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Eight novel mutations detected from eight Chinese patients with isovaleric acidemia

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ABSTRACT

Background: Isovaleric acidemia (IVA), a rare autosomal recessive disorder in leucine metabolism caused by defected *IVD* gene, is characterized by episodes of acute metabolic crisis and psychomotor development retardation. This study aimed to determine the clinical, biochemical, and mutation spectrum of patients with IVA from mainland China.

Methods: Eight patients (three boys and five girls) from eight unrelated families were collected, *IVD* gene mutations and phenotypes were examined.

Results: The patients were admitted because of vomiting, feeding difficulty, psychomotor retardation and "dirty sock" odor. Elevated blood isovaleryl (C5)-carnitine and urine isovalerylglycine were detected from all our patients. Fourteen mutations of the *IVD* gene were detected, eight of them are novel, c.145C > T (p.Q49Ter), c.359G > A (p.R120Q), c.424C > T (p.R142C), c.458T > C (p.L153P), c.466-1G > T, c.676_677insA (p.T226Nfs*13), c.1039G > A (p.A347T) and c.1076A > G (p.D359G). With this study, a total of 34 alleles were studied in the Chinese population. c.1208A > G (p.Y403C), the common mutation in Taiwan, accounts for 9/34 alleles (7 in previous reports and 2 in this study).

Conclusions: We described eight novel mutations detected from eight unrelated Chinese patients and provided evidence to support that the p.Y403C is the hotspot mutation in this population.

1. Introduction

Isovaleric acidemia (IVA, OMIM #243500) is a rare autosomal recessive disorder that is characterized by impaired leucine metabolism, and is caused by isovaleryl-CoA dehydrogenase (*IVD*) gene defect [1]. It is the first organic acidemia discovered in 1966 and is associated with significant morbidity and mortality [1]. The incidence of IVA varies among geographic regions, e.g., it is estimated to be 1 in 62,500 in Germany [2], 1 in 250,000 in the United States [3], and 1 in 365,000 in Taiwan [4]. The incidence in Mainland China has not been reported.

IVD gene encodes the mitochondrial enzyme isovaleryl-CoA dehydrogenase (IVD, 175 kDa, E.C.1.3.99.10), a flavin adenine dinucleotide (FAD)-containing enzyme consisting of four identical subunits. This enzyme regulates the conversion of isovaleryl-CoA to 3-methylcrotonyl-CoA in leucine catabolism. A defected enzyme IVD leads to accumulation of isovaleryl-CoA derivatives in cells, blood, and urine, such as isovaleric acid, 3-hydroxyisovaleric acid, isovaleryl (C5)-carnitine, and isovalerylglycine (IVG). The deposition of these metabolites subsequently damages organs. The clinical features of IVA include feeding difficulty, vomiting, lethargy, developmental delay, neuropathologic implications, metabolic acidosis, hypoglycemia, hypocalcemia, and sweaty feet odor [1,5]. According to the clinical manifestations, IVA is categorized into three subtypes; (1) acute neonatal type with symptoms appearing within the first two weeks of life; (2) chronic intermittent type which manifests as non-specific failure to thrive and/or developmental delay. During the metabolic crisis, its phenotypes resemble

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those of the acute neonatal form; and (3) asymptomatic type which presents with mild clinical symptoms or mild biochemical abnormalities [1,5].

IVA can be identified in the newborn by screening, and it is one of the treatable metabolic diseases. However, only ten patients have been documented in the Chinese population based on genetic diagnosis [4,6–9], and seven of them came from Taiwan and Hongkong. As the general populations among Mainland China (1.4 billion), Taiwan (23.4 million) and Hongkong (7.4 million) vary differently, the mutation spectrum of Taiwanese and Hongkongese can not represent the whole Han Chinese population, the study of Mainland China is crucially important. Here, we report the clinical, biochemical and mutation spectra of eight IVA patients from Mainland China, and review all the reported literature of IVA in this population.

2. Materials and methods

2.1. Research subjects

Eight patients (three boys and five girls) from eight unrelated Chinese families were diagnosed with IVA at the Department of Pediatrics, Peking University First Hospital, Beijing, China, between February 2006 and November 2018. Six patients (except Patient 4 and 5) came to our hospital because of vomiting, lethargy, coma, and psychomotor development retardation. The parents of Patient 4 and 5 visited us for genetic diagnosis after the death of the probands. The parents of all patients were healthy and non-consanguineous. This study was approved by the institutional review board of Peking University First Hospital, and it was conducted in conformity with the Declaration of Helsinki guidelines.

2.2. Routine tests and metabolic examinations

Routine laboratory tests for blood and urine, liver and renal function, serum electrolytes, glucose, ammonia, ketones, creatine kinase, creatine kinase isoenzymes, and blood gas analyses were conducted.

Urinary organic acids were analyzed by gas chromatography–mass spectrometry (GC–MS) using a GCMS-QP2010 analyzer (Shimadzu, Japan) and the differential diagnosis of organic acidurias was performed using the Inborn Errors of Metabolism Screen System software [10,11].

Analysis of blood amino acids, free carnitine, and acylcarnitines was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Applied Biosystems, USA) using an Applied Biosystems API 3200 analyzer and ChemoView software for differential diagnosis of amino acid defects, fatty acid metabolic disorders, and other organic acidurias [12].

2.3. IVD gene analysis

Genetic analysis was performed after obtaining informed consent from the parent of each patient. Genomic DNA was extracted from peripheral blood lymphocytes of patients and their parents using the TIANamp Blood DNA Kit (Tiangen Biotech, China). Exons and flanking intronic regions of the *IVD* gene were amplified using PCR and then sequenced. The results were compared with the reference sequences of *IVD* (NM_000016) deposited in UCSC genome (http://genome.ucsc.edu/). Sequencing data were compared with an integrated set of variants (http://www.hgmd.cf.ac.uk), genotypes, and haplotypes from the 1000 Genomes Project (www.1000genomes.org) to identify mutations.

Multiple sequence alignments were performed to verify the degree of conservation. PolyPhen-2 and Mutationtaster program were used to predict the impact of missense alterations on protein function (http://genetics.bwh.harvard.edu/pph/, http://www.mutationtaster.org/). Multiple sequence alignments were obtained using BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi).

3. Results

3.1. Clinical data

Clinical data and the results of laboratory examinations are presented in Table 1. The age of disease onset ranged from 1 day to 2 years, and the age of diagnosis ranged from 25 days to 6 years, two patients got their diagnosis after their death. Five patients had acute neonatal form whereas three patients had a chronic intermittent form. All the patients' birth histories were normal, and five of the acute neonatal form cases occurred during the neonatal period, with vomiting,

Table 1
Clinical and laboratory data of eight Chinese patients with IVA.

Patients	1	2	3	4	5	6	7	8	Normal range	Units
Gender	Female	Male	Female	Female	Male	Female	Male	Female		
Age of onset	2 d	1 d	4 d	2 d	3 d	1 y	1 y8 m	2 y		
Age of diagnosis	1 y6 m	25 d	1 y8 m	After death	After death	1 y	4 y9 m	6 y		
Present age	2 y6 m	7 y	8 y	Died at 15 d	Died at 11 d	5 m	11 y	18 y		
Symptoms and signs										
Vomiting	+	+	+	+	+	+	+			
Hypothermia	−	+	+	+	−	−	−			
Coma	−	+	+	+	+	−	−			
Epilepsy	−	+	−	+	−	−	+			
Attack times	1	2	2	1	1	1	2	1		
Trigger events	Infection	Infection	Vaccine/ infection	Infection	N/A	High protein diet/ infection	Infection	Infection		
Laboratory findings (during the attack)										
WBC	4.25↓	2.62↓	3.69↓	0.84↓	2.17↓	3.71↓	4.84↓	4.54↓	*	$\hat{}10^9$/L
RBC	3.16↓	4.93	3.38↓	2.97↓	5.08	4.69	2.57↓	5.12	*	$\hat{}10^{12}$/L
HGB	103↓	162	99↓	87↓	79↓	108↓	108	122	*	g/L
PLT	218	174	30↓	9↓	103↓	286	413↑	187	150–350	$\hat{}10^9$/L
Metabolic acidosis	+	+	+	+	+	+	+	+		
Hyperammonemia	−	+	+	+	+	+	+	−		
Pathoglycemia	−	−	+	+	+	−	−	−		
Urine IVG	1291.73↑	1537.94↑	1859.94↑	258.3↑	1859.94↑	367.1↑	1783.56↑	759.22↑	< 0.4	mmol/mmol creatinine
Blood C5-carnitine	9.79↑	20.7↑	8.41↑	10.23↑	8.41↑	8.2↑	4.6↑	6.93↑	< 0.5	μmol/L

Note: M, male; F, female; y, years; m, months; d, days; N/A, not available; *: the normal range various from ages.

consciousness, and "dirty sock" odor occurring 1–4 days after birth. Among them, two patients (Patient 4 and 5) who had acute neonatal form died in their early life. Patient 4 presented with fever, vomiting, and feeding difficulty at 2 days old. She was diagnosed with pneumonia and received anti-infection treatment, and died at 15 days after birth. Patient 5 showed lethargy and persistent vomiting at 3 days, metabolic acidosis was found, and epilepsy was developed. Ten days after birth, the boy had a severe infection and died on the 11th day. The other three chronic intermittent form cases had a late-onset which occurred between 1 and 2 years old, presenting with psychomotor retardation and decompensation metabolic crisis attack. The trigger events of the acute attack were infections, vaccine injection, and high dietary intake of protein food.

3.2. Laboratory examinations

All patients had metabolic acidosis, leukopenia, aglobulia, or thrombocytopenia during acute episodes; one patient (Patient 4) had pancytopenia. Six patients (Patient 2–7) had hyperammonemia, and three patients had hypoglycemia (Patient 3–5).

Blood C5-carnitine level was significantly increased in all patients (4.6–20.7 μmol/L, normal value < 0.5 μmol/L) and the concentration of urinary IGV was elevated (258.3–1783.56 mmol/mmol creatinine, normal value < 0.4 mmol/mmol creatinine).

3.3. Molecular analysis

Fourteen mutations were identified in the eight patients (Table 2). Among them, there were eight novel mutations belonging to *IVD* gene, include c.145C > T (p.Q49Ter), c.359G > A (p.R120Q), c.424C > T (p.R142C), c.458T > C (p.L153P), c.466-1G > T, c.676_677insA (p.T226Nfs*13), c.1039G > A (p.A347T) and c.1076A > G (p.D359G) (Fig. 1a). The changes in codons and amino acids of these mutations are listed in Table 3. Also, six mutations were reported previously, include c.157C > T (p.R53C), c.158G > A (p.R53H), c.214G > A (p.D72N), c.1183C > T (p.R395G), c.1193G > A (p.R398Q) and c.1208A > G (p.Y403C).

Except for the de novel mutation p.R120Q (the mutation was not detected from the proband's parents), all mutations found in the patients were detected in their parents heterozygously. Only two novel mutations p.R120Q and p.R142C are detected in the 1000 Genomes Project (www.1000genomes.org) databases (rs145725101 and rs376324882).

Sequence alignment of enzyme IVD revealed that Q49 and L153 amino acids are conserved in mammals and R223 is conserved in vertebrates. The amino acids of other four novel missense mutation including R120, R142, A347, and D359 are highly conserved in humans, *M. mulatta*, *Mus musculus*, *Trubripes*, *Danio rerio*, and *Xenopus tropicalis* (Table 4). All missense mutations were predicted to be "probably damaging, possibly damaging" and "disease-causing" using PolyPhen-2.0 and MutationTaster tools, respectively. Insertion and termination mutations were predicted to be "disease-causing" by MutationTaster (Table 2).

3.4. Treatment and prognosis

After diagnosis, except for Patient 4 and 5, all patients were treated with L-carnitine, glycine, and leucine free formula. After treatment, all patients were relatively stable, three of them (Patient 2, 3 and 7) had a recurrence of acute metabolic crisis due to infection whereas the other three patients (Patient 1, 6 and 8) did not develop acute decompensation attack again till now. Two patients (Patient 1 and 2) developed psychomotor retardation, whereas four patients (Patient 3, 6–8) were healthy.

Table 2
Mutations detected in *the IVD* gene among eight Chinese patients with IVA.

Patients	Mutation at the nucleotide level	Mutation type	Mutation at the protein level	PolyPhen-2.0 prediction and score	MutationTaster prediction and score	Conservation	Allele frequency*
1	c.359G > A, rs145725101	heterozygous	p.R120Q	Possibly damaging (1)	Disease-Causing (0.99)	Yes	1/16
	c.458T > C	heterozygous	p.L153P	Probably damaging (1)	Disease-Causing (0.99)	Conserved in mammals	1/16
2	c.145C > T	heterozygous	p.Q49Ter	N/A	Disease-Causing (1)	Conserved in mammals	1/16
3	c.1193G > A	heterozygous	p.R398Q	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16
	c.1208A > G	heterozygous	p.Y403C	Probably damaging (0.99)	Disease-Causing (0.99)	Yes	2/16
4	c.158G > A, rs2229311	heterozygous	p.R53H	Probably damaging (1)	Disease-Causing (1)	Yes	1/16
	c.676_677insA	heterozygous	p.T226Nfs*13	N/A	Disease-Causing (1)	Conserved in vertebrates	1/16
5	c.424C > T, rs376324882	heterozygous	p.R142C	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16
	c.466-1G > T	heterozygous	splicing	N/A	Disease-Causing (1)	N/A	1/16
6	c.157C > T, rs34695403	heterozygous	p.R53C	Probably damaging (0.99)	Disease-Causing (0.99)	Yes	1/16
	c.1039G > A	heterozygous	p.A347T	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16
7	c.1183C > G	heterozygous	p.R395G	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16
	c.1208A > G	heterozygous	p.Y403C	Probably damaging (1)	Disease-Causing (1)	Yes	2/16
8	c.214G > A, rs747273828	heterozygous	p.D72N	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16
	c.1076A > G	heterozygous	p.D359G	Probably damaging (1)	Disease-Causing (0.99)	Yes	1/16

Note: *Allele FFrequency: in this study, allele frequency represent the mutated alleles frequency among 16 alleles; N/A, not available;

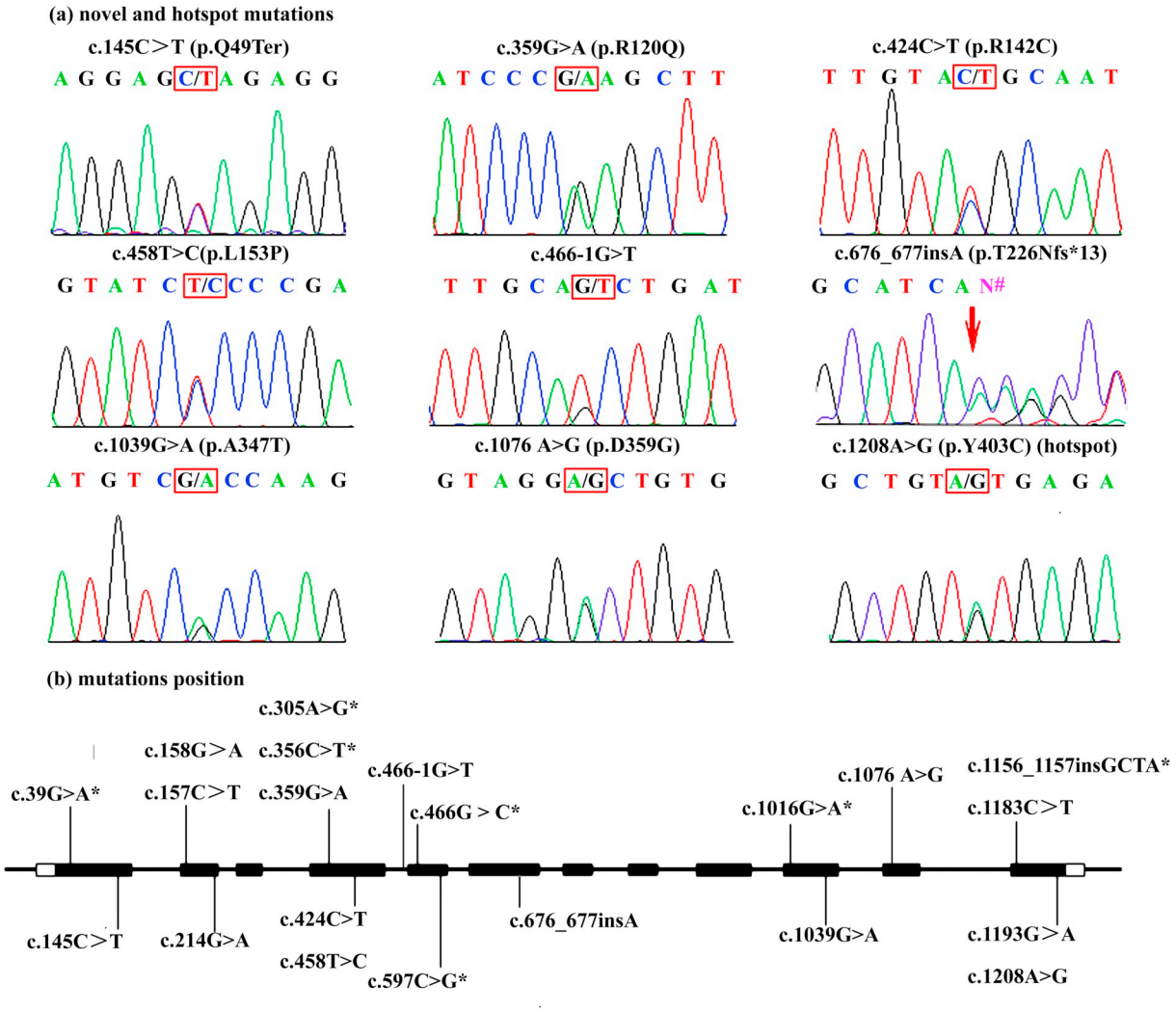

Fig. 1. *IVD* mutations analysis results. (a) Novel and hotspot *IVD* mutations in this study; (b) *IVD* mutation distribution in the Chinese population. Red square frame: missense mutations changes; red arrow: bases insertion position. N#: doublet of bases, including C/A A/C G/A C/G C/C T/C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

To date, few IVA cases had been reported in the Chinese population [4,6–9]. Recently, it was reported that p.Y403C is a potential hotspot mutation in Taiwanese [4]. However, the majority of reports on IVA involving Han Chinese patients (seven patients) are from Taiwan and Hongkong [4,7]. Only three reports are from Mainland China [6–9]. Which means, the previous study lack of data of Mainland China. In this study, we present eight IVA cases from Mainland China and describe their clinical findings, treatment, and response. This study provides a clinical basis of reference for future cases of IVA in the Chinese population. Here, eight novel mutations associated with IVA were identified, and the mutation p.Y403C was confirmed to be common in the Han Chinese population (both Mainland China and outside Mainland China).

Accumulation of isovaleric acid and its derivatives is associated with disease development. In healthy individuals, the plasma isovaleric acid concentration is < 10 μM, whereas it is 10–450 μM in IVA patients, reaching 600–5000 μM during acute episodes. High levels of metabolites damage multiple organs, making acute episodes attacks and multiple organ injuries happen. Similar to previous reports, acute metabolic attack, the main cause of sudden death due to IVA, was detected in our patients as the diagnostic clue. Two of our patients died during the initial catabolic episode in their early life, and the others had the metabolic attack between 1 day to 2 years old. This type of metabolic crisis attack is more likely to occur during infancy and early childhood [13], the latest metabolic crisis attack occurred at the age of 7 years in this study.

The most affected system due to the accumulation of toxic metabolite is the nervous system. The cause of brain cell damage is oxidative stress induced by metabolites, such as isovaleric acid [14]. Besides, isovaleric acid significantly inhibits Na, K-ATPase channel in brain cortical homogenate [15]. In a previous report, 44% of IVA patients had mild motor dysfunction, and 19% had cognitive deficits, suggesting that the patients' intelligence quotient was not related to the intensity of catabolic attack but to the age at diagnosis [13].In our study, nervous system injury is typical, the majority of patients exhibited lethargy, and some had epilepsy and coma. Also, two patients (Patient 1 and 2) experienced psychomotor retardation because of brain damage even after treatment. Patient 1 showed the moderate cognitive defect and mild motor development, whereas Patient 2 showed mild psychomotor retardation. This may because Patient 1 was diagnosed at a relatively late stage when she was 1 year and 6 months old, while Patient 2 received therapy much earlier (at 25 days old).

Other symptoms such as pancytopenia, pathoglycemia, cytopenia, hyperammonemia, and some rare symptoms including acute

Table 3
Comparison of codons and amino acids sequence of seven novel and one hotspot *IVD* mutations.

Variant	Codons and amino acids sequence of the mutant	Codons and amino acids sequence of wild-type
c.145C>T	codon: GAG GAG **T**AG AGG CAG	codon: GAG GAG **C**AG AGG CAG
(p.Q49Ter)	aa[1]: E E * N/A N/A	aa: E E **Q** R Q
c.359G>A	codon: ATA TCC C**A**A GCT TCC	codon: ATA TCC C**G**A GCT TCC
(p.R120Q)	aa: I S **Q** A S	aa: I S R A S
c.424C>T	codon: CTT GTA **T**GC AAT GGG	codon: CTT GTA **C**GC AAT GGG
(p.R142C)	aa: L V C N G	aa: L V **R** N G
c.458T>C	codon: AAG TAT C**C**C CCG AAG	codon: AAG TAT C**T**C CCG AAG
(p.L153P)	aa: K Y P P K	aa: K Y L P K
c.676_677insA	codon: GGC ATC A[2]**A**C AGC CTT	codon: GGC ATC A[2]CA GCC TTC
(p.T226Nfs*13)	aa: **G I N S L**	aa: G I **T A F**
c.1039G>A	codon: AAT GTC **A**CC AAG GCC	codon: AAT GTC **G**CC AAG GCC
(p.A347T)	aa: N V **T** K A	aa: N V **A** K A
c.1076A>G	codon: GCT AAG G**G**C TGT GCA	codon: GCT AAG G**A**C TGT GCA
(p.D359G)	aa: A K **G** C A	aa: A K **D** C A
c.1208A>G	codon: AAG CTG T**G**T GAG ATA	codon: AAG CTG T**A**T GAG ATA
(p.Y403C)	aa: K L **C** E I	aa: K L **Y** E I

Note: [1], amino acid; [2], insertion position; N/A, not available. The base and amino acid differences between wild-type and mutant are highlighted in bold. The splicing mutation (c.466-1G > T) is not listed above because it does not lead to amino acid changes directly.

pancreatitis, have been reported previously [16]. The hematological system is easy to be affected by toxic metabolites, pancytopenia and other cytopenias induced by myelosuppression [17] are common in IVA patients and were found in all patients of this study. Pathoglycemia is common, especially during acute episodes because the metabolic crisis attack is related to energy support system dysfunction. Three patients (Patient 3–5) had hypoglycemia due to physiological stress and fasting; hyperglycemia was not detected.

Due to similar clinical and biochemical phenotype, IVA can be easily misdiagnosed as pylori abnormalities, diabetic ketoacidosis, and bone marrow hematopoietic disorders [18]. In some patients, gastrostomy was performed because of persistent vomiting [6]. Thus, the differential biochemical diagnosis should be carried out. Blood C5-carnitine level assay is frequently used in newborn screening and differential diagnosis for IVA. It has been suggested that C5-carnitine has a prognostic value. However, a recent study revealed that urine IVG level is a more effective predictor of disease condition and prognosis [19]. In our study, all patients had high levels of blood C5-carnitine and urine IVG. However, the urine IVG levels in the patients who had severe phenotype and bad prognosis (Patient 5 and 6) were not more concentrated than other patients. It may because that urine samples were collected close to the time of their death; the precursor of IVG was not abundant because the patients did not have enough intake during that time.

Treatment for this disease is primarily aimed at reducing the formation of isovaleryl-CoA from leucine catabolism and thereby preventing acute episodes of attack. IVA patients require life-long dietary restrictions, for instance, leucine free intake combined with L-carnitine and glycine supplementation helps to reduce the level of toxic chemicals by enhancing the conversion of isovaleric acid to non-toxic conjugates which are excreted in urine [1,2,5]. Early diagnosis and treatment are crucial to reducing sudden deaths and benefiting the prognosis. In our patients, none of them was detected by newborn screening, two patients died in their neonatal period before diagnosis, two patients had psychomotor retardation, and other four patients responded well to treatment presented with normal developmental development and very little metabolic crisis times.

The human *IVD* gene is located on chromosome 15q14-15 and consists of 12 exons [20]. 92 mutations on *IVD* gene have been reported so far (http://www.hgmd.cf.ac.uk/ac/index.php). Among the pathogenic mutations, the distribution of hotspot *IVD* mutations varies among populations: p.A282V missense mutation is common in Caucasian IVA patients, especially in individuals with asymptomatic form [21]. Other hotspot mutations include p.G123R [22] for Caucasians in South Africa, p.R50T and p.Y403C for Taiwanese [4] and c.457-3_2CA > GG for Koreans [23].

So far, ten IVA patients have been reported in the Chinese population by genetic diagnosis [4,6–9]. Two of them were siblings [4],

Table 4
Amino acid alignment in IVD residues (highlighted in bold).

Species	p.Q49	p.R120	p.R142	p.L153	p.T226	p.A347	p.D359
Humans	GLSEE**Q**RQLRQ	VMEEIS**R**ASGAV	CINQLV**R**NGNEA	AQKEKY**L**PKLISG	PASRGI**T**AFIVE	YVYNV**A**KACD	GHCTAK**D**CAGVI
M. mulatta	GLSEE**Q**RQLRQ	VMEEIS**R**ASG - - - -	CVNQLV**R**NGNEA	AQKEKY**L**PKLTSG	PASRGI**T**AFIVE	YVYNV**A**KACD	GHCTAK**D**CAGV -
Mus musculus	GLNEE**Q**KQLRH	VMEEIS**R**ASGAVGL	CINQIV**R**NGNEA	AQKEKY**L**PKLISG	PASRGI**T**AFIVE	YVYNV**A**KACD	GHI IPK**D**CAGVI
Trubripes	GLTEE**Q**IQLRQ	VLEEMS**R**VSGGIAL	CINQMV**R**HANEK	- - - - -**Y**M**P**KLLTG	- HQRGI**T**AFIVE	YLYNV**A**RACD	GHVSTK**D**CAGVI
Danio rerio	GLTDD**Q**IQLRQ	IMEEIS**R**VSAAIAL	CINQLT**R**HG - - -	KQKEKY**M**PKLISY	- - ARGI**T**AFIVE	YLYNV**A**RACD	GHFSAK**D**CAGVI
Xenopus tropicalis	GLNDE**E**IALRQ	VVEEIS**R**ASAAVGL	CINQIV**R**NGNEA	AQKEKY**L**PKLISG	PASHGI**T**AFL VE	YLYMV**A**KAAD	GNVSNK**D**CAGVI

implying that a total of 18 alleles were studied. A total of 34 alleles were identified among the Chinese population (18 in previous and 16 in this study), and 21 mutations were found. The distribution of mutations is shown as Fig. 1b, exons 4 and 12 are the most frequently mutated region. Among the mutations, p.R53H accounts for 4 of 34 alleles (3 in previous [4,8], and 1 in this study), and p.Y403C accounts for 9 of 34 alleles (7 in previous [4,7] and 2 in this study). Thus p.Y403C is the most common mutation, whereas p.R53H is relatively common in this population. As the previous studies about IVA were mostly from Taiwan (six patients) and Hongkong (one patient), only three Mainland China patients were found previously. Thus this study provided evidence to support that the p.Y403C is the hotspot mutation in the Han Chinese population.

p.Y403C interferes the binding of FAD to enzyme IVD [4], resulting in mild and severe phenotypes. Among Chinese IVA cases with p.Y403C mutation, a boy with homozygous p.Y403C (who had a neonatal onset) died at 16 days old although was diagnosed and given IVA treatment [9]. Four patients with the heterozygous p.Y403C mutation had neonatal-onset form, and one had a late-onset form [4,7]. In this study, this mutation was identified in two cases (a severe case of Patient 4 and a mild case of Patient 8). Thus the genotype-phenotype relationship for p.Y403C is not yet clear. Seven novel missense mutations identified in this study are either highly or partially conserved among species.

In summary, this study reveals eight new mutations associated with IVA in the Chinese population. p.Y403C is the hotspot mutation in this population, which may be used for genetic diagnosis of this disease in the future. Further large-scale studies are required to investigate the full scope of mutations in disease-causing genes associated with IVA in the Chinese population.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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