Mini-Review

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Methylmalonic acidemia: Current status and research priorities

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Summary **Methylmalonic acidemia (MMA) is a lethal, severe heterogeneous disorder of methylmalonate and cobalamin (cbl; vitamin B12) metabolism with poor prognosis. Two main forms of the disease have been identified, isolated methylmalonic acidurias and combined methylmalonic aciduria and homocystinuria, which is respectively caused by different gene mutations. Here, we review the improvement of pathogenesis, diagnosis and treatment in MMA. Importantly, the reported epidemiological data of MMA patients in China and the hot mutation sites in Chinese patients are listed, which will aid in improving healthcare of Chinese patients in the future. c.729_730insTT was the most common mutation in Chinese isolated MMA patients, while c.609G>A and c.658_660delAAG were in Chinese cblC type patients according to unrelated studies. The estimated newborn screening incidence was reported to be 1:26,000, 1:3,920, 1:11,160, 1:6,032 respectively in Beijing and Shanghai, Shandong province, Taian district, and Henan province of China. Alternatively, when patients with suspected inherited metabolic diseases were used as the screened sample, the relatively high incidence 0.3% and 1.32% were respectively obtained in southern China and throughout all the provinces of mainland China and Macao with the exception of five provinces (Hainan, Neimenggu, Tibet, Ningxia, and Hong Kong).**

Keywords: Methylmalonic acidemia, hot mutation sites, diagnosis, treatment, epidemiological data, China

1. Introduction

Methylmalonic acidemia (MMA) is a lethal, severe and multi-systems injured disease of abnormal metabolism. It was first reported in 1967 (*1*). According to phenotype, two main forms of the disease have been identified, including isolated methylmalonic acidurias and combined methylmalonic aciduria and homocystinuria. Isolated methylmalonic acidurias is due to defects of methylmalonyl-CoA mutase or the synthesis of the MUT coenzyme adenosylcobalamin (AdoCbl), while combined methylmalonic aciduria and homocystinuria is characterized by elevated plasma homocysteine and decreased levels of the coenzymes adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl). Additionally, some benign MMA subtypes have been described.

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MMA has a wide clinical spectrum, ranging from a benign condition to fetal neonatal disease. Onset of the manifestations of MMA ranges from the neonatal period to adulthood. Affected children usually exhibit anorexia, failure to thrive, hypotonia, developmental delay, progressive renal failure, functional immune impairment, optic nerve atrophy, and hematologic abnormalities. 4 children with combined MMA and homocysteinemia were recently reported to present predominantly with late-onset diffuse lung diseases (DLD) (*2*). Atypical and "benign"/adult MMA are associated with increased, albeit mild, urinary excretion of methylmalonate; however, it is uncertain whether individuals with these conditions will develop symptoms.

Patients with MMA experience significant morbidity and mortality, and the prognosis for long-term survival is poor. The mortality of mut MMA was 60-88% in the first reports in the 1980s and 1990s (*3*) and has improved somewhat to ~40% by the first decade in the 2000s (*4*). A prospective cohort study was conducted in 45 Chinese pediatric patients diagnosed

with methylmalonic acidemia with homocystinuria between 2006 and 2012, which show that elevated urea and urinary methylmalonic acid are predictors of adverse outcomes for the patients (*5*).

2. Etiology and pathogenesis

Methylmalonic aciduria is a genetically heterogeneous disorder of methylmalonate and cobalamin (cbl; vitamin B12) metabolism.

Isolated methylmalonic acidurias have also been classified by complementation groups: MMA 'mut' , caused by mutation in the *MUT* gene on chromosome 6p21; MMA cblA, caused by mutation in the *MMAA* gene on 4q31; MMA cblB, caused by mutation in the *MMAB* gene on 12q24; and Tcblr type, caused by mutation in the *CD320* gene on chromosome 19p13.2. MMA 'mut' involves two subtypes: Mut0 mutations result in no detectable MCM activity; Mut- mutations result in low residual enzyme activity. MCM, an adenosylcobalamin-dependent enzyme, have a function in isomerization of L-methylmalonyl-CoA to succinyl-CoA, for entry into the tricarboxylic acid cycle for energy production. All mutations of *MMAA* strongly decreased functional association with MUT and interfered with gating the transfer of AdoCbl from MMAB to MUT, which is a disease-causing mechanism of cblA type MMA (*6*). The *MMAB* gene encodes the mitochondrial enzyme ATP: cobalamin adenosyl transferase (ATR), which catalyzes transfer of an adenosyl group from ATP to cobalamin (I) to form AdoCbl. *CD320* encodes a transcobalamin receptor that binds TCN2-cobalamin at the plasma membrane and internalizes the complex by endocytosis.

Combined MMA and homocystinuria is a genetically heterogeneous disorder of cobalamin (cbl; vitamin B12) metabolism. The defect causes decreased levels of the coenzymes adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), which results in decreased activity of the respective enzymes methylmalonyl-CoA mutase and methyltetrahydrofolate–homocysteine methyltransferase, also known as methionine synthase. Different forms of the disorder have been classified: cblC, cblD, cblF, cblX, cblJ. CblC type is caused by mutation in the *MMACHC* gene on chromosome 1p34. The MMACHC protein may act as an intracellular cobalamin-trafficking chaperone and has been shown to act, in part, to catalyze the reductive decyanation of cyanocobalamin, generating cob(II)alamin, which is the substrate for assimilation into the active cofactor forms methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl). CblD type is caused by mutation in the *MMADHC* gene on chromosome 2q23, which plays a role in directing cobalamin to the 2 cobalamin-dependent enzymes, Methylmalonyl CoA mutase and Methionine synthase. CblF type is caused by mutation in the *LMBRD1* gene on chromosome 6q13. cblX type is an X-linked recessive metabolic disorder, caused by mutation in the *HCFC1* gene on chromosome Xq28, mutations of which inhibit its function in the transcriptional activation of *MMACHC* (*7*). cblJ type is caused by mutation in the *ABCD4* gene on chromosome 14q24, which is involved in the lysosomal release of cbl into the cytoplasm. Finally, deficiency of the enzyme methylmalonyl-CoA epimerase and ADP-forming succinyl-CoA synthetase caused by mutation of the *MCEE* gene on chromosome 2p13.3 and mutation in the *SUCLA2* gene on chromosome 13q14.2 can lead to benign subtypes.

The expression of miR-9 was recently found to be significantly down-regulated in MMA patient plasma and sensitively changed after VitB12 treatment, and up-regulation of miR-9 reduced neural apoptosis induced by methylmalonate via targeting BCL2L11. The above results show that miR-9 may act as a potential "competitor" of gas chromatography-mass spectrometry for the diagnosis of MMA and a monitor of changes in MMA and might provide new insights into a therapeutic entry point for treating MMA (*8,9*).

3. Diagnosis

Acidosis, ketosis, hyperammonemia, hypoglycemia, hyperglycemia, and neutropenia are main symptoms of MMA. Major secondary complications of MMA include developmental delay (variable), tubulointerstitial nephritis with progressive renal failure, "metabolic stroke" (acute and chronic basal ganglia involvement), disabling movement disorder with choreoathetosis, dystonia and para/quadriparesis, pancreatitis, growth failure, functional immune impairment, and optic nerve atrophy.

Combined with the above clinical features, MMA needs to be diagnosed by some laboratory methods. The basic laboratory markers suggestive of MMA include low bicarbonate levels less than 22 mmol/L in infants and less than 17 mmol/L in neonates, ketones in the urine, blood ammonia levels greater than 150 µg/dL in neonates, 70 µg/dL in infants, and 35-50 µg/dL in older children and adults, blood glucose levels less than 40 mg/mL in infants and less than 60 mg/mL in children, and absolute neutrophil counts less than 1,500/mm^3. Also, C3 values greater than 7 µmol/L and C3:C2 ratios greater than 0.2 measured by tandem mass spectroscopy (MS/MS) show suspected disorders of cobalamin or propionate metabolism. Finally, relatively high concentrations of methylmalonic acid and methyl citrate in urine from patients' gas chromatography/mass spectrometry (GC/MS) can lead to definitive diagnosis of the disorder.

Plasma homocysteine can be measured to identify gene types involved in MMA. Patients with very high concentrations of methylmalonic acid in urine, but normal homocysteine, have mutations in at least one

Table1. The hot mutation sites of Chinese patients

Number of Patients	Disease type	Pathogenic gene	Frequent mutation site	*Ref.*
16	cblC	*MMACHC*	c.609G>A /c.658_660delAAG	(*12*)
43	isolated MMA	*MUT*	c.729_730insTT	(*10*)
79	cblC	*MMACHC*	c.609G>A, c.658_660delAAG, c.482G>A, c.394C>T, c.80A>G	(*11*)

of the MUT (mut-, mut 0), cblB, cblA and cblD (var 2) subtypes. Patients with abnormally high concentrations of methylmalonic acid in urine and homocysteine in plasma have mutations in at least one of the cblC, cblF, or cblD (var 1) subtypes. Patients with slightly elevated methylmalonic acid in urine, but normal homocysteine, have mutations in at least one of the MCEE, SUCLA2 and benign MMA subtypes.

Mutation analysis is not only the gold standard diagnosis of MMA but also can aid in the choice of treatment strategy, B12 responsive or unresponsive. Several studies reported the gene mutation spectrum in Chinese patients with isolated MMA and cblC type MMA. The frequent mutation sites are presented below (Table 1). c.729_730insTT was the most frequent mutation among the 43 recruited Chinese isolated MMA patients (*10*). c.609G>A, c.658_660delAAG, c.482G>A, c.394C>T, c.80A>G accounted for 80% of disease alleles in 79 unrelated Chinese cblC type MMA patients (*11*). It was also reported by another recent study that c.609G>A and c.658_660delAAG were the most common mutations detected in 13 (81%) out of 16 patients (*12*).

Timely diagnosis and adequate treatment greatly improve the prognosis. In the past 15 years, many Chinese patients with this condition have achieved favorable treatment outcomes, and some of them have reached childbearing age. In 2015, the first case of a Chinese woman with cblC was reported to experience a successful pregnancy and deliver a healthy boy (*13*).

4. Treatment

Since the long-term neurodevelopmental outcome is strongly influenced by the duration of coma and peak blood ammonia concentrations, therapy must not be delayed and therefore the diagnostic workup and the initial medical treatment should proceed simultaneously: stabilize the patient; stop protein intake; start intravenous glucose; and seek expert metabolic advice. While waiting for the laboratory diagnosis, drugs including L-carnitine, hydroxocobalamin,biotin,sodium phenylbutyrate, l-arginine-Hcl and N-carbamyl-glutamate should be properly used.

Standard therapy of long-term management includes: L-carnitine; antibiotics to reduce intestinal flora; vitamin B12 in responsive MMA patients; low-protein diet; precursor-free amino acid and/or isoleucine/valine supplementation; vitamin and mineral supplementation; caring for special situations and provision of an emergency regimen in recurrent illnesses (*14*). Generally, cblc type is almost all B12-responsive, mut type is B12-unresponsive, and other types is partly responsive to B12. B12-responsive cblC type MMA is most common in China.

The use of specialized amino acid formulations containing minimal to no valine, isoleucine, methionine and threonine in the treatment for MMA has become widely implemented. However, two recent follow-up studies found that the excessive use of medical foods, especially in the setting of reduced natural protein intake, resulted in iatrogenic amino acid deficiencies and was associated with poor growth outcomes in a large cohort of isolated MMA and cblC type MMA patients (*15,16*). So, it is proposed that medical foods and dietary guidelines for MMA should be revised based on well-controlled and sufficiently powered clinical studies to support their efficacy and safety.

Because of the poor prognosis despite of the above treatment, centers started to pursue elective liver and combined liver-kidney transplantation as a treatment for the metabolic instability that eventually causes death since 1990s (*17*). Solid organ transplantation can eliminate the frequent episodes of metabolic acidosis, but has numerous practical limitations that include procedural availability, surgical mortality and morbidity, expense, a finite donor pool, and the need for life-long immune suppression. End stage renal disease (ESRD) is a cardinal manifestation of methylmalonic acidemia (MMA) Patients and MMA patients who have been treated by orthotopic liver transplantation. $Mut^{-/-}$;$Tg^{INS\text{-}Alb\text{-}Mut}$ mice model had been established to suggest that proximal tubular mitochondrial dysfunction is a key pathogenic mechanism of MMA-associated kidney disease, and identified lipocalin-2 as a biomarker of increased oxidative stress in the renal tubule, defining an approach for the treatment and monitoring of kidney disease in patients with MMA (*18*).

Due to limitations of organ transplantation, researchers studied viral gene therapy as treatment for MMA, using preclinical cellular and animal models since 2007. The treated $Mut^{-/-}$ mice have near-normal long-term survival and growth parameters, demonstrate enzymatic activity longer than one year after a single treatment with an AAV8 or AAV9 vector (*19,20*). Although genotoxicity was observed in the mouse studies with some vectors, it has been demonstrated that manipulating regulatory elements and AAV dosing could reduce the genotoxicity (*21*). Lack of neutralizing

Table 2. The reported epidemiological data of MMA patients in China

District	Time period	Number Screened	Number of Patients	incidence	Disease subtype	*Ref.*
Beijing,Shanghai	Before 2011	400,000	15	1:26,000	unknown	(*25*)
Shandong province	May 2011 to May 2014	35,291	9	1:3,920	cblC	(*26*)
Southern China	January 2009 to March 2012	16,075 with suspected inherited metabolic diseases	48	0.3%	Unknown	(*27*)
Throughout all the provinces of mainland China and Macao, with the exception of five provinces (Hainan, Neimenggu, Tibet, Ningxia, and Hong Kong)	February 2002 to June 2012	18,303 with suspected inherited metabolic diseases	242	1.32%	Unknown	(*28*)
Taiwan	August 2013 to December 2014	44,639	4	1:11,160	cb1C/mut	(*29*)
Henan Province	January 2013 to March 2016	349,858	58	1:6,032	Unknown	(*30*)

antibodies against AAV in MMA patients shown in a recent study (*4*) allow for the potential clinical application of systemic *AAV* gene delivery as a new treatment for mut MMA.

5. Epidemiological data of MMA patients in China

Estimates of incidence for MMA is reported to be 1/50,000 in Japan (*22*), 1/250,000 in Germany (*23*), and 1/85,000 in Taiwan of China (*24*).

MMA was first described in mainland China in 2000 (*25*). Along with the wide application of tandem mass spectrometry and gas chromatography/mass spectrometry, newborn screening for this metabolism error have been available in most parts of China. Some statistical results had been reported on the basis of screening data in different districts of China. As is shown below (Table 2), 15 MMA patients of the screened 400,000 babies before 2011 were diagnosed in Beijing and Shanghai, that is, the estimated incidence is 1:26,000 (*25*); 9 MMA patients were identified among 35,291 newborns in Shandong province screened between May 2011 and May 2014, giving an estimated incidence of 1:3,920 live births for MMA, and all were classified as cblC disease (*26*); in southern China, 16,075 urine samples were collected from patients who were highly suspected of having inborn errors of metabolism, among which 48 MMA patients were detected (*27*). Similarly, 242 MMA patients were confirmed in 18,303 patients with suspected inherited metabolic diseases throughout all the provinces of mainland China and Macao, with the exception of five provinces (Hainan, Neimenggu, Tibet, Ningxia, and Hong Kong) (*28*). 4 MMA patients were detected in 44,639 newborns from Taian district of China, and the estimated incidence is 1/11,160 (*29*); the reported prevalence of MMA in Henan province was 1/6,032, according to 349,858 screening results of newborns from January 2013 to March 2016 in this province (*30*).

Overall, cblC type is the most common type in Chinese patients. The data differ significantly in different districts of China, which may be related to the onset date of MMA screening. Additionally, the screening bases used by the above research vary. Specifically, the screening bases of two statistics were the number of patients with suspected inherited metabolic diseases in southern China and throughout all the provinces of mainland China and Macao with the exception of five provinces (Hainan, Neimenggu, Tibet, Ningxia, and Hong Kong), while the other statistical data are newborn screening results.

6. Conculsion

In conclusion, MMA is a severe genetic disease with poor prognosis. It can be defined by MS/MS and GC-MS. However, the importance of mutation analysis should lead to more sufficient awareness, which will guide the choice of treatment strategy. The efficacy and safety of low-protein diet for treatment of MMA should be reconsidered because of poor growth outcomes. Also, liver transplant, kidney transplant and combined liver-kidney transplant have been reported for the treatment of methylmalonic aciduria. However, organ transplantation for MMA remains controversial because the criteria for solid organ transplantation in MMA have not been well established and the high risk of complications. Viral gene therapy has been successfully tried at the animal model level, and will be a new potential treatment for mut MMA.

The reported epidemiological data on MMA in China was limited in local districts and the results differ significantly among them. The lack of national epidemiological data on MMA in China, which has been reported in other countries, may hinder improvement in healthcare for MMA. So, it is urgent to obtain accurate

data all over China based on the application of newborn screening strategy.

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