Combined Malonic and Methylmalonic Aciduria Due to ACSF3 Variants Results in Benign Clinical Course in Three Chinese Patients

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Introduction: Combined malonic and methylmalonic aciduria (CMAMMA) is a rare metabolic disease caused by biallelic variants in ACSF3 gene. The clinical phenotype is highly heterogeneous in this disorder, ranging from asymptomatic to severe symptoms. No cases with CMAMMA were reported in China.

Materials and Methods: In this study, three Chinese pediatric patients were diagnosed with CMAMMA unexpectedly while being treated for other ailments. To better characterize CMAMMA in a Chinese population, we made a multidimensional analysis with detailed clinical phenotype, semi-quantitative detection of urine organic acid, and analysis of ACSF3 gene variants.

Results: The clinical presentation of these patients is quite different; their main complaints were anemia, jaundice, or abnormal urine test, respectively. They showed no symptoms of the classic methylmalonic academia, but urine organic acid analysis showed elevated malonic acid and methylmalonic acid in all the patients repeatedly. Variants were found at four sites in ACSF3 gene. Patient 1 carried the compound heterogeneous variant c.689G>A (p.Trp230∗)/c.1456G>A (p.Ala486Thr). A compound heterozygous variant c.473C>T (p.Pro158Leu)/c.1456G>A (p.Ala486Thr) was identified in patient 2. Patient 3 harbored a novel homozygous variant c.1447A>G (p.Lys483Glu).

Conclusions: Three Chinese patients were diagnosed with CMAMMA caused by ACSF3 variants. Their clinical course revealed that CMAMMA can be a benign condition that does not affect individual growth and development, but severe clinical phenotype may appear when other triggers exist. This study systematically elaborates CMAMMA in a Chinese population for the first time, broadens the spectrum of gene variant, and provides a strong basis for the etiological study of this disorder.

Keywords: combined malonic and methylmalonic aciduria, ACSF3 gene, benign condition, Chinese population, novel variant
INTRODUCTION

Methylmalonic academia (MMAemia) is a common inborn error of metabolism characterized by abnormal accumulation of methylmalonic acid in body fluids, resulting in many serious clinical manifestations. MMAemia is mainly caused by the defect of methylmalonyl-CoA mutase (MUT) or its coenzyme cobalamin (Cbl) (1). Combined malonic and methylmalonic aciduria (CMAMMA) is a rare atypical form of MMAemia featured with increased concentrations of malonic acid (MA) and methylmalonic acid (MMA) in urine (2). CMAMMA is caused by biallelic variants in the AcylCoA synthetase family member 3 (ACSF3, OMIM:614265) gene. ACSF3 encodes a mitochondrial acyl-CoA synthetase, which is essential for the synthesis of malonyl-CoA as well as methylmalonyl-CoA (3). Patients who carried ACSF3 gene variants excrete more MMA than MA in reported cases, which make them distinct from the patients with classical MMAemia (4).

The gene of ACSF3 is located on chromosome 16q24.3. It consists of 11 exons and encodes a 576-amino-acid protein with the first 83 residues representing the predicted mitochondrial transit peptide (4). To date, more than 50 patients worldwide have been reported to have CMAMMA caused by homozygous or compound heterozygous variants in ACSF3, including missense, nonsense, deletion, frameshift, and splice site variants. The clinical presentation of CMAMMA is quite controversial. Signs and symptoms reported so far are involved in neurological abnormalities in adults and infection induced encephalopathy in pediatric patients. Nevertheless, asymptomatic patients with normal outcomes strongly suggest that CMAMMA can be a benign state (2, 4–9). No case of the disease has been reported in China so far.

In this study, we summarized the clinical course, urine organic acid screening results, and analysis of ACSF3 gene variants in three Chinese patients with CMAMMA diagnosed in our hospital. We identified four variants inherited from the parents separately, among which c.1447A>G (p.K483E) is a novel variant. Based on the present study and literature review, we speculate that the CMAMMA is a benign state when other triggers are absent in Chinese population.

MATERIALS AND METHODS

Participants

Three patients at the age of 11 days to 8 months were diagnosed as CMAMMA in Tianjin Children’s Hospital in China. There was no family history in these patients. The informed consents from guardians and the approval of the Medical Ethics Committee of Tianjin Children’s Hospital were obtained.

Routine Tests and Metabolic Assay

Routine tests were conducted in three patients, such as physical examination, routine blood, urine and stool test, blood gas analysis, biochemical test, imaging examination, and etiological examination for infected patients. Semi-quantitative analysis of organic acid in urine was performed using gas chromatography–mass spectrometry (GC/MS).

RESULTS

Clinical Data

Patient 1 was a 3-month-old girl. She was admitted to the hospital due to pale face for 2 weeks, fever, and cough for 2 days. Detailed physical examination and laboratory tests revealed that the patient had anemia, splenomegaly, hepatomegaly, thrombocytopenia, and bronchitis. Widened interval outside the cerebrum was noted in computed tomography. These clinical features were mainly attributed to suspected Evans syndrome and viral infection. The patient is 5 years old now and shows no sign of physical or psychomotor retardation.

Patient 2 was an 11-day-old boy. He was born at term by normal delivery. He was referred to the hospital due to...
TABLE 1 Genetic and phenotypic finding of patients with CMAMMA.

| Patient | Age and sex | Clinical feature | Variants | References |
|---------|-------------|------------------|----------|------------|
| 1       | 3mo, F      | Anemia, splenomegaly, hepatomegaly, thrombocytopenia, and bronchitis | c.689G>A<sup>a</sup> (p.W230*) | Present study |
| 2       | 11d, M      | Jaundice, neonatal omphalitis, myocardial damage, testicular hydrocele, and liver function damage | c.473C>T<sup>a</sup> (p.K486T) | Present study |
| 3       | 8mo, M      | Urinary tract infection, pneumonia, and diarrhea | c.1447A>G<sup>a</sup> (p.K483E) | Present study |
| 4       | 14y, M      | Asymptomatic | c.1411C>T<sup>a</sup> (p.R471W) | Present study |
| 5       | 4y, F       | Asymptomatic | c.1075G>A<sup>a</sup> (p.R471Q) | Present study |
| 6       | 2y, M       | Asymptomatic | c.1075G>A<sup>a</sup> (p.R471Q) | Present study |
| 7       | 43y, F      | Ocular migraine, memory problems, T2 hyperintensities on brain MRI | c.1386A>C<sup>a</sup> (p.K462T) | Present study |
| 8       | 51y, M      | Complex partial seizures, and memory problems | c.1672C>T<sup>a</sup> (p.R558W) | Present study |
| 9       | 55y, F      | Psychiatric symptoms, T2 hyperintensities on brain MRI | c.1672C>T<sup>a</sup> (p.R558W) | Present study |
| 10      | 22mo, F     | Seizure, encephalopathy, ketoacidosis | c.1672C>T<sup>a</sup> (p.R558W) | Present study |
| 11      | 4y, F       | Hypoglycemia, acidosis, poor weight gain, diarrhea episodes | c.857G>A<sup>a</sup> (p.E286*) | Present study |
| 12      | 66y, F      | Incontinence, mild memory problems | c.1411C>T<sup>a</sup> (p.R471W) | Present study |
| 13      | 6mo, M      | Failure to thrive, elevated transaminases | c.1411C>T<sup>a</sup> (p.R471W) | Present study |
| 14      | 17mo, M     | Psychomotor delay, microcephaly, dystonia, axial hypotonia, and speech delay | c.593T>G<sup>a</sup> (p.M198R) | Present study |
| 15      | 26mo, M     | Psychomotor delay, hypotonia, and loss of speech | – | (Continued) |
| 16      | 6y, F       | Asymptomatic until 6y, encephalopathic event upon an influenza infection | c.1075G>A<sup>a</sup> (p.E359K) | Present study |
| 17      | 5.5y, F     | Asymptomatic until 5.5y, encephalopathic event upon an influenza infection | c.1075G>A<sup>a</sup> (p.E359K) | Present study |
| 18      | 2y, F       | Mild developmental delay and seizure disorder | c.1672C>T<sup>a</sup> (p.R558W) | Present study |
| 19      | 73y,M       | Late-onset neurologic syndrome, rare axonal degeneration of segmental myelin thinning. | c.634G>C<sup>a</sup> (p.E212L) | Present study |
| 20      | 10y, F      | Significant developmental and speech delays | c.1470G>C<sup>a</sup> (p.R494Q) | Present study |
| 21      | 8mo, F      | Persistent elevation of MMA | c.1239+2T>G<sup>a</sup> | Present study |
| 22      | 3y, F       | Recurrent vomiting, febrile seizures | Chr16:87441993:89171912deletion (spanning AC5F3) | Present study |
| 23      | n/a         | Asymptomatic at 3y | c.1468_1474delCA<sup>a</sup> (p.Y487*) | Present study |
| 24      | n/a         | Hearing loss, psychomotor delay | c.1075G>A<sup>a</sup> (p.E359K) | Present study |

(Continued)
| Patient | Age and sex | Clinical feature | variants | References |
|---------|-------------|------------------|----------|------------|
| 25      | n/a         | Seizures, dystonia | c.1075G＞A (p.E359K) | c.1672C＞T (p.R558W) | (7) |
| 26      | n/a         | Asymptomatic at 7 m | c.820C＞T (p.G274A) | c.820C＞T (p.G274A) | (7) |
| 27      | 2y, M       | Hydronephrosis, several renal cysts | c.1718delT | c.1672C＞T (p.R558W) | (6) |
| 28      | 7y, F       | None              | n/a      | n/a        | (8) |
| 29      | 6y, F       | None              | c.1146_1147delCA (p.Y482*) | c.1075G＞A (p.E359K) | (6) |
| 30      | 24y, F      | None              | c.1075G＞A (p.E359K) | c.1470G＞C (p.E490D) | (6) |
| 31      | 5y, F       | Growth at 3rd percentile, consistent with parental heights, allergic rhinitis, eczema | c.1239+2T＞G | c.1672C＞T (p.R558W) | (6) |
| 32      | 2y, M       | Growth at 3–15th percentile, consistent with parental heights | n/a      | n/a        | (6) |
| 33      | 11y, M      | Hypopigmented spots | n/a      | n/a        | (8) |
| 34      | 27y, F      | Recurrent urinary tract infection, hives, and endometriosis | c.1672C＞T (p.R558W) | c.1672C＞T (p.R558W) | (6) |
| 35      | 24y, M      | Hepatomegaly and bovine protein intolerance in infancy, bilateral vesicoureteral reflux | c.1075G＞A (p.E359K) | c.1075G＞A (p.E359K) | (6) |
| 36      | 22y, F      | Attention deficit hyperactivity disorder | n/a      | n/a        | (6) |
| 37      | 20y, M      | Nissen fundoplication for GERD in infancy | n/a      | n/a        | (6) |
| 38      | 23y, F      | Neonatal cutaneous lupus erythematosus | c.1553C＞A (p.A518D) | c.1553C＞A (p.A518D) | (6) |
| 39      | 20y, F      | Slightly smaller right kidney, and migraines | n/a      | n/a        | (6) |
| 40      | 7y, M       | None              | c.1075G＞A (p.E359K) | c.1672C＞T (p.R558W) | (6) |
| 41      | 13y, M      | None              | c.1075G＞A (p.E359K) | c.1672C＞T (p.R558W) | (6) |
| 42      | 6mo, F      | Severe bronchiolitis/pneumonia at 1 month, lactic acidosis, mild valvular pulmonary stenosis, height and weight 0.1–3rd percentile | c.1075G＞A (p.E359K) | c.1075G＞A (p.E359K) | (6) |
| 43      | 7y, M       | None              | c.774_775del (p.W259Gfs*2) | c.1672C＞T (p.R558W) | (7) |
| 44      | 14y, M      | Attention deficit hyperactivity disorder, dysorthography | c.1470G＞C (p.E490D) | c.1075G＞A (p.E359K) | (6) |
| 45      | 16y, M      | Eczema, scoliosis, glaucoma and cataract, mild language and fine motor delay in 1st years, corrected by school age, cyclic vomiting in adolescence with no metabolic derangement | c.1239+2T＞G | c.1075G＞A (p.E359K) | (6) |
| 46      | 30y, F      | Dehydration and metabolic acidosis in the context of severe bloody diarrhea at 4 weeks of age, otherwise healthy | c.1075G＞A (p.E359K) | c.1075G＞A (p.E359K) | (6) |
| 47      | 19y, M      | Attention deficit hyperactivity disorder | c.1411C＞T (p.R471W) | c.1411C＞T (p.R471W) | (6) |
| 48      | 8y, F       | Bilateral congenital dacryostenosis | c.1075G＞A (p.E359K) | c.1075G＞A (p.E359K) | (6) |

(Continued)
jaundice for 7 days. Detailed physical examination showed neonatal omphalitis, myocardial damage, testicular hydrocele, and liver function damage. Laboratory tests showed that the direct bilirubin level was 17.1 µmol/L (normal: 0–5 µmol/L), and indirect bilirubin level was 184.4 µmol/L (normal: 3.4–10.3 µmol/L). These clinical manifestations were mainly caused by jaundice and infection. He is 3 years old now and attains a development milestone.

Patient 3 was an 8-month-old boy. He was born at term by cesarean section. He was admitted because of abnormal urine test for 3 months with red urine and urinary frequency. Urinary tract infection, pneumonia, and diarrhea were found in this patient. No obvious abnormality in his growth development was present. Infection factors were the main cause of his clinical presentations. He shows no sign of psychomotor retardation at the age of 5 in our recent follow-up.

Urine Analysis
GC/MS demonstrated elevated MA and MMA in all the patients repeatedly (Figure 1), which suggested the suspected diagnosis of MMAemia. However, the patients did not present with the clinical symptoms of classic MMAemia.

Gene Variation Analysis
All the patients were detected to carry variants in ACSF3 gene (NM_174917.4) by WES. Patient 1 carried a compound heterozygous variant. The variant c.689G>A (p.Trp230*) was inherited from her mother and the other variant c.1456G>A (p.Ala486Thr) was inherited from her father. Besides, the compound heterozygous variant, c.473C>T (p.Pro158Leu)/c.1456G>A (p.Ala486Thr), was identified in the ACSF3 gene in patient 2. The two heterozygous variants were inherited from the mother and father, respectively. Patient 3 harbored a homozygous variant of c.1447A>G (p.Lys483Glu) in ACSF3 gene, which was inherited from his parents (Table 1). Sanger sequencing was used to confirm the variants (Figures 2–4). The homozygous variant c.1447A>G in ACSF3 gene is a novel discovery.

DISCUSSION
CMAMMA is a rare inborn error of metabolism characterized by high excretion of MMA than MA in urine. The pathogenic gene ACSF3 catalyzes the initial reaction in intramitochondrial fatty acid synthesis by activating MA and MMA into their respective CoA thiosteres. To date, about 52 patients with 31 homozygous or compound heterozygous variants of ACSF3 have been reported worldwide, including missense, nonsense, deletion, frameshift, and splice site variants. The locations of these variants in ACSF3 are graphically displayed in Figure 5. Most of the variants are missense, mainly located at the carboxyl terminal of the protein (Table 1) (2, 4–9). The most common ACSF3 variants reported so far are c.1075G>A (p.E359K) and c.1672C>T (p.R558W), which are also the most common variants in asymptomatic patients. It indicates that the two variants are more likely to have a mild effect on gene function. Patients who carried the heterozygous variant c.1470G>C (p.E490D) have mild clinical symptoms, while the homozygous variant can result in significant developmental and speech delays.

Patients who carried ACSF3 variants exhibit controversial clinical phenotypes. Based on the literature review we did, severe clinical manifestations are mainly reported in adults, with the most common symptoms of neurological problems and psychiatric features (2). Because of the long-term damage accumulation, older people are at high risk for neurological illness even without ACSF3 variants. The phenotype of pediatric patients is relatively mild or even asymptomatic. Three pediatric patients diagnosed with CMAMMA due to ACSF3 mutation were reported to be clinically asymptomatic, and they had age-appropriate development (4). A retrospective study described the course of 25 CMAMMA individuals and suggested that CMAMMA is probably a benign condition (8). Another study showed that infection factors could provoke metabolic dysregulation in pediatric patients with CMAMMA, and normal levels of development could be obtained at follow-up (5). Therefore, CMAMMA may be considered as a risk factor instead of a disease, which could lead to clinical symptoms when other influencing factors exist.
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FIGURE 2 | Sanger sequencing of the genomic DNA from patient 1 and her parents. (A) Patient 1 carried with a heterozygous variant of c.689G>A, her father with a normal genotype, and mother with a heterozygous variant of c.689G>A. (B) Patient 1 carried with a heterozygous variant of c.1456G>A, her father with a heterozygous variant of c.1456G>A, and mother with a normal genotype.

FIGURE 3 | Sanger sequencing of the genomic DNA from patient 2 and his parents. (A) Patient 2 with a heterozygous variant of c.473C>T, his father with a normal genotype, and mother with a heterozygous variant of c.473C>T. (B) Patient 2 with a heterozygous variant of c.1456G>A, his father with a heterozygous variant of c.1456G>A, and mother with a normal genotype.

c.473C>T, c.1447A>G, c.311A>T, c.1239+2T>G, c.1446_1447delCA, c.424C>T, c.820C>T, c.1553C>A, c.774_775del, and c.1081G>A. Patients carrying these variants were asymptomatic or had infection-induced symptoms that disappeared after treatment (2, 4–9). Besides, although the variant c.1456G>A has a high frequency in population, our present study showed that patients carrying this variant had repeatedly elevated biochemical phenotype without obvious clinical phenotype, which could also be observed in other inherited metabolic disease. The β-ureidopropionase deficiency patients carrying the homozygous or compound heterozygous c.977G>A variant (high prevalence in normal Japanese
population) in UPBI gene could be asymptomatic as well (10). We speculate that the c.1456G>A variant in ACSF3 may act in a similar way. High frequency of c.1456G>A in population indicates that CMAMMA may not be as rare as generally considered, and this kind of variant may contribute to the benign condition in patients. Accumulations of free MA and MMA were neurotoxic in vitro, while the concentrations might be insufficient to produce disease in CMAMMA patients (8). Actually, we cannot completely exclude the possibility that the accumulation of metabolites is associated with the occurrence of neurological symptoms as the patients grow up, or that the people with the ACSF3 gene variants are more genetic susceptible to neurological problems later in life. Increased dependency on β-oxidation for energy production were observed in fibroblasts from CMAMMA patients. As a result, the subsequent increased risk for hypoxia and oxidative stress may be crucial for the onset of neurological symptoms in the long run (11).

CONCLUSIONS

In conclusion, our study included three Chinese pediatric patients under the age of 4 years. Their clinical features were mainly caused by infection or other reasons. They showed no sign of physical or psychomotor retardation in our follow-up. Combined with reported cases, our study strongly suggested that the CMAMMA is a benign clinical course, especially for patients carrying certain variants. In addition, the present study had some limitations. Firstly, this study only involved the correlation analysis between genotype and phenotype. Functional verification of variants in vitro will help to elucidate the pathogenesis of CMAMMA, especially for the novel variant c.1447A>G (p.K483E), while functional studies were not available in this study due to the limitations of experimental condition. Secondly, a longer follow-up period is needed to better assess the growth and development of the patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Tianjin Children’s
Hospital. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

**AUTHOR CONTRIBUTIONS**

PW and JS conceived the concept and wrote the manuscript. CG contributed to literature review and revised the manuscript. XY provided clinical diagnosis. JZ contributed to interpretation of the results. CZ performed the analysis. CC participated in supervision of the project. All authors read and approved the final manuscript.

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