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Very long-chain acyl-coenzyme A dehydrogenase deficiency in Chinese patients: Eight case reports, including one case of prenatal diagnosis

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ABSTRACT

Objective: Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) is a rare mitochondrial fatty acid β-oxidation disorder. We aimed to explore the clinical, biochemical, and genetic findings, treatments and outcomes in eight Chinese VLCADD patients.
Methods: Eight patients from six unrelated Chinese families with symptomatic VLCADD were diagnosed in the past 4 years. The clinical features and *ACADVL* gene mutations were analyzed.
Results: One patient underwent newborn screening and has been treated timely, she hardly had any symptoms. The remaining seven patients were found because of edema, diarrhea, coma, liver damage and psychomotor retardation. Seven patients had fatty liver. Five had myopathy. All patients had elevated blood tetradecanoylcarnitine. Nine heterozygous mutations of the *ACADVL* gene were found. Three (c.1102C > T, c.1795G > A and IVS10, +6T > A) were novel. Seven patients completely recovered after treatment. One patient died before diagnosis due to cardiomyopathy. His mother underwent amniocentesis for prenatal diagnosis. The fetus had the same gene mutation of the proband and markedly elevated tetradecanoylcarnitine in amniotic fluid. The boy has been treated after birth and he is healthy now.
Conclusions: Dietary treatment usually leads to good outcomes to VLCADD patients. Amniocytes *ACADVL* mutations and amniotic fluid tetradecanoylcarnitine analysis are useful for the prenatal diagnosis.

1. Introduction

Very long-chain acyl-coenzyme A dehydrogenase (VLCAD; EC: 1.3.99.13) deficiency (MIM #201475; VLCADD) is a rare autosomal recessive metabolic disorder that affects mitochondrial fatty acid β-oxidation, which in turn impacts heart, liver and muscle function [Aoyama et al., 1995]. Fatty acid oxidation defects (FAODs), a group of severe inherited metabolic disorders with significantly heterogeneous phenotypes, are responsible for 5% of all sudden and unexpected deaths in infants [Bennett and Powell, 1994; Yamamoto et al., 2011]. Among FAODs, VLCADD is a common cause of sudden infant deaths. The incidence of VLCADD is estimated to be 1 in 85, 000 newborns for Australia, Germany and the US combined [Lindner et al., 2010].

VLCADD is categorized into three types according to organ-system involvement and age at onset: neonatal-onset form, infantile-onset form and late-onset form. The neonatal-onset form is the most severe form and can be fatal owing to cardiac and multiorgan dysfunction and failure. The infantile-onset form always presents with recurrent hypoketotic hypoglycemia and hepatomegaly. A noteworthy feature of the late-onset form is myopathies, which manifest as myalgia, muscle weakness and recurrent episodes of rhabdomyolysis. If patients are not diagnosed and treated in time, VLCADD can be fatal.

Thus far, only a few cases of VLCADD have been documented in mainland China [Zhang et al., 2014]. Moreover, prenatal diagnosis of this disease has not yet been performed in China. Here, we report the clinical, biochemical and mutation spectra of eight Chinese VLCADD patients. Furthermore, prenatal diagnosis of VLCADD was performed in one fetus via *ACADVL* gene analysis of the amniocytes and amniotic fluid tetradecanoylcarnitine assay.

2. Materials and methods

2.1. Subjects

Eight patients (six boys, two girls) from six unrelated families were diagnosed with VLCADD in the past 4 years. Four patients (1

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and 2, and 4 and 5) were siblings. VLCADD was detected via newborn screening in patient 8 and via prenatal diagnosis in patient 5. In six patients, the clinical onset occurred between the ages of 2 days and 1.5 years. Patients were admitted with suspected diagnoses of liver diseases, epilepsy, cardiomyopathy and metabolic disorders due to positive family histories (Table 1).

The parents of the patients were healthy and non-consanguineous. For the genetic analyses, we obtained 200 DNA samples from healthy Chinese volunteers, who served as normal controls. Informed consents were obtained from the parents and the controls.

2.2. Routine tests

Routine tests of blood and urine, and tests for liver and renal function, serum electrolytes, glucose, ammonia, ketones, creatine kinase and isoenzymes of creatine kinase were conducted. Abdominal ultrasonography and echocardiography were performed to examine the liver and heart. On abdominal ultrasonography, increased echogenicity was observed in the entire liver, indicating fatty infiltration of the liver. Amino acid and acylcarnitine profiles were analyzed using liquid chromatography/tandem mass spectrometric assay (LC/MS/MS). These profiles were measured in dried blood spots by using LC/MS/MS, an Applied Biosystems API 3200 MS/MS analyzer and ChemoView software (AB Sciex Pte. Ltd, US). The sample preparation, processing procedures and analysis were based on previously reported methods [Zytkovicz et al., 2001].

2.3. ACADVL gene analysis

Genomic DNA was extracted from the peripheral blood lymphocytes of the patients, their parents and the 200 normal controls, by using the TIANamp Blood DNA Kit (Tiangen Biotech Co. Ltd., Beijing, China). The exons and flanking intronic regions of the *ACADVL* gene were amplified and analyzed via direct sequencing. The sequence results were then compared with the *ACADVL* gene sequence (GenBank: NM_000018). The sequence data were compared with an integrated set of variants, genotypes, and haplotypes from the 1000 Genomes Project (www.1000genomes.org) to identify mutations.

2.4. cDNA sequence analysis

RNA was extracted from peripheral leukocytes by using the Trizol method. Reverse transcription was accomplished using the TIANamp Reverse Transcription kit (Tiangen Biotech Co. Ltd.). *ACADVL* gene cDNA was amplified and sequenced.

2.5. Prediction of the effects of novel ACADVL gene mutations

Multiple sequence alignments to verify the degree of conservation were performed. The PolyPhen and Mutationtaster programs were used to predict the impact of missense alterations on protein function (http://genetics.bwh.harvard.edu/pph/, http://www.mutationtaster.org/).

3. Results

3.1. Clinical features

All eight patients were born at term. Their antenatal and postnatal histories were unremarkable. Only one patient was a firstborn with a normal family history (patient 8). The remaining seven patients (1–7) were the second, third or fourth child.

As shown in Table 1, only one patient underwent newborn screening for VLCADD. The presenting symptoms in the remaining patients occurred between the ages of 2 days and 1.5 years. Six patients had recurrent diarrhea. Three had vomiting. Four patients presented with hypoketotic hypoglycemia during acute attack episodes. Four had neurological symptoms. One patient died at the age of 5 months. All patients had liver, muscle or heart damage, and under therapy.

Table 1
Clinical and laboratory data of eight patients with VLCAD deficiency.

Patients	1	2	3	4	5	6	7	8	Normal range	Units
Gender	M	M	M	M	M	M	F	F		
Age of onset	2 d	1 y 6 m	1 y 2 m	5 d	2 m	2 m	1 y	25 d		
Age of diagnosis	1 y 6 m	1 y 8 m	1 y 10 m	after death	prenatal	1 y 3 m	5 y	3 d		
Present Age	6 y	4 y	3 y 4 m	dead	8 m	3 y	5 y 7 m	1 y 5 m		
Symptoms and signs										
lethargy	+	−	+	+	−	−	+	−		
weakness	−	−	−	+	−	−	−	−		
seizures	+	−	+	+	−	−	+	−		
cyanosis	+	−	−	+	−	−	−	−		
psychomotor retardation	−	−	−	+	−	−	−	−		
hepatomegaly	+	+	+	+	+	+	+	−		
Trigger events	diarrhea	diarrhea	diarrhea	N/A	pneumonia	diarrhea	diarrhea	diarrhea		
Positive family history	Sibling of patient 2	Sibling of patient 1	+	Sibling of patient 5	Sibling of patient 4	+	+	−		
Liver dysfunction	+	+	+	+	+	+	+	+		
Hypoglycemia	+	−	+	+	+	−	+	−		
Cardiomyopathy	−	−	−	+	−	+	−	−		
Serum CK	362 ↑	12	1047 ↑	10043 ↑	143	1029 ↑	3156 ↑	18	26–164	U/L
Serum CK-MB	6.8	3.2	50.3 ↑	372 ↑	27	55 ↑	25	4.5	0–30	U/L
Blood C14:1	5 ↑	2.46 ↑	2.69 ↑	4.19 ↑	4.06 ↑	6.65 ↑	0.85 ↑	2.8 ↑	0.01–0.5	μmol/L
Blood C0	26.0	27.4	7.2 ↓	3.17 ↓	31.88	21.4	2.44 ↓	21.5	20–60	μmol/L
Outcome	healthy	healthy	healthy	dead	healthy	healthy	healthy	healthy		

Note: M, male; F, female; y, years; m, months; d, days; CK, creatine kinase; CK-MB, creatine kinase MB.