Metabolic Brain Disease (2019) 34:451–458
https://doi.org/10.1007/s11011-018-0351-y

ORIGINAL ARTICLE

Hydrocephalus in cblC type methylmalonic acidemia

Kaihui Zhang[1] · Min Gao[1] · Guangyu Wang[2] · Yingying Shi[3] · Xiaoying Li[4] · Yvqiang Lv[1] · Guangye Zhang[1] · Zhongtao Gai[1] · Yi Liu[1]

Received: 24 May 2018 / Accepted: 22 November 2018 / Published online: 19 December 2018

Abstract

Methylmalonic acidemia (MMA) is a typical type of organic acidemia caused by defects in methylmalonyl-CoA mutase or adenosyl-cobalamin synthesis. Hydrocephalus (HC), results from an imbalance between production and absorption of cerebrospinal fluid (CSF), causeing enlarged cerebral ventricles and increased intracranial pressure, is a condition that requires urgent clinical decision-making. MMA without treatment could result in brain damage. However, HC in MMA was rarely reported. In this study, 147 MMA were identified from 9117 high risk children by gas chromatography mass spectrometry (GC/MS) for organic acidurias screening in urine samples and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for amino acids detection in blood samples. Totally 10 cases with MMA and HC were determined by brain MRI/CT, as well as gene mutation testing either by high throughput sequencing or Sanger sequencing. Besides, homocysteine was also analyzed for the 10 MMA with HC. Out of them, 9 cases carry out compound heterozygous mutations or homozygous mutation in *MMACHC* gene, and 1 case has *MUT*mutation. The mutation c.609G > A in *MMACHC* was the most common in the cbl type patients. Although MMA has a high incidence in Shandong province of China, especially cblC type. All of the 10 patients were not correctly diagnosed before developing HC. As a result, when a child develops progressive and refractory HC, the screening for inherited metabolic diseases should be immediately conducted.

Keywords Methylmalonic academia · Hydrocephalus · *MUT* · *MMACHC* · cblC type

Introduction

Hydrocephalus (HC) is a condition in which an accumulation of cerebrospinal fluid (CSF) is within the brain, and it results from an imbalance betweenproduction and absorption of CSF mostly caused by craniocerebral disease. HC typically causes enlarged cerebral ventricles and increased intracranial pressure, which requires urgent clinical decision-making. Untreated or undertreated HC could result in significantly neurological manifestations, developmental disability, and even death(Gmeiner et al. 2018). Particularly, children with HC are unable to express their discomfort which was mostly used for clinical neurological assessment of HC. Besides, HC in children is usually caused by underlying diseases, such as cerebrovascular shunt malfunction, inherited metabolic disease, and so on (Ma et al. 2018).

Inherited metabolic diseases (IMDs), also named as inborn errors of metabolism (IEM), are genetic disorders that disrupt enzyme activity, cellular transport, or energy production (Villoria et al. 2016). Methylmalonic acidemia (MMA) is a classical type of organic acidemia usually caused by a deficiency of methylmalonyl-CoA mutase (MUT, EC 5.4.99.2). Defects can be in the metabolic pathway where methylmalonyl-CoA is converted into succinyl-CoA by MUT. MUT is a mitochondrial enzyme encoded by *MUT* gene, with an important cofactor, adenosyl-cobalamin from which the activity of MUT is influenced by a defect in transport or synthesis (Han et al. 2017). Heretofore there are six subtypes, cblA, cblB,

✉ Zhongtao Gai
gaizhongtao@sina.com

✉ Yi Liu
liuyi-ly@126.com

[1] Pediatric Research Institute, Qilu Children's Hospital of Shandong University, 23976 Jingshi Road, Jinan 250022, Shandong, China

[2] Department of Neurosurgery, Qilu Children's Hospital of Shandong University, Jinan 250022, Shandong, China

[3] Department of Imaging, Qilu Children's Hospital of Shandong University, Jinan 250022, Shandong, China

[4] Department of Neonatology, Qilu Children's Hospital of Shandong University, Jinan 250022, Shandong, China

cblC, cblF, cblD and cblX, associated with disturbance of adenosyl-cobalamin metabolism (Keyfi et al. 2016). The deficiency of enzymes, involved in the propionyl-CoA to succinyl-CoA conversion pathway, and methylmalonyl-CoA epimerase, as well as ADP-forming succinyl-CoA synthetase are also found in patients with MMA. So far, 13 genes are associated with MMA, such as *MUT*, *MMACHC*, *MMADHC*, *MMAA*, *MMAB*, *MCEE*, *ACSF3*, *LMBRD1*, *HCFC1*, *ABCD4*, and *SUCLA2* (Pupavac et al. 2016).

Methylmalonic acid derived from the degradation of isoleucine, valine, methionine, threonine, odd-chain fatty acids, and cholesterol side chains, is ranked poisonous to the body, which could be accumulated in the tissues of patients with MMA. The clinical characteristics of MMA vary, including acidosis, ketosis, hyperammonemia, hypoglycemia or hyperglycemia, and neutropenia. Moreover, the neurological dysfunction caused by degeneration of striatal neurons and cerebral cortical atrophy is the most significant and common, manifesting coma, drowsiness, convulsions, encephalopathy, and so on (Han et al. 2017; Carrillo-Carrasco et al. 2012). HC in patients with MMA has been rarely reported so far, andcan be used as an early indication of timely treatment, such as prompt surgery. In this study, we present clinical characteristics and mutational analyses of 10 Han Chinese patients with MMA identified by gas chromatography mass spectrometry (GC/MS) for organic acidurias screening in urine samples and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for amino acids detection in blood samples as well as High throughput sequencing for gene mutation testing. The relationship between HC and genotype of MMA is analyzed and summarized.

Material and methods

Compliance with ethical standards

The work was approved by Medical Ethics Committee of Qilu Children's Hospital of Shandong University. The parents of the patients gave their written informed consent before clinical and laboratory examinations. All the relevant regulations and institutional polices were followed strictly with the Declaration of Helsinki. The patients' information was anonymized prior to submission.

Patients

Totally 10 children (6 males and 4 females) from 9 unrelated Chinese families were diagnosed as MMA with HC at pediatric intensive care unit and neurosurgery department of the Qilu Children's Hospital of Shandong University screened from 147 MMA by LC/MS-MS and GC/MS between May 2011 and Jun 2018. The parents of the patients were all healthy and non-consanguineous. Blood samples were obtained from the patients and their parents in accordance with informed consent in the study.

Routine tests and metabolic analysis

Routine physical examination was conducted for height, weight, and head circumference, muscular tension, mental condition and so on. Biochemical lab tests were also performed for hepatic and renal function, glucose, ammonia, lactic acid and blood gas analysis, and so on by using blood, urine and cerebrospinal fluid. MRI/CT was conducted to all index patients.

The levels of organic acidurias were measured by gas chromatography mass spectrometry (GC/MS) using a GCMS-QP2010 analyzer (Shimadzu, Tokyo, Japan) and the Inborn Errors of Metabolism Screening System software (Shimadzu, Tokyo, Japan). Meanwhile, the levels of blood carnitine and amino acids including propionylcarnitine (C3), propinonylcarnitine/free-carnitine (C3/C0), propionylcarnitine/acetylcarnitine (C3/C2), and methionine (Met) in dried blood spots were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an Applied Biosystems API 3200 analyzer (ABSCIEX, Foster City, USA) and the ChemoView software(ABSCIEX, Foster City, USA).

Targeted captured sequencing and bioinformatics analysis

Peripheral blood samples were collected from the patients and their parents for DNA extraction using a DNA extraction kit (TIANamp Blood Genomic DNA Purification Kit; Tiangen Biotech, Beijing, China).

Whole exome targeted capture sequencing were used for the mutation screening of the patients. A total of 712 genes associated with inherited metabolic diseases were selected and their coding regions were captured using a custom exome enrichment kit (Agilent, Santa Clara, USA). Fifteen microgram of DNA from the proband was used to generate index libraries (average size 350-450 bp, including adapter sequences) for HiSeq2000 sequencer (Illumina, San diego, USA). Totally 90 sequencing cycles per read were carried out. The obtained mean exome coverage was more than 98%, with more than 99% of variants accuracy. Potential effects of the mutations on function were assessed using the following databases: UCSC Genome Bioinformatics (http://genome.ucsc.edu/) (comparing with the reference sequence of *MUT* gene and *MMACHC* gene); Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) (comparing with an integrated set of variants); 1000 Genomes Project (www.1000genomes.org) and ExAC (http://exac.broadinstitute.org/about), and so on.

Verification and detection of mutations

Sanger sequencing was applied to verify and detect the potential MMA mutations in the patients. The gene-specific primers were designed according to the sequences of the *MMACHC* gene (NM_015506), *MUT* gene (NM_000255) from the UCSC Genome browser database. The primers used for *MMACHC* and *MUT* gene are shown in Table 1. The total volume used for PCR reactions was 25 μl and the reaction conditions were 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 55–65 °C for 30s, extension at 72 °C for 45 s, and final extension at 72 °C for 5 min. The PCR amplification products were purified and sequenced using an ABI Prism 3700 automated sequencer (Applied Biosystems, Foster City, CA).

Result

Clinical characteristics and laboratory tests

Totally 147 MMA were carried out the gene mutation detections, in which 30 patients have been tested by NGS, and others were gene-screening of *MUT* gene and *MMACHC* gene by Sanger sequencing. Out of them, 123 patients carrythe compound heterozygous mutations or homozygous mutations in *MUT* gene or *MMACHC* gene inherited from their parents. Three patients were found to have one mutation in *MMACHC* gene, and 6 patients had one mutation in *MUT* gene. While no mutations were detected in 15 cases including 7 cases tested by whole exome targeted capture sequencing (Table 2).

Ten cases with MMA accompanied by HC were identified among 147 MMA, and the incidence of HC to cblC type is 8.82% (9/102). All the 10 cases had increased methylmalonate in their urine and propionyl carnitine (C3) in their dried blood spots. Four of the patients (case 4, 6, 9 and 10) had lower level of methionine (Met) (Table 3).

Clinical data of 10 cases were collected and showed in Table 2. All patients were less than six months at the time of first onset, and most of them showed the symptoms at about one month old, including poor feeding, failure to thrive, pneumonia, and so on, but no vomiting and the head circumference of 10 cases was normal. There is no significant difference between HC group and those that did not develop it in the age distribution to early onset type of cblC.

The homocysteine was higher in 9 patients (cases 2–10). Case 1, 9 and 10 had elevated blood ammonia, but other indicators were normal. All patients had low hemoglobin, cases 2, 3, 4, 7, 8, 9 and 10 showing moderate anemia, cases 7 and 8 with severe anemia. Cases 2, 4, 5, 6, 9 and 10 presented low neutrophils. Increasing lactic acid was found in cases 1 and 2.

Cranial MRI/CT showed severe ventriculomegaly and extensive cerebral parenchyma atrophy in cases 2–8. Case 1 had mild ventriculomegaly, but had severe subdural effusion and cerebral atrophy (Fig. 1).

Genetic analysis

Nine patients were identified to carry mutations in *MMACHC* with compound heterozygous mutation or homozygous mutation, and one patient had the *MUT* mutations. The most common mutation was c.609G > A in *MMACHC*. Moreover, the high-frequent mutations, including c.567dupT, c.658_660delAAG in *MMACHC,* and c.729_730insTT in *MUT*, which have been reported before (Wu et al. 2017; Worgan et al. 2006) were also found in these patients with HC (Table 4). Out of them, the six mutations were reported to be pathogenic in HGMD (The Human Gene Mutation Database) (Lerner-Ellis et al. 2009).

Table 1 The primers of PCR for exons of *MMACHC* and *MUT*

Gene	Forward	Reverse	Products(bp)
MMACHC-1	GGGATACCGTGATGATACGC	GAACCCAGGAGGATCAGAGG	680
MMACHC-2	TGCATCACATAGCGTCAGTG	AGCCTGGCTTTAGGGTATCA	467
MMACHC-3	TCATGTTTTCCCTTCTGAGGA	CAAAGCTAATTTGTTCTGGGTTG	395
MMACHC-4	ATGATGGCAGTTGACTTGGT	CCTAACTTCCTTGAGCCTTGT	634
MUT-1	CAGACCTACACTCACAGTTGACATC	CTTAAGGCTCAGAAGTTCTCAGTC	1182
MUT-2	TGGCATCTTCATCATGAAGTC	ACTGGTTTGCAAGGAGGTAGTTC	549
MUT-3	GTCAGTGGCTACATACCAGTTAC	AATACAAGTAAACAAATTAGGTGC	404
MUT-4	AAGGAAATTAAGCTCCCAGTAG	CAGCAACCTTCAGGTACTTGTC	503
MUT-5	CCTGGTGATATACTTGAGAGTCC	TCAGCACTATTATTGCCCTTC	684
MUT-6	CTGGTTGTCTATTTATCCTGAG	AAGCTACTGGGTATTCATTCTC	649
MUT-7	TGGTTAGACATCCACACACACT	CACTGTCTGTTTTCTGCCTTC	788
MUT-8	GTATCTTACTCCAGGAAGCAGG	TACTGCAATAGAAGCAATGGC	550
MUT-9	TAATGAAGGGCATAGGAAG	TAACCACCAGAGGGAGACA	657
MUT-10	GCTGGAGACAAGATATTCCTC	GTAGGTACAGTCCTGATGATGG	600
MUT-11	ATGTAAGTAGAAGACCTCCCTGA	CACAAGGGAAACAACTGACTC	831
MUT-12	GGGCATCATCTAAACTATCTTC	TGAGTAGCTCCTATTTCCCAC	833
MUT-13	AACCTAAGGAGAAGGCAGAGAG	GCTATCCTCAAATGCAAACTG	623

Table 2 the summary for mutation genotype of 147 patients and age of onset

Number	Positive metabolic screening	cblC type	mut type	One mutation in MMACHC gene	One mutation in MUT gene	Undetermined type
Patients	147	102	21	3	6	15
Early onset (<1 year)	128	92	17	3	6	12
Late onset(≥1 year)	19	10	4	0	0	3

Diagnosis, treatment and follow up

The clinical diagnosis was made timely for 7 of the patients (cases 1–6 and 10) based on their symptoms, physical examination, laboratory test, MRI/CT and genetic analysis. A ventricular peritoneal shunt surgery was performed immediately on 7 patients (cases 3–8 and 10) after their diagnosis of HC.

Unfortunately, three cases of 4, 7 and 8 died after operation due to delayed MMA diagnosis, and more sadly, cases 7 and 8 were siblings from a healthy couple. Case 4 and 8 died of severe pneumonia and respiratory failure, while case 7 died of severe respiratory acidosis with metabolic acidosis and respiratory failure. Case 3, 5, 6 and 10 showed improvement of symptoms after surgery and subsequent medical treatment. At one year follow-up, case 3 could sit and climb. His symptoms of anemia, severe pneumonia, and malnutrition disappeared. Brain MRI/CT revealed that the volume of the brain tissue had significantly improved. Other two patients (cases 5 and 6), however, died from severe infection and metabolic crisis several months after their parents abandoned treatment.

The operation was not performed for case 1, 2 and 9 as their HC was neither very severe, nor had their parents consent. Case 1, who carried the mutations in *MUT* gene that is vitamin B12-non-responsive type with the protein-restricted diet, was dead from severe infection due to the metabolic disregulation two months later. Case 2 was treated with the non-surgical medication including hydroxocobalamin (OH-Cbl), betaine, folate, L-carnitines, and protein-restricted diet. The patient was followed up regularly in his local clinic. At one-year follow-up by telephone, his father narrated that his brain MRI showed a gradual improvement in his brain tissue and HC volume, but the child showed mild developmental delay with low body weight, and he could not walk and speak at that time. Case 9 was admitted to the hospital due to moderate anemia, and her parents gave up the surgery and medication. Laterwhen she was followed-up by phone, her father narrated the patient died when she was sleeping at 4-month-10-day old.

The drug treatment strategy for the cohort was that L-carnitine (300 mg/(kg.d)), and OH-Cbl (1 mg/d) were provided in the acute period and after operation, and L-carnitine (100 mg/(kg.d)), OH-Cbl (0.1 mg/(kg.d)), betaine (200 mg/(kg.d)) and folate (10 mg/d) were used in the long-term maintenance therapy. Arginine (250 mg/(kg.d)) was prescribed for Case 1, 9 and 10.

Discussion

There are two active types of cobalamin (Cbl, also named vitamin B12) in cells, methyl-cobalamin in the cytosol and adenosyl-cobalamin in the mitochondrion, both of which are transformed through hydroxylcobalamin (Rossi A et al.). Adenosyl-cobalamin is the co-enzyme for MUT, and the deficiency of which influences the catalytic effect of MUT and results in the MMA. Meanwhile, methyl-cobalamin is the co-factor for N5-methyltetrahydrofolate-homocysteine methyltransferase which could transfer homocysteine to methionine, and the deficiency could cause the increase of homocysteine and the decrease of methionine. Thus, the deficiency of Cbl upstream metabolic pathway in the cytosol, including cblC, cblD and cblF, has two biochemical hallmarks, methylmalonic aciduria and homocystinuria. CblC type is the most common subtype of Cbl metabolic deficiency. There are two distinct clinical phenotypes of methylmalonic aciduria and homocystinuria (MMA-HC), which are different with age of onset, type of neurologic and systemic impairment and outcome (Fischer et al. 2014; Rossi et al. 2001a, b). Rosenblatt et al. (Rosenblatt et al. 1997) has reported the patients present within 12 months of age, including neonate onset, as early onset and above 4 years of age as late onset. All of the 10 patients in our study are early onset, even under six months of age. Moreover, the patients with early-onset MMA-HC could present a host of severe neurologic, hematologic, and gastrointestinal abnormalities with a significant percentage of deaths and residual moderate to severe neurologic deficit in survivors (Rossi et al. 2001a, b; Rosenblatt et al. 1997; Fischer et al. 2014). Furthermore, there is non- specificity in radiological findings of MMA (Radmanesh et al. 2008) such as ventricular dilation, cortical atrophy, periventricular white matter abnormality, thinning of the corpus callosum, subcortical white matter abnormality, cerebellar atrophy, basal ganglionic calcification, and myelination delay, and so on.

The pathophysiologic mechanism of hydrocephalus in patients with MMA-HC remains obscures, about which Rossi A et al. (Rossi et al. 2001a, b) proposed a new theory that increased arterial stiffness can cause pulse pressure to be propagated downstream undamped, elevating the intracerebral pressure and leading to ventricular dilatation and higher intraventricular pulse pressure, thereby producing hydrocephalus. Besides, hydrocephalus was also reported in other metabolic

Table 3 The clinical and laboratory features of the 8 patients with MMA

No.	1	2	3	4	5	6	7	8	9	10
Sex	M	M	M	M	F	M	F	M	F	F
Subtype	mut	cblC	cblC	cblC	cblC	cblC	cblC	cblC	cblC	cblC
Age at onset	5M10D	1 M27D	1 M	4 M	1 M	1 M10D	1 M	20D	2M8D	2M20D
Age at examination	5M16D	2M27D	1 M26D	6M17D	2M12D	2M16D	2M3D	1 M25D	2M9D	3 M5D
Current age	died at 7M20-D	1Y6M	1Y1 M	died at 7M20D	6M20D	8M7D	died at 2M29D	died at 2M10D	died at 4 M10D	4 M12D
Head circumference (cm)	62	36	37	42	35.5	36	36	36	37	38
Clinical features	cough, asth-ma	choking, pneumonia, jaundice	congenital heart disease, anemia, severe pneumonia, malnutrition.	poor feeding, failure to thrive, hypotonia, pneumonia	pneumonia	poor feeding, failure to thrive	poor feeding, failure to thrive	poor feeding, severe pneumonia	Sleepiness, anemia	convulsion
Brain MRI/CT										
Ventricular dilation	+	++	++	+++	+++	++	++	+++		
Cerebral parenchyma atrophy	++	++	++	+++	++	++	++	++		
Subdural effusion	++	++	++	++	–	–	–	–		
Cerebrospinal fluid										
Color	N	N	N	N	N	N	N	N	N	N
Protein	N	N	N	N	N	N	N	N	N	N
Pressure (10—14 mm)	N.D.	N.D.	18	16	12	14	12	15	N.D.	15
Blood BCAA and carnitine										
C0 (7–51.4uM)	4.78	5.55	8.66	4.95	10.23	21.98	17.25	12.38	9.57	12.96
C3(0.5–4.7uM)	10.7	6.77	6.61	5.27	12.37	10.7	15.4	17.23	5.45	5.41
C3/C0(0.01–0.2)	2.24	1.22	0.76	1.06	1.21	0.49	0.89	1.39	0.57	0.42
C3/C2(0.03–0.2)	–	0.87	0.51	1.04	0.98	0.57	0.71	1.02	0.54	0.43
Met(8–50 uM)	23.45	9.12	11.11	5.67	13.21	5.8	11.32	13.47	4.52	5.96
Urinary organic acid										
Methylmalonic acid(0.2–3.6 mmol/mol creatinine)	256.7	59.33	258	332	207.8	290.6	372.9	51.67	145.1	155.46
Laboratory tests										
Hemoglobin(110-170 g/L)	97	79	67	80	91	98	52	46	60	72
The number of Neutrophils(2–7.7 10*9/L)	2.27	0.82	7.72	0.41	1.43	1.29	2.74	3.21	1.26	0.39
Blood lactic acid(0.5~1.7 mmol/L)	1.97	2.34	1.63	1.69	0.92	1.54	0.79	0.94	1.51	1.23
Homocysteine(0-10 μmol)	7.2	119.6	132.7	174.1	145.4	203.2	204.5	198.7	166.2	69.3
Blood ammonia(18-72umol/L)	89	51	51	42	20	36	48	53	79	78
Treatment										
Age at drug treatment onset	6M20D	3 M	1 M2D	–	2M15D	2M20D	–	–	2M20D	3 M25D
Dietary protein restriction	+	+	–	–	–	–	–	–	–	–
Drug treatment	+	+	+	–	+	+	–	–	+	+
Ventricular peritoneal shunt surgery	–	–	+	+	+	+	+	+	–	+

N.D., Not Detectable

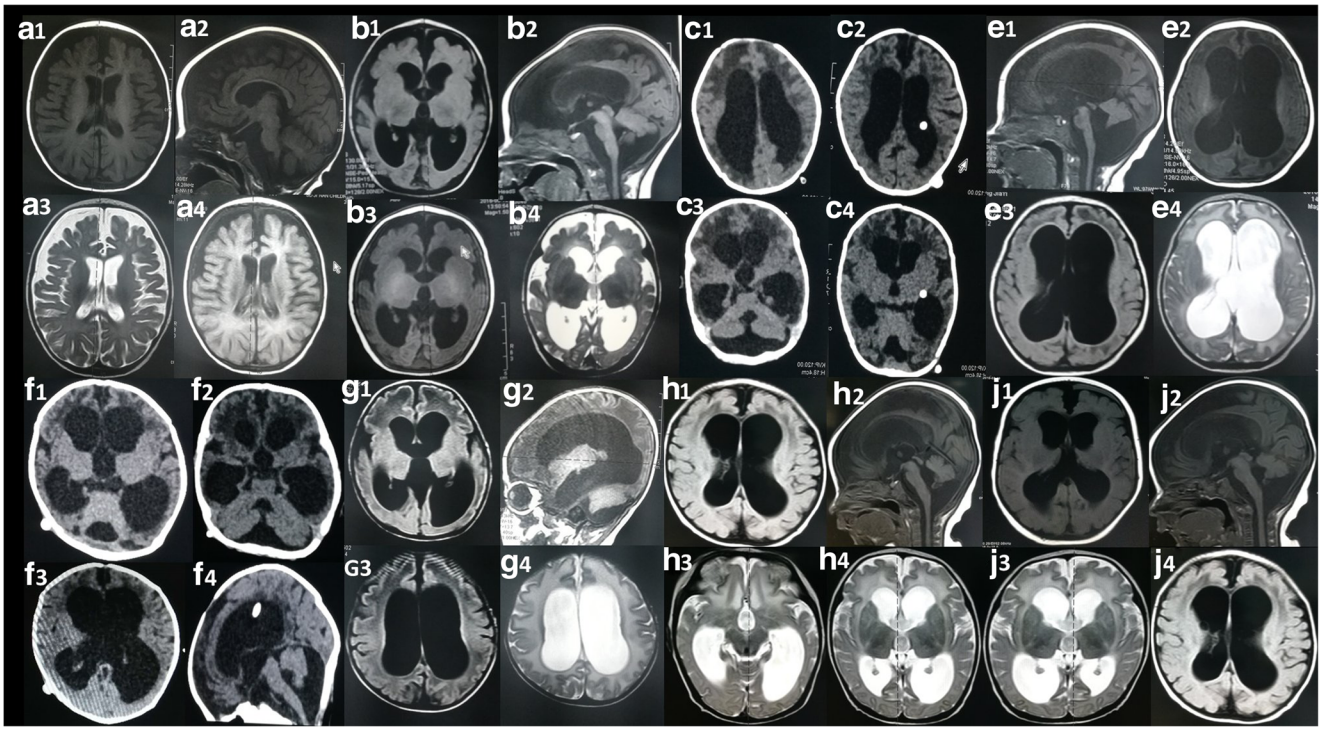

Fig. 1 Magnetic resonance/CT imaging of brain in case 1, 2, 3, 5, 6, 7, 9, 10. A1–4: case 1; **B1–4:** case 2; **C1–4:** case 3; **E1–4:** case 5; **F1–4:** case 6; **G1–4:** case 7; H1–4: case 9; J1–4: case10. All of them are accord with the manifestation of infant Hydrocephalus. Cranial MRI/CT showed severe ventriculomegaly and widespread cerebral parenchyma atrophy in case 2, 3, 5, 6, 7, 9 and 10. Case 1 have mild entriculomegaly, but with severe subdural effusion and cerebral atrophy. Red Arrows show the position of the catheter postoperatively. Hydrocephalus in Case 3 and 6 were detected by CT, and in other cases were by MRI

diseases with homocysteine. It is noteworthy that the high level of homocysteine in the blood could cause cardiovascular and cerebrovascular diseases, of which mainly pathophysiological mechanism is endothelial cell injury further leading to inflammation in the blood vessels (Baethmann et al. 2000; Sosvorová et al. 2014). Thus, homocysteine with a toxic effect to the arterial wall reduces the compliance of extracerebral intracranial arteries. Interestingly, hydrocephalus internus also

Table 4 Genotype profile of eight patients with MMA

Patient	Gene	Nucleotide substitution	Amino acid substitution	Parental origin	NOVEL/ Reported	Pathogenicity**	Sequencing method
Case 1	*MUT*	c.729_730insTT	p.Asp244Leufs	Father	Reported	pathogenic	Sanger
		c.322C > T	p. Arg108Cys	Mother	Reported	pathogenic	
Case 2	*MMACHC*	c.C217 > T	p. Arg73Ter	Father	reported	pathogenic	Sanger
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 3	*MMACHC*	c.609G > A	p. Trp203Ter	Father	reported	pathogenic	Sanger
		c.658_660del AAG	p. Lys220del	Mother	Reported	pathogenic	
Case 4	*MMACHC*	c.567dupT	p.Ile190Tyrfs	Father	Reported	pathogenic	Sanger
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 5	*MMACHC*	c.609G > A	p. Trp203Ter	Father	reported	pathogenic	Sanger
		c.658_660del AAG	p. Lys220del	Mother	reported	pathogenic	
Case 6	*MMACHC*	c.609G > A	p. Trp203Ter	Father	reported	pathogenic	Sanger
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 7	*MMACHC*	c.567dupT	p.Ile190Tyrfs	Father	reported	pathogenic	exome
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 8	*MMACHC*	c.567dupT	p.Ile190Tyrfs	Father	reported	pathogenic	Sanger
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 9	*MMACHC*	c.609G > A	p. Trp203Ter	Father	reported	pathogenic	exome
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	
Case 10	*MMACHC*	c.567dupT	p.Ile190Tyrfs	Father	reported	pathogenic	exome
		c.609G > A	p. Trp203Ter	Mother	reported	pathogenic	

**Pathogenicity analysis was done according to ACMG standards

happened in patients with 5, 10-methylenetetrahydrofolate reductase deficiency, of which the biochemical hallmarks are hyperhomocystinemia, homocystinuria and low levels of plasma methionine (Baethmann et al. 2000; Huemer et al. 2016).

Herein, we described clinical and genetic characteristics of 10 cases of MMA with HC. In the acute phase, patients with MMA often present lethargy, coma, vomiting, failure to thrive, muscular hypotonia, progressive neurological deterioration and kidney failure. To the best of our knowledge, MMA patients with obvious HC are very rare. Not only is HC caused by MMA hard to diagnose early, but also the primary disease of MMA is easily overlooked as most surgeons are unfamiliar with MMA, which would lead to surgical failure (Gmeiner et al. 2018; Ma et al. 2018).

Shandong province in China, where all the 10 cases are from, is a high incidence area for MMA, and the estimated local incidence of MMA is as high as 1:3000, which the similar situations also occurred in the neighboring provinces of Hebei and Henan (Han et al. 2016). There have been so far 13 genes associated with MMA. Thereinto, *MMACHC* gene is the cause of cblC type which is also the most common subtype (Carrillo-Carrasco et al. 2012) and is Cobalamin-responsive (Fraser and Venditti 2016). Therefore, early diagnosis and treatment for MMA is vital. The *MMACHC* mutation, c.609G > A (p.W203X) (Lerner-Ellis et al. 2006), has been reported to be the most common in Chinese patients, especially in Shandong province (Liu et al. 2010). In this research, nine index cases with cblC type all had the c.609G > A mutation, in which the probands 6 and 9 had the homozygous mutations. Founder effect could explain the high frequency mutations.

This is the first report about the patient with *MUT* mutation developing hydrocephalus though the symptom of HC is not serious. Moreover, the patient showed severe subdural effusion and brain atrophy, despite the patient receiving special diet and medications. From our data, the complication of HC occurred mainly in cblC type (8.82%), of which 102 children with two *MMACHC* gene mutations type were diagnosed. Meanwhile, only 21 patients with mut type caused by *MUT* gene mutation were found. The important reason was that the frequency of mut type is very low. In addition, the patient with *MUT* gene mutation had the more severe condition so that there was no time to develop the complication of HC.

Ma et al. (Ma et al. 2018) reviewed eight patients of MMA with severe HC in previous literature. Definitely, three patients benefitted from the surgical shunting. As it is known, timely surgical shunting is vital for severe hydrocephalus and severe ventriculomegaly with progressive intracracial hypertention. Moreover, infants with HC often present the typical characteristics of intracranial hypertension, including increased head circumference and bulging anterior fontanelle. However, the typical features of HC (Yaeger and Marin 2017), especially intracranial hypertension, were not observed in our cases, which is consistent with the previous reports (Ma et al. 2018). The possible explanation is that ventriculomegaly gradually develops in MMA patients, and brain atrophy could take on much pressure from HC. In our study, seven patients underwent the ventricular peritoneal shunt, of which three (cases 4, 7 and 8) died of multiple organ failure after operation due to delayed MMA diagnosis.

Case 2 was not as severe as case 3, while he only received medicine treatment due to his parents' refusal to surgery. His development was delayed when he was followed up after one-year. MMA patients with severe hydrocephalus could benefit from timely surgery for the recovery of brain atrophy is faster and more effective (Ma et al. 2018; Kraemer et al. 2017). Unfortunately, cases 5 and 6, who had improvement after shunting surgery and timely medical treatment, died from severe infection and metabolic crisis several months after their parents withdrew treatment without permission.

High-throughput sequencing has been increasingly accessible in the clinical diagnosis (Ye et al. 2018). Target region capture sequencing is developed mainly to enrich and sequence specific regions of particular interest, such as specific exons or entire exomes on the basis of high-throughput sequencing. We used a panel of target region capture sequencing containing 712 genes associated with inherited metabolic diseases in which 13 genes necessary for high-risk MMA were included, such as *MUT*, *MMACHC*, *MMADHC*, *MMAA*, *MMAB*, *MCEE*, *ACSF3*, *LMBRD1*, *HCFC1*, *ABCD4*, *SUCLA2*, *SUCLG1* and *TCN2* (Devi and Naushad 2017). Nine patients were identified to have compound heterozygous mutations or homozygous mutations in *MMACHC* gene, and 1 were *MUT* mutations respectively. The mutation of c.609G > A in *MMACHC* was the most common mutation found in the cblC type patients.

In conclusion, we presented 10 cases of MMA with hydrocephalus. The cblC type with HC is more common and has bigger ventriculomegaly than MMA caused by mut type. It seemed that mut type with HC involved whole brain range, especially, severe subdural effusion. It still needs to accumulate more cases to differentiate the HC between cblC type and mut type. Surgery is a good choice for MMA with severe HC, but it is necessary to evaluate appropriately the overall condition of the patients before surgery.

Acknowledgments This work was financially supported by the Science and technology development project of Shandong Health Department (2013WS0009) and Natural Science Training Foundation of Shandong Province (ZR2014HP051). The authors are grateful to the patients and their parents for their contribution to the study. We would like to thank SinoPath (Beijing) Medical Laboratory for technical support.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

References

Baethmann M, Wendel U, Hoffmann GF, Göhlich-Ratmann G, Kleinlein B, Seiffert P, Blom H, Voit T (2000) Hydrocephalus internus in two patients with 5,10-methylenetetrahydrofolate reductase deficiency. Neuropediatrics 31(6):314–317

Carrillo-Carrasco N, Chandler RJ, Venditti CP (2012) Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis 35(1):91–102

Devi AR, Naushad SM (2017) Targeted exome sequencing for the identification of complementation groups in methylmalonic aciduria: a south Indian experience. Clin Biochem 50(1–2):68–72

Fischer S, Huemer M, Baumgartner M, Deodato F, Ballhausen D, Boneh A, Burlina AB, Cerone R, Garcia P, Gökçay G, Grünewald S, Häberle J, Jaeken J, Ketteridge D, Lindner M, Mandel H, Martinelli D, Martins EG, Schwab KO, Gruenert SC, Schwahn BC, Sztriha L, Tomaske M, Trefz F, Vilarinho L, Rosenblatt DS, Fowler B, Dionisi-Vici C (2014) Clinical presentation and outcome in a series of 88 patients with the cblC defect. J Inherit Metab Dis 37(5):831–840

Fraser JL, Venditti CP (2016) Methylmalonic and propionic acidemias: clinical management update. Curr Opin Pediatr 28(6):682–693

Gmeiner M, Wagner H, van Ouwerkerk WJR, Senker W, Holl K, Gruber A (2018) Abdominal pseudocysts and peritoneal catheter revisions: surgical long-term results in pediatric hydrocephalus. World Neurosurg 111:e912–e920

Han B, Cao Z, Tian L, Zou H, Yang L, Zhu W, Liu Y (2016) Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic acidemia and homocysteinemia (cblC type) in Shandong province, China. Brain and Development 38(5):491–497

Han LS, Huang Z, Han F, Wang Y, Gong ZW, Gu XF (2017) Eight novel MUT loss-of-function missense mutations in Chinese patients with isolated methylmalonic academia. World J Pediatr 13(4):381–386

Huemer M, Mulder-Bleile R, Burda P, Froese DS, Suormala T, Zeev BB, Chinnery PF, Dionisi-Vici C, Dobbelaere D, Gökcay G, Demirkol M, Häberle J, Lossos A, Mengel E, Morris AA, Niezen-Koning KE, Plecko B, Parini R, Rokicki D, Schiff M, Schimmel M, Sewell AC, Sperl W, Spiekerkoetter U, Steinmann B, Taddeucci G, Trejo-Gabriel-Galán JM, Trefz F, Tsuji M, Vilaseca MA, von Kleist-Retzow JC, Walker V, Zeman J, Baumgartner MR, Fowler B (2016) Clinical pattern, mutations and in vitro residual activity in 33 patients with severe 5, 10 methylenetetrahydrofolate reductase (MTHFR) deficiency. J Inherit Metab Dis 39(1):115–124

Keyfi F, Talebi S, Varasteh AR (2016) Methylmalonic Acidemia diagnosis by laboratory methods. Rep Biochem Mol Biol 5(1):1–14

Kraemer MR, Sandoval-Garcia C, Bragg T, Iskandar BJ (2017) Shunt-dependent hydrocephalus: management style among members of the American Society of Pediatric Neurosurgeons. J Neurosurg Pediatr 20(3):216–224

Lerner-Ellis JP, Tirone JC, Pawelek PD, Dore C, Atkinson JL, Watkins D, Morel CF, Fujiwara TM, Moras E, Hosack AR, Dunbar GV, Antonicka H, Forgetta V, Dobson CM, Leclerc D, Gravel RA, Shoubridge EA, Coulton JW, Lepage P, Rommens JM, Morgan K, Rosenblatt DS (2006) Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nat Genet 38(1):93–100

Lerner-Ellis JP, Anastasio N, Liu J, Coelho D, Suormala T, Stucki M, Loewy AD, Gurd S, Grundberg E, Morel CF, Watkins D, Baumgartner MR, Pastinen T, Rosenblatt DS, Fowler B (2009) Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Hum Mutat 30(7):1072–1081

Liu MY, Yang YL, Chang YC, Chiang SH, Lin SP, Han LS, Qi Y, Hsiao KJ, Liu TT (2010) Mutation spectrum of MMACHC in Chinese patients with combined methylmalonic aciduria and homocystinuria. J Hum Genet 55(9):621–626

Ma M, Wu M, Li Y, Wu D, Zhang B (2018) Shunt surgery for early-onset severe hydrocephalus in methylmalonic acidemia: report on two cases and review of the literature. Childs Nerv Syst 34(7):1417–1421

Pupavac M, Tian X, Chu J, Wang G, Feng Y, Chen S, Fenter R, Zhang VW, Wang J, Watkins D, Wong LJ, Rosenblatt DS (2016) Added value of next generation gene panel analysis for patients with elevated methylmalonic acid and no clinical diagnosis following functional studies of vitamin B12 metabolism. Mol Genet Metab 117(3):363–368

Radmanesh A, Zaman T, Ghanaati H, Molaei S, Robertson RL, Zamani AA (2008) Methylmalonic acidemia: brain imaging findings in 52 children and a review of the literature. Pediatr Radiol 38(10):1054–1061

Rosenblatt DS, Aspler AL, Shevell MI, Pletcher BA, Fenton WA, Seashore MR (1997) Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). J Inherit Metab Dis 20(4):528–538

Rossi A, Cerone R, Biancheri R, Gatti R, Schiaffino MC, Fonda C, Zammarchi E, Tortori-Donati P (2001a) Early-onset combined methylmalonic aciduria and homocystinuria: neuroradiologic findings. AJNR Am J Neuroradiol 22(3):554–563

Rossi A, Biancheri R, Tortori-Donati P (2001b) The pathogenesis of hydrocephalus in inborn errors of the single carbon transfer pathway. Neuropediatrics 32(6):335–336

Sosvorová L, Bešťák J, Bičíková M, Mohapl M, Hill M, Kubátová J, Hampl R (2014) Determination of homocysteine in cerebrospinal fluid as an indicator for surgery treatment in patients with hydrocefalus. Physiol Res 63(4):521–527

Villoria JG, Pajares S, Lopez RM, Marin JL, Ribes A (2016) Neonatal screening for inherited metabolic diseases in 2016. Semin Pediatr Neurol 23(4):257–272

Worgan LC, Niles K, Tirone JC, Hofmann A, Verner A, Sammak A, Kucic T, Lepage P, Rosenblatt DS (2006) Spectrum of mutations in Mut methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. Hum Mutat 27(1):31–43

Wu LY, An H, Liu J, Li JY, Han Y, Zhou AH, Wang F, Jia JP (2017) Manic-depressive psychosis as the initial symptom in adult siblings with late-onset combined Methylmalonic aciduria and Homocystinemia, cobalamin C type. Chin Med J 130(4):492–494

Yaeger SK, Marin JR (2017) An infant diagnosed with hydrocephalus by point-of-care ultrasound. Pediatr Emerg Care 33(4):287–289

Ye B, Smerin D, Gao Q, Kang C, Xiong X (2018) High-throughput sequencing of the immune repertoire in oncology: applications for clinical diagnosis, monitoring, and immunotherapies. Cancer Lett 416:42–56