Genotype-Phenotype Correlation Analysis of Children with Methylmalonic Academia: A Retrospective Study in China

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Introduction. Methylmalonic acidemia (MMA) is the most common type of congenital organic acidemia, which can result from a deficiency of methylmalonyl coenzyme A mutase or abnormalities in the metabolism of adenosylcobalamin.

Objective. This study aimed to investigate the genotype, phenotype, and their correlations in Chinese MMA patients carrying mutations in the MMACHC or MMUT genes.

Methods. Thirty-four unrelated patients diagnosed with MMA were retrospectively analyzed. Clinical features, biochemical index, and mutation spectrum were described. Genotype-phenotype correlations were investigated for different pathogenic genes and common mutations within the genes.

Results. Patients with mutations in the MMACHC gene demonstrate a higher incidence of movement disorders. Patients with mutations in the MMUT gene have significantly elevated levels of C3/C2 ratio and ammonia. The most common mutations of the MMACHC gene include c.609G>A (69%) and c.80A>G (38%), while the most common mutation of the MMUT gene is c.1677-1G>A (24%). Patients with MMACHC c.80A＞G have more severe anemia, whereas patients with MMUT c.1677-1G＞A tend to have less severe anemia. Patients with MMACHC c.80A＞G have a lower C3/C2 ratio and methylmalonic acid than those without the variant. Patients with MMUT c.1677-1G＞A are more likely to have cognitive impairment, ST-T abnormalities, and abnormal renal function.

Conclusions. This study showed that the genotype and phenotype of MMA are correlated in the Chinese population, creating distinct clinical manifestations.

Keywords. Methylmalonic Acidemia; DNA Mutational Analysis; Genotype-Phenotype Correlation; MMACHC; MMUT

INTRODUCTION

Methylmalonic acidemia (MMA), also known as methylmalonic aciduria, is the most common type of congenital organic acidemia with an autosomal recessive inheritance pattern with multifactorial origins. Based on a meta-analysis, the pooled MMA detection rates were estimated to be 0.79, 1.12, 1.22, and 6.04 per 100,000 newborns in the regions of Asia-Pacific, Europe, North America, and the Middle East and North Africa, respectively [1]. MMA is characterized by the accumulation of methylmalonic acid, 3-hydroxypropionic acid, and methylcitric acid in the bloodstream, potentially leading to diverse clinical manifestations through complex mechanisms [2,3].

MMA can arise from two main classes of genetic defects: the deficiency of methylmalonyl coenzyme A mutase (MCM), referred to as the "mut" class, or abnormalities in the metabolism of adenosylcobalamin (AdoCbl), referred to as the "cbl" class. The genetic landscape of MMA is highly heterogeneous, with the identification of at least 10 pathogenicity genes. The most commonly implicated genes include MMUT (mut type), MMAA (cblA type), MMAB (cblB type), MMACHC (cblC type), MMADHC (cblD type), LMBRD1 (cblF type), ABCD4 (cblJ type), and HCFC1 (cblX type) [4–6].

MMA can be classified into two types: "isolated MMA" and "combined MMA" (MMA combined with homocysteinemia), based on the biochemical manifestations. In China, isolated MMA accounts for approximately 30%, while combined MMA is the most prevalent type, accounting for nearly 70% of all affected patients [7].

The isolated form of MMA is mainly caused by a complete or partial deficiency of the MCM enzyme [8]. This form comprises four subtypes, namely MMUT defect, cblA (MMAA gene), cblB (MMAB gene), and cblD

(MMADHC gene) defect [9–11]. MMUT gene deficiency is the most common in the Chinese population, accounting for 93.5% of isolated MMA cases. The MMUT gene is located in the chromosome region 6p12.3, contains 13 exons, and encodes a protein consisting of 750 amino acids. There is a wide spectrum of mutations in the MMUT gene [12]. Common clinical presentations of isolated MMA include lethargy, vomiting, hepatomegaly, hypotonia, biochemical disturbances (metabolic acidosis and hyperammonemia), and hyperammonemic encephalopathy. Additionally, many individuals with this condition may experience feeding problems (typically anorexia), impaired neurological function (comatose state and metabolic stroke), failure to thrive, and developmental delay [2,8]. Mut-type patients are particularly susceptible to the development of end-stage renal failure [13].

There are five subtypes of the combined form of MMA, encompassing cblC (MMACHC gene), cblD (MMADHC gene), cblF (LMBRD1 gene), cblJ (ABCD4 gene), and cblX (HCFC1 gene) deficiencies [14,15]. The most frequently occurring subtype is the MMACHC gene-induced cblC defect [16]. The MMACHC gene is located on chromosome 1p34.1 and contains five exons encoding a protein of 282 amino acids. The clinical manifestations of combined MMA include lethargy, poor feeding, dehydration, failure to thrive, and developmental delay among infants. Progressive encephalopathy can develop in some patients as they age [3]. Neurological deficits can manifest at any stage of life, often leading to irreversible impairments [17]. Atypical hemolytic uremic syndrome, related to thrombotic microangiopathy, is known to develop in cblC patients.

Numerous studies have conducted genotypic analyses, which have reported the mutation spectrum of the defective genes [7,12,18]. Genotype-phenotype correlation has also been explored in several studies. Isolated MMA children were found to have higher blood propionylcarnitine (C3), C3/acetylcarnitine (C2), urine methylmalonic acid, methylcitric acid, and serum total homocysteine (tHcy) levels compared to those with combined MMA [19]. Generally, the clinical manifestations of mut are more severe compared to cblC and other

types of isolated MMA [12]. In a recent study, the authors compared the response to treatment in patients carrying different mutations. The study identified the c.729_730insTT, c.1280G>A, c.323G>A, and c.1630_1631delGGinsTA groups as treatment-resistent, while the c.1663G>A variation group showed treatment responsiveness [18]. A study specifically analyzing the rare c.1663G>A mutation showed that patients with this mutation exhibited a later onset of symptoms, a milder clinical phenotype, less severe biochemical abnormalities, improved responsiveness to vitamin B12 treatment, reduced morbidity, easier metabolic control, and, consequently, a more favorable prognosis in comparison to patients with other mutations in the MMUT gene [20]. The genotype caused by compound heterozygous mutations of two alleles c.609 G>A and c.658_660delAAG was shown to have severe microcephaly and eye diseases, which were not improved after the treatment [21]. Despite ongoing efforts, there are still numerous mutations that have not been fully characterized in terms of their phenotypic profiles.

In this study, we investigated the genotype-phenotype correlation in Chinese MMA patients by examining 34 patients who carried mutations in the MMUT or MMACHC genes. A comprehensive retrospective analysis of their clinical data was conducted, encompassing molecular diagnosis, metabolite profiles, and prognosis. Specifically, we compared the clinical characteristics between patients with mutations in the MMUT gene and those with mutations in the MMACHC gene. Furthermore, we explored the potential association between specific mutations and clinical features within the patient cohort. The primary objective of this study was to provide a comprehensive overview of the clinical manifestations associated with specific genotypes in Chinese patients diagnosed with MMA.

MATERIALS AND METHODS

Patients

This study included 34 unrelated patients who met the following criteria: (1) diagnosed with isolated or combined MMA after clinical presentation of malnutrition, anemia, seizures, lethargy, or developmental delay at a tertiary hospital in China; and (2) carrying at least one variant allele of the MMUT gene or the MMACHC gene. Demographic, clinical, laboratory, and genetic testing data of the patients were collected retrospectively after obtaining written informed consent from the guardians of the participants. The study was approved by the Ethics Committee of our hospital and was conducted in accordance with the principles outlined in the Declaration of Helsinki.

MMA was diagnosed based on the criteria outlined in accordance with the guideline [6].

The diagnostic process involved the following criteria: (1) evaluation of patients presenting with clinical symptoms consistent with MMA; (2) confirmation by the presence of significantly elevated levels of urine methylmalonic acid or an increased C3 or C3/C2 ratio; and (3) exclusion of secondary factors such as vitamin B12 deficiency. Patients with tHcy levels exceeding 15 μM were categorized as having combined MMA, while other patients were diagnosed with isolated MMA.

Biochemical Examination

The study measured the concentrations of 18 amino acids and 45 acylcarnitines in dried blood spots using tandem mass spectrometry and high-performance liquid chromatography techniques. Additionally, the concentrations of 132 organic acids, including methylmalonic acid, methylcitric acid, and 3-hydroxypropionic acid, were measured in urine using gas chromatography-mass spectrometry with the urease-liquid-liquid extraction method. The serum tHcy levels were determined using the enzyme cycling method.

Gene Mutation Analysis

After obtaining informed consent from the guardians, we collected approximately 3 mL of peripheral blood from the patients and their parents in ethylenediaminetetraacetic acid anticoagulant tubes for genomic DNA extraction. We used a whole-exome capture kit and followed the sequencing analysis workflow of the Baylor College of Medicine's Exome Sequencing Process (Baylor process) for candidate variant screening. The candidate gene mutation sites identified were subsequently validated through Sanger sequencing within the family. The pathogenicity of the variants was assessed based on the classification criteria of the American College of Medical Genetics and Genomics (ACMG), considering multiple factors. Verification techniques such as multiple ligation-dependent probe amplification or fluorescence quantitative PCR were used for suspected copy number variations.

Statistical Analysis

All statistical analysis was performed using R (version 4.1.2). Continuous variables were presented as median (Interquartile range; IQR) and compared using Wilcoxon test. Categorical variables were presented as count (%) and compared using χ^2 tests or proportion tests as appropriate. All statistical tests were two-sided. $P < 0.05$ was considered statistically significant unless otherwise stated.

RESULTS

Study Population

Thirty-four patients clinically diagnosed after onset were included in the analysis. Of these patients, 16 (47%) were male and 18 (53%) were female. Clinical onset ranged from the age of one day to seven years, with 28 (82%) experiencing early onset. Among the patients, 13 (38%) cases were combined MMA with mutations in the MMACHC gene, and 21 (62%) cases were isolated MMA with mutations in the MMUT gene. The general features of the patients are presented in Table 1.

Table 1 General features of the MMA patients.

Characteristic	Overall N = 34	MMACHC N = 13	MMUT N = 21	*P*-value
Sex				0.7869
Male	16 / 34 (47%)	7 / 13 (54%)	9 / 21 (43%)	
Female	18 / 34 (53%)	6 / 13 (46%)	12 / 21 (57%)	
Disease onset time				>0.9
Early-onset	28 / 34 (82%)	11 / 13 (85%)	17 / 21 (81%)	
Late-onset	6 / 34 (18%)	2 / 13 (15%)	4 / 21 (19%)	
Nervous impairment				
Seizure	8 / 34 (24%)	5 / 13 (38%)	3 / 21 (14%)	0.2305
Cognitive impairment	17 / 34 (50%)	6 / 13 (46%)	11 / 21 (52%)	>0.9
Movement disorder	7 / 34 (21%)	7 / 13 (54%)	0 / 21 (0%)	0.0008
Hematological abnormality				
Anemia				>0.9
Mild	7 / 34 (21%)	3 / 13 (23%)	4 / 21 (19%)	
Moderate	12 / 34 (35%)	5 / 13 (38%)	7 / 21 (33%)	
Severe	4 / 34 (12%)	1 / 13 (7.7%)	3 / 21 (14%)	
Neutropenia	13 / 34 (38%)	5 / 13 (38%)	8 / 21 (38%)	>0.9
Thrombocytopenia	5 / 34 (15%)	1 / 13 (7.7%)	4 / 21 (19%)	0.6816
Hypoalbuminemia	8 / 34 (24%)	2 / 13 (15%)	6 / 21 (29%)	0.6420
Hypokalemia	3 / 34 (8.8%)	0 / 13 (0%)	3 / 21 (14%)	0.4208
Hyponatremia	2 / 34 (5.9%)	1 / 13 (7.7%)	1 / 21 (4.8%)	>0.9
Hypocalcemia	2 / 34 (5.9%)	0 / 13 (0%)	2 / 21 (9.5%)	0.6914

Metabolic crises				
Metabolic acidosis	27 / 31 (87%)	10 / 11 (91%)	17 / 20 (85%)	>0.9
Vomiting	11 / 34 (32%)	1 / 13 (7.7%)	10 / 21 (48%)	0.04123
Poor feeding	6 / 34 (18%)	2 / 13 (15%)	4 / 21 (19%)	>0.9
Cardiovascular				
QTc interval prolongation	4 / 30 (13%)	0 / 13 (0%)	4 / 17 (24%)	0.1813
ST-T abnormality	9 / 30 (30%)	3 / 13 (23%)	6 / 17 (35%)	0.7478
Myocardial enzyme abnormality	16 / 34 (47%)	5 / 13 (38%)	11 / 21 (52%)	0.6623
Abnormal liver function	9 / 34 (26%)	3 / 13 (23%)	6 / 21 (29%)	>0.9
Abnormal renal function	4 / 34 (12%)	2 / 13 (15%)	2 / 21 (9.5%)	>0.9
Biochemical characteristics				
C3 (μmol/L)	14 (6, 19)	9 (5, 12)	17 (6, 24)	0.1695
C3/C2 ratio	1.16 (0.36, 0.99)	0.44 (0.20, 0.51)	1.59 (0.47, 2.07)	0.0054
Methylmalonic acid (mmol/mol)	1,346 (25, 1,166)	263 (19, 410)	1,996 (35, 2,324)	0.0763
Ammonia (μmol/L)	137 (37, 128)	42 (32, 60)	196 (81, 167)	0.0001
Lactic acid (mmol/L)	2.56 (1.51, 3.36)	2.15 (1.30, 2.30)	2.82 (1.72, 3.78)	0.3124
Failure to thrive	23 / 29 (79%)	11 / 12 (92%)	12 / 17 (71%)	0.3603

P < 0.05 is considered statistically significant and is shown in bold.

Patients with mutations in the MMACHC and MMUT genes were compared. Results showed that the patients differ in nervous impairment and that patients with mutations in the MMACHC gene demonstrate a significantly higher incidence of movement disorders (as shown in Table 1 and Figure 1a). Patients with mutations in the MMUT gene have significantly elevated levels of C3/C2 ratio and ammonia, and numerically higher C3 levels and urine methylmalonic acid levels (as shown in Table 1 and Figure 1b). Additionally, they exhibit a significantly higher proportion of patients who presented with vomiting.

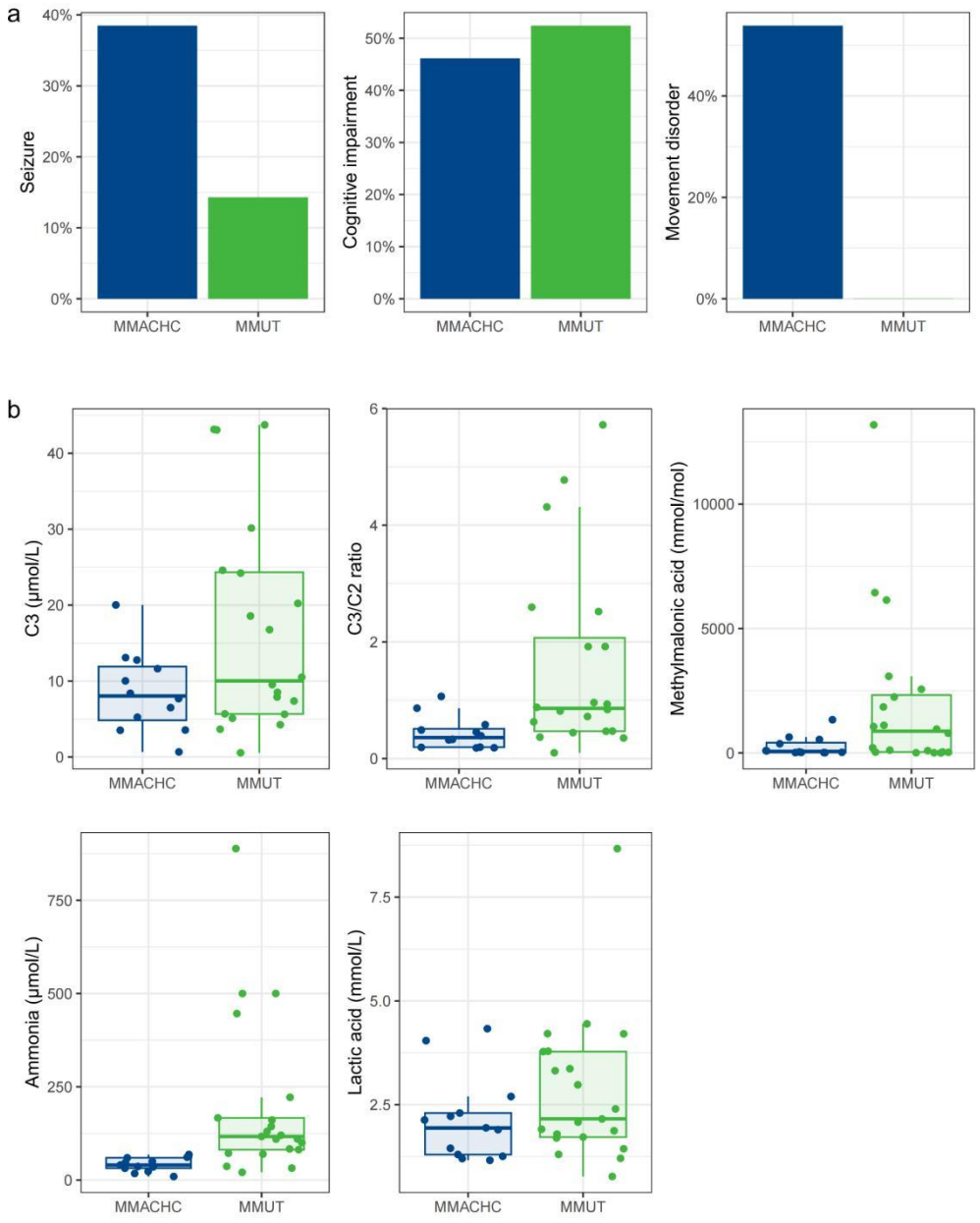

Figure 1 Differences in nervous impairment (a) and biochemical characteristics (b) between patients with mutations in the MMACHC and MMUT genes.

MMA-related Gene Variant Spectrum

The mutations identified in this study are shown in Table 2. In total, 35 mutations were detected, comprising 9 in the MMACHC gene and 26 in the MMUT gene. The most frequent mutations in the MMACHC gene were

c.609G>A identified in 9 patients and c.80A>G identified in 5 patients. Mutations in the MMUT gene exhibited a broader spectrum, with c.1677-1G>A being the most frequent mutation (identified in 5 patients). Most of the mutations had previously been detected in the Chinese population. The variant MMACHC c.90G>A was not previously observed in the Chinese population but has been reported in a study conducted in Spain [22]. We found six novel variants in the MMUT genes: c.1438G>T, c.1676+11A>G, c.3850T>G, c.508dupA, and c.688C>T, each present in one patient. We verified these novel variants using the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Table 2 The variation spectrum of the MMACHC and MMUT genes in the study population.

Gene	cDNA change	N	Reference
MMACHC	c.609G>A	9	[28]
MMACHC	c.80A>G	5	[28]
MMACHC	c.658_660del	3	[28]
MMACHC	c.567dupT	2	[28]
MMACHC	c.365A>T	1	[28]
MMACHC	c.616C>T	1	[28]
MMACHC	c.445_446delTG	1	[21]
MMACHC	c.90G>A	1	[22]
MMACHC	c.689G>A	1	ClinVar: RCV000723210
MMUT	c.1677-1G>A	5	[12,18]
MMUT	c.323G>A	3	[18]
MMUT	c.729__730insTT	3	(Kang et al., 2020; Liang et al., 2023)
MMUT	c.914T>C	3	(Kang et al., 2020; Liang et al., 2023)
MMUT	c.1106G>A	2	(Kang et al., 2020; Liang et al., 2023)
MMUT	c.1630G>T	2	[12]
MMUT	c.1663G>A	2	(Kang et al., 2020; Liang et al., 2023)

MMUT	c.1630_1631delGGinsTA	2	(Kang et al., 2020; Liang et al., 2023)
MMUT	c.1233_1235del	1	[18]
MMUT	c.1280G>A	1	[12,18]
MMUT	c.1531C>T	1	[12,18]
MMUT	c.1677-1G>C	1	[12,18]
MMUT	c.1741C>T	1	[12,18]
MMUT	c.2080C>T	1	[12,18]
MMUT	c.322C>T	1	[12,18]
MMUT	c.424A>G	1	[12,18]
MMUT	c.599T>C	1	[12,18]
MMUT	c.613G>A	1	[18]
MMUT	c.755dupA	1	[12,18]
MMUT	c.944dupT	1	[18]
MMUT	c.1438G>T	1	Novel
MMUT	c.1676+11A>G	1	Novel
MMUT	c.3850T>G	1	Novel
MMUT	c.508dupA	1	Novel
MMUT	c.688C>T	1	Novel

Genotype-Phenotype Correlation Analysis

Genotype-phenotype correlation analysis was performed on the most common variants: MMACHC c.609G＞A, MMACHC c.80A＞G, and MUT c.1677-1G＞A. For each variant, patients with the mutation were compared with patients without the mutation but with other mutations in the gene. The results are shown in Table 3. Due to the limited sample size, we highlighted comparisons with a P-value of less than 0.2. Differences in the severity of anemia were observed. As shown in Figure 2, patients with MMACHC c.80A＞G have more severe anemia, whereas patients with MMUT c.1677-1G＞A tend to have less severe anemia. Other phenotypes that showed differences in the genotype subgroups include that patients with and without MMACHC c.609G＞A differ in their time of disease onset. Patients without the variant are more likely to be late-onset. Patients with MMACHC c.80A＞G have a lower C3/C2 ratio and methylmalonic acid than those without the variant. Patients with MMUT c.1677-1G＞A are more likely to present with cognitive impairment, ST-T abnormality, and abnormal renal function.

Table 3 Genotype-phenotype correlation analysis results.

Characteristic	MMACHC c.609G>A			MMACHC c.80A>G			MMUT c.1677-1G>A		
	Negative N = 4	Positive N = 9	*P*-value	Negative N = 8	Positive N = 5	*P*-value	Negative N = 16	Positive N = 5	*P*-value
Sex			0.4306			0.8259			>0.9
Male	1 / 4 (25%)	6 / 9 (67%)		5 / 8 (63%)	2 / 5 (40%)		7 / 16 (44%)	2 / 5 (40%)	
Female	3 / 4 (75%)	3 / 9 (33%)		3 / 8 (38%)	3 / 5 (60%)		9 / 16 (56%)	3 / 5 (60%)	
Disease onset time			0.1407			0.6705			>0.9
Early-onset	2 / 4 (50%)	9 / 9 (100%)		6 / 8 (75%)	5 / 5 (100%)		13 / 16 (81%)	4 / 5 (80%)	
Late-onset	2 / 4 (50%)	0 / 9 (0%)		2 / 8 (25%)	0 / 5 (0%)		3 / 16 (19%)	1 / 5 (20%)	
Nervous impairment									
Seizure	2 / 4 (50%)	3 / 9 (33%)	>0.9	4 / 8 (50%)	1 / 5 (20%)	0.6201	2 / 16 (13%)	1 / 5 (20%)	>0.9
Cognitive impairment	3 / 4 (75%)	3 / 9 (33%)	0.4306	3 / 8 (38%)	3 / 5 (60%)	0.8259	6 / 16 (38%)	5 / 5 (100%)	0.0537
Movement disorder	3 / 4 (75%)	4 / 9 (44%)	0.6765	5 / 8 (63%)	2 / 5 (40%)	0.8259	0 / 16 (0%)	0 / 5 (0%)	——
Hematological abnormality									
Anemia			0.8694			0.0221			0.4418
Mild	1 / 4 (25%)	2 / 9 (22%)		3 / 8 (38%)	0 / 5 (0%)		3 / 16 (19%)	1 / 5 (20%)	
Moderate	2 / 4 (50%)	3 / 9 (33%)		1 / 8 (13%)	4 / 5 (80%)		6 / 16 (38%)	1 / 5 (20%)	

Severe	0 / 4 (0%)	1 / 9 (11%)		0 / 8 (0%)	1 / 5 (20%)		3 / 16 (19%)	0 / 5 (0%)	
Neutropenia	2 / 4 (50%)	3 / 9 (33%)	>0.9	2 / 8 (25%)	3 / 5 (60%)	0.4990	5 / 16 (31%)	3 / 5 (60%)	0.5300
Thrombocytopenia	0 / 4 (0%)	1 / 9 (11%)	>0.9	1 / 8 (13%)	0 / 5 (0%)	>0.9	3 / 16 (19%)	1 / 5 (20%)	>0.9
Hypoalbuminemia	1 / 4 (25%)	1 / 9 (11%)	>0.9	0 / 8 (0%)	2 / 5 (40%)	0.2482	4 / 16 (25%)	2 / 5 (40%)	0.9354
Hypokalemia	0 / 4 (0%)	0 / 9 (0%)	——	0 / 8 (0%)	0 / 5 (0%)	——	1 / 16 (6.3%)	2 / 5 (40%)	0.2500
Hyponatremia	1 / 4 (25%)	0 / 9 (0%)	0.6645	0 / 8 (0%)	1 / 5 (20%)	0.805	0 / 16 (0%)	1 / 5 (20%)	0.5286
Hypocalcemia	0 / 4 (0%)	0 / 9 (0%)	——	0 / 8 (0%)	0 / 5 (0%)	——	1 / 16 (6.3%)	1 / 5 (20%)	0.9669
Metabolic crises									
Metabolic acidosis	3 / 3 (100%)	7 / 8 (88%)	>0.9	6 / 6 (100%)	4 / 5 (80%)	0.9237	13 / 15 (87%)	4 / 5 (80%)	>0.9
Vomiting	0 / 4 (0%)	1 / 9 (11%)	>0.9	0 / 8 (0%)	1 / 5 (20%)	0.8050	8 / 16 (50%)	2 / 5 (40%)	>0.9
Poor feeding	0 / 4 (0%)	2 / 9 (22%)	0.8476	2 / 8 (25%)	0 / 5 (0%)	0.6705	3 / 16 (19%)	1 / 5 (20%)	>0.9
Cardiovascular									
QTc interval prolongation	0 / 4 (0%)	0 / 9 (0%)	——	0 / 8 (0%)	0 / 5 (0%)	——	2 / 13 (15%)	2 / 4 (50%)	0.4513
ST-T abnormality	0 / 4 (0%)	3 / 9 (33%)	0.5462	1 / 8 (13%)	2 / 5 (40%)	0.6395	3 / 13 (23%)	3 / 4 (75%)	0.1929
Myocardial enzyme abnormality	2 / 4 (50%)	3 / 9 (33%)	>0.9	2 / 8 (25%)	3 / 5 (60%)	0.4990	7 / 16 (44%)	4 / 5 (80%)	0.3661
Abnormal liver function	1 / 4 (25%)	2 / 9 (22%)	>0.9	1 / 8 (13%)	2 / 5 (40%)	0.6395	4 / 16 (25%)	2 / 5 (40%)	0.9354

Abnormal renal function	1 / 4 (25%)	1 / 9 (11%)	>0.9	0 / 8 (0%)	2 / 5 (40%)	0.2482	0 / 16 (0%)	2 / 5 (40%)	0.0740
Biochemical characteristics									
C3 (μmol/L)	6.4 (3.6, 7.2)	9.7 (7.4, 11.9)	0.3677	8.3 (5.9, 10.8)	9.0 (3.5, 13.1)	>0.9	19 (5, 27)	10 (7, 11)	0.4973
C3/C2 ratio	0.33 (0.29, 0.37)	0.49 (0.20, 0.65)	0.4962	0.54 (0.36, 0.68)	0.29 (0.18, 0.32)	0.0735	1.62 (0.47, 2.22)	1.47 (0.82, 0.96)	0.9304
Methylmalonic acid (mmol/mol)	189 (18, 227)	301 (21, 410)	0.8081	427 (50, 587)	35 (14, 36)	0.1490	2,216 (62, 2,095)	1,336 (32, 2,246)	0.9328
Ammonia (μmol/L)	34 (30, 39)	45 (35, 61)	0.3301	46 (34, 62)	36 (23, 50)	0.4351	198 (71, 180)	189 (110, 144)	0.7411
Lactic acid (mmol/L)	1.55 (1.27, 1.66)	2.41 (1.90, 2.70)	0.2601	2.38 (1.39, 3.04)	1.78 (1.30, 2.13)	0.6216	2.44 (1.72, 3.33)	4.00 (2.16, 4.21)	0.2310
Failure to thrive	4 / 4 (100%)	7 / 8 (88%)	>0.9	7 / 8 (88%)	4 / 4 (100%)	>0.9	9 / 14 (64%)	3 / 3 (100%)	0.5934

P < 0.2 is shown in bold.

		No	Mild	Moderate	Severe
MMACHC c.609G>A	Negative	25%	25%	50%	0%
	Positive	33%	22%	33%	11%
MMACHC c.80A>G	Negative	50%	38%	13%	0%
	Positive	0%	0%	80%	20%
MMUT c.1677-1G>A	Negative	25%	19%	38%	19%
	Positive	60%	20%	20%	0%

Figure 2 Differences in anemia severity in different genotype subgroups.

DISCUSSION

In a nationwide report conducted in China, it was observed that the proportion of hospitalized pediatric patients with MMA exhibited an increasing trend from 2013 to 2017, placing a growing burden on the national healthcare system [23]. The clinical manifestations of MMA are diverse and lack specificity, depending on the type of disease and the degree of enzyme deficiency. It can vary in severity and potential involvement of single or multiple organ systems. The absence of specific signs and symptoms in MMA clinical presentations highlights the impact of this condition on multiple systems, including the nervous, digestive, circulatory, and urinary systems, resulting in a complex and multifaceted syndrome [24]. Apart from the phenotypic diversity, MMA also demonstrates genotypic diversity. The genetic landscape of MMA is highly complex, with the identification of over 10 pathogenicity genes and hundreds of associated mutations. Moreover, the mutation spectrum varies significantly among different racial populations [18]. Considering the combined genotypic and phenotypic heterogeneity, a comprehensive understanding of the clinical and biochemical characteristics of patients carrying specific mutations is crucial for unraveling the mysteries surrounding this disease. In this study, we retrospectively analyzed 34 unrelated patients diagnosed with MMA, aiming to investigate the

genotype, phenotype, and their correlations in Chinese patients with MMA who carry mutations in the MMACHC or MMUT genes.

In our study, patients carrying mutations in the MMUT gene exhibited significantly increased levels of C3/C2 ratio and ammonia, as well as numerically higher C3 levels and urine methylmalonic acid levels, when compared to patients with mutations in the MMACHC gene. The findings are consistent with a previous phenotypic-genotypic analysis conducted on children with MMA, where all the indicators of isolated MMA were higher than those of combined MMA [19]. Similarly, an Indian study reported that patients with isolated MMA had a higher frequency of hyperammonemia and elevated lactate as compared to those with combined MMA [25]. This has been attributed to the fact that the defect in the MCM enzyme, which constitutes the main component of the enzyme's active center, exerts a much greater impact on metabolism pathways and cell functions than its coenzyme [19]. The MMUT enzyme plays a critical role in mitochondrial metabolism, and its deficiency leads to severe clinical consequences. Patients affected by this deficiency often exhibit various symptoms, including poor feeding, vomiting, progressive lethargy, and a clinical presentation resembling sepsis [26]. In line with this theory, our study reveals a notable observation: patients carrying mutations in the MMUT gene display a significantly higher prevalence of vomiting as a presenting symptom. It has also been observed that patients with mutations in the MMACHC gene have a higher incidence of movement disorders. This is likely due to the direct impact of MMACHC gene mutations on vitamin B12 metabolism, which significantly affects the function of the basal ganglia. In contrast, mutations

in the MMUT gene primarily result in the accumulation of toxic metabolites, such as methylmalonic acid, which may not have the same direct effect on the basal ganglia.

The mutation MMACHC c.609 G>A, which results in a premature termination codon at amino acid residue 203 located in the C-terminal region of MMACHC, has been identified as the most common mutation in cblC patients from China [27,28]. This mutation is also the most frequent in our current study. According to a case series study, MMACHC c.609G>A is mainly associated with early-onset cblC [29], which aligns with our current finding. A previous study found that the compound heterozygous mutations of c.609G>A and c.658_660delAAG caused by both variants have the most severe phenotype and the worst prognosis. Affected children with this mutation exhibit microcephaly and eye problems, which do not show significant improvement after treatment [21]. This was not confirmed in our study due to the limited number of patients with both variants.

In our study, we observed that patients with the MMACHC c.80A>G variant exhibited a lower C3/C2 ratio and reduced levels of methylmalonic acid compared to those without the variant. Despite these biochemical differences, we found that these individuals experienced more severe anemia and displayed greater abnormalities in renal function. These findings align with previous studies that have also reported an association between the MMACHC c.80A>G variant and renal complications [30]. However, the underlying mechanism driving this relationship remains unclear and warrants further investigation.

In our study, we found that patients carrying the MMUT c.1677-1G>A variant had a higher likelihood of experiencing cognitive impairment, ST-T abnormality, and abnormal renal function. This variant is classified as a splice site mutation, leading to disruption in the splicing process of the MMUT gene, which is involved in methylmalonic acid metabolism. The resulting accumulation of toxic metabolites may contribute to cognitive impairments and neurological abnormalities. Additionally, the variant's impact on renal function can be attributed to the systemic effects of increased methylmalonic acid levels. However, further research is needed to gain a better understanding of the underlying mechanisms driving these associations.

Except for the common variants, we identified six novel variants in the MMUT genes: c.1438G>T, c.1676+11A>G, c.3850T>G, c.508dupA, and c.688C>T, each present in one patient.

The major limitation of our study is the insufficient sample size, rendering many of the statistical tests in the current study underpowered. More patients should be enrolled to investigate the relationship between more rare genotypes and clinical symptoms. Another limitation is that our current study focuses on characterizing genotype-specific clinical manifestations. Treatment response was not included in the current genotype-phenotype correlation analysis due to incomplete data.

In conclusion, a cohort of 34 Chinese MMA patients was studied. Clinical features, biochemical index, and mutation spectrum were described. Genotype-phenotype correlations were investigated for different pathogenic genes and common mutations within the genes. This study

showed that the genotype and phenotype of MMA are correlated in the Chinese population, creating distinct clinical manifestations.

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