM-IS subjects were included in this study if they had a diagnosis of VLCAD deficiency and were ascertained by abnormal newborn screening. Eligible cases enrolled between March 2007 and November 2014 were included, and data entered into the IBEM-IS through December 15, 2015 were queried. For each individual meeting these criteria, available data regarding diagnostic evaluation, pregnancy, cardiac evaluations, and dietary management at initial and interval visits were obtained for analysis. "Intake" was defined as the time the child was first entered into the IBEM-IS database. Data reported as having occurred prior to that time were thus reported retrospectively. Serial "interval" data referred to post-intake data from the interval between the prior and most recent clinic evaluations. "Neonatal" symptoms were those reported by the center to occur in the neonatal period. The IBEM-IS data dictionary specifies that the neonatal period spans the first 28 days of life. "Initial evaluation" symptoms were those reported at the first metabolic evaluation after the abnormal newborn screen results were known (and may have occurred in the first month of life or later).

The data set analyzed for this study did not include direct or indirect identifiers or protected health information from any subject in the database. The study was reviewed and granted an exemption by the Institutional Review Board for Clinical Investigations at Duke University. Data were tabulated and analyzed using IBM SPSS Statistics software version 23.0. Analysis of most parameters consisted of frequency tabulation and descriptive statistics of data collected. Values for C14 and C14:1 acylcarnitines from NBS samples were compared using Independent Samples Mann Whitney *U* Test. Conflicting or unclear information was clarified with the originating center via the IBEM-IS Coordinating Center, Michigan Public Health Institute. Genotype information was collated. For variants not previously published in publicly available databases or in the literature, we utilized several open source prediction software programs (Mutation Taster, www.mutationtaster.org; SIFT, http://sift.jcvi.org/) to annotate pathogenicity. Ensembl transcript ID ENST00000356839 served as the reference sequence (accessed December 8, 2015).

Molecular modeling was performed on a Silicon Graphics Fuel workstation (Mountain View, CA) using the Insight II 2000 software package (Dassault Systèmes, BIOVIA Corp – formerly Accelrys Technologies, San Diego, CA) and the published atomic coordinates of recombinant human VLCAD [22]. The disordered region of the C-terminus in the crystal structure was further modeled using the Homology module that is part of the software. Mutated residues were positioned on the molecule as described to assess their possible effect [23,24].

A case was considered "symptomatic" if the subject was reported to show symptoms potentially attributable to VLCAD deficiency at the initial evaluation or later (as described under "Results"). Creatine kinase (CK) levels <250 μM were considered normal in the first 30 days of life; rhabdomyolysis was defined as CK ≥ 1000 μM at any age. Because jaundice is common in neonates, jaundice alone did not qualify a subject as "symptomatic". NBS blood spot C14:1 was the only biochemical marker utilized to compare symptomatic versus asymptomatic cases, as ratios using other acylcarnitines do not appear more discriminatory than C14:1 alone [25].

3. Results

3.1. General characteristics

Eighty patients with a diagnosis of VLCAD deficiency were identified in the IBEM-IS. Of these, 67 were ascertained by NBS and therefore met the inclusion criteria for this study. Nine of the cases ascertained via newborn screening were excluded due to lack of follow up diagnostic and clinical information entered in the IBEM-IS. An additional six cases were excluded after the authors considered NBS, diagnostic and molecular information and agreed that the cases likely represented carrier individuals, all of whom were alive at entry into the study (see below). Thus, 52 patients remained in the study cohort after the above exclusions, including a patient that was diagnosed prenatally. Clinical and biochemical data for these 52 patients were analyzed.

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