Molecular Genetics and Metabolism Reports 39 (2024) 101088

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

Case Report

Newborn screening for isovaleric acidemia: A case report of a Chinese patient with novel variants

Huizhong Li, Fang Shao, Wei Zhou *

Neonatal Disease Screening Center, The Affiliated Xuzhou Maternity and Child Health Care Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China

A B S T R A C T

Isovaleric acidemia (IVA) is a rare autosomal recessive disorder that manifests as a deficiency of isovaleryl-CoA dehydrogenase (IVD), a key enzyme in leucine metabolism. The clinical presentations associated with IVD deficiency are variable and include feeding intolerance, vomiting, metabolic acidosis, ketonemia, "sweaty feet" odor, lethargy, coma and even death. Tandem mass spectrometry (MS/MS) and gas chromatography–mass spectrometry (GC/MS) methods were used to perform organic acid analysis of blood and urine samples from IVA patients, and the genetic analysis included next generation sequencing (NGS) and Sanger sequencing of the *IVD* gene. Here, we report the case of an almost seven-year-old male patient from a Chinese family who was asymptomatic during the newborn period, including the clinical manifestations and examination results. Genetic analysis revealed a previously unreported compound heterozygous variant in the *IVD* gene: c.593G > C (p.W198S) and c.859C > T (p.R287W).

1. Introduction

Isovaleric acidemia (IVA, OMIM #243500) is an autosomal recessive and heterogeneous disorder attributed to isovaleryl CoA dehydrogenase (IVD, E.C.1.3.99.10) deficiency, which results in the accumulation of isovaleric acid (C5-carnitine), 3-hydroxyisovaleric acid and isovalerylglycine (IVG) [1–4].

IVA, the first identified organic acidemia disorder, was initially described in 1966 through the detection of urinary metabolites by gas chromatography–mass spectrometry (GC/MS) [5]. Global epidemiology statistics for IVA indicate considerable differences in IVA depending on ethnicity and dietary variables [6–9]. Following the implementation of newborn screening (NBS) by MS/MS in mainland China, the incidence of IVA has been reported to range from 1:84,469 to 1:160,000 in the Chinese population [9].

The clinical presentation of IVA varies greatly and includes the early-onset acute neonatal form, the late-onset chronic intermittent form and the asymptomatic form. Individuals with IVA typically present with feeding intolerance, vomiting, metabolic acidosis, ketonemia, "sweaty feet" odor, lethargy, coma and even death [10]. For patient with more severe disease, the current treatment is a low-protein diet with leucine restriction combined with L-carnitine and L-glycine administration to scavenge accumulated toxins [11].

Here, we reported the case of a child who was diagnosed with mild, chronic IVA. We identified two novel variants of the *IVD* gene in this patient.

2. Case presentation

The patient, an almost 7-year-old boy from nonconsanguineous healthy parents, was delivered at 40 weeks of gestation under normal birth conditions in Xuzhou. The boy weighed 3.5 kg at birth and was the mother's first pregnancy. After an uncomplicated delivery, the child exhibited acceptable cardiorespiratory adaptation and breastfed adequately. The first NBS was performed by a local community pediatrician at the age of 5 days, and the findings were abnormal.

The initial NBS revealed a C5-carnitine concentration of 11.45 mol/L (reference range, 0.04–0.6 mol/L), and repeated screening following admittance to the pediatric outpatient unit showed an increase to 15.25 mol/L at 30 days of age. Simultaneously, urine organic acid analysis by GC/MS revealed increased excretion of IVG (80.54–89.80 mmol/mmol creatinine, normal value<0.4 mmol/mmol creatinine), and the patient was sent home with medication and an outpatient follow-up appointment.

Two previously unreported compound heterozygous variants of the *IVD* gene, c.593G > C (p.W198S) and c.859C > T (p.R287W), were confirmed through genetic analysis. Sanger sequencing was used to validate the inheritance of the variants (Fig. 1). The p.W198S variant was inherited from his mother, whereas the p.R287W variant was inherited from his father. Three-dimensional protein modeling of the

* Corresponding author at: Neonatal Disease Screening Center, The Affiliated Xuzhou Maternity and Child Health Care Hospital of Xuzhou Medical University, No.46 Heping Road, Xuzhou, Jiangsu 221009, China.
E-mail address: wei0743916@163.com (W. Zhou).

https://doi.org/10.1016/j.ymgmr.2024.101088
Received 18 March 2024; Received in revised form 24 April 2024; Accepted 29 April 2024

variations and assessment of the conservation of the identified amino acids across numerous species were also performed. Online tools, including PolyPhen2, Sorting Intolerant From Tolerant (SIFT), and Mutation Taster, and the American College of Medical Genetics and Genomics (ACMG) classification system, were used to calculate the pathogenicity scores (Table 1).

After the diagnosis of IVA, the patient was administered 100 mg/kg/day of L-carnitine, which stabilized the free carnitine plasma concentration at 30–60 μmol/L. In addition, the parents were provided with instructions to implement a balanced dietary protocol for daily protein restriction while avoiding low-protein malnutrition. This patient benefited from the use of leucine-free special formula milk powder. To date, the findings from clinical follow-up visits have been unremarkable, and longitudinal biochemical monitoring of blood C5-carnitine levels during L-carnitine treatment was performed. The GC/MS results showed steadily normalizing urinary excretion of IVG during treatment.

3. Discussion

IVA, a common type of organic acidemia, is an inborn error caused by a deficiency of IVD in leucine catabolism. The clinical manifestations of IVA exhibit diverse presentations, including asymptomatic, chronic intermittent, and acute neonatal forms. Chronic intermittent IVA commonly manifests as developmental delay or failure to thrive with onset in infancy or childhood. However, early identification and intervention play a critical role in preventing mental impairment and improving IVA patient outcomes.

The *IVD* gene encodes a 421-amino acid protein with a molecular weight of 46.5 kDa. The IVD protein comprises various functional structures, and the amino acid regions 123–159 and 356–403 corresponding to α-helices in the N-terminal domain near the monomer center and the C-terminal domain end, respectively, are conserved across different species. FAD binding sites were identified in the UniProt database within the amino acid regions 165–174 and 380–384, and the pathogenic variant distribution mainly clustered near these FAD binding sites. Moreover, asymptomatic hot regions at amino acids 282–318, which are distant from the FAD binding sites, may have minimal impact on protein function [12].

To date, over 90 the pathogenic variants of *IVD* have been identified, and the sites vary dramatically in different ethnic groups worldwide [13,14]. For example, according to recent studies, c.1208 A > G (p.Y403C) is considered as a potential common variant in the Han Chinese population [15]. The variant hotspot sites in patients from mainland China and Taiwan are residues 53, 120, 214, 339, 371, 395, 398, and 403 [16]. However, these findings are limited by a lack of reports from mainland China, suggesting that previous studies on IVA need to be

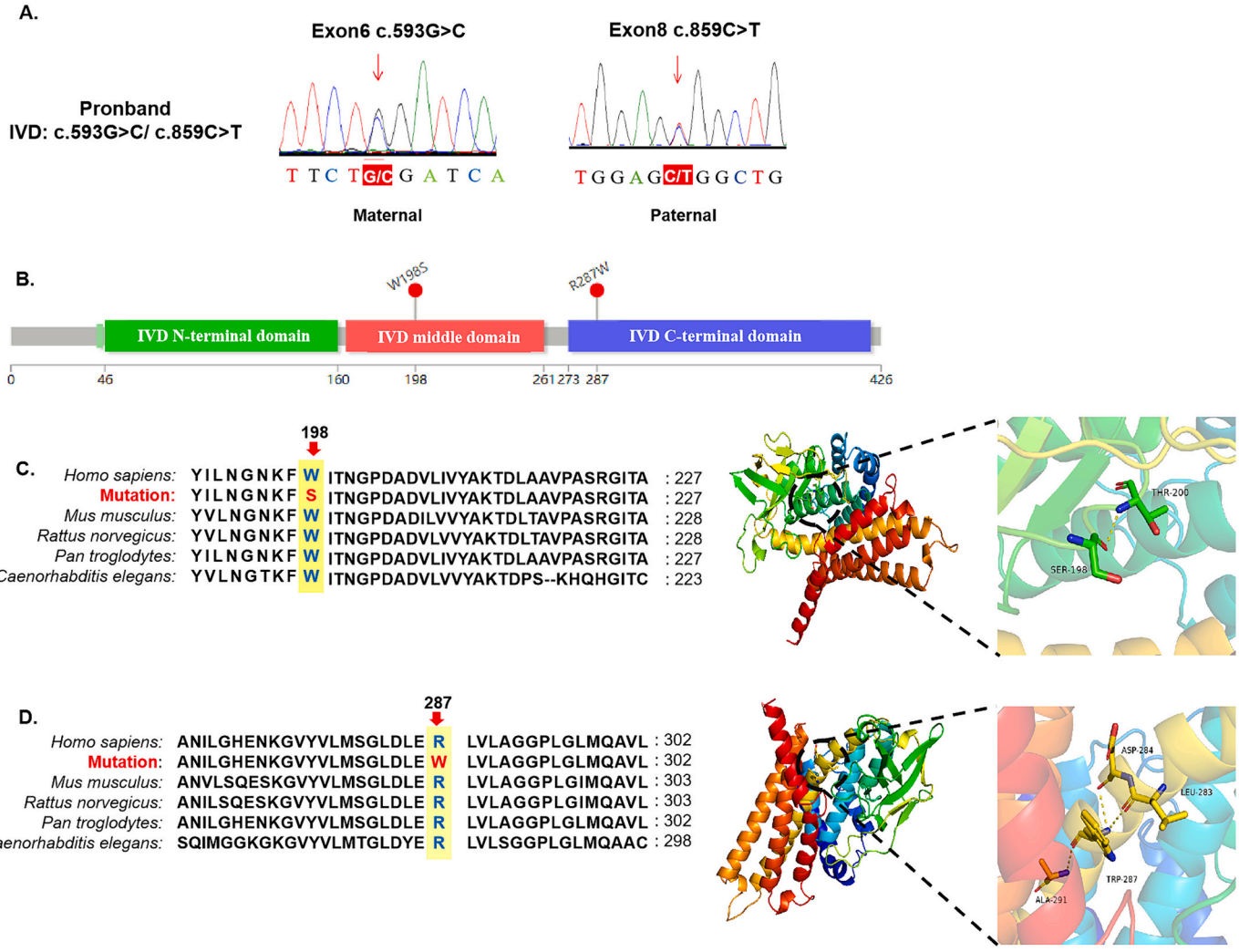

Fig. 1. Bioinformatics analysis of IVD variants in an individual with confirmed IVA. A. The gene variations were validated using Sanger sequencing. B. Structure of the IVD protein. C—D. Conservation of the identified amino acids across diverse species and the 3D models for wild-type IVD and IVD with the identified missense variants at site 198 and site 287 in our patient with isovaleric acidemia (IVA).

Table 1
The pathogenicity predicted using online tools.

Nucleotide change	Amino acid change	Parental derivation	Pathogenicity prediction			
			Polyphen2 (score)	SIFT	Mutation taster (score)	ACMG
c.593G > C	p.W198S	Maternal	Probably damaging (1.000)	Deleterious	Disease causing (~1.000)	Uncertain
c.859C > T	p.R287W	Paternal	Probably damaging (1.001)	Deleterious	Disease causing (1.000)	Uncertain

further expanded. Here, two novel variants located in the *IVD* gene were identified, expanding the molecular genetic spectrum associated with IVA.

The novel variants p.W198S and p.R287W of the *IVD* gene resulted in significant accumulation of the toxic metabolite C5-carnitine in the blood, which was identified by NBS in our center. The patient with confirmed compound heterozygous variants presented with a neonatal-onset form of IVA because the variants interfered with the activity of the IVD enzyme. However, the genotype–phenotype relationship for the two new missense variants is not yet known [3]. Remarkably, positions 198 and 287 of the *IVD* gene are highly conserved among different species. In our protein model analysis, the tryptophan at position 198 was altered to serine, which decreased steric hindrance in the absence of a benzene moiety. Simultaneously, replacement of the conserved basic amino acid arginine with neutral tryptophan at position 287 resulted in abnormal folding of the IVD protein. The surface charge alterations and steric hindrance caused by tryptophan might inactivate enzymes [17].

4. Conclusion

In summary, we report an individual with IVA who was identified with NBS. Sequencing analysis confirmed that the compound heterogeneous *IVD* variants c.593G > C (p.W198S) and c.859C > T (p.R287W) are novel variants, and these two missense variants may inactivate enzymes by altering conserved amino acids.

CRediT authorship contribution statement

Huizhong Li: Writing – original draft, Investigation. **Fang Shao:** Validation, Methodology. **Wei Zhou:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

References

[1] F. Keyfi, M. Nasseri, S. Nayerabadi, A. Alaei, A. Mokhtariye, A. Varasteh, Frequency of inborn errors of metabolism in a northeastern Iranian sample with high consanguinity rates, Hum. Hered. 83 (2) (2018) 71–78.
[2] M. Wajner, Neurological manifestations of organic acidurias, Nat. Rev. Neurol. 15 (5) (2019) 253–271.
[3] O.K. Zaki, C.G. Priya Doss, S.A. Ali, et al., Genotype-phenotype correlation in patients with isovaleric acidaemia: comparative structural modelling and computational analysis of novel variants, Hum. Mol. Genet. 26 (16) (2017) 3105–3115.
[4] M. Alfadhel, A. Babiker, Inborn errors of metabolism associated with hyperglycaemic ketoacidosis and diabetes mellitus: narrative review, Sudan J. Paediatric. 18 (1) (2018) 10–23.
[5] Q. Yang, S.S. Lin, J.T. Yang, L.J. Tang, R.Q. Yu, Detection of inborn errors of metabolism utilizing GC-MS urinary metabolomics coupled with a modified orthogonal partial least squares discriminant analysis, Talanta 165 (2017) 545–552.
[6] R. Ensenauer, R. Fingerhut, E.M. Maier, et al., Newborn screening for isovaleric acidemia using tandem mass spectrometry: data from 1.6 million newborns, Clin. Chem. 57 (4) (2011) 623–626.
[7] D.M. Frazier, D.S. Millington, S.E. McCandless, et al., The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005, J. Inherit. Metab. Dis. 29 (1) (2006) 76–85.
[8] M.L. Couce, L. Aldamiz-Echevarría, M.A. Bueno, et al., Genotype and phenotype characterization in a Spanish cohort with isovaleric acidemia, J. Hum. Genet. 62 (3) (2017) 355–360.
[9] W.D. Lin, C.H. Wang, C.C. Lee, C.C. Lai, Y. Tsai, F.J. Tsai, Genetic mutation profile of isovaleric acidemia patients in Taiwan, Mol. Genet. Metab. 90 (2) (2007) 134–139.
[10] E. Sag, A.H. Cebi, G. Kaya, G. Karaguzel, M. Cakir, A rare cause of recurrent acute pancreatitis in a child: Isovaleric Acidemia with novel mutation, Pediatric Gastroenterol. Hepatol. Nutrit. 20 (1) (2017) 61–64.
[11] Y. Chinen, S. Nakamura, K. Tamashiro, et al., Isovaleric acidemia: therapeutic response to supplementation with glycine, l-carnitine, or both in combination and a 10-year follow-up case study, Mol. Genetics Metabol. Reports. 11 (2017) 2–5.
[12] W. Chen, C. Miao, Y. Li, et al., A case report of a novel Isovaleryl-CoA dehydrogenase gene mutation in a Chinese family with Isovaleric Acidemia, Clin. Lab. 69 (9) (2023).
[13] A.N. Adhikari, R.J. Currier, H. Tang, et al., Genomic analysis of historical cases with positive newborn screens for short-chain acyl-CoA dehydrogenase deficiency shows that a validated second-tier biochemical test can replace future sequencing, Intern. J. Neonatal Screen. 6 (2) (2020).
[14] E. Szymańska, A. Jezela-Stanek, A. Bogdańska, et al., Long term follow-up of polish patients with Isovaleric aciduria. Clinical and molecular delineation of Isovaleric Aciduria, Diagnostics (Basel, Switzerl.) 10 (10) (2020).
[15] Y. Li, M. Shen, Y. Jin, et al., Eight novel mutations detected from eight Chinese patients with isovaleric acidemia, Clin. Chim. Acta 498 (2019) 116–121.
[16] X. Liu, X. Liu, W. Fan, et al., Analysis of the genotype-phenotype correlation in isovaleric acidaemia: a case report of long-term follow-up of a chinese patient and literature review, Front. Neurol. 13 (2022) 928334.
[17] I. Ibarra-González, C. Fernández-Lainez, S. Guillén-López, et al., Molecular analysis using targeted next generation DNA sequencing and clinical spectrum of Mexican patients with isovaleric acidemia, Clin. Chim. Acta 501 (2020) 216–221.