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Genetic analyses of very long-chain acyl-coenzyme A dehydrogenase deficiency: A case report with a novel ACADVL variant

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ABSTRACT

Background: Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) is a rare autosomal recessive disease associated with variants in the *ACADVL* gene.
Methods: In December 2021, a neonate with VLCADD was identified via newborn screening in Xuzhou, China. Genetic testing and genetic family verification were performed via high-throughput sequencing combined with Sanger sequencing. The pathogenicity and functional impacts of novel variants were predicted using bioinformatics methods.
Results: Initial results obtained from tandem mass spectrometry blood screening were suggestive of VLCADD. Two compound heterozygous variants, c.753 T > G (p.S251R) and c.1276G > A (p.A426T), inherited from the father and mother, respectively, were detected in the *ACADVL* gene of this individual. The c.753 T > G variant is novel and unreported.
Conclusion: These findings broaden the known mutational spectrum of the *ACADVL* gene in a Chinese population.

1. Introduction

Very long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) (OMIM #201475) is a rare autosomal recessive inherited disease in which *ACADVL* variants (OMIM #609575) induce a functional deficiency in very long-chain acyl-coenzyme A dehydrogenase (VLCAD) [1]. Given that VLCAD serves as the key enzyme in the first step of long-chain acylcarnitine (LCA) β-oxidation, variants may lead to the impairment of mitochondrial fatty acid β-oxidation. This ultimately results in pathological changes in multiple organs and tissues, such as the myocardium, liver, and skeletal muscles, or even death. VLCADD presents with clinical heterogeneity and a wide age range at onset, ranging from neonatal to adult. The severe neonatal form is typically characterized by cardiomyopathy and hypoglycemia, whereas later-onset forms manifest with rhabdomyolysis and muscle weakness. A mild or asymptomatic form may also be observed. All phenotypes are associated with a risk of life-threatening hypoketotic hypoglycemia during metabolic decompensation, often triggered by fasting or illness, highlighting the critical importance of early diagnosis. In severe cases, metabolic decompensation disrupts energy production, potentially leading to death if untreated. Understanding the underlying metabolic mechanisms of this crisis is essential for improving individual outcomes. VLCADD can be broadly classified based on the affected tissues and time of onset as follows: the myocardial form (severe early-onset), hepatic form (hypoketotic hypoglycemic form), and myopathic form (late-onset).

Newborn screening (NBS) for VLCADD has been implemented in many countries to facilitate early detection and timely treatment. In China, NBS for VLCADD began in 2002. Since its introduction, the incidence of VLCADD has been subject to change, with a potential increase in detection rates due to the expanded screening program. Understanding the impact of NBSs on the incidence and clinical outcomes of VLCADD in China is essential for assessing the effectiveness of public health interventions and optimizing individual management strategies. Incidence rates of 1:70,424 to 1:236,655 have been reported in China, with a particularly high incidence in the Suzhou region [2,3]. Herein, we report the case of a neonate who exhibited significantly elevated blood levels of multiple types of long-chain acylcarnitine (LCA) (particularly C14:1) during newborn screening for inherited metabolic diseases via tandem mass spectrometry (MS/MS) in December 2021 at the Xuzhou Newborn Screening Center. Subsequent genetic testing, bioinformatics analyses of variants, and follow-up were performed. The results of this

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study are expected to contribute to research on VLCADD in the Chinese population and provide a scientific basis for future diagnosis and treatment.

2. Case report

2.1. Case description

The individual was a girl born with a birth weight of 3590 g via vaginal delivery at a gestational age of 39 weeks and 4 days in Xuzhou, China. Her parents were healthy and nonconsanguineous and denied any family history of genetic diseases. Dried blood samples from this individual were collected 4 days after birth for initial MS/MS screening. The results revealed abnormally elevated levels of multiple types of LCAs (including C12, C14, C14:1, and C18). In particular, the C14 concentration was 2.41 μmol/L (reference range: 0.04–0.37 μmol/L), the C14:1 concentration of 3.07 μmol/L (reference range: 0.02–0.22 μmol/L), and the C14:1/C16 and C14:1/C8:1 ratios were abnormally increased. These results prompted an immediate recall examination of the child, which revealed C14 and C14:1 concentrations of 0.52 μmol/L and 1.24 μmol/L, respectively. Based on the two sets of MS/MS blood screening results, the child was suspected of having VLCADD. The child is currently undergoing periodic monitoring and treatment at our center, and no obvious abnormalities were observed during the most recent return visit. The most recent MS/MS screening results indicated that the levels of different LCAs were essentially normal, with a C14:1 concentration of 0.19 μmol/L (Table 1). This individual's condition was managed with a high-carbohydrate, low-fat diet that restricted long-chain fatty acids and included medium-chain triglyceride (MCT) supplementation, aimed at preventing metabolic decompensation such as hypoglycemia and rhabdomyolysis, while ensuring adequate caloric intake. The infant followed a controlled fasting protocol with fasting intervals to maintain metabolic stability. Cardiac assessments, including electrocardiogram (ECG) and echocardiogram, showed normal results, and liver function tests were within normal limits, with no evidence of hepatomegaly or elevated liver enzymes. The individual has remained clinically stable without significant metabolic disturbances under the current management plan. *Re*-screening of blood samples via MS/MS and physical examinations has been performed on a periodic basis. The individual is currently 29 months old and has shown normal growth and development.

2.2. Genetic analyses

Compound heterozygous variants of the *ACADVL* gene, namely, c.753 T > G (p.S251R) in exon 9 and c.1276G > A (p.A426T) in exon 13, were detected in the child; both were missense variants. On the basis of American College of Medical Genetics and Genomics (ACMG) guidelines, the c.753 T > G (p.S251R) variant was initially classified as a variant of uncertain significance (VUS) based on the following criteria: PM1 (the variant is located in a mutational hotspot), PM2 (the variant is absent in the general population, indicating it is a low-frequency variant), and PP3 (bioinformatics tools predict a deleterious effect). Specifically, the REVEL tool indicated a damaging effect, while SIFT, PolyPhen-2, MutationTaster, and GERP+ also predicted a harmful impact. There are no relevant reports of this variant in the literature, and no pathogenicity data were available in the ClinVar database. Family analysis revealed that the patient's father carries the variant in a heterozygous state, while the mother does not carry this mutation. Moreover, the c.1276G > A (p.A426T) was initially classified as having uncertain clinical significance according to the ACMG guidelines (PM2 + PP3). PM2 indicates a low-frequency variant with no observed allele frequency in general population databases, whereas PP3 is supported by bioinformatics predictions with multiple tools (SIFT, PolyPhen_2, MutationTaster, GERP+), suggesting a damaging effect on protein function. Although the S251R variant has not been previously reported in the literature, two other variants at the same codon, S251G and S251N, have been documented in the gnomAD database. Despite their presence in the general population, these variants do not provide definitive clinical insights, thereby supporting the classification of S251R as a variant of uncertain significance. Given the lack of comparable reports in the literature and pathogenicity results, the c.753 T > G variant is not present in the ClinVar database, and that the c.1276G > A variant is present with conflicting interpretations of pathogenicity. Although there are no reports in the literature linking this variant to disease, ClinVar classified its pathogenicity as uncertain. Genetic family verification revealed that the father of the proband does not carry the variant, whereas the mother is heterozygous. Genetic family verification via Sanger sequencing revealed that the compound heterozygous variants c.753 T > G (p.S251R) and c.1276G > A (p.A426T) were inherited from the child's father and mother, respectively (Fig. 1).

The prediction results from three bioinformatics algorithms indicated that both variants are deleterious mutations, as summarized in Table 2. c.753 T > G (p.S251R) was predicted to be deleterious using PolyPhen-2 (score: 0.999) and SIFT (score: 0.001), pathogenic using MutationTaster (score: 110), and deleterious using REVEL, an ensemble prediction software for protein function. c.1276G > A (p.A426T) was predicted to be deleterious using PolyPhen-2 (score: 0.998) and SIFT (score: 0.012), pathogenic using MutationTaster (score: 58), and deleterious using REVEL.

Sequence homology analysis revealed that serine and alanine are the 251st and 426th amino acids encoded by ACADVL across five species (*Homo sapiens, Mus musculus, Macaca fascicularis, Danio rerio,* and *Bos taurus*), suggesting that the two sites are highly conserved and possess important biological functions. Using PyMOL v2.5 software, a three-dimensional structural simulation of the ACADVL protein revealed that the spatial hindrance and charge of the variant residues differ from those of the wild-type residues and may contribute to disrupting hydrogen bonds and thereby altering the tertiary structure of the protein (Fig. 1).

Table 1
The detection results of the individual with VLCADD by MS/MS (μmol/L).

Age	C14 (0.04–0.37)	C14:1 (0.02–0.22)	C16 (0.35–6.2)	C16:1 (0.02–0.47)	C18 (0.18–1.9)	C18:1 (0.34–2.7)
4 days	2.41	3.07	7.08	0.88	2.72	2.58
13 days	0.52	1.24	1.4	0.22	0.96	0.73
20 days	0.39	1.12	0.87	0.18	0.62	0.58
Over 3 months	0.66	1.56	1.53	0.35	0.79	0.85
Over 5 months	0.8	0.34	0.9	0.15	0.72	0.59
Over 8 months	0.39	0.68	1.16	0.13	0.92	0.63
1 year and 1 month	1.15	3.08	1.73	0.64	1.73	0.64
1 year and 4 months	0.79	2.18	1.44	0.44	1.13	1.03
1 year and 8 months	0.52	1.31	1.56	0.28	1.04	0.91
1 year and 9 months	0.14	0.13	0.93	0.04	0.66	0.38
2 years and 1 month	0.24	0.45	1.15	0.1	0.69	0.48
2 years and 5 month	0.15	0.19	0.93	0.06	0.57	0.39

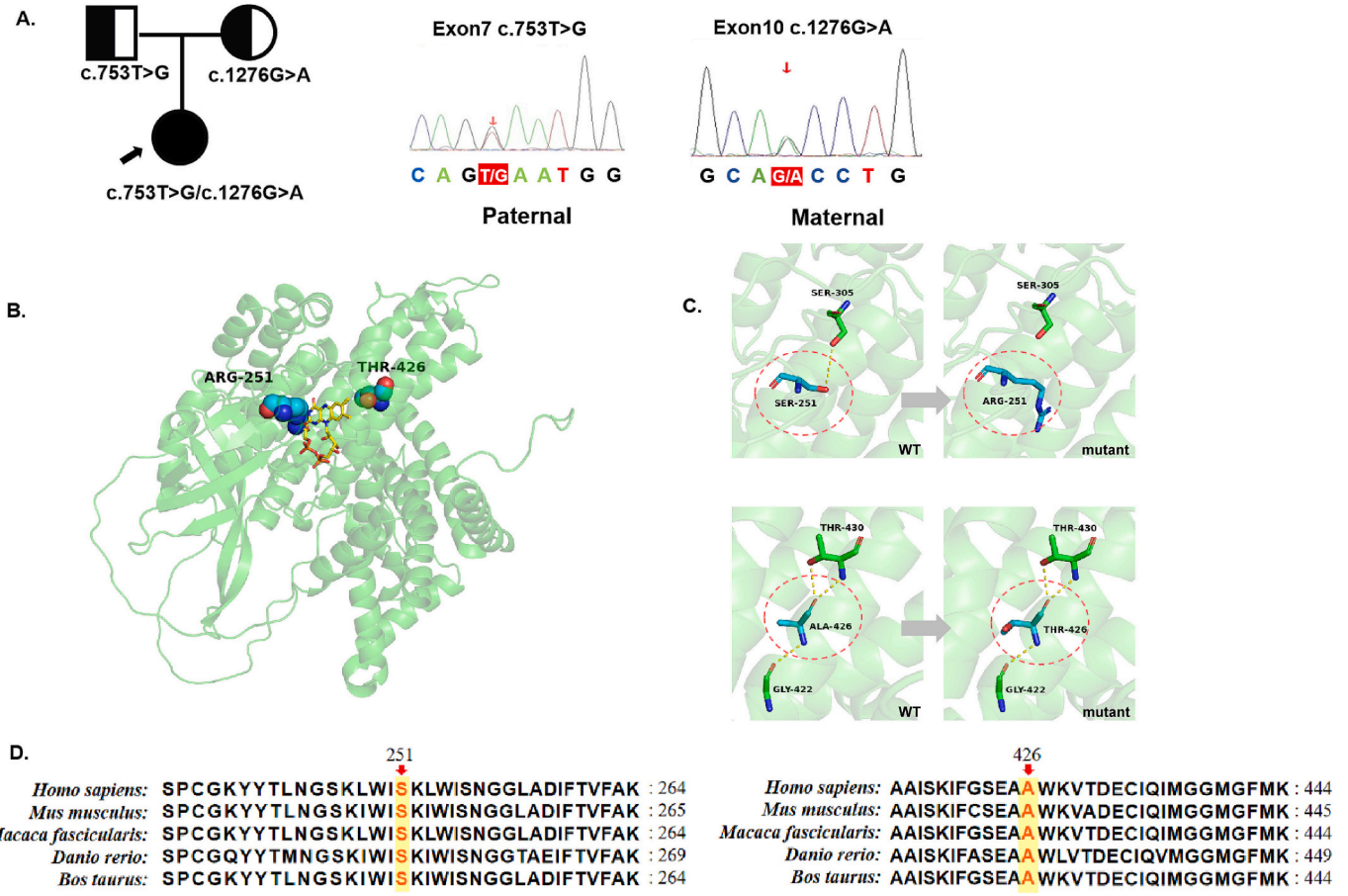

Fig. 1. Genetic, stability and molecular conservation analyses of *ACADVL* variants in the confirmed VLCADD individual. A. The gene variations were validated using Sanger sequencing. B. Location of the two variants on the dimeric structure of wild-type (WT) VLCAD (PDB ID: 2UXW) with its cofactor, flavin adenine dinucleotide (FAD). 3D-model of VLCAD is represented in green. The cofactor FAD is marked in yellow. The two mutant residues are marked in sky-blue. C. Structural predictions in WT and mutant residues in sky-blue. The yellow dashed lines represent the electrostatic interactions of mutant and WT residues, respectively. D. Conservation of the identified amino acids across diverse species of site 251 and site 426 in our individual with VLCADD.

Table 2
The pathogenicity predicted using bioinformatic tools for the two variants.

Nuceoride change	Amino acid change	Parental derivation	Pathogenicity prediction			
			PolyPhen-2 (Score)	SIFT (Score)	MutationTaster (Score)	REVEL
c.753 T > G	p.S251R	Paternal	Probably damaging (0.999)	Deleterious (0.001)	Disease causing (110)	Deleterious
c.1276G > A	p.A426T	Maternal	Probably damaging (0.998)	Deleterious (0.012)	Disease causing (58)	Deleterious

3. Discussion

VLCADD is one of the 121 diseases included in China's First National List of Rare Diseases released in 2018. It is a mitochondrial fatty acid β-oxidation disorder with a distinct etiology, clinical manifestations, and diagnosis and treatment protocols. Epidemiological studies have indicated that the incidence of VLCADD in neonates in Europe and the United States ranges from approximately 1:30,000 to 1:100,000 [4,5], which is considerably higher than the incidence rates of 1:380,000 to 1:400,000 reported in Asia [3]. Furthermore, researchers have reported an incidence of 1:70,424 in Suzhou, China, which is markedly higher than the overall incidence in Asia. VLCADD is the second most common fatty acid oxidation disorder in this region [6]. As of the end of December 2023, a total of 691,712 neonates had been screened, and only one (the individual described in this case study) received a confirmed diagnosis of VLCADD, which indicates a cumulative incidence rate of only 1:691,712 in Xuzhou. The observed differences in the incidence of VLCADD between Suzhou and Xuzhou, despite both being located in Jiangsu, China, may be attributed to regional genetic and demographic characteristics. Additionally, disparities in sample sizes of newborn screening, diagnostic practices, and health care infrastructure between the two regions could also contribute to the differences in reported cases. Further study is needed to elucidate the underlying factors and provide a more accurate estimate of VLCADD incidence across different regions.

Located on chromosome 17p13.1, the *ACADVL* gene consists of 20 exons that encode a 655-amino-acid homodimeric protein with a molecular weight of 70 kDa. The primary functional domains of the human VLCAD protein include the acyl-coenzyme A (acyl-CoA)-binding-, NAD-binding-, and FAD-binding domains, which serve as binding domains for

the coenzymes NAD+ and FAD and play important roles in promoting oxidative metabolic processes [7]. Catalytically active sites located in the acyl-CoA binding domain of the protein are responsible for catalyzing the dehydrogenation of long-chain fatty acids. In this case, we identified the p.S251R variant, which, to the best of our knowledge, has not been reported in the literature. The 251st amino acid residue of ACADVL, encoded by exon 9, is located in the FAD-binding domain of the VLCAD protein. This variant may influence the binding of VLCAD to its coenzyme FAD, thereby modulating its enzymatic activity. We found S251 to be highly conserved among the assessed vertebrate species, and multiple prediction algorithms indicated that the p.S251R variant is pathogenic. An analysis of secondary/tertiary protein structures also revealed the presence of significant differences between the wild-type and mutant proteins, with the variant leading to the disruption of existing hydrogen bonds. However, although these finding support the pathogenicity of the novel variant, further analyses are needed to determine the degree of pathogenicity. Morover, splicing variants near the c.753 T > G (p.S251R) position of the *ACADVL* gene have been shown to disrupt normal mRNA processing, leading to exon skipping or the activation of cryptic splice sites. These alterations often result in reduced or absent ACADVL enzyme activity, contributing to the pathogenesis of VLCADD. Further research is needed to better understand the molecular mechanisms involved and inform potential therapeutic strategies.

Another variant, c.1276G > A (p.A426T), has been reported to affect ACADVL function [7–9]. The 426th amino acid residue of the VLCAD protein is encoded by exon 13 of ACADVL, and we found that the alanine (A426) residue at this site is also highly conserved across species. Secondary/tertiary structure analysis indicated that compared with the wild-type protein, the variant increases the spatial hindrance in this amino acid and results in a change from the nonpolar hydrophobic alanine to the polar neutral tyrosine, which may alter protein stability, thereby indicating the pathogenic potential of the variant.

To date, 458 variants in the *ACADVL* gene have been reported, with likely pathogenic variants accounting for the majority. Moreover, racial and regional differences have been found with respect to variants in this gene [10]. For example, the c.848 T > C variant has been identified as being the most common variant in European and American populations, whereas c.65C > A variant is highly prevalent in Middle Eastern populations, and c.1349G > A variant has a relatively high rate of detection in Asian and Chinese populations [10–14]. *ACADVL* variants lead to VLCAD enzyme deficiency, with nonsense or splice-site variants resulting in a complete loss of enzymatic activity, which is associated with more severe clinical manifestations in affected individuals. This generally leads to the development of the severe early-onset myocardial form of VLCADD. However, the uncertain significance variants (VUS) identified in the *ACADVL* gene, including missense mutations, require further experimental evaluation to assess their impact on VLCAD enzyme activity [15]. While computational predictions suggest potential functional effects, the clinical relevance of these variants remains uncertain without additional functional assays. Further studies are needed to clarify the effects of these VUS on enzyme function and clinical phenotypes.

Newborn screening based on MS/MS is acknowledged as a key approach for the early detection of VLCADD. Characteristic abnormal changes in acylcarnitines are associated with elevated levels of multiple types of LCAs (C14, C14:1, C16, C18, and C18:1) and their corresponding ratios. In particular, the concentration of C14:1 has been established as the most sensitive indicator for VLCADD when screening using MS/MS [16,17]. In VLCADD, levels of C14:1 decrease with time [18]. In this case, the initial C14:1 level was 3.07 μmol/L; however, by the time of the first follow-up, the level had decreased to 1.24 μmol/L. This reduction is consistent with the expected temporal decline in the concentration of C14:1, as well as factors such as the timing of blood collection, which occurred after the child had been bathed, approximately four days post-birth. At this time point, there may have been insufficient maternal milk production and a relatively long interval since the previous feeding. Under such conditions, the child may have been in a relatively fasting state, and consequently, the breakdown of fats may have contributed to a significant elevation in the levels of C14:1. VLCADD is relatively rare in China, and in certain individuals, the concentrations of C14:1 may differ significantly between initial screening and re-examination; accordingly, clinicians should take such factors into consideration when making a diagnosis. C14:1 is a key metabolic biomarker in individuals with VLCADD, and alterations in its concentration play a pivotal role in both diagnosis and therapeutic monitoring. Variations in the levels of C14:1 offer valuable insights into disease progression and treatment response, underscoring its importance in clinical management. Currently, the child lacks any obvious symptoms. In addition to complying with medical advice to avoid fasting, the child has adhered to a low-fat diet and is being periodically monitored and followed up at our center [11].

4. Conclusions

VLCADD is an inherited metabolic disorder that can be detected via MS/MS screening. Current clinical treatment mainly aims to ensure the avoidance of fasting, fatigue, and excessive exercise; adherence to a low-fat diet; and timely supplementation with high-carbohydrate foods and medium-chain triglycerides. This case report expands the mutational spectrum of the ACADVL gene in VLCADD, providing valuable insights that may improve genetic testing and prenatal diagnosis for at-risk families. The identification of novel mutations enhances the accuracy of genetic counseling and may contribute to more personalized diagnostic and treatment strategies, ultimately assisting in the early detection and management of VLCADD in future pregnancies. In conclusion, the identification of novel mutations in VLCADD, along with the clinical treatment data from our center, contributes valuable insights into this disorder in the Chinese population. These findings may improve early diagnosis and inform personalized treatment strategies for VLCADD in similar patient groups.

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CRediT authorship contribution statement

Wei Zhou: Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Huizhong Li:** Software, Methodology, Data curation. **Li Yang:** Writing – review & editing, Validation.

Declaration of competing interest

None.

Data availability

Data will be made available from the corresponding author upon reasonable request.

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