Brief report

Clinical characteristics of hemolytic uremic syndrome secondary to cobalamin C disorder in Chinese children

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Background: **The present study was undertaken to investigate the clinical characteristics of hemolytic uremic syndrome (HUS) secondary to cobalamin C disorder (cbl-C disorder).**

Methods: **We reviewed retrospectively the medical records of 3 children with HUS secondary to cbl-C disorder who had been treated between April 1, 2009 and October 31, 2013.**

Results: **The 3 patients with HUS secondary to cbl-C disorder presented with progressive hemolytic anemia, acute renal failure, thrombocytopenia, poor feeding, and failure to thrive. Two of the 3 patients once had high blood pressure. The mutations of c.609G>A (p.W203X), c.217C>T (p.R73X) and c.365A>T (p.H122L) in the methylmalonic aciduria (cobalamin deficiency) cbl-C type, with homocystinuria gene were detected in the 3 patients. In these patients the levels of lactate dehydrogenase and homocysteine in serum were elevated and the level of methylmalonic acid (MMA) in urine was also elevated. After treatment with hydroxocobalamin, 2 patients were discharged with no obvious abnormal growth and neurological development and 1 patient died of multiple organ failure.**

Conclusions: **The results of this study demonstrated that cbl-C disorder should be investigated in any child presenting with HUS. The high concentrations of homocysteine and MMA could be used for timely recognization of the disease. Once the high levels of plasma homocystein and/or plasma or urine MMA are detected, the treatment with parenteral hydroxocobalamin should be prescribed immediately. The early diagnosis and treatment would contribute to the good prognosis of the disease.**

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Key words: **children;**
cobalamin C disorder;
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Introduction

Cobalamin C disorder (cbl-C disorder) is an inborn error of intracellular cobalamin metabolism.[1] Weisfeld-Adams et al[2] reported the incidence of cbl-C disorder in New York State is 1/100 000 approximately. Over 300 cbl-C patients have been reported globally to date.[3] The incidence of this disorder in Chinese patients has not yet been reported.[4] But mutations in the gene of methylmalonic aciduria (cobalamin deficiency) cbl-C type, with homocystinuria (*MMACHC*) were found as the cause of cbl-C disorder in Chinese patients by impairing intracellular synthesis of adenosylcobalamin and methylcobalamin, which are the cofactors for the methylmalonyl coenzyme A mutase and methionine synthase enzymes.[5,6]

Hemolytic uremic syndrome (HUS) is characterized by the triads of mechanical intravascular hemolytic anemia with microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure (ARF). The gastrointestinal infection with enterohemorrhagic *Escherichia coli* (e.g., O_{157}:H_7) usually took place in patients with typical HUS, and few patients with atypical HUS were infected by enterohemorrhagic *Escherichia coli*.[7] Among patients with cbl-C disorder, HUS has been reported in white children.[8-10] In addition, recent studies[11,12] have reported that approximately 10% of patients with cbl-C disorder develop HUS and the occurrence of HUS is at least as frequent in late forms of cbl-C defect as in neonatal forms.[11] In the present report, we presented with clinical characteristics of 3 Chinese children with HUS secondary to cbl-C disorder.

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Methods

Subjects

We consecutively collected the medical records of the 3 children with HUS secondary to cbl-C disorder who had been admitted to Beijing Children's Hospital between April 1, 2009 and October 31, 2013. The data of the children were collected by trained investigators using a standard case report form, which included demographic characteristics, family status, clinical presentation, treatment follow-up and outcome. The study was approved by the Ethics Committee of Beijing Children's Hospital. Written informed consents were obtained from the parents of the 3 children.

Biological assays and genetic screening

Pre-treatment biochemical parameters in blood were analyzed by a hematology analyzer and a clinical chemistry analyzer. The levels of methylmalonic acid in urine were analyzed by gas chromatography mass spectrometry, whereas those of homocystine in serum were analyzed by a clinical immune analyzer. Genomic DNA was amplified for *MMACHC* using polymerase chain reaction. Amplimers, including individual exons, splice donor and acceptor site, were subjected to double-stranded DNA sequence analysis on an ABI 3730XL Genetic Analyzer. The references of *MMACHC*-primers were as follows: 5'-GGGATACCGTGATGATACGC-3' and 5'-GAACCCAGGAGGATCAGAGG-3' for Exon 1; 5'-TGCATCACATAGCGTCAGTG-3' and 5'-AGCCTGGCTTTAGGGTATCA-3' for Exon 2; 5'-TCATGTTTTCCCTTCTGAGGA-3' and 5'-CAAAGCTAATTTGTTCTGGGTTG-3' for Exon 3; 5'-AGGCCTAGCTTGCAATGATG-3' and 5'-GAAGGCAGATGGGAATTCTG-3' for Exon 4a; 5'-TTTGGCAAAGCAAAAGGTT-3' and 5'-CAAGATGGGTGGATCACGA-3' for Exon 4b; 5'-AGCCTGGCCAATACAGTGAA-3' and 5'-AGCCTTCCCTTGGTTCTAGC-3' for Exon 5a; and 5'-ACCATTTTGGGAGGCTGAG-3' and 5'-GGGCAGGCTACTGGTTTGTA-3' for Exon 5b.

Potential pathogenicity and evolutionary conservation of genetic alterations were checked in the literature and several in silico prediction programs. Influence on splicing was assessed using the splice site prediction software Human Splicing Finder (www.umd.be/HSF/) and Splice-Site Finder (http://www.genet.sickkids.on.ca/cftr/app).

Follow-up

In the three patients, patient 1 was followed up for 3, 6, 9, 15, and 21 months and patient 2 was followed up for 3, 6, 9 and 12 months after discharge by interview at the outpatient department.

Results

In total, 2 of the 3 patients with HUS secondary to cbl-C disorder were identified by clinical and genetic diagnosis and 1 patient was diagnosed by clinical manifestattions. The clinical and biochemical characteristics at presentation with HUS secondary to cbl-C disorder are shown in Table. Patient 1 was complicated by megaloblastic anemia. The other two patients did not present any extra-renal symptoms in association with HUS. Their genetic detection and laboratory findings are shown in Fig. 1. The values of hemoglobin, platelet, serum creatinine and major clinical events in acute phase and subsequent follow-up are shown in Fig. 2.

Patient 1 was a 1.5-year-old boy of Chinese-Mongolian ethnic. His clinical manifestations were poor feeding, failure to thrive, anemia, edema of eyelids, and hematuria. Neither he had a history of diarrhea nor his parents had a consanguineous marriage. His elder brother was found to have cbl-C disorder by newborn screening and mutation screening of the *MMACHC* gene. His elder brother of patient 1 died of cbl-C disorder at age of 10 months. Before admission to our hospital, the patient had a 6-month history of anemia and he was treated at a local hospital. His blood pressure was 120/70 mmHg. His family history and special clinical manifestations

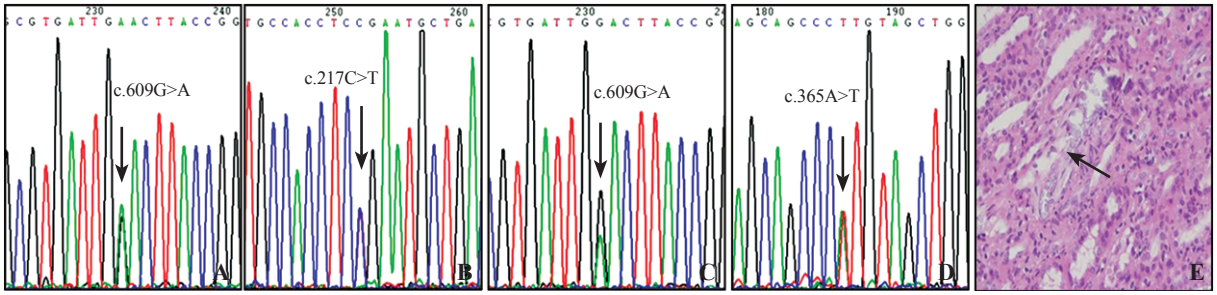

Fig. 1. A&B: The heterozygous mutations of the *MMACHC* gene in patient 1 were c.609G>A and c.315C>T (black arrow); **C&D:** The heterozygous mutations of the *MMACHC* gene in patient 2 were c.609G>A and c.365A>T (black arrows); **E:** Homocysteine crystals (black arrow) were in the kidney tubule of patient 2 (hematoxylin-eosin staining, original magnification × 40). *MMACHC*: methylmalonic aciduria (cobalamin deficiency) cbl-C type, with homocystinuria.

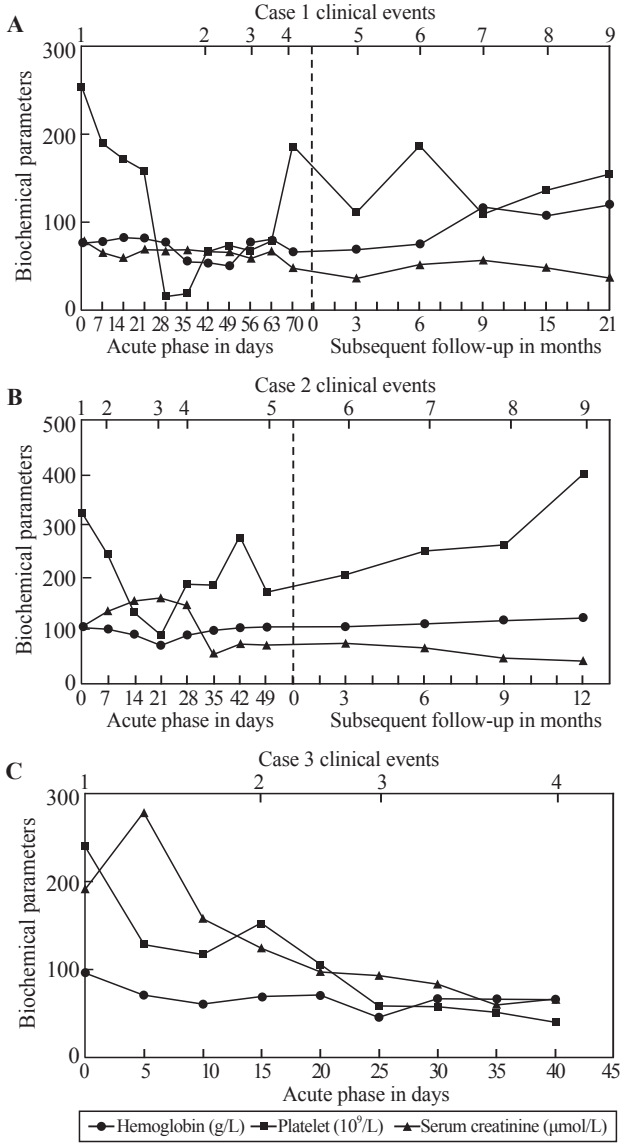

Fig. 2. Hemoglobin, platelet count and serum creatinine of each patient indicated by y axis; the lower x axis shows time (acute phase in days or weeks, subsequent follow-up in months) until the last follow-up. The upper x axis shows major events during the acute phase and follow-up. **A:** Event 1 shows that patient 1 admitted to the local hospital. Event 2 shows that patient 1 was admitted to Beijing Children's Hospital. Event 3 shows that hydroxocobalamin treatment was initiated according to high concentrations of homocysteine and MMA in blood and urine respectively. Event 4 shows that the patient was diagnosed with HUS secondary to cbl-C by detection of the *MMACHC* gene. Events 5-9 show that the patient was followed up for 3, 6, 9, 15, 21 months; **B:** Event 1 shows that patient 2 visited the Nephrology Outpatient of Beijing Children's Hospital. Event 2 shows that patient 1 admitted to Beijing Children's Hospital. Event 3 shows that hydroxocobalamin treatment was initiated according to high homocysteine in blood and high MMA in urine. Event 4 shows renal biopsy for patient 2. Event 5 shows that the patient was diagnosed with HUS secondary to cbl-C by *MMACHC* gene detection. Events 6-9 show that the patient was followed up for 3, 6, 9, 12 months; **C:** Event 1 shows that patient 3 was admitted to a local hospital. Event 2 shows that patient 3 was admitted to intensive care unit of Beijing Children's Hospital. Event 3 shows that hydroxocobalamin treatment was initiated according to the high concentration of homocysteine in blood and the high concentration of MMA in urine. Event 4 shows the death of patient 3. MMA: methylmalonic acid; HUS: hemolytic uremic syndrome; cbl-C: cobalamin C; *MMACHC*: methylmalonic aciduria (cobalamin deficiency) cbl-C type, with homocystinuria.

Table. Clinical and biochemical characteristics at presentation with HUS secondary to clb-C disorder

Characteristics	Patients		
	Case 1	Case 2	Case 3
Age at initial presentation (y)	1.5	3.3	2.7
Gender	Male	Female	Male
Weight (kg)	8.8	13.5	14.0
Height (cm)	73	94	100
Initial symptoms	Anemia Edema Hematuria Poor feeding Failure to thrive	Anemia Fever Edema Poor feeding Failure to thrive	Anemia Poor feeding Failure to thrive
Family history	+	-	-
Blood pressure (mmHg)	120/70	160/105	170/110
RBC (3.5-5.5×10^{12}/L)	1.8	2.2	1.3
HGB (110.0-160.0 g/L)	56.0	71.0	44.0
Analysis of bone marrow	Megaloblastic anemia	-	-
Hematuria	+	+	+
Proteinuria	+	+	+
BUN (1.7-7.1 mmol/L)	13.0	18.9	25.7
CR (27.0-130.0 μmol/L)	79.3	161.2	279.1
UA (119.0-416.0 μmol/L)	735.3	891.3	899.0
ALT (5.0-40.0 U/L)	36.0	17.1	191.0
AST (5.0-40.0 U/L)	21.0	28.3	60.0
CK-MB (0-25.0 U/L)	12.0	13.0	41.0
LDH (50.0-240.0 U/L)	459.0	1363.0	984.0
HBDH (80.0-220.0 U/L)	381.0	979.0	850.0
MMA (<0.001 mmol/creatinine)	0.412	2.804	0.550
HCY (1.90-12.98 mmol/L)	97.58	150.38	134.15
VB12 (140.0-960.0 pg/mL)	1200.0	557.0	1500.0
Outcome	Survived	Survived	Died

cbl-C: cobalamin C; RBC: red blood cells; HGB: hemoglobin; BUN: blood urea nitrogen; CR: creatinine; UA: uric acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK-MB: creatine kinase-MB; LDH: lactate dehydrogenase; HBDH: α-hydroxybutyrate dehydrogenase; MMA: methylmalonic acid; HCY: homocysteine; VB12: vitamin B12.

including poor feeding and failure to thrive led us to take consideration of cbl-C disorder. Because of the disease history of his brother, mutations of the *MMACHC* gene as c.609G>A and c.217C>T were also detected (Fig. 1A and B). At last, patient 1 was diagnosed with HUS secondary to cbl-C disorder.

Patient 2 was a 3.3-year-old girl of Chinese-Mongolian ethnic origin who presented with edema in face, poor feeding, failure to thrive, anemia and fever when she was admitted to the hospital. Her blood pressure was 160/105 mmHg. She had no history of diarrhea. The concentration of methylmalonic acid (MMA) was 0.42 mmol/creatinine (normal level: <0.001 mmol/creatinine). The concentration of homocystine increased to 97.58 mmol/L (normal level: <12.98 mmol/L). With increasing levels of serum urea, creatinine and serum uric, renal biopsy performed on the 20th day after admission showed homocysteine crystals in kidney tubules (Fig. 1E) but few lesions of thrombotic microangiopathy. Genetic diagnosis revealed the mutations of the *MMACHC* gene as c.609G>A and c.365A>T (Fig. 1C and D). She was diagnosed with HUS secondary to cbl-C disorder.

Once high levels of plasma homocystein and plasma or urine MMA were detected, the two children were treated immediately according to the standardized treatment reported previously:[13,14] intramuscular injection of vitamin B12 0.5-1 mg/day once or twice weekly and L-carnitine (500 mg/day) to increase the excretion of MMA; folic acid 2.5-5 mg/day and betaine 500-2000 mg/day to lower the level of homocystein. After therapy, the levels of MMA in urine declined and patients 1 and 2 were discharged. The two children were interviewed after discharge respectively and their concentration of urinary MMA was kept below 0.001 mmol/creatinine. Their values of hemoglobin, platelet and serum creatinine during a follow-up are shown in Fig. 2. The follow-up showed significant improvement of renal and hematological manifestations. In addition, no abnormal growth and neurological development were noted in the two patients. Their blood pressure was within the normal range (110-70 mmHg).

Patient 3 was admitted to Beijing Children's Hospital earlier than patients 1 and 2. He was a 2.7-year-old boy of Chinese Han ethnic. He suffered from iron deficiency anemia for 12 months which was diagnosed at a local hospital. Previously he had no diarrhea. Because delayed healing and recurrent anemia, he visited the outpatient department of our hospital. Because of progressive anemia, poor feeding, failure to thrive and ARF, he was admitted to the intensive care unit of the hospital. His blood pressure was 170/110 mmHg. High concentration of urinary MMA (0.55 mmol/creatinine) and serum homocystein (134.15 mmol/L) were detected, and immediate treatment with parenteral hydroxocobalamin was prescribed. Biochemical parameters proved the occurrence of multiple organ injury (Table). The patient died of multiple organ failure despite the level of MMA in urine declined (0.05 mmol/creatinine) after treatment.

Discussion

We described the clinical characteristics of 3 pediatric patients with hemolytic uremic syndrome secondary to cbl-C disorder. The 3 patients were less than 4 years old and were not in neonatal forms. It is inconsistent with the report that the occurrence of HUS is at least as frequent in late forms of cbl-C disorder as in neonatal forms.[11] Different race has different occurrence of HUS in neonatal forms. A large number of cases of the disease are required to confirm this speculation. In our study, all patients presented the common clinical manifestations of HUS including hemolytic anemia, ARF and thrombocytopenia. No patients had diarrhea which was the most important clinical manifestation in typical HUS, they were diagnosed with HUS. The patients with HUS secondary to cbl-C disorder had some special clinical manifestations including poor feeding, failure to thrive, and high blood pressure. Poor feeding and failure to thrive were possibly related to MMA accumulated and metabolic disturbance of fatty acids, e.g., the formation of odd numbered fatty acids.[15] High blood pressures probably resulted in the injury of blood vessels by homocysteine.[16,17] In general, neurological manifestations are seen commonly in patients with early cbl-C disorder.[1] However, 2 patients in the study did not show any abnormal neurological development in a short-term follow up. Possibly it was due to the immediate treatment. But we were not sure that there was no obvious abnormal neurological development in the two patients. Fischer et al[11] reported the improvement of biochemical abnormalities, non-neurological signs and mortality of patients after treatment with parenteral hydroxocobalamin, betaine, folate/folinic acid and carnitine, but the improvement of long-term neurological and ophthalmological outcome was not significant. Hence a long-term follow up is necessary to confirm the findings in neurological development. Although the two patients in our study were of Chinese Mongolian ethenic origin, whether HUS secondary to cbl-C disorder was related to different ethenics should be clarified.

Biological assays may confirm the diagnosis of cbl-C disorder. Researchers[18-21] reported the elevated levels of MMA and homocystein as the biochemical hallmarks of cbl-C disorder. The increased levels of urea and creatinine were related to kidney injury. Zwickler et al[22] reported the elevated level of uric acid; we also found the elevated level of uric acid might be related to the increase of MMA and renal dysfunction. In addition, the level of plasma lactate dehydrogenase (LDH) in the three patients increased continuously, which was consistent with Davin et al's finding that elevation of LDH level may contribute to ongoing low-grade chronic thrombotic microangiopathy.[23]

After treatment with hydroxocobalamin, the outcomes of our patients were different. Patient 3 had poor outcome because of delayed treatment with hydroxocobalamin. This patient suffered from iron deficiency anemia for 12 months until it was diagnosed at a local hospital. At the time of worsening of the condition, he visited our hospital. However, multiple organ failure had already occurred. Obviously, good outcomes of patients 1 and 2 attributed to timely diagnosis and immediate treatment with hydroxocobalamin.

Three mutations of the *MMACHC* gene were detected in patients 1 and 2. The definite diagnosis of HUS secondary to cbl-C disorder was dependent on the detection of mutations of the *MMACHC* gene, We concluded that: 1) Any child with HUS should be investigated for cbl-C disorder by the high plasma concentration of homocystein and/or high concentration

of plasma or urine MMA; 2) Treatment with parenteral hydroxocobalamin should be given immediately if the levels of homocysteine and MMA are markedly increased; 3) Genetic screening may subsequently confirm the diagnosis of cbl-C disorder, but treatment should be given early; 4) Renal biopsy (homocysteine crystals in kidney tubules but show few lesions of thrombotic microangiopathy) revealed that injury to the kidney should be controlled and thrombotic microangiopathy should be refrained by immediate treatment with hydroxocobalamin.

In conclusion, cbl-C disorder should be investigated in children with HUS. The high concentrations of homocysteine and MMA could be used to timely detect the disease. Once the high levels of plasma homocystein and/or plasma or urine MMA were detected, the treatment with parenteral hydroxocobalamin should be prescribed immediately. The early diagnosis and treatment would contribute to the good prognosis of the disease.

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Ethical approval: The study was approved by the Ethics Committee of Beijing Children's Hospital. Written informed consents were obtained from the parents of the study subjects.
Competing interest: None declared.
Contributors: Li QL proposed the study and wrote the draft. Song WQ was involved in the study design and was the guarantor. Peng XX analyzed and interpreted the data of the study. All authors contributed to the design and interpretation of the study.

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