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Case Report

Clinical features and *GCDH* gene variants in three Chinese families with glutaric aciduria type 1: A case series and literature review

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ABSTRACT

Aim: To analyze the clinical phenotype and genetic etiology of three cases of glutaric aciduria type 1 (GA1) in Chinese children.

Methods: We performed genetic and metabolic testing using tandem mass spectrometry (MS/MS) and gas chromatography–mass spectrometry (GC/MS), followed by trio whole-exome sequencing (trio-WES) and Sanger sequencing. A literature review on glutaric aciduria type 1 (GA1) in Chinese patients was also conducted.

Results: Sequencing results showed each case had compound heterozygous variants in *GCDH*(NM_000159.4): c.214C > G (p.Arg72Gly) and c.411C > G (p.Tyr137Term) (Case 1), c.214C > G (p.Arg72Gly) and c.1204C > T (p.Arg402Trp) (Case 2), and c.1228G > T (p.Val410Leu) and c.395G > A (p.Arg132Gln) (Case 3). These variants were inherited from their respective parents. Notably, the c.214C > G variant found in two children was a novel variant not previously reported. A review of the literature revealed that, clinically, the majority of patients experienced onset in infancy and early childhood (82%). Additionally, 38.36% were diagnosed through newborn screening, with the primary reasons for the initial visit being delayed development (32.43%) and infections (21.61%). The most common clinical manifestations included increased head circumference (77.19%) and motor developmental delay (65.15%). Biochemically, patients exhibited significant elevations in C5DC (98.51%) and C5DC/C8 (94.87%) in blood, as well as GA (94.37%) and 3OHGA (69.39%) in urine. Radiographically, patients showed a high prevalence of abnormalities in cranial MRI (86.15%) and EEG (73.33%). Genetically, 67 distinct *GCDH* gene variants were identified among 73 patients, with missense variants being the most prevalent type (73.97%). The most frequent variant was c.1244-2 A > C, observed in 17.12% of cases. Additionally, the majority of variant sites were located in exons 11 (25.37%) and 6 (22.39%).

Conclusion: *GCDH* variants were identified as the causative factors in the three children. The discovery of the novel variant (c.214C > G) expands the spectrum of pathogenic *GCDH* variants. These findings facilitate the diagnosis and treatment of affected children and provide a basis for genetic counseling and prenatal diagnosis for their families.

1. Introduction

Glutaric acidemia (GA) is a rare genetic metabolic disorder with three types identified to date. Glutaric aciduria type 1 (GA1, OMIM 231670) is an autosomal recessive neuro-metabolic disorder caused by variants in the glutaryl-CoA dehydrogenase (GCDH) gene. These variants disrupt the metabolism of lysine, hydroxylysine, and tryptophan, leading to the accumulation of glutaric acid (GA), 3-hydroxyglutaric acid (3OHGA), and glutaryl carnitine (C5DC) in body tissues [1]. The structural similarity between elevated levels of glutaric acid and 3-

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hydroxyglutaric acid in brain tissue and the excitatory neurotransmitter glutamate can lead to the overactivation of glutamate receptors. This overactivation inhibits the synthesis of gamma-aminobutyric acid (GABA), resulting in diminished inhibitory neurotransmission and the induction of oxidative stress responses. Additionally, both glutaric acid and 3-hydroxyglutaric acid can inhibit the activity of neuronal alpha-ketoglutarate dehydrogenase, leading to disturbances in energy metabolism and neuronal damage [2]. The accumulation of these metabolites can result in acute striatal damage, muscle tone disorders, and clinical manifestations including spastic paralysis, seizures, acidosis, and hyperammonemia [3]. Glutaric aciduria type 2 (GA2, OMIM 231680) is an autosomal recessive genetic disease that affects the metabolism of fatty acids, amino acids, and choline. It is also known as multiple acyl-CoA dehydrogenase deficiency (MADD) [4]. The most common causes are variants in the genes encoding electron transfer flavoprotein dehydrogenase (ETFDH) or electron transfer flavoprotein (ETF), which impair the function of multiple organs, including the heart, liver, brain, and skeletal muscles [5]. GA2 has a lower incidence in newborns globally compared to GA1 [6]. Glutaric aciduria type 3 (GA3, OMIM 231690) is a rare metabolic disorder caused by variants in the *SUGCT* (C7orf10) gene, encoding succinate-hydroxymethylglutarate CoA-transferase. Children with GA3 usually have no clinical symptoms or consistent clinical manifestations. Compared to GA1 and GA2, GA3 is less known and rarely occurs, often being considered a likely "non-disease" [7].

The incidence of GA1 varies by ethnicity and region. The global incidence is approximately 1:100,000; however, it is significantly higher among specific populations such as the Pennsylvania Amish, Irish, Canadians, and Black South Africans, ranging from 1:200 to 1:2，300 [8–10]. The incidence rate in the Chinese population is less well understood, with some studies indicating an incidence of 1:185,971 [11] newborns in China. Rates in southern Zhejiang Province (1:221,053) are much lower compared to Quanzhou City (1:47,044) and Jiangsu Province (1:89,335) [1]. In northern Xi'an City, the incidence rate is 1:73,076 [9].

This study investigated three suspected cases of GA1 in Gansu Province through blood and urine mass spectrometry, cranial MRI, trio-WES, and Sanger sequencing to clarify their diagnosis and molecular genetic causes. It also reviewed the literature on Chinese patients with GA1 reported domestically to provide references for the diagnosis and treatment of this disease.

2. Case report

2.1. Case 1

The patient, a 15-month-old boy born at term via spontaneous vaginal delivery, presented to the local pediatrician with intermittent convulsions and developmental delays for 4 months. He had been previously diagnosed with epilepsy. The patient can pronounce single words such as "Dad" and "Mom". His birth weight was 3.35 kg. He has a history of birth asphyxia, but his Apgar score is available. The physical examination showed a head circumference of 48.6 cm (ref. P_{25}: 46.0 ~ P_{75}: 47.7 cm) and hypotonia in all four limbs. Other physical examinations showed no significant abnormalities. The Gesell Developmental Diagnostic Scale (GDS) report suggested a mild developmental delay. Brain MRI (Fig. 1 A-C) revealed widened extracerebral spaces at the bilateral frontal-parietal regions, widened lateral sulci, and abnormal signals around the bilateral basal ganglia and lateral ventricles. The electroencephalogram (EEG) was abnormal (Fig. 1 D-G), showing generalized tonic-clonic seizures. During the same period, the background EEG showed a gradual evolution from widespread spiking rhythmic emission to widespread spiking slow complex wave emission, lasting for about 1 min and 20 s. This was followed by a phase of widespread low voltage, with the background rhythm returning to normal after about 1 min.

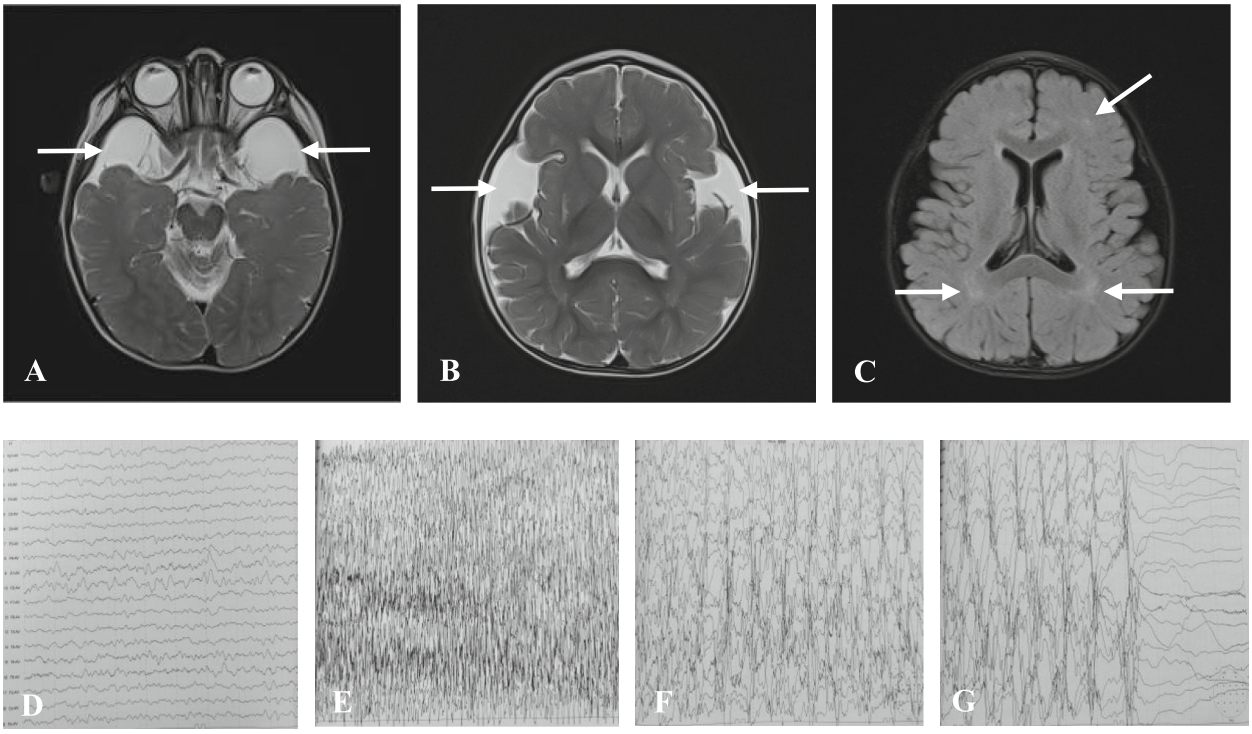

Fig. 1. Cranial MRI (A-C) and EEG (D-G) of Case 1.
Widening of the subarachnoid space in the bilateral temporal regions (see white arrows). B) Widening of the lateral fissure pools bilaterally (see white arrows). C) Multiple abnormal high signals around the bilateral lateral ventricles (see white arrows). D) No abnormal wave discharge observed during the interictal period. E) Onset of seizure showing widespread spike-wave discharge. F) Gradually evolves into widespread spike-slow and multiple spike-slow wave discharges. G) Post-seizure, there was a transition from tonic-clonic seizure lasting approximately 50 s to clonic seizure of the limbs lasting about 30 s, followed by a return to normal.

2.2. Case 2

The patient, a 9-month-old boy born at term via spontaneous vaginal delivery, presented with "developmental regression for 1 month." One month prior, he experienced a fever and diarrhea, after which he was unable to sit independently, had poor head control, and could not roll over. Occasionally, he experienced have brief episodes of staring, lasting about 2–3 s, without convulsions. No family history of hereditary diseases was reported. His birth weight was 3.24 kg. Physical examination showed a weight of 10.8 kg (ref. P_{25}: 8.7 ~ P_{75}: 10.1 kg), a length of 74.0 cm (ref. P_{25}: 71.4 ~ P_{75}: 74.7 cm), and a head circumference of 46.5 cm (ref. P_{25}: 44.3 ~ P_{75}: 46.0 cm). He had weak neck muscles, poor head control, unstable head holding, and hypotonia in all four limbs. Other physical examinations showed no significant abnormalities. The Gesell Developmental Diagnostic Scale (GDS) report indicated a severe developmental delay. Brain MRI revealed bilateral frontal-temporal atrophy, reduced white matter, bilateral subdural effusion in the temporal region, widened lateral fissures, and abnormal signals around the bilateral basal ganglia and anterior horns of the lateral ventricles (Fig. 2). EEG showed low-amplitude sharp waves and sharp-slow complex waves in the bilateral occipital and right frontal-temporal regions.

2.3. Case 3

The patient, a 2-month-old male infant, presented to a local pediatrician with elevated levels of glutaryl carnitine (C5DC) detected by tandem mass spectrometry newborn screening. The physical examination of the patient revealed a weight of 6.1 kg (ref. P_{25}: 5.4 ~ P_{75}: 6.2 kg), a length of 61.5 cm (ref. P_{25}: 57.5 ~ P_{75}: 60.4 cm), and a head circumference of 41 cm (ref. P_{25}: 38.3 ~ P_{75}: 39.9 cm); other examinations showed no significant abnormality.

The three patients were not related to each other by consanguinity. Informed consent was obtained from all parents, and the study was reviewed and approved by the hospital's Ethics Committee (Approval No. 30 of 2018).

3. Methods

3.1. Genetic metabolic disease mass spectrometry analysis

Blood and urine samples were collected, and amino acids and acylcarnitines in the blood were quantified using the Waters TQD tandem mass spectrometer and NeoBase™ Non-derivatized MSMS Kit (PerkinElmer, USA). Urinary organic acids were quantified using gas chromatography–mass spectrometry (GC/MS) with the Shimadzu GC/MS system (Japan).

3.2. Trio whole-exome sequencing (trio-WES)

Three to five milliliters of peripheral venous blood were collected from each child and their parents, using EDTA as an anticoagulant, and stored at 4 °C. DNA was extracted using the TIANGEN TIANamp Genomic DNA Kit (Tiangen Biotech, China), and its purity and concentration were measured with a Nanodrop 2000 spectrophotometer (Thermo Fisher, USA). The DNA concentration was adjusted to 50–250 ng/μL and stored at −20 °C. Family-based whole-exome sequencing (WES) was conducted, achieving an average depth of 220× and coverage >20× across all regions. Sequencing was performed using the xGen Exome Research Panel v2.0 capture probes (IDT, USA) in PE150 mode on the Illumina NovaSeq 6000 platform (San Diego, CA). Sequencing coverage was ≥99%. The raw sequencing data were screened to remove reads that did not meet quality control (QC) criteria and to exclude duplicates. The remaining reads were then subjected to statistical analysis, and candidate variants were identified using GATK v3.70 (Genome Analysis Toolkit).

3.3. Bioinformatics analysis

Candidate variants were queried in databases including The Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/ac/index.php), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and PubMed (https://pubmed.ncbi.nlm.nih.gov/) to assess their novelty. Bioinformatics tools, including PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/), were employed to evaluate the potentially deleterious effects of these variants. Amino acid sequences were queried in the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/), and sequence conservation analysis for the *GCDH* gene variant c.214C > G (p.Arg72Gly) was conducted using T-Coffee (https://tcoffee.crg.eu/apps/tcoffee/do:regular). SWISS-MODEL (https://swissmodel.expasy.org/) was utilized to predict and analyze the three-dimensional structure of the mutant protein. InterVar (http://wintervar.wglab.org/) was employed to assess the pathogenicity of all variants based on the ACMG (American College of Medical Genetics and Genomics) standards and guidelines [12].

3.4. Sanger sequencing verification

Specific primers for the candidate variant sites in the *GCDH* (NM_000159.4) gene were designed using the NCBI Primer-BLAST online primer design database (https://www.ncbi.nlm.nih.gov/primer-blast) (Table 1). PCR amplification of DNA fragments from the region of the variant site was performed. The PCR products were purified using a Tiangen Midi Purification Kit (Tiangen Biotech, Beijing, China)

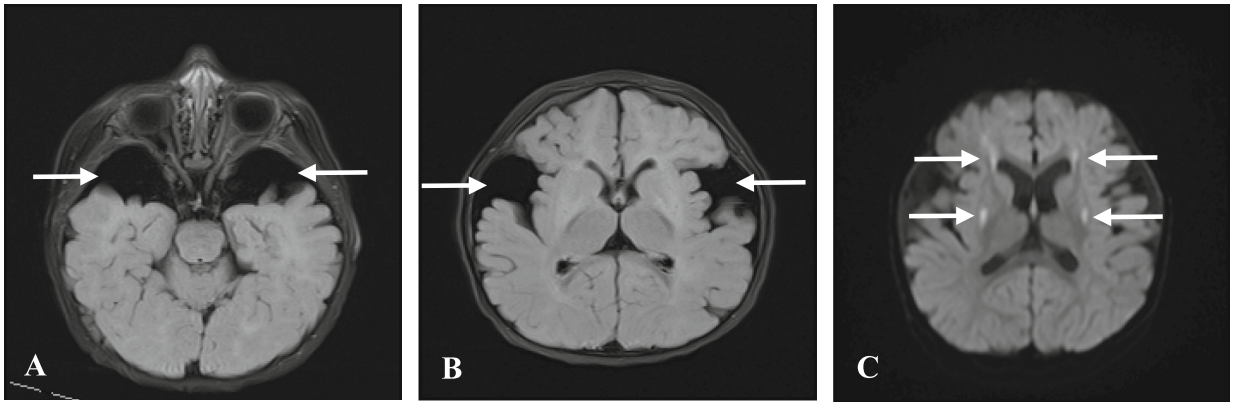

Fig. 2. Cranial MRI of Case 2.
Bilateral frontal-temporal atrophy, decreased cerebral white matter, subdural effusion in both temporal regions. B) Widened cerebral fissures. C) Abnormal signals near the bilateral basal ganglia and anterior horns of the lateral ventricles. (see white arrows).

Table 1
Sanger sequencing primers for the *GCDH* gene.

Case	Variant site	Forward primer sequence(5′-3′)	Reverse primer sequence(5′-3′)	Product length(bp)
1	c.214C > G	AATTCCCCTTCCCAGCCTC	TTGAGGTGGGGTGGCTATG	224
	c.411C > G	TGCCTCCTTGTGTGTCCTTA	TGTTCCTCGCTGCCATAGG	243
2	c.214C > G	AATTCCCCTTCCCAGCCTC	TTGAGGTGGGGTGGCTATG	224
	c.1204C > T	CAGGATCCCCAGTCCTTGTT	GAGGTCCAGCTCCTACCTTC	218
3	c.395G > A	TCTGAAAGTGGCTGTGGAGA	ACTCATCGCCGACCTGTAG	248
	c.1228G > T	ATCCCCAGTCCTTGTTACCC	TGAGGTCCAGCTCCTACCTT	215

from 2% agarose gels and then bi-directionally sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on an ABI-3500DX Genetic Analyzer (Applied Biosystems, USA). Finally, the Sanger sequencing results were aligned and analyzed with the *GCDH* (NM_000159.4) reference sequence using the SeqMan v7.1 sequence alignment software.

3.5. A review of the literature

A literature search was conducted using the "China National Knowledge Infrastructure" (CNKI, https://www.cnki.net/), "Wanfang Database" (http://med.wanfangdata.com.cn/), and "PubMed," employing the Chinese search term "Glutaric Aciduria Type 1" and the English search terms "Glutaric Aciduria Type 1" AND "*GCDH*" AND "Chinese." The search covered the period from January 2012 to May 2023. A retrospective analysis was conducted on 14 papers (including this study) reporting a total of 73 cases [1,3,6,13–22], which documented the genotype and clinical phenotype characteristics of Chinese patients with GA1.

4. Results

4.1. Genetic metabolic disease mass spectrometry analysis

The blood levels of C5DC, the C5DC/C8 ratio, the C5DC/C3 ratio, as well as urinary glutaric acid and 3-hydroxyglutaric acid, were elevated in all patients except for the C5DC/C8 ratio in Patient 2. (Table 2).

4.2. Trio-WES and sanger sequencing verification

Trio-WES results revealed that each case harbored compound heterozygous variants in *GCDH* (NM_000159.4): c.214C > G (p.Arg72Gly) and c.411C > G (p.Tyr137Term) (Case 1), c.214C > G (p.Arg72Gly) and c.1204C > T (p.Arg402Trp) (Case 2), and c.1228G > T (p.Val410Leu) and c.395G > A (p.Arg132Gln) (Case 3). Sanger sequencing validation confirmed that the variants were inherited from the respective fathers and mothers of the three children (Fig. 3A), consistent with the autosomal recessive (AR) inheritance pattern.

4.3. Pathogenicity analysis

Patients in Cases 1 and 2, who are unrelated by consanguinity, both carried the c.214C > G variant. The c.214C > G variant has not been recorded in the HGMD, ClinVar, and PubMed databases, indicating its novelty. This missense variant results in the substitution of arginine at position 72 with glycine (p.Arg72Gly). This variant is located in an exon functional area or pathogenic hotspot (presence of >3 deleterious variants within 10 bp around the variant) (PM1) and has a frequency of <0.0005 in normal population databases, classifying it as a low-frequency variant (PM2_Supporting). In the context of autosomal recessive (AR) disease, a likely pathogenic variant in the trans position (confirmed in trans) was detected in each of the two families in this study: c.411C > G (case 1) and c.1204C > T (case 2). Therefore, the evidence score for PM3 is 1 (PM3_Moderate). Bioinformatics analyses using tools such as PolyPhen-2, PROVEAN, and Mutation Taster indicate deleterious effects (PP3). This variant affects an arginine that is highly conserved across species at position 72 (Fig. 3B) and protein modeling revealed that the variant protein has one fewer hydrogen bond (Fig. 3C). According to the ACMG guidelines [12], the variant is classified as likely pathogenic: PM1 + PM2_Supporting+PM3_Moderate+PP3. Other variants, such as c.411C > G, c.1204C > T, and c.395G > A, are documented in the HGMD database. Although c.1228G > T is not listed in HGMD, variants with other amino acid changes at the same position (e.g., c.1228G > A, p.Val410Met) are recorded. These variants are preliminarily classified as follows: c.411C > G (p.Tyr137*) as likely pathogenic (PVS1 + PM2_Supporting); c.1204C > T (p.Arg402Trp) as likely pathogenic (PS3 + PM1 + PM2_Supporting+PP3); c.395G > A (p.Arg132Gln) as likely pathogenic (PM3_Strong+PM1 + PM2_Supporting+PP3); and c.1228G > T (p.Val410Leu) as likely pathogenic (PM1 + PM2_Supporting+PM5 + PP3).

4.4. Clinical features and GCDH variant characteristics of Chinese GA1 patients

The clinical characteristics and *GCDH* variant features of Chinese GA1 patients were analyzed through a literature review, with 14 reports including this study covering 73 patients and 146 variants (Table 3).

1) Clinical Features: proportion of males at the onset of GA1 was slightly higher at 63.27%. The majority of cases presented in infancy and early childhood (82.00%), with only a small percentage of cases emerging in adulthood (4.00%). Among the 50 patients with documented ages, 2 were diagnosed in adulthood. One such patient was an 18-year-old female [14], who sought diagnosis after her sister was diagnosed with GA1. She presented with fever and diarrhea at approximately 1 year of age and subsequently had residual motor deficits. At that time, cranial magnetic resonance imaging (MRI) revealed

Table 2
The mass spectrometry analysis and genotype of three GA1 children.

Case	Age at diagnosis	Head circumference	MS/MS			GC/MS		Genotypes		
	(months)	(cm)	C5DC (μmol/L)	C5DC/C8	C5DC /C3	GA (μmol/L)	3OHGA (μmol/L)	Child	Father	Mother
1	15	48.6	5.2	26	1.69	957.26	12.65	c.214C > G/c.411C > G	c.214C > G	c.411C > G
2	9	46.5	0.85	8.5	1.18	1012.64	7.33	c.214C > G/c.1204C > T	c.214C > G	c.1204C > T
3	2	41	3.6	120	3.19	607.29	6.74	c.1228G > T/c.395G > A	c.395G > A	c.1228G > T

Notes: MS/MS tandem mass spectrometry, GC/MS gas chromatography–mass spectrometry, C5DC glutarylcarnitine (ref. 0.05–0.41), C5DC/C8 glutarylcarnitine/capryloyl carnitine (ref. 0.83–14.00), C5DC/C3 glutarylcarnitine/propionylcarnitine (ref. 0.03–0.48), GA glutaric acid (ref. 0.00–8.44), 3OHGA 3-hydroxyglutaric acid (ref. 0.00–0.50).

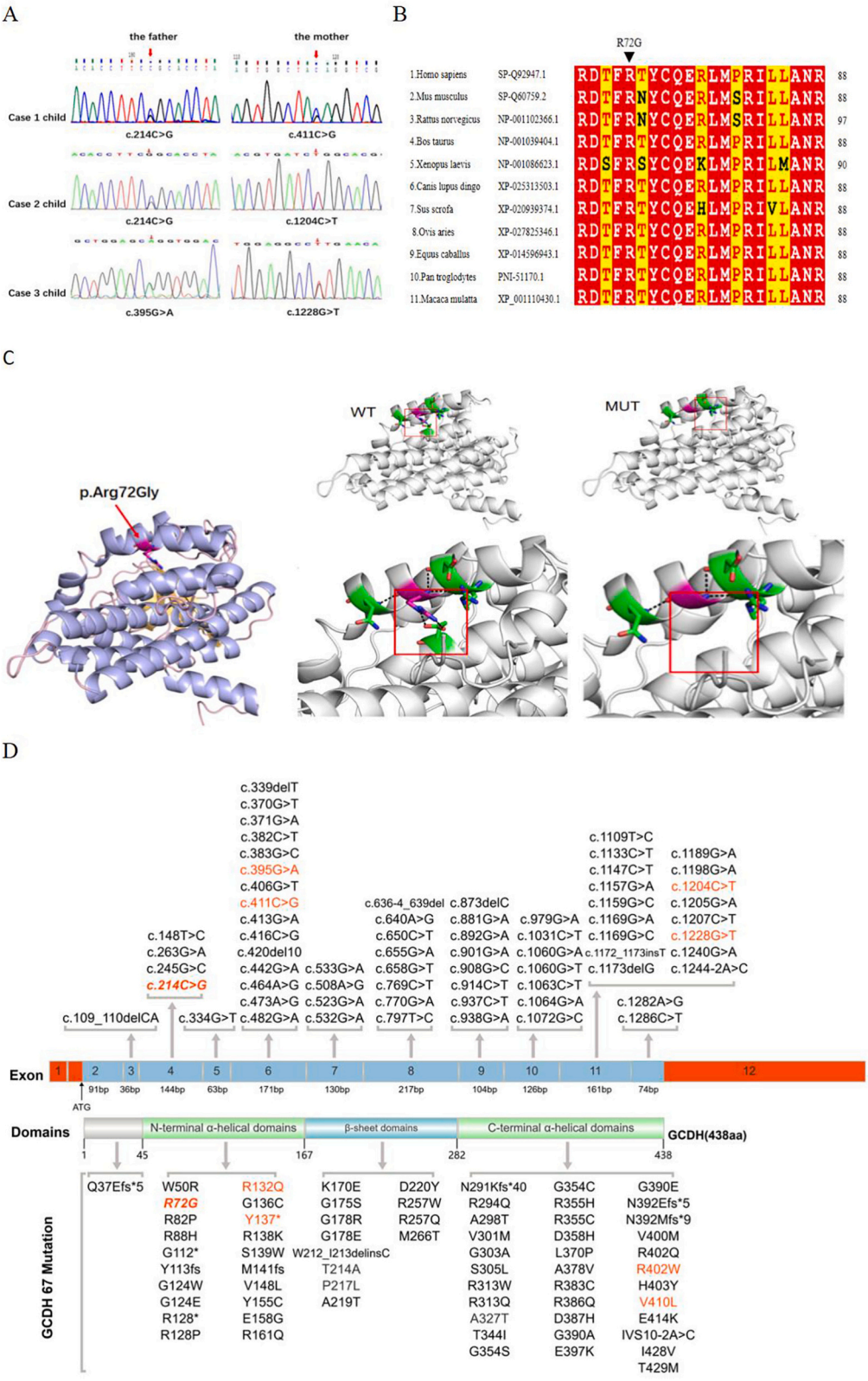

Fig. 3. Sanger Sequencing and Bioinformatics Analysis of the Patients.
GCDH sequencing results revealed that each case had compound heterozygous variants. These compound heterozygous variants were inherited from their clinically normal mothers and fathers, as indicated by red arrows. B) Conservation analysis of variant sites across different species. Arginine at position 72 is highly conserved across species, as indicated by black triangles. C) Protein modeling showed the normal GCDH protein structure and the specific location of the p.Arg72Gly variant. It also showed that the variant protein is reduced by one hydrogen bond in comparison to the normal protein (indicated by red box). D) Distribution of *GCDH* variants in exons and protein domains in GA1 patients. (Exon: Red bands show 5'-UTR and 3'-UTR regions and blue bands show coding regions. Domains: Red display indicates variants from this article, with red bolded and slanted indicate new variant.)

Table 3
Clinical, Biochemical, Radiographic and Genetic Variant Features of Chinese GA1 Patients.

	Our study ($N = 3$)	Literature ($N \leq 70$)*	Total ($N \leq 73$)*
Clinical			
Sex			
Male	3/3 (100.00)	28/46 (60.87)	31/49 (63.27)
Female	0/3(0.00)	18/46 (39.13)	18/49 (36.73)
Age of onset			
≤1 year old	2/3 (66.67)	28/47 (59.57)	30/50 (60.00)
1–3 years old (include 3 years old)	1/3 (33.33)	10/47 (21.28)	11/50 (22.00)
3–18 years old	0/3(0.00)	7/47(14.89)	7/50 (14.00)
≥18 years old	0/3(0.00)	2/47(4.26)	2/50 (4.00)
Newborn screening			
Yes	1/3 (33.33)	27/70 (38.57)	28/73 (38.36)
No and not mentioned	2/3 (66.67)	43/70 (61.43)	45/73 (61.64)
Reasons for first visit			
Developmental delay	1/3 (33.33)	11/34 (32.35)	12/37 (32.43)
Infections	1/3 (33.33)	7/34(20.59)	8/37 (21.62)
Diarrhea	0/3(0.00)	5/34(14.71)	5/37 (13.51)
Seizures	1/3 (33.33)	3/34(8.82)	4/37 (10.81)
Head circumference increased	0/3(0.00)	3/34(8.82)	3/37 (8.11)
Overeating or intracranial hemorrhage or rash or vaccination or vomiting	0/3(0.00)	5/34(14.71)	5/37 (13.51)
The clinical phenotype			
Head circumference increased	3/3 (100.00)	41/54 (75.93)	44/57 (77.19)
Delayed motor development	3/3 (100.00)	40/63 (63.49)	43/66 (65.15)
Seizures	3/3 (100.00)	25/58 (43.10)	28/61 (45.90)
Hypotonia	2/3 (66.67)	27/68 (39.71)	29/71 (40.85)
Biochemical			
Tandem mass spectrometry (MS/MS)			
Glutarylcarnitine (C5DC) ↑	3/3 (100.00)	63/64 (98.43)	66/67 (98.51)
Glutarylcarnitine/capryloyl carnitine (C5DC/C8) ↑	2/3 (66.67)	35/36 (97.22)	37/39 (94.87)
Free carnitine (C0) Normal	0/3(0.00)	20/36 (55.56)	20/39 (51.28)
Free carnitine (C0) ↓	2/3 (66.67)	16/36 (44.44)	18/39 (46.15)
Free carnitine (C0) ↑	1/3 (33.33)	0/36(0.00)	1/39 (2.56)
Gas chromatography-mass spectrometry (GC/MS)			
Glutaric acid (GA) ↑	3/3 (100.00)	64/68 (94.12)	67/71 (94.37)
3-Hydroxyglutaric acid (3OHGA) ↑	3/3 (100.00)	31/46 (67.39)	34/49 (69.39)
Radiographic			
Cranial magnetic resonance imaging (MRI) Abnormal	3/3 (100.00)	53/62 (85.48)	56/65 (86.15)
Electroencephalography (EEG) Abnormal	3/3 (100.00)	8/12(66.67)	11/15 (73.33)
Genetic Variant			
Type of variant			
Missense variant	5/6 (83.33)	103/140 (73.57)	108/146 (73.97)
Splice site variant	0/6(0.00)	25/140 (17.86)	25/146 (17.12)
Frameshift variant	0/6(0.00)	8/140(5.71)	8/146 (5.48)
Nonsense variant	1/6 (16.67)	4/140(2.86)	5/146 (3.42)
Variation frequency[a]			
c.1244_2 A > C (IVS10-2 A > C)	0/6(0.00)	25/140 (17.86)	25/146 (17.12)
c.532G > A (G178R)	0/6(0.00)	5/140(3.57)	5/146 (3.42)
c.1147C > T (R383C)	0/3(0.00)	5/140(3.57)	5/146 (3.42)
c.416C > G (S139W)	0/6(0.00)	5/140(3.57)	5/146 (3.42)
c.1064G > A (R355H)	0/6(0.00)	5/140(3.57)	5/146 (3.42)
c.395G > A (R132Q)	1/6 (16.67)	4/140(2.86)	5/146 (3.42)

* Number of cases with indicated feature/total number for whom we have relevant information(% in parentheses).
[a] Only the top 2 variant frequencies are listed among the 67 variants.
↑ Increase.

abnormalities. Despite being evaluated for inherited metabolic diseases on two separate occasions (at 16½ and 18 years of age), no significant abnormalities were found. Currently, the patient exhibits no notable issues aside from claudication in the left lower limb. The other case involved a 51-year-old male patient [16] who presented with a deep coma following alcohol abuse and binge drinking. Cranial MRI revealed cerebral infarction. At the time of diagnosis, both patients demonstrated normal intelligence. The primary reasons for the initial diagnosis were developmental delay (32.43%) and infection (21.62%). Additionally, overeating, intracranial hemorrhage, rash, vaccinations, and vomiting may have contributed to the initial visit. Clinical manifestations included increased head circumference (77.19%), motor developmental delay (65.15%), seizures (45.90%), and hypotonia (40.85%). Of the 73 patients, 5 clearly reported experiencing an encephalopathic crisis, triggered by infection in 4 cases and diarrhea in 1 case.

2) *GCDH* Variant Characteristics: Of the 146 variants, missense variants were the most common, constituting 73.97%, followed by splice-site variants at 17.12%. A total of 67 variants were identified in 73 patients. The c.1244-2 A > C variant had the highest frequency, occurring at 17.12%, and was the most prevalent splice-site variant. The second highest frequency variants were c.532G > A, c.1147C > T, c.416C > G, c.1064G > A, and c.395G > A, each accounting for 3.42%, and they were the most common missense variants. Additionally, exon 11 had the highest distribution of variant sites at 25.37%, followed by exon 6 at 22.39%. The variants identified in this study are located in exons 4, 6, and 11. Specifically, R72G, R132Q, and Y137* are situated in the N-terminal regions of the protein domains, while R402W and V410L are found in the C-terminal regions. (Fig. 3D).

4.5. Treatment and follow-up management

Based on the combined analysis of the children's medical histories,

clinical symptoms, auxiliary examinations, and genetic diagnoses, all three cases were confirmed as glutaric aciduria type 1. Cases 1 and 2 have been receiving long-term treatment with oral L-carnitine, vitamin D, and a special formula diet that is free from lysine and low in tryptophan. Currently, both children show significant clinical improvement, with no epileptic seizures and motor and speech development that is comparable to their peers. Case 3, after discontinuing drugs for two months, was treated three times (at 8 months old, 1 year and 2 months old, and 1 year and 8 months old) for acute onset of pneumonia and bronchitis at external hospitals. Cranial MRI at 8 months old showed widening of the subarachnoid space at the bilateral frontal-temporal-parietal regions and bilateral cerebral atrophy. During the course of the disease, the child exhibited progressive increase in head circumference, increased muscle tone, limb seizures, tongue-biting injuries, and abnormal EEG findings. Indicators related to the final hospitalization (at 1 year and 8 months old) revealed multiple organ damage, including electrolyte disturbances, hypoalbuminemia, liver and kidney damage, and anemia. The child subsequently died five days later.

5. Discussion

GCDH, the causative gene for GA1, is located on chromosome 19p13.13, spanning approximately 7 kb and consisting of 12 exons (of which the latter 11 are coding exons [23]) and 10 introns, encoding 438 amino acids [6,17]. To date, the Human Gene Mutation Database (HGMD) has recorded 304 variants in this *gene*, with missense variants being the most common (76.64%), followed by frameshift variants (8.88%) and splice site variants (6.58%). This study found that in the Chinese population, missense variants are also the most common, frameshift variants are relatively stable, and splice site variants are slightly more prevalent compared to those recorded in HGMD. Additionally, *GCDH* variants exhibit genetic heterogeneity, with common variants varying among different geographical and ethnic populations. For instance, the c.1093G > A variant is found in the Irish population [24,25], c.1204C > T is prevalent in the European population [24,26,27], c.298C > T is common in Australia [24], and c.914C > T is observed in Japan [28].Common variants in Taiwan and Hong Kong, China, include c.1244-2 A > C [25,29], which is also reported as a hotspot variant in southern China [1,6,9]. Both Case 1 and Case 2 in this study harbor the variant c.214C > G in exon 4, a novel variant identified in children from Gansu, China, thereby expanding the spectrum of pathogenic variants in *GCDH*.

The *GCDH* gene encodes glutaryl-CoA dehydrogenase (GCDH), which resides in the mitochondrial matrix as a homotetramer consisting of four identical monomers. Each monomer is composed of a precursor protein of 438 amino acids and includes three structural domains: the N-terminal α-helical domain (positions 45–167, containing six α-helices, A to F), the middle β-sheet domain (positions 168–281, with seven β-sheet structures, 1 to 7), and the C-terminal α-helical domain (positions 282–438, containing five α-helices, G to K) [6,23]. The initial 44 amino acids of the N-terminus function as a mitochondrial targeting signal and are cleaved after mitochondrial entry [23]. Within the mitochondria, the three domains interact with the coenzyme flavin adenine dinucleotide (FAD) to form the tetramer, facilitating the enzymatic activity of GCDH [30]. In our study, protein modeling revealed that the novel variant (p. Arg72Gly) present in Cases 1 and 2 results in one fewer hydrogen bond compared to the wild type, potentially impacting protein function.

The clinical manifestations of glutaric aciduria type 1 (GA1) are primarily characterized by elevated levels of organic acids, such as glutaric acid and 3-hydroxyglutaric acid, with a predominant impact on the nervous system. Children typically exhibit symptoms between 3 months and 3 years of age, presenting with a highly variable clinical phenotype during infancy and early childhood. This may include macrocephaly [6], movement disorders, muscle tone disturbances, and epileptic seizures [31]. Without treatment, 80% to 90% of infants may suffer irreversible acute striatal damage during the vulnerable period of brain development (mostly between 3 and 36 months), due to acute encephalopathic crises triggered by infections, fever, or surgical interventions. This striatal damage often leads to complex movement disorders, primarily characterized by muscle tone disorders and/or chorea, with superimposed muscular hypotonia. Severe movement disorders can evolve into persistent muscle tone disorders, potentially akin to akinetic-rigid parkinsonism or spasticity, which can significantly impact the life expectancy of these children [32,33]. Macrocephaly is one of the earliest and most common clinical manifestations [1]. In our study, 73 Chinese children mainly presented with increased head circumference (77.19%), motor developmental delay (66.15%), seizures (45.90%), and hypotonia (40.85%) (Table 3). Both Case 1 and Case 2 exhibited these clinical manifestations before diagnosis. Therefore, it is crucial to give significant attention to patients presenting with specific neurological abnormalities such as increased head circumference, seizures, and developmental delays, and to focus on screening for GA1. Meanwhile, timely screening before the onset of symptoms is strongly recommended to facilitate the early diagnosis of GA1.

GA1 is an autosomal recessive metabolic disorder, where most infants are either asymptomatic or present with nonspecific neurological symptoms, such as hypotonia and delayed motor development, which makes clinical identification challenging [32]. The implementation of tandem mass spectrometry (MS/MS) and gas chromatography–mass spectrometry (GC/MS) in newborn metabolic disease screening is essential for the early detection of GA1, enabling timely identification and effective prevention of neurological symptoms in 80%–90% of affected children [31]. Unfortunately, in our study, Cases 1 and 2 were not screened for neonatal inherited metabolic disorders at birth and were diagnosed only after presenting with epileptic seizures and developmental delays affecting the nervous system at 1 year and 8 months of age, respectively.

GA1 patients frequently exhibit abnormal metabolic indicators, including elevated blood C5DC, C5DC/C8, and/or C5DC/C3 ratios, as well as elevated urinary glutaric acid (GA) and 3-hydroxyglutaric acid (3OHGA), along with abnormal cranial MRI and EEG findings [31]. Consistent with these findings, our study observed significantly elevated biochemical indicators in Chinese GA1 patients, with most also exhibiting radiographic abnormalities (Table 3). Genetic diagnosis is essential for evaluating patients with normal urinary 3-hydroxyglutaric acid concentrations [20]; additionally, genetic variant analysis is crucial for genetic counseling, prenatal screening, and early postnatal diagnosis, offering a sensitivity of 98%–99% [32]. Our study, utilizing metabolic disease mass spectrometry analysis (blood/urine) in conjunction with whole exome sequencing (WES), identified specific biochemical abnormalities and compound heterozygous *GCDH* variants that were consistent with the clinical phenotype, thereby definitively diagnosing GA1.

Currently, there is no cure for GA1; however, dietary intervention involving lysine restriction and supplementation with levocarnitine (L-carnitine) can reduce the incidence of encephalopathic crises in infants and young children by approximately two-thirds [6]. If treatment is initiated according to guidelines before the onset of symptoms during the newborn period, it can effectively prevent neurological damage and significantly reduce the incidence of the disease in these patients [25,32]. In our study, Cases 1 and 2 demonstrated favorable clinical outcomes following timely intervention upon diagnosis. However, Case 3, although diagnosed promptly after an abnormality was detected through newborn screening, may have succumbed to an encephalopathic crisis resulting from the discontinuation of medication.

6. Conclusions

In conclusion, newborn metabolic disease screening is crucial for the early diagnosis and treatment of GA1. For symptomatic patients, pediatricians should perform comprehensive examinations rather than relying solely on clinical phenotypes. Integrating genetic metabolic disease mass spectrometry analysis with genetic analysis facilitates a

timely and accurate diagnosis. Our study identified three cases of GA1, which allowed for timely and effective treatment. Additionally, we identified the new variant (c.214C > G) in the *GCDH* gene in two patients, expanding the spectrum of pathogenic variants and providing a basis for future pregnancies, prenatal diagnosis, and preventive measures for the patients' families. However, our study has limitations. Firstly, we did not conduct further functional validation or investigate the pathogenic mechanism of the novel variant (c.214C > G). Additionally, the small sample size of diagnosed GA1 cases limits our ability to establish a definitive relationship between this variant and its prevalence in our region.

CRediT authorship contribution statement

Yunxi Chen: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Qinghua Zhang:** Writing – review & editing, Visualization, Funding acquisition. **Lei Cao:** Methodology, Visualization. **Xuan Feng:** Conceptualization. **Pengwu Lin:** Investigation. **Shaohua Zhu:** Methodology. **Furong Liu:** Methodology, Investigation. **Xing Wang:** Data curation, Validation. **Shengju Hao:** Supervision, Validation. **Yafei Cao:** Investigation. **Hongyan Wang:** Conceptualization. **Yali Ni:** Conceptualization, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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