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ORIGINAL ARTICLE

Different mutations in the *MMUT* gene are associated with the effect of vitamin B12 in a cohort of 266 Chinese patients with mut-type methylmalonic acidemia: A retrospective study

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Abstract

Background: To summarize the relationship between different *MMUT* gene mutations and the response to vitamin B12 in MMA.

Methods: This was a retrospective study of patients diagnosed with mut-type MMA. All patients with mut-type MMA were tested for responsiveness to vitamin B12.

Results: There were 81, 27, and 158 patients in the completely responsive, partially responsive, and nonresponsive groups, respectively, and the proportions of symptom occurrence were 30/81 (37.0%), 21/27 (77.8%), and 131/158 (82.9%), respectively ($p < .001$). The median levels of posttreatment propionyl carnitine (C3), C3/acetyl carnitine (C2) ratio in the blood, and methylmalonic acid in the urine were all lower than pretreatment, and the median level of C3/C2 ratio in the completely responsive group was within the normal range. In 266 patients, 144 different mutations in the *MMUT* gene were identified. Patients with the

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mutations of c.1663G>A, c.2080C>T, c.1880A>G, c.1208G>A, etc. were completely responsive and with the mutations of c.1741C>T, c.1630_1631GG>TA, c.599T>C, etc. were partially responsive. The proportions of healthy/developmental delay outcomes in the three groups were 63.0%/23.5%, 33.3%/40.7%, and 13.3%/60.1%, respectively ($p < .001$).

Conclusion: Different mutations in the *MMUT* gene are associated with the effect of vitamin B12 treatment.

KEYWORDS

genotype–phenotype correlation, methylmalonic acidemia, methylmalonyl-CoA mutase, vitamin B12

1 | INTRODUCTION

Methylmalonic acidemia (MMA, OMIM# 251000) is a rare, autosomal, recessive, multi-systemic genetic metabolic disease caused by an enzyme deficiency during the conversion of methylmalonyl-CoA into succinyl-CoA (Villani et al., 2017). The worldwide incidence of MMA ranges between 1:20,000 and 1:125,000 (Chapman et al., 2018; Shibata et al., 2018), but a multicenter screening study in China showed an incidence of 1:35,734 (Yang et al., 2019), varying widely among regions, with 1:107,000 in Taiwan (Shibata et al., 2018), 1:21,515 in southern China (Lin et al., 2019), 1:22,358 in eastern China (Yang et al., 2020), 1:6,264 in Xinxiang City (Ma et al., 2020), and 1:5589 in Jining City (Yang et al., 2020). The expanded screening program for newborns by tandem mass spectrometry (MS/MS) is currently performed in an increasing number of regions in China.

According to the different biochemical manifestations, two main forms can be identified: isolated MMA and combined MMA and homocystinuria. About 30% of patients with MMA in China are isolated MMA (Liu et al., 2018), which is primarily caused by the partial or total deficiency of methylmalonyl-CoA mutase (MCM, EC 5.4.99.2) or its cofactor, 5′-deoxyadenosylcobalamin (AdoCbl). The main gene affected in isolated MMA is *MMUT*. The disease onset of patients with isolated MMA ranges from the neonatal period to adulthood. The accumulation of methylmalonic acid and other toxic metabolites causes a variety of clinical signs and symptoms such as difficulty feeding, developmental retardation, lethargy, coma, convulsions, and metabolic acidosis (Villani et al., 2017). Untimely treatment may lead to multi-systemic complications and even death. The current standard therapy includes a protein-restricted diet, L-carnitine, folic acid, and, pivotally, vitamin B12 in responsive patients (Baumgartner et al., 2014). Cobalamin, the cofactor of MCM, can increase the residual enzyme activity, reduce the frequency of metabolic decompensations, and improve neurologic complications and outcomes.

Nevertheless, different patients have different responses to vitamin B12 (Hvas et al., 2001; Rajan et al., 2002), but the specific influencing factors and mechanisms are still unclear. This study aims to summarize the relationship between different *MMUT* gene mutation sites and the responsiveness and curative effect of vitamin B12.

2 | METHODS

2.1 | Patients

This was a retrospective study of patients with a confirmed diagnosis of mut-type MMA between February 2007 and January 2020 at multiple Chinese hospitals and clinical centers. Patients were included if mut-type MMA was confirmed by mutation analysis of the *MMUT* gene (Han et al., 2015; Hu et al., 2018; Xie et al., 2016). Those with incomplete data were excluded.

2.2 | Biochemical examinations

The levels of blood amino acids, free carnitine, and acylcarnitines were detected in dried blood spots using tandem mass spectrometry (MS/MS, API 4000, American Biosystems Inc.). The levels of organic acids in urine were measured using gas chromatography-mass spectrometry (GC-MS, QP2010, Shimadzu Corp.). Sample preparation and detection procedures were based on methods reported previously (Han et al., 2008).

2.3 | MMUT gene mutation detection

Gene mutations were detected by Sanger sequencing or next-generation sequencing. Mutations were identified using the normal human *MMUT* sequence as a reference (GenBank, NC_000006.12). We used the ClinVar

database, the HGMD database, and the literature to identify whether the mutations had been reported. The pathogenicity of novel variants was evaluated based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines (Richards et al., 2015). The pathogenicity of missense mutations was predicted using the Mutation Taster (http://www.mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Provean (http://provean.jcvi.org/index.php), and SIFT (https://sift.bii.a-star.edu.sg/) software.

2.4 | Treatment and follow-up

All patients underwent a vitamin B12 loading test. In this test, children who had been diagnosed and had not been treated received an intramuscular injection of 1 mg of hydroxycobalamin every day for 5 consecutive days. Blood tandem mass spectrometry and urine gas chromatography were performed before and 5 days after injection, and the results of the two tests were compared (Han & Yang, 2018). The vitamin B12 loading test is used to identify the effective children who need a continuous intramuscular injection of hydroxycobalamin. Among them, fully effective children are injected once every 5–10 days, 1 mg each time. Partially effective children are injected once every 2–7 days, 1 mg each time, supplemented with L-carnitine at the same time, 50–100 mg/kg/day, and a protein-restricted diet, with limited amounts of isoleucine, valine, threonine, and methionine. Children whose vitamin B12 load test proves to be ineffective may not use hydroxy cobalamin, and they have to limit their protein intake and receive drugs such as levocarnitine.

Asymptomatic and stable patients were treated using vitamin B12, L-carnitine, and a protein-restricted diet. Patients in the acute phase had to stop protein intake, were injected with a large dose of hydroxocobalamin, and had given L-carnitine therapy. After the acute phase, those patients performed the vitamin B12 loading test again. All doses of drugs except vitamin B12 were maintained during treatment.

We distinguished three distinct clinical groups based on clinical manifestations and biochemical findings during treatment. Complete vitamin B12 responsive was defined as a reduction of more than 50% in the blood C3/C2 ratio or urinary level of methylmalonic acid after vitamin B12 treatment (Baumgartner et al., 2014). If the blood level of C3/C2 ratio or urine level of methylmalonic acid were decreased by <50% but >30% after vitamin B12 treatment, it was deemed to be partially responsive (Han & Yang, 2018). The nonresponsive patients had a decrease value in the C3/C2 ratio by <30%. The clinical and biochemical phenotypes and genotypes of patients from the three groups were compared.

The changes in the levels of C3 and C3/C2 ratio in the blood and methylmalonic acid in the urine, as well as mental development, were monitored routinely before and after treatment. The developmental quotient (DQ) was evaluated using the Gesell Developmental Schedules for patients <42 months, which provides a developmental profile in five domains, namely, adaptability, gross motor, fine motor, language, and personal–social domains. According to the average score of DQ, the development of the children was classified as: normal ($DQ \geq 85$), deficient ($DQ < 75$), and borderline ($75 \leq DQ < 85$) (You et al., 2019). The intelligence quotient (IQ) was assessed by the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) for children who were 4–6.5 years old and the Wechsler Intelligence Scale for Children (WISC) for children who were 6–16 years old. Low intelligence was defined as an IQ score of <80.

2.5 | Statistical analysis

Statistical analysis was performed using SAS 8.0 (SAS Institute). Continuous data with a nonnormal distribution (according to the Kolmogorov–Smirnov test) were presented as medians with range. Categorical data were presented as frequencies (rates). Intragroup comparisons were performed using the paired t test. Nonnormally distributed data were analyzed using the Kruskal–Wallis H test with the Nemenyi post hoc test. All tests were two-tailed, and $p < .01$ were considered to be statistically significant.

3 | RESULTS

3.1 | Characteristics of the patients

A total of 266 patients (157 males and 109 females) were included in this study. Among them, 170 patients (64%) were diagnosed through newborn screening, and the remaining patients were diagnosed because of disease onset. The general information of the three groups is summarized in Table 1. There were 81, 27, and 158 patients in the completely responsive, partially responsive, and nonresponsive groups, respectively. The median age at diagnosis was 23.3 months for the completely responsive group, 8.43 months for the partially responsive group, and 3.0 months for the nonresponsive group ($p = .001$). In the completely responsive, partially responsive, and nonresponsive groups, patients identified through newborn screening accounted for 58/81

TABLE 1 Clinical characteristics of the patients

Characteristics	Nonresponse ($n = 158$)	Partial response ($n = 27$)	Complete response ($n = 81$)	*P*
Age at diagnosis (months), median (range)	3.04 (0.03–131.33)	8.40 (0.12–75.00)	23.29 (0.07–162.00)[ab]	.001
Sex, *n* (%)				
Male	95 (60.1)	14 (51.9)	48 (59.3)	.721
Female	63 (39.9)	13 (48.1)	33 (40.7)	
Screening rate, *n* (%)	93 (58.9)	19 (70.4)	58 (71.6)	.116
Onset of first symptoms, *n* (%)	131 (82.9)	21 (77.8)	30 (37.0)[a,b]	<.001
Initial symptoms, *n* (%)				
Difficult feeding	97 (73.7)	14 (51.9)	13 (16.0)[a,b]	<.001
Vomiting	53 (30.5)	13 (48.1)	15 (18.5)[a,b]	.006
Diarrhea	33 (20.9)	9 (33.3)	5 (6.2)[a,b]	.001
Jaundice	59 (37.3)	9 (33.3)	6 (7.4)[a,b]	<.001
Seizure	45 (28.5)	5 (18.5)	8 (9.9)[a,b]	.004
Lethargy	55 (34.8)	8 (29.6)	13 (16.0)[a]	.010
Coma	41 (25.9)	7 (25.9)	8 (9.9)[a]	.013
Motor disturbance	46 (29.1)	11 (40.7)	8 (9.9)[a,b]	.001
Muscle weakness	47 (29.7)	6 (22.2)	10 (12.3)[a]	.011
Biochemical features at baseline, median (range)				
C3 level (upper limit: 0.5–4.0 μmol/L)	12.59 (0.72–120.50)	11.27 (3.65–46.77)	6.72 (1.93–48.50)[a,b]	<.001
C3/C2 ratio (upper limit: 0.04–0.25)	0.72 (0.21–2.51)	0.93 (0.14–2.89)[a]	0.51 (0.12–2.83)[a,b]	<.001
Urinary MMA (upper limit: 0.2–3.6 mmol/mol)	326.8 (0–6961.07)	203.43 (0–2200.00)	90.60 (4.62–2976.25)[a]	<.001
Biochemical features after vitamin B12 treatment, median (range)				
C3 level (upper limit: 0.5–4.0 μmol/L)	21.58 (1.59–84.22)	10.78 (0.11–51.61)[a]	4.46 (0.34–37.82)[a,b]	<.001
C3/C2 ratio (upper limit: 0.04–0.25)	0.69 (0.15–2.07)	0.43 (0.08–1.46)[a]	0.20 (0.06–0.95)[a,b]	<.001
Urinary MMA (upper limit: 0.2–3.6 mmol/mol)	296.55 (0–3632.49)	98.22 (0–1591.00)	13.55 (0–545.47)[a,b]	<.001
Outcomes at follow-up, *n* (%)				
Healthy	21 (13.3)	9 (33.3)[a]	51 (63.0)[a,b]	<.001
Delay	95 (60.1)	11 (40.7)	19 (23.5)	
Death	6 (3.8)	1 (3.7)	1 (1.2)	
Missing data	36 (22.8)	6 (22.2)	10 (12.3)	

Abbreviation: MMA, methylmalonic acid.

[a]$P < .05$ versus nonresponsive group.

[b]$P < .05$ versus partially responsive group.

(71.6%), 19/27 (70.4%), and 93/158 (58.9%), respectively, whereas the remaining patients were diagnosed because of symptoms. All patients received treatment after diagnosis confirmation.

3.2 | Disease presentation

There were significant differences in symptoms presentation and clinical severity among the three groups. Before and during this study period, the proportions of symptom occurrence (any combination of difficult feeding, vomiting, diarrhea, muscle weakness, lethargy, coma, jaundice, anemia, metabolic decompensation, and/or progressively developmental delay) were 30/81 (37.0%) in the completely responsive group, 21/27 (77.8%) in the partially responsive group, and 131/158 (82.9%) in the nonresponsive group ($p < .0001$) (Table 1). The initial symptoms were variable, including different combinations and degrees of difficult feeding,

vomiting, diarrhea, muscle weakness, lethargy, coma, jaundice, anemia, metabolic decompensation, and progressively developmental delay.

3.3 | Biochemical features of the patients

The levels of C3 and the C3/C2 ratio in blood and methylmalonic acid in the urine of patients in each group are presented in Table 1. The median levels of methylmalonic acid, C3, and C3/C2 ratio before therapy in all groups were higher than the upper limit values (0.2–3.6 mmol/mol, 0.5–4.0 μmol/L, and 0.04–0.25, respectively). The levels of pretreatment methylmalonic acid, C3, and C3/C2 ratio in the completely responsive group were lower than in the nonresponsive group (all $p < .05$); the pretreatment levels of C3 and C3/C2 ratio were lower in the completely responsive group than in the partially responsive group (both $p < .05$). The C3/C2 ratio was lower in the partially responsive group than in the nonresponsive group ($p < .05$). The median levels of posttreatment methylmalonic acid, C3, and C3/C2 ratio (Table 1) were all lower in the completely responsive group than in the two other groups (all $p < .05$). The median level of C3/C2 in the completely responsive group was within the normal range. The median levels of posttreatment C3 and C3/C2 ratio (Table 1) were all lower in the partially responsive group than in the nonresponsive groups (all $p < .05$). The difference between pretreatment and posttreatment about the level of C3/C2 in the blood was statistically significant both in responsive and partially responsive groups (all $p < .01$). The level of C3, C3/C2 ratio, and methylmalonic acid in pretreatment, posttreatment, and difference values were all different among the three groups (all $p < .001$).

3.4 | Mutation analysis

In 266 patients with mut-type MMA, 144 different mutations in the *MMUT* gene were identified (Tables 2–4). The numbers of different mutations in the completely responsive, partially responsive, and nonresponsive groups were 35, 14, and 95, respectively. Among the 35 mutations in the completely responsive group (Table 2), the two most frequent were c.1663G>A (29 patients) and c.2080C>T (10 patients). Among the 14 mutations in the partially responsive group (Table 3), the three most frequent were c.1741C>T (seven patients), c.1630_1631GG>TA (six patients), and c.599T>C (six patients). Among the 95 mutations in the nonresponsive group (Table 4), the most common mutations included c.729_730insTT (32 patients), c.323G>A (30 patients), c.1106G>A (21 patients), c.914T>C (15 patients), and c.1677-1G>A (12 patients).

Of the 35 different mutations in the completely responsive and partially responsive group, 31 (88.6%) were missense mutations, one was a deletion/duplication/insertion, two were nonsense mutations, and none was splice-site mutation; in the nonresponsive group, those numbers 63, 13, 18, and 8, respectively (Figure 1). Nearly, all insertion/deletion mutations and frameshift mutations (p.S23*, p.Q35*, p.K121*, p.G133Vfs*6, p.K210*, p.D244Lfs*39, p.H252Qfs*6, p.G454Efs*6, p.R467*, c.R511*, p.E593*, p.V704*, and p.R727*) were observed in the nonresponsive group.

Although mutations relevant to phenotypes were found in every exon (except for the noncoding exon 1), the distribution was not equal (Figure 2), and the mutations were predominantly found in exons 2, 3, and 6 (50%). The DNA sequences encoding the N-terminal domain and the C-terminal domain showed comparable mutation rates. The proportion of mutations in the N-terminal domain was higher in the nonresponsive group (65/95, 68.4%) than in the completely responsive group (14/26, 53.0%). Among the linker region, only one nonsense mutation (c.1531C>T) in exon 8 was found in the nonresponsive group. The residue p.Ile521–p.Ala558 encoded by exon 9, p.A555T (c.1663G>A), was the most frequently found mutation in the completely responsive group. Other contiguous mutations in exon 9 (p.R532H (c.1595G>A), p.L537Q (c.1610T>A), and p.G544E (c.1632G>A)) were all in the completely responsive and partly responsive groups.

3.5 | Prognosis

A significant difference was detected in the prognosis of the three groups ($p < .0001$). As for the current health condition, 51 patients (50/71, 70.4%) in the completely responsive group and nine (9/21, 42.9%) in the partially responsive group lived a normal life asymptomatically. Among those healthy patients, nine patients in the completely responsive group and six in the partially responsive group had experienced disease onsets but were asymptomatic after treatment. In the nonresponsive group, 95 patients (95/122, 77.9%) had developmental delay, and 21 (21/122, 17.2%) regained health.

A total of 45 patients were assessed for DQ (16, 7, and 22, respectively, in the completely responsive, partially responsive, and nonresponsive groups). In the completely responsive group, DQ was normal in eight patients, borderline in two, and deficient in six for an abnormal rate of 50% (8/16). In the partially responsive group, DQ was normal in four patients, borderline in two, and deficient in one for a total abnormal rate of 42.9% (3/7). In the nonresponsive group, DQ was normal in 10 patients, borderline in two, and deficient in

TABLE 2 Mutations of patients completely responsive to vitamin B12

	Alleles 1			Alleles 2			
Gene	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	***N***
MMUT	Exon2	c.278G>A	p.R93H	Exon3	c.567T>G	p.N189K	1
MMUT	Exon2	c.278G>A	p.R93H	Exon10	c.1784_1785 delAA	p.K595Rfs*11	1
MMUT	Exon2	c.295A>C	p.M99L	Exon3	c.626dupC	p.K210*	1
MMUT	Exon2	c.295A>C	p.M99L	Exon5	c.914T>C	p.L305S	1
MMUT	Exon2	c.346G>A	p.V116M	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon2	c.346G>A	p.V116M	Intron9	c.1677-5_1690del TTCAG	p.C560fs	1
MMUT	Exon3	c.389G>C	p.G130A	Exon2	c.323G>A	p.R108H	1
MMUT	Exon3	c.389G>C	p.G130A	Exon3	c.659A>T	p.N220V	1
MMUT	Exon3	c.441T>A	p.D147E	Exon2	c.323G>A	p.R108H	1
MMUT	Exon3	c.441T>A	p.D147E	Exon3	c.441T>A	p.D147E	1
MMUT	Exon3	c.446A>G	p.D149G	Exon3	c.729_730insTT	p.D244Lfs*39	1
MMUT	Exon3	c.446A>G	p.D149G	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon3	c.457G>A	p.V153I		Undetected		1
MMUT	Exon3	c.461G>A	p.R154H		Undetected		1
MMUT	Exon3	c.482G>T	p.G161V	Exon3	c.482G>T	p.G161V	1
MMUT	Exon3	c.556A>G	p.M186V	Exon3	c.419T>C	p.L140P	1
MMUT	Exon3	c.556A>G	p.M186V	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon3	c.694A>T	p.I232F	Intron9	c.1677-1G>A	Splicing	1
MMUT	Exon3	c.724A>T		Exon4	c.788G>T	p.G263V	1
MMUT	Intron3	c.753+3A>G	Splicing	Exon2	c.323G>A	p.R108H	1
MMUT	Intron3	c.753+3A>G	Splicing	Exon4	c.755dupA	p.H252Qfs*6	2
MMUT	Exon4	c.865A>G	p.R289G	Exon3	c.729_730insTT	p.D244Lfs*39	1
MMUT	Exon6	c.1142G>A	p.G381E	Exon6	c.1153_1154del	p.L385fs	2
MMUT	Exon6	c.1208G>A	p.R403Q	Exon2	c.91C>T	p.R31*	1
MMUT	Exon6	c.1208G>A	p.R403Q	Exon3	c.556A>G	p.M186V	1
MMUT	Exon6	c.1208G>A	p.R403Q	Exon6	c.1208G>A	p.R403Q	1
MMUT	Exon9	c.1610T>A	p.L537Q	Exon2	c.323G>A	p.R108H	1
MMUT	Exon9	c.1632G>A	p.G544E	Exon3	c.729_730insTT	p.D244Lfs*39	1

(Continues)

TABLE 2 (Continued)

Gene	Alleles 1			Alleles 2			N
	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	
MMUT	Exon9	c.1663G>A	p.A555T	Exon10	c.1679G>A	p.C560Y	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon12	c.2009G>T	p.G670V	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon13	c.2131G>T	p.E711*	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon13	c.2131G>T	p.E711*	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon13	Deletion		1
MMUT	Exon9	c.1663G>A	p.A555T	Exon2	c.2T>C	P.M1T	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon3	c.424A>G	p.T142A	2
MMUT	Exon9	c.1663G>A	p.A555T	Exon3	c.494A>G	p.D165G	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon3	c.613G>A	p.E205K	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon3	c.626dupC	p.K210*	2
MMUT	Exon9	c.1663G>A	p.A555T	Exon3	c.729_730insTT	p.D244Lfs*39	7
MMUT	Exon9	c.1663G>A	p.A555T	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon5	c.914T>C	p.L305S	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon6	c.1106G>A	p.R369H	3
MMUT	Exon9	c.1663G>A	p.A555T	Exon6	c.1207C>T	p.R403*	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon6	c.1233_1235delCAT	p.I410-	1
MMUT	Exon9	c.1663G>A	p.A555T	Exon6	c.1280G>A	p.G427D	1
MMUT	Exon9	c.1663G>A	p.A555T		Undetected		2
MMUT	Exon11	c.1847G>A	p.R616H		Undetected		1
MMUT	Exon11	c.1880A>G	p.H627R	Exon5	c.914T>C	p.L305S	1
MMUT	Exon11	c.1880A>G	p.H627R	Exon6	c.1280G>A	p.G427D	1
MMUT	Exon11	c.1880A>G	p.H627R	Exon9	c.1630_1631 GG>TA	p.G544*	1
MMUT	Exon11	c.1880A>G	p.H627R	Exon11	c.1919A>T	p.D640V	1
MMUT	Exon12	c.2011A>G	p.I671V	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon12	c.2080C>T	p.R694W	Intron2	c.385+5G>T	Splicing	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon3	c.424A>G	p.T142A	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon3	c.454C>T	p.R152*	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon3	c.682C>T	p.R228*	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon3	c.729_730insTT	p.D244Lfs*39	1

(Continues)

TABLE 2 (Continued)

Gene	Alleles 1			Alleles 2			N
	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	
MMUT	Exon12	c.2080C>T	p.R694W	Exon6	c.1105C>T	p.R369C	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon6	c.1233_1235 delCAT	p.I410-	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon10	c.1741C>T	p.R581*	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon12	c.2106delA	p.G702Gfs*3	1
MMUT	Exon12	c.2080C>T	p.R694W	Exon13	c.2179C>T	p.R727*	1
MMUT	Exon13	c.2168G>A	p.G723D	Exon5	c.920_923delTCTT	p.F307Sfs*6	2
MMUT	Exon13	c.2206C>T	p.L736F	Exon5	c.914T>C	p.L305S	1
MMUT	Exon13	c.2216T>C	p.I739T	Exon3	c.729_730insTT	p.D244Lfs*39	2
MMUT	Exon13	c.2454delA		Exon13	c.2454delA		1

Abbreviation: N, number of patients.

10 for a total abnormal rate of 54.6% (12/22). Among 13 patients who had a WPPSI or WSIC, three patients (3/5) in the completely responsive group, three (3/3) in the partially responsive group, and three (3/5) in the nonresponsive group were normal.

4 | DISCUSSION

In this study, we described the relationship between *MMUT* gene mutations and the response to vitamin B12 therapy, which, to date, is the single largest study for mut-type MMA. Vitamin B12 is used in the management of MMA (Baumgartner et al., 2014), but the patients display different responses to treatment and the reason why is unknown (Hvas et al., 2001; Rajan et al., 2002). All 266 patients in our cohort had a vitamin B12 loading test, and the results strongly suggest that the response to vitamin B12 should be assessed in every patient and the treatments tailored accordingly. Most importantly, the present study showed that the different mutations found in the *MMUT* gene are associated with the effect of vitamin B12 treatment according to the ratio of C3/C2 in blood and the level of methylmalonic acid in urine after treatment.

MMA is an autosomal recessive disease that is typically diagnosed in the neonatal period and frequently after an acute metabolic decompensation (Kölker et al., 2015). Newborn screening can shorten the diagnostic process and improve prognosis (Heringer et al., 2016). In recent years, more and more children were diagnosed through newborn screening. It has become evident that metabolic crises are more common in patients with mut-type MMA (Kang et al., 2019). Hörster et al. (2007) reported that mut^- patients usually present with a milder phenotype and lower occurrence of long-term complications compared with mut^0 patients. In the present cohort, the patients in the completely responsive and partially responsive groups had milder clinical manifestations and more ideal biochemical measurements. In the completely responsive and partially responsive groups, 77 patients (77/108, 71%) were diagnosed by newborn screening, and 51 patients (51/77, 66%) were asymptomatic. On the contrary, patients not responsive to vitamin B12 had an early onset (<1 year old), and the first symptom included lethargy, coma, and seizures, among others. In the nonresponsive group, 93 patients (93/158, 59%) were diagnosed by newborn screening, but only 17 patients (17/93, 18%) were not affected clinically. After symptom onset, and about 50% of patients had developmental delays.

The patients, whether they are identified through screening or clinical onset, should start treatments as soon as possible, without waiting for biochemical and genetic results. The primary aim in treating MMA is to decrease

TABLE 3 Mutations of patients partially responsive to vitamin B12

	Alleles 1			Alleles 2			
Gene	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	***N***
MMUT	Exon2	c.268delinsAA	p.P99Nfs*14	Exon5	c.1070C>G	p.S357*	1
MMUT	Exon3	c.554C>T	p.S185F	Exon12	c.2062G>T	p.E688*	1
MMUT	Exon3	c.599T>C	p.I200T	Exon3	c.599T>C	p.I200T	1
MMUT	Exon3	c.599T>C	p.I200T	Exon3	c.693C>G	p.Y231*	1
MMUT	Exon3	c.599T>C	p.I200T	Exon4	c.755dupA	p.H252Qfs*6	2
MMUT	Exon3	c.599T>C	p.I200T	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon3	c.599T>C	p.I200T	Exon10	c.1741C>T	p.R581*	1
MMUT	Exon5	c.925T>G	p.W309G	Exon12	c.2107G>A	p.G703R	1
MMUT	Exon6	c.1138G>A	p.G380R	Exon3	c.1286A>G	p.Y429C	1
MMUT	Exon9	c.1630_1631GG>TA	p.G544*	Exon2	c.323G>A	p.R108H	2
MMUT	Exon9	c.1630_1631GG>TA	p.G544*	Exon3	c.494A>G	p.D165G	1
MMUT	Exon9	c.1630_1631GG>TA	p.G544*	Exon3	c.729_730insTT	p.D244Lfs*39	1
MMUT	Exon9	c.1630_1631GG>TA	p.G544*	Exon3	c.753G>T	p.K251N	1
MMUT	Exon9	c.1630_1631GG>TA	p.G544*	Exon5	c.925T>G	p.W309G	1
MMUT	Exon10	c.1741C>T	p.R581*	Exon3	c.742T>A	p.Y248N	1
MMUT	Exon10	c.1741C>T	p.R581*	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon10	c.1741C>T	p.R581*	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon10	c.1741C>T	p.R581*	Exon6	c.1107dupT	p.T370fs	1
MMUT	Exon10	c.1741C>T	p.R581*	Exon13	c.2179C>T	p.R727*	1
MMUT	Exon10	c.1741C>T	p.R581*		Undetected		2
MMUT	Exon11	c.1846C>T	p.R616C	Exon3	c.454C>T	p.R152*	1
MMUT	Exon11	c.1943G>A	p.G648D	Exon9	c.1630_1631GG>TA	p.G544*	2
MMUT	Exon11	c.1943G>A	p.G648D	Intron9	c.1677-1G>A	Splicing	1

Abbreviation: N, number of patients.

TABLE 4 Mutations of patients not responsive to vitamin B12

	Alleles 1			Alleles 2			
Gene	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	**Exon/intron**	**Nucleotide mutation**	**Amino acid alteration**	***N***
MMUT	Exon2	c.68C>G	p.S23*		Undetected		1
MMUT	Exon2	c.91C>T	p.R31*	Exon11	c.1850T>G	p.L617R	1
MMUT	Exon2	c.103C>T	p.Q35*	Exon2	c.322C>T	p.R108C	1
MMUT	Exon2	c.103C>T	p.Q35*	Exon3	c.613G>A	p.E205K	1
MMUT	Exon2	c.103C>T	p.Q35*	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon2	c.141C>T	p.G138R	Exon2	c.217C>T	p.R73X	1
MMUT	Exon2	c.260G>A	p.G87E	Exon3	c.433G>A	p.G145S	1
MMUT	Exon2	c.322C>T	p.R108C	Exon2	c.323G>A	p.R108H	1
MMUT	Exon2	c.322C>T	p.R108C	Exon3	c.581C>T	p.P194L	1
MMUT	Exon2	c.323G>A	p.R108H	Exon2	c.22delC	p.L8Ffs*9	1
MMUT	Exon2	c.323G>A	p.R108H	Exon2	c.323G>A	p.R108H	3
MMUT	Exon2	c.323G>A	p.R108H	Exon2	c.326A>G	p.Q109R	1
MMUT	Exon2	c.323G>A	p.R108H	Exon3	c.424A>G	p.T142A	4
MMUT	Exon2	c.323G>A	p.R108H	Exon3	c.682C>T	p.R228*	1
MMUT	Exon2	c.323G>A	p.R108H	Exon6	c.1280G>A	p.G427D	1
MMUT	Exon2	c.323G>A	p.R108H	Exon8	c.1514T>C	p.I505T	1
MMUT	Exon2	c.360dupT	p.K121*	Exon7	c.1349A>G	p.E450G	1
MMUT	Exon3	c.398_399delGA	p.G133Vfs*6		Undetected		1
MMUT	Exon3	c.421G>A	p.A141T	Exon4	c.811_812 insGG	p.A271Gfs*12	1
MMUT	Exon3	c.424A>G	p.T142A	Exon3	c.419T>C	p.L140P	1
MMUT	Exon3	c.424A>G	p.T142A	Exon3	c.544dupA	p.M182Nfs*29	1
MMUT	Exon3	c.424A>G	p.T142A	Exon8	c.1537_1538 insT		1
MMUT	Exon3	c.494A>G	p.D165G	Exon2	c.323G>A	p.R108H	1
MMUT	Exon3	c.494A>G	p.D165G	Exon6	c.1330G>A	p.K444*	1
MMUT	Exon3	c.613G>A	p.E205K	Exon5	c.982C>T	p.L328F	1
MMUT	Exon3	c.613G>A	p.E205K	Exon6	c.1280G>A	p.G427D	1
MMUT	Exon3	c.613G>A	p.E205K	Intron9	c.1677-1G>A	Splicing	1
MMUT	Exon3	c.626dupC	p.K210*	Exon2	c.323G>A	p.R108H	1

(Continues)

TABLE 4 (Continued)

	Alleles 1			Alleles 2			
Gene	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	*N*
MMUT	Exon3	c.626dupC	p.K210*	Exon3	c.494A>G	p.D165G	1
MMUT	Exon3	c.626dupC	p.K210*	Exon5	c.1049A>G	p.H350R	1
MMUT	Exon3	c.626dupC	p.K210*	Intron5	c.1084-33 delTTTC	Splicing	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon2	c.91C>T	p.R31*	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon2	c.323G>A	p.R108H	5
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.424A>G	p.T142A	2
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.467A>T	p.D156V	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.654A>C	p.Q218H	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.655A>G	p.N219D	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.699dup	p.P234Sfs*11	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon3	c.729_730insTT	p.D244Lfs*39	4
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon5	c.914T>C	p.L305S	2
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon6	c.1105C>T	p.R369C	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon6	c.1106G>A	p.R369H	3
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon6	c.1286A>G	p.Y429C	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon8	c.1540C>T	p.Q514*	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon10	c.1760A>G	p.Y587C	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon11	c.1847A>C	p.D625A	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon12	c.2107G>A	p.G703R	1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39	Exon13	deletion		1
MMUT	Exon3	c.729_730insTT	p.D244Lfs*39		Undetected		1
MMUT	Intron3	c.754-1G>C	Splicing	Exon5	c.1061C>T	p.S354F	1
MMUT	Intron3	c.754-1G>C	Splicing	Exon10	c.1718T>C	p.F573S	1
MMUT	Intron3	c.754-1G>C	Splicing	Exon12	c.2009G>T	p.G670V	1
MMUT	Intron3	c.754-1G>C	Splicing	Exon13	c.2150G>T	p.G717V	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon2	c.29dupT	p.L10Ffs*39	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon3	c.590C>A	p.A197E	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon4	c.755dupA	p.H252Qfs*6	1

(Continues)

TABLE 4 (Continued)

	Alleles 1			Alleles 2			
Gene	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	*N*
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Intron4	c.912-2A>T	Splicing	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon5	c.920_923delTCTT	p.F307Sfs*6	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon5	c.947A>C	p.Y316S	1
MMUT	Exon4	c.755dupA	p.H252Qfs*6	Exon6	c.1280G>A	p.G427D	2
MMUT	Exon5	c.914T>C	p.L305S	Exon2	c.323G>A	p.R108H	3
MMUT	Exon5	c.914T>C	p.L305S	Exon3	c.424A>G	p.T142A	1
MMUT	Exon5	c.914T>C	p.L305S	Exon5	c.970G>A	p.A324T	1
MMUT	Exon5	c.914T>C	p.L305S	Exon5	c.975_976 dflTA	Splicing	1
MMUT	Exon5	c.914T>C	p.L305S	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon5	c.914T>C	p.L305S	Exon10	c.1687G>C	p.G563R	1
MMUT	Exon5	c.914T>C	p.L305S	Exon10	c.1806T>G	p.L617R	1
MMUT	Exon5	c.914T>C	p.L305S	Exon12	c.2062G>T	p.E688*	1
MMUT	Exon5	c.1009T>C	p.F337L	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon5	c.1009T>C	p.F337L	Exon9	c.1581_1582 insA	p.L385W	1
MMUT	Exon5	c.1038_1040 delTCT	p.L346_347del		Undetected		1
MMUT	Exon5	c.1038_1040 delTCT	p.L346_347del	Exon6	c.1141G>A	p.G381R	
MMUT	Exon6	c.1105C>T	p.R369C	Exon2	c.323G>A	p.R108H	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon2	c.278G>A	p.R93H	2
MMUT	Exon6	c.1106G>A	p.R369H	Exon2	c.322C>T	p.R108C	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon2	c.323G>A	p.R108H	3
MMUT	Exon6	c.1106G>A	p.R369H	Exon2	c.349G>T	p.E117*	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon3	c.424A>G	p.T142A	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon3	c.470T>A	p.V157D	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon3	c.494A>G	p.D165G	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon3	c.544dupA	p.M182Nfs*29	1

(Continues)

TABLE 4 (Continued)

	Alleles 1			Alleles 2			
Gene	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	*N*
MMUT	Exon6	c.1106G>A	p.R369H	Exon7	c.1439A>G	p.D480G	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon8	c.1530_1531 insTT		1
MMUT	Exon6	c.1106G>A	p.R369H	Exon10	c.1807A>T	p.R603W	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon12	C.2062G>T	P.E688*	1
MMUT	Exon6	c.1106G>A	p.R369H	Exon13	c.2131G>T	p.E711*	1
MMUT	Exon6	c.1141G>A	p.G381R	Exon3	c.398_399 delGA	p.Q131Pfs*8	1
MMUT	Exon6	c.1159A>C	P.T387P	Exon2	c.323G>A	p.R108H	1
MMUT	Exon6	c.1159A>C	p.T387P	Exon3	c.693C>G	p.Y231*	1
MMUT	Exon6	c.1280G>A	p.G427D	Exon2	c.1A>G	p.M1V	1
MMUT	Exon6	c.1280G>A	p.G427D	Exon3	c.454C>T	p.R152*	1
MMUT	Exon6	c.1280G>A	p.G427D	Exon3	c.567T>G	p.N189K	1
MMUT	Exon6	c.1280G>A	p.G427D	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon6	c.1280G>A	p.G427D	Intron10	c.1809-1G>A	Splicing	1
MMUT	Exon6	c.1280G>A	p.G427D	Intron5	c.1084-10A>G	Splicing	1
MMUT	Exon6	c.1295A>C	p.E432A	Exon2	c.278G>A	p.R93H	1
MMUT	Exon6	c.1295A>C	p.E432A	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon6	c.1295A>C	p.E432A		Undetected		1
MMUT	Exon6	c.1295A>C	p.E432A	Intron9	c.1677-1G>A	Splicing	1
MMUT	Exon7	c.1359delT	p.G454Efs*6	Exon4	c.861C>G	p.Y287*	1
MMUT	Exon7	c.1359delT	p.G454Efs*6	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon7	c.1399C>T	p.R467*	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon7	c.1399C>T	p.R467*	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon8	c.1531C>T	c.R511*	Exon3	c.626dupC	p.K210*	1
MMUT	Exon8	c.1531C>T	c.R511*	Exon3	c.683G>A	p.R228Q	1
MMUT	Exon8	c.1531C>T	c.R511*	Exon4	c.755dupA	p.H252Qfs*6	1
MMUT	Exon9	c.1595G>A	p.R532H	Exon6	c.1280G>A	p.G427D	1

(Continues)

TABLE 4 (Continued)

	Alleles 1			Alleles 2			
Gene	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	*N*
MMUT	Exon9	c.1595G>A	p.R532H	Exon12	c.2011A>G	p.I671Y	1
MMUT	Intron9	c.1677-1G>A	Splicing	Exon13	c.2156_2156delC	P.N720fs	1
MMUT	Intron9	c.1677-1G>A	Splicing	Exon2	c.323G>A	p.R108H	1
MMUT	Intron9	c.1677-1G>A	Splicing	Exon3	c.569G>T	p.G190V	1
MMUT	Intron9	c.1677-1G>A	Splicing	Exon3	c.578T>A	p.L193N	1
MMUT	Intron9	c.1677-1G>A	Splicing	Exon3	c.729_730 insTT	p.D244Lfs*39	2
MMUT	Intron9	c.1677-1G>A	Splicing	Exon5	c.914T>C	p.L305S	3
MMUT	Intron9	c.1677-1G>A	Splicing	Exon6	c.1280G>A	p.G427D	1
MMUT	Intron9	c.1677-1G>A	Splicing	Intron8	c.1560+2 T>A	Splicing	1
MMUT	Exon10	c.1777G>T	p.E593*	Exon3	c.494A>G	p.D165G	1
MMUT	Exon10	c.1777G>T	p.E593*	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon10	c.1777G>T	p.E593*	Exon13	c.2179C>T	p.R727*	1
MMUT	Exon11	c.1850T>G	p.L617R	Exon2	c.91C>T	p.R31*	1
MMUT	Exon11	c.1850T>G	p.L617R	Exon5	c.914T>C	p.L305S	1
MMUT	Exon11	c.1850T>G	p.L617R	Exon6	c.1280G>A	p.G427D	1
MMUT	Exon11	c.1850T>G	p.L617R	Exon10	c.1679G>A	p.C560Y	1
MMUT	Exon11	c.1853T>C	p.L618P	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon11	c.1853T>C	p.L618P	Exon6	c.1106G>A	p.R369H	1
MMUT	Exon12	c.2009G>T	p.G670V	Exon3	c.729_730 insTT	p.D244Lfs*39	1
MMUT	Exon12	c.2106delA	p.V704*	Exon10	c.1790T>G	p.I597R	1
MMUT	Exon13	c.2179C>T	p.R727*	Exon2	c.323G>A	p.R108H	1
MMUT	Exon13	c.2179C>T	p.R727*	Exon3	c.424A>G	p.T142A	1
MMUT	Exon13	c.2179C>T	p.R727*	Exon9	c.1673C>T	p.A558V	1
MMUT	Exon13	c.2179C>T	p.R727*	Intron9	c.1677-1 G>A	spicing	1

(Continues)

TABLE 4 (Continued)

Gene	Alleles 1			Alleles 2			N
	Exon/intron	Nucleotide mutation	Amino acid alteration	Exon/intron	Nucleotide mutation	Amino acid alteration	
MMUT	Exon13	c.2179C>T	p.R727*	Exon10	c.1690G>A	p.E564K	1
MMUT	Exon13	c.2445delA			Undetected		1

Abbreviation: N, number of patients.

the toxic metabolites, increase the disposal of toxic metabolites, and achieve normal development (Hörster & Hoffmann, 2004). In 1968, Lindblad et al. (1969) and Rosenberg et al. (1968) reported simultaneously that vitamin B12 could decrease the levels of methylmalonic acid in the urine compared with only diet therapy. Since then, intramuscular hydroxycobalamin has been increasingly used for patients with B12-responsive MMA (Fraser & Venditti, 2016). Cobalamin, the cofactor of MCM, can increase the residual enzyme activity, reduce the frequency of metabolic decompensations, and improve neurologic complications and outcomes.

Isolated MMA could be divided into two subclasses (mut^- and mut^0) (Willard & Rosenberg, 1980), based on the presence (mut^-) or absence (mut^0) of residual enzyme activity in the fibroblasts of the patients by the PI assay to supplementation with hydroxocobalamin (OHCbl) (Forny et al., 2016). Nearly all cblA, one-third of cblB, and cblD-variant patients and mut^- patients usually have a greater response to vitamin B12 supplementation (Fowler et al., 2008; Matsui et al., 1983; Tanpaiboon, 2005; Willard & Rosenberg, 1980). Nevertheless, there are inconsistent results in vivo and in vitro (Fowler et al., 2008). Response to vitamin B12 should be assessed by vitamin loading tests in every MMA patient, and, for responders, vitamin B12 should be used as a long-term treatment (Baumgartner et al., 2014). In the present study, all eligible patients received the vitamin B12 loading test in time, and the results indicated the patient's responsiveness to vitamin B12 and served as a reference for the primary treatment. The gene mutations carried by the patients in the nonresponsive group were all exclusive to the absence of response to vitamin B12. In the meantime, some patients who carried one of those mutations showed a response to vitamin B12, suggesting that the other allele plays a decisive role.

The outcome of MMA has a close relationship with the enzymatic subgroup, cobalamin responsiveness, and age at onset (Hörster et al., 2009). Compared with the nonresponsive group, the completely responsive and partially responsive groups showed lower morbidity, less developmental delay, and thereby better prognosis. There was a close relationship between these clinical phenotypes and genotypes. Among patients with the c.1663G>A mutation, who were responsive to vitamin B12, only five (5/29, 17%) showed symptoms, and the ratio of C3/C2 in blood after treatment in 26 patients (26/29, 90%) was normal (<0.25). The mutation c.729_730insTT, which is the locus with the highest mutation rate in the Chinese population in this study and in other previous studies (Han et al., 2015; Hu et al., 2018; Kang et al., 2019), was found in the nonresponsive group. Among patients with c.729_730insTT, 26 patients (26/32, 81%) were diagnosed after onset, two patients

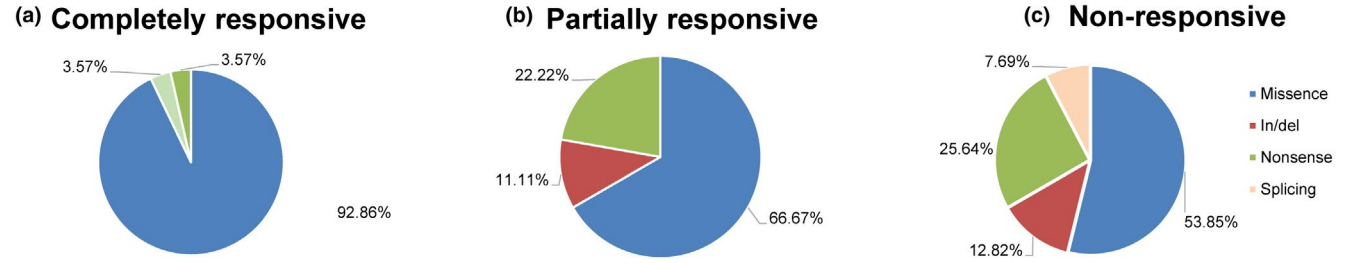

FIGURE 1 Pie chart summarizing the types of MMUT mutations in patients with mut-type methylmalonic acidemia. (a) Completely responsive group, (b) partially responsive group, and (c) no responsive group

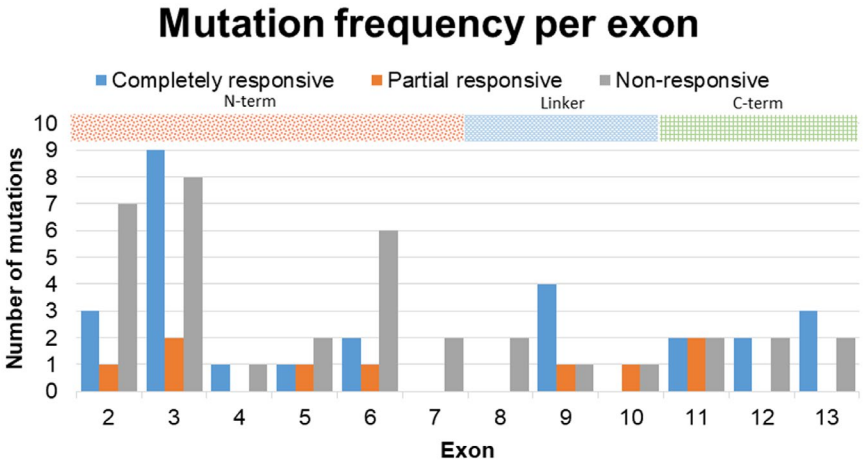

FIGURE 2 The relative mutation frequency for each individual exon in the different response groups (no exon 1, since it is noncoding; only the coding region of exons 2 and 13 were calculated)

died, and nearly all remaining patients showed developmental delay. After treatment, the ratio of C3/C2 in the blood of all patients with c.729_730insTT was >0.5.

At present, the underlying mechanism of the correlation between the different mutations and vitamin B12 responsiveness is unclear. One potential influencing factor might be the different types of mutations. In the present study, 92.9% of the mutations in the responsive group were missense mutations. At the same time, nearly all insert/deletion mutations and frameshift mutations that usually led to a dysfunctional enzyme were found in the non-responsive group. p.S23*, p.Q35*, p.K121*, p.K210*, p.R467*, c.R511*, p.E593*, p.V704*, and p.R727* are nonsense mutations that result in a premature stop codon and usually nonfunctional protein, and the patients carrying those mutations were all non-responsive to vitamin B12. The MCM enzyme has two polypeptide chains of 750 amino acids and assembles as a homodimer composed of two domains. Both the N-terminal (residue 1–481) and the C-terminal (residue 482–585) domains bind the essential cofactor 5′-deoxyadenosylcobalamin (AdoCbl) and are interconnected by a linker region (residue 586–750). The amino acids encoded by exons 8–10 correspond almost fully to the protein linker region, which does not contribute residues to either the catalytic center or the ligand-binding pockets (Froese et al., 2010). The linker region is a nonfunctional area and has less impact on enzyme activity. It might be an explanation of why patients with mutations located in this region (p.R532H, p.L537Q, p.G544E, and p.A555T) are responsive to vitamin B12. The *MMUT* gene, which encodes the methylmalonyl-CoA mutase, lies on chromosome 6p 12.3 (Ledley et al., 1988) and spans over 13 exons, with the first exon being noncoding. In addition, 68% of the mutations in the nonresponsive group were located in the N-terminal region, suggesting damage to the active site, whereas 43% of the mutations were in the C-terminal region in the responsive and partially responsive group, which is considered to elicit less effect on the MCM functions. Splice mutations may lead to entire exons being spliced out of the mRNA or translating into intron regions aberrantly (Furuya et al., 2018). In the present study, the donor splice site c.753+3A>G was responsive to vitamin B12, whereas the acceptor splice sites c.-39-2A>G, c.754-1G>C, and c.1677-1G>A were nonresponsive; this is only an observation and no conclusion can be made on donor/acceptor splice mutations at this point. In addition, the same phenotype may be the result of a combination of factors.

MMA is an autosomal recessive disorder, and the phenotype then depends upon the two mutated alleles.

It was speculated that the mutation sites with lighter phenotype were mostly located in the nonfunctional domain of the MMUT protein and that complex heterozygous mutations containing mutations with a lighter phenotype will have less impact on the protein function; that is, more enzyme activity will be retained. The c.729_730insTT allele indeed appears to be associated with vitamin B12 nonresponsive MMA but was also found in many fully- or partially vitamin B12 responsive patients. The possession of one copy of this allele would not provide useful information for phenotype prediction. The second allele plays an important role in the phenotype.

From previous studies and case reports, we know that many MMA cases caused by MMUT gene mutations are unresponsive to vitamin B12 (Hvas et al., 2001; Rajan et al., 2002). Therefore, the identification of mutations that are responsive to vitamin B12 will guide management. In this study, we used genetic testing results and the vitamin B12 loading test results to identify patients with compound heterozygous mutations that appeared to lead to a lighter clinical presentation. In such patients, the treatment effect of vitamin B12 was good, and their prognosis was better than for other patients.

This study has limitations. Some mutations were found in only one or two patients, and it cannot be concluded at this point that those uncommon mutations belong to only one phenotype. Furthermore, individual differences could not be fully excluded. In future, we will collect more cases and continue to follow them in order to supplement, verify, and correct the present results. Moreover, a biochemical assay of PI or determination of enzymatic activity will be performed.

In conclusion, the correlations between genotypes and phenotypes in a cohort of 266 Chinese patients with mut-type MMA were studied. The results suggest that specific *MMUT* mutations belong to a specific phenotypic group of response to vitamin B12. The mutations c.1663G>A, c.2080C>T, c.1880A>G, and c.1208G>A were found in patients responsive to vitamin B12. Patients with the c.1663G>A mutation usually had a better prognosis as long as they could be diagnosed and treated in time. The mutations c.1741C>T, c.1630_1631GG>TA, and c.599T>C were found in partially responsive patients to vitamin B12. The mutations c.729_730insTT, c.1106G>A, c.323G>A, c.1677-1G>A, and c.914T>C were found in patients not responsive to vitamin B12. The patients in the completely and partially responsive groups had milder clinical phenotypes and more favorable biochemical measurements. Therefore, gene sequencing and vitamin B12 loading tests should be performed in every MMA patient, while vitamin B12 should be used as a long-term treatment in responders.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

ETHICAL COMPLIANCE

This study was approved by the ethical committee of Xin Hua Hospital (lead center) (XHEC-D-2020-159). The patients or their legal guardians signed an informed consent form, approving the analysis of their clinical records and publication of the anonymous data.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

Baumgartner, M. R., Hörster, F., Dionisi-Vici, C., Haliloglu, G., Karall, D., Chapman, K. A., Huemer, M., Hochuli, M., Assoun, M., Ballhausen, D., Burlina, A., Fowler, B., Grünert, S. C., Grünewald, S., Honzik, T., Merinero, B., Pérez-Cerdá, C., Scholl-Bürgi, S., Skovby, F., … Chakrapani, A. (2014). Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. *Orphanet Journal of Rare Diseases*, *9*, 130. https://doi.org/10.1186/s13023-014-0130-8

Chapman, K. A., Gramer, G., Viall, S., & Summar, M. L. (2018). Incidence of maple syrup urine disease, propionic acidemia, and methylmalonic aciduria from newborn screening data. *Molecular Genetics and Metabolism Reports*, *15*, 106–109. https://doi.org/10.1016/j.ymgmr.2018.03.011

Forny, P., Schnellmann, A. S., Buerer, C., Lutz, S., Fowler, B., Froese, D. S., & Baumgartner, M. R. (2016). Molecular Genetic Characterization of 151 Mut-Type Methylmalonic Aciduria Patients and Identification of 41 Novel Mutations in MUT. *Human Mutation*, *37*(8), 745–754. https://doi.org/10.1002/humu.23013

Fowler, B., Leonard, J. V., & Baumgartner, M. R. (2008). Causes of and diagnostic approach to methylmalonic acidurias. *Journal of Inherited Metabolic Disease*, *31*(3), 350–360. https://doi.org/10.1007/s10545-008-0839-4

Fraser, J. L., & Venditti, C. P. (2016). Methylmalonic and propionic acidemias: clinical management update. *Current Opinion in Pediatrics*, *28*(6), 682–693. https://doi.org/10.1097/mop.0000000000000422

Froese, D. S., Kochan, G., Muniz, J. R. C., Wu, X., Gileadi, C., Ugochukwu, E., Krysztofinska, E., Gravel, R. A., Oppermann, U., & Yue, W. W. (2010). Structures of the human GTPase

MMAA and vitamin B12-dependent methylmalonyl-CoA mutase and insight into their complex formation. *Journal of Biological Chemistry*, *285*(49), 38204–38213. https://doi.org/10.1074/jbc.M110.177717

Furuya, M., Kobayashi, H., Baba, M., Ito, T., Tanaka, R., & Nakatani, Y. (2018). Splice-site mutation causing partial retention of intron in the FLCN gene in Birt-Hogg-Dubé syndrome: A case report. *BMC Medical Genomics*, *11*(1), 42. https://doi.org/10.1186/s12920-018-0359-5

Han, L.-S., Huang, Z., Han, F., Ye, J., Qiu, W.-J., Zhang, H.-W., Wang, Y. U., Gong, Z.-W., & Gu, X.-F. (2015). Clinical features and MUT gene mutation spectrum in Chinese patients with isolated methylmalonic acidemia: Identification of ten novel allelic variants. *World Journal of Pediatrics*, *11*(4), 358–365. https://doi.org/10.1007/s12519-015-0043-1

Han, L. S., & Yang, Y. L. (2018). Expert consensus on dietary therapy and nutrition management of isolated methylmalonic aciduria. *Chinese Journal of Practical Pediatrics*, *33*, 481–486. https://doi.org/10.19538//.e/2018070601

Han, L. S., Ye, J., Qiu, W. J., Gao, X. L., Wang, Y., Jin, J., & Gu, X. F. (2008). Diagnosis of inborn errors of metabolism using tandem mass spectrometry and gas chromatography mass spectrometry. *Zhonghua Yi Xue Za Zhi*, *88*(30), 2122–2126.

Heringer, J., Valayannopoulos, V., Lund, A. M., Wijburg, F. A., Freisinger, P., Barić, I., Baumgartner, M. R., Burgard, P., Burlina, A. B., Chapman, K. A., i Saladelafont, E. C., Karall, D., Mühlhausen, C., Riches, V., Schiff, M., Sykut-Cegielska, J., Walter, J. H., Zeman, J., Chabrol, B., & Kölker, S. (2016). Impact of age at onset and newborn screening on outcome in organic acidurias. *Journal of Inherited Metabolic Disease*, *39*(3), 341–353. https://doi.org/10.1007/s10545-015-9907-8

Hörster, F., Baumgartner, M. R., Viardot, C., Suormala, T., Burgard, P., Fowler, B., Hoffmann, G. F., Garbade, S. F., Kölker, S., & Baumgartner, E. R. (2007). Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut0, mut-, cblA, cblB). *Pediatric Research*, *62*(2), 225–230. https://doi.org/10.1203/PDR.0b013e3180a0325f

Hörster, F., Garbade, S. F., Zwickler, T., Aydin, H. I., Bodamer, O. A., Burlina, A. B., Das, A. M., De Klerk, J. B. C., Dionisi-Vici, C., Geb, S., Gökcay, G., Guffon, N., Maier, E. M., Morava, E., Walter, J. H., Schwahn, B., Wijburg, F. A., Lindner, M., Grünewald, S., … Kölker, S. (2009). Prediction of outcome in isolated methylmalonic acidurias: combined use of clinical and biochemical parameters. *Journal of Inherited Metabolic Disease*, *32*(5), 630. https://doi.org/10.1007/s10545-009-1189-6

Hörster, F., & Hoffmann, G. F. (2004). Pathophysiology, diagnosis, and treatment of methylmalonic aciduria-recent advances and new challenges. *Pediatric Nephrology*, *19*(10), 1071–1074. https://doi.org/10.1007/s00467-004-1572-3

Hu, S., Mei, S., Bai, Y., & Kong, X. (2018). Analysis of MUT gene mutations and prenatal diagnosis for 20 pedigrees affected with isolated methylmalonic aciduria. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, *35*(4), 471–474. https://doi.org/10.3760/cma.j.issn.1003-9406.2018.04.002

Hvas, A. M., Ellegaard, J., & Nexo, E. (2001). Increased plasma methylmalonic acid level does not predict clinical manifestations of vitamin B12 deficiency. *Archives of Internal Medicine*, *161*(12), 1534–1541. https://doi.org/10.1001/archinte.161.12.1534

Kang, L., Liu, Y., Shen, M., Liu, Y. I., He, R., Song, J., Jin, Y., Li, M., Zhang, Y., Dong, H., Liu, X., Yan, H., Qin, J., Zheng, H., Chen, Y., Li, D., Wei, H., Zhang, H., Sun, L., … Yang, Y. (2019). A study on a cohort of 301 Chinese patients with isolated methylmalonic acidemia. *Journal of Inherited Metabolic Disease*, https://doi.org/10.1002/jimd.12183

Kölker, S., Cazorla, A. G., Valayannopoulos, V., Lund, A. M., Burlina, A. B., Sykut-Cegielska, J., Wijburg, F. A., Teles, E. L., Zeman, J., Dionisi-Vici, C., Barić, I., Karall, D., Augoustides-Savvopoulou, P., Aksglaede, L., Arnoux, J.-B., Avram, P., Baumgartner, M. R., Blasco-Alonso, J., Chabrol, B., … Burgard, P. (2015). The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: The initial presentation. *Journal of Inherited Metabolic Disease*, *38*(6), 1041–1057. https://doi.org/10.1007/s10545-015-9839-3

Ledley, F. D., Lumetta, M. R., Zoghbi, H. Y., VanTuinen, P., Ledbetter, S. A., & Ledbetter, D. H. (1988). Mapping of human methylmalonyl CoA mutase (MUT) locus on chromosome 6. *American Journal of Human Genetics*, *42*(6), 839–846.

Lin, Y., Zheng, Q., Zheng, T., Zheng, Z., Lin, W., & Fu, Q. (2019). Expanded newborn screening for inherited metabolic disorders and genetic characteristics in a southern Chinese population. *Clinica Chimica Acta*, *494*, 106–111. https://doi.org/10.1016/j.cca.2019.03.1622

Lindblad, B., Lindstrand, K., Svanberg, B., & Zetterström, R. (1969). The effect of cobamide coenzyme in methylmalonic acidemia. *Acta Paediatrica*, *58*(2), 178–180. https://doi.org/10.1111/j.1651-2227.1969.tb04704.x

Liu, Y., Liu, Y. P., Zhang, Y., Song, J. Q., Zheng, H., Dong, H., & Yang, Y. L. (2018). Heterogeneous phenotypes, genotypes, treatment and prevention of 1 003 patients with methylmalonic acidemia in the mainland of China. *Zhonghua Er Ke Za Zhi*, *56*(6), 414–420. https://doi.org/10.3760/cma.j.issn.0578-1310.2018.06.003

Ma, S., Guo, Q., Zhang, Z., He, Z., Yue, A., Song, Z., Zhao, Q., Wang, X., & Sun, R. (2020). Expanded newborn screening for inborn errors of metabolism by tandem mass spectrometry in newborns from Xinxiang city in China. *Journal of Clinical Laboratory Analysis*, *34*(5), 23159. https://doi.org/10.1002/jcla.23159

Matsui, S. M., Mahoney, M. J., & Rosenberg, L. E. (1983). The natural history of the inherited methylmalonic acidemias. *The New England Journal of Medicine*, *308*(15), 857–861. https://doi.org/10.1056/NEJM198304143081501

Rajan, S., Wallace, J. I., Brodkin, K. I., Beresford, S. A., Allen, R. H., & Stabler, S. P. (2002). Response of elevated methylmalonic acid to three dose levels of oral cobalamin in older adults. *Journal of the American Geriatrics Society*, *50*(11), 1789–1795. https://doi.org/10.1046/j.1532-5415.2002.50506.x

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Rosenberg, L. E., Lilljeqvist, A., & Hsia, Y. E. (1968). Methylmalonic aciduria: Metabolic block localization and vitamin B 12 dependency. *Science*, *162*(3855), 805–807. https://doi.org/10.1126/science.162.3855.805

Shibata, N., Hasegawa, Y., Yamada, K., Kobayashi, H., Purevsuren, J., Yang, Y., Dung, V. C., Khanh, N. N., Verma, I. C., Bijarnia-Mahay,

S., Lee, D. H., Niu, D.-M., Hoffmann, G. F., Shigematsu, Y., Fukao, T., Fukuda, S., Taketani, T., & Yamaguchi, S. (2018). Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening. *Molecular Genetics and Metabolism Reports*, *16*, 5–10. https://doi.org/10.1016/j.ymgmr.2018.05.003

Tanpaiboon, P. (2005). Methylmalonic acidemia (MMA). *Molecular Genetics and Metabolism*, *85*(1), 2–6. https://doi.org/10.1016/j.ymgme.2005.03.008

Villani, G. R., Gallo, G., Scolamiero, E., Salvatore, F., & Ruoppolo, M. (2017). "Classical organic acidurias": diagnosis and pathogenesis. *Clinical and Experimental Medicine*, *17*(3), 305–323. https://doi.org/10.1007/s10238-016-0435-0

Willard, H. F., & Rosenberg, L. E. (1980). Inherited methylmalonyl CoA mutase apoenzyme deficiency in human fibroblasts: Evidence for allelic heterogeneity, genetic compounds, and codominant expression. *The Journal of Clinical Investigation*, *65*(3), 690–698. https://doi.org/10.1172/JCI109715

Xie, B., Luo, J., Fan, X., Chen, R., Wang, J., Zhang, S., & Chen, S. (2016). Identification of two novel mutations of MUT gene in a Chinese family affected with isolated methylmalonic acidemia. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, *33*(2), 135–139. https://doi.org/10.3760/cma.j.issn.1003-9406.2016.02.002

Yang, C., Zhou, C., Xu, P., Jin, X., Liu, W., Wang, W., Huang, C., Jiang, M., & Chen, X. (2020). Newborn screening and diagnosis of inborn errors of metabolism: A 5-year study in an eastern Chinese population. *Clinica Chimica Acta*, *502*, 133–138. https://doi.org/10.1016/j.cca.2019.12.022

Yang, Y., Wang, L., Wang, B., Liu, S., Yu, B., & Wang, T. (2019). Application of next-generation sequencing following tandem mass spectrometry to expand newborn screening for inborn errors of metabolism: A multicenter study. *Frontiers in Genetics*, *10*, 86. https://doi.org/10.3389/fgene.2019.00086

You, J., Shamsi, B. H., Hao, M. C., Cao, C. H., & Yang, W. Y. (2019). A study on the neurodevelopment outcomes of late preterm infants. *BMC Neurology*, *19*(1), 108. https://doi.org/10.1186/s12883-019-1336-0

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