Easily Misdiagnosed *cblC* Deficiency in Adolescents: the Clinical and Metabolic Studies

Zhehui Chen
Peking University First Hospital
Hui Dong
Peking University First Hospital
Yupeng Liu
Peking University People's Hospital
Ruxuan He
Beijing Children's Hospital Capital Medical University
Jinqing Song
Peking University First Hospital
Ying Jin
Peking University First Hospital
Mengqiu Li
Peking University First Hospital
Yi Liu
China-Japan Friendship Hospital
Xueqin Liu
Peking University First Hospital
Hui Yan
Peking University First Hospital
Jianguang Qi
Peking University First Hospital
Fang Wang
Peking University First Hospital
Huijie Xiao
Peking University First Hospital
Hong Zheng
Henan University of TCM: Henan University of Chinese Medicine
Lulu Kang
Zhengzhou University First Affiliated Hospital
Dongxiao Li
Henan Children's Hospital: Zhengzhou Children's Hospital
Yao Zhang

Peking University First Hospital

Yanling Yang (organic.acid@vip.126.com)

Peking University First Hospital

Research

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Abstract

Purpose: Adolescents are easily attacked by potential inherited metabolic disorders. cblC deficiency is the most common type of methylmalonic aciduria in China. The late-onset patients present with varied non-specific symptoms and usually being misdiagnosed. The purpose of this study is to investigate the clinical features of patients with adolescence-onset cblC deficiency and explore the prevention and control strategies.

Methods: Fifty-seven patients (34 males and 23 females) with adolescence-onset cblC deficiency were admitted in our clinic from 2002 to September 2021. The diagnosis was confirmed by metabolic and genetic tests. The clinical and biochemical features, disease triggers, outcome and genotypes-phenotypes correlation were examined.

Results: The onset ages ranged from 10 to 25 years old (median age was 12 years). 16 cases (28.0%) presented with symptoms after infection or sports training. 46 patients (80.7%) had neuropsychiatric diseases. 14 patients (24.6%) displayed cardiovascular diseases. Five cases (8.9%) showed pulmonary hypertension. Renal damage was observed in seven cases (12.3%). 23 mutations were identified from the MMACHC gene of 57 patients. 37 patients demonstrated c.482G>A (64.9%) and 16 cases had c.609G>A (26.3%). Among 13 patients that exhibited spastic paraplegia as a main manifestation, 10 patients had c.482G>A (76.9%). Five patients presented with psychotic disorders and spastic paraplegia with c.482G>A. All patients improved after metabolic treatment with cobalamin, L-carnitine, and betaine. 30 school-aged patients returned to school. Two patients were married and had healthy babies.

Conclusion: Patients with adolescence-onset cblC deficiency presented with varied neuropsychiatric symptoms or multiple organ damage. Metabolic studies and individualized treatment are keys to improve the outcome of the patients.

Background

Methylmalonic aciduria (MMA) is the most common organic acid metabolic disorders in China, and MMA combined with homocystinuria caused by cblC deficiency accounts for 70%. In Japan, the incidence of MMA is about 1/50000[1]. In China, the data of Newborn Screening showed that the prevalence of MMA in Northern China is higher than in Southern China [2–3]. The symptoms of MMA is different, resulting in multiple organs damage [4]. Additionally, the disease could happened at any age. At neonatal-onset, the patient could be critically ill, progress quickly, and have a high mortality. At infant-onset, infection and starvation are the mostly common triggers for MMA. The infant usually presents with developmental delays, seizures, and confusion. If the infant cannot get timely treatment, patients has neurological sequelae [5]. At adolescence-onset, most teenagers present with mental and/or behavioral abnormalities, mental regression, or movement disorders [6]. These symptoms are non-specific, and patients are easily misdiagnosed. Plasma total homocysteine (tHCY), blood amino acids, acyl-carnitines analysis, and genetic study should be used for final diagnosis [7]. Following metabolic treatment with cobalamin, L-

carnitine, and betamine, patients may fully recover. For late onset MMA, most patients have non-specific symptoms that can lead to incorrect diagnosis, causing delay of treatment. We analyzed clinical features, triggers, metabolites, and genotypes, including the relationship between genotypes and phenotypes, in adolescence-onset of cblC deficiency to gain understanding for early intervention of this treatable and reversible disease.

Methods

Patients

From 2002 to May 2021, 57 Chinese patients with adolescence-onset cblC deficiency were diagnosed and followed up at Peking University First Hospital. Diagnosis was confirmed by biochemical and genetic analysis.

Biochemical assays

Amino acids, free carnitine, and acyl-carnitines in dried blood spots were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, API3200, Triple Quad 4500, Applied Biosystems, CA, USA). The concentrations of the metabolites were calculated automatically using Chemoview software.

Gas chromatography and mass spectrometry (GC/MS) by GCMS-QP 2010 (Shimadzu Corporation, Kyoto, Japan) was used to analyze urine organic acids, according to a previous established protocol [8–10]. Data were collected using GC/MS solution software. Plasma tHCY was detected by chemiluminescent immunoassay (Abbott I2000, USA).

Genetic analysis

Peripheral blood samples were collected from the patients and their parents. DNA was extracted using a DNA Isolation Kit (Bioteke, China, AU1802). Purified DNA samples were sent to Running Gene Inc. (Beijing, China) or Berry Genomics Corporation (Beijing, China) for next generation sequencing to screen mutations in patients. The pathogenicity of each novel variant was evaluated according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines [11].

Routine examination

Body weight, height, secondary sex characteristics, genitals, and gonads were recorded to evaluate the growth and sexual development of the patients. Blood pressure, electrocardiography, and echocardiography were used for cardiovascular monitoring. Routine examination of blood, urine, glucose, insulin, and hepatic and renal functions were conducted in all patients. Bone density and blood vitamin D were measured. Cranial MRI or/and CT scans were used to evaluate brain imaging.

Treatment

For patients in the acute decompensated stage, initial therapy included cobalamin intramuscular or intravenous injections (1 to 10 mg/d), L-carnitine (2 to 3 g/d), intravenous fluid therapy with glucose and electrolytes, oral betaine (3 to 9 g/d), folate, and symptomatic treatment. The individual long-term metabolic treatment was adjusted according to their clinical condition [7, 12].

Results

Among the 57 patients, 34 (59.7%) were males and 23 (40.4%) were females (Table 1).

Table 1
Clinical manifestations of 57 cblC-deficient patients with the onset around puberty.

Clinical manifestations	Age of onset									
	10 - 12 years		13 - 15 years		16 - 18 years		19 years-		Total	
	n	%	n	%	n	%	n	%	n	%
Neuropsychiatric diseases	24	42.1	12	21.1	4	7	6	10.5	46	80.7
Movement disorders	16	28.1	11	19.3	2	3.5	4	7.0	33	57.9
Psychotic behavior disorders	13	22.8	2	3.5	3	5.3	3	5.3	21	36.8
Mental regression	11	19.3	4	7.0	0	0	4	7.0	19	33.3
Seizures	6	10.5	4	7.0	1	1.8	3	5.3	14	24.6
Spastic paralysis	5	8.8	5	8.8	1	1.8	2	3.5	13	22.8
Visual impairments	0	0	1	1.8	0	0.0	1	1.8	2	3.5
Lethargy/coma	1	1.8	4	7.0	0	0.0	1	1.8	6	10.5
Cardiovascular disease	5	8.8	6	10.5	0	0.0	0	0	11	19.3
Pulmonary hypertension	3	5.3	3	5.3	0	0.0	0	0	6	10.5
hypertension	2	3.5	1	1.8	0	0.0	0	0	3	5.3
Cardiomyopathy	1	1.8	1	1.8	0	0.0	0	0	2	3.5
Thrombus	0	0	1	1.8	0	0.0	0	0	1	1.8
Arrythmia	0	0	2	3.5	0	0.0	0	0	2	3.5
Kidney Disease	2	3.5	4	7.0	0	0.0	0	0	6	10.5
Proteinuria	2	3.5	4	7.0	0	0.0	0	0	6	10.5
Renal insufficiency	1	1.8	0	0	0	0.0	0	0	1	1.8
Others	10	17.5	5	8.8	2	3.5	0	0	17	29.8
Anemia	4	7.0	4	7.0	0	0.0	0	0	8	14.0
Anorexia	3	5.3	0	0	1	1.8	0	0	4	7.0
obesity	3	5.3	1	1.8	0	0.0	0	0	4	7.0
Liver damage	0	0	0	0	1	1.8	0	0	1	1.8
Total	**31**	**54.4**	**16**	**28.1**	**4**	**7.0**	**6**	**10.5**	**57**	**100.0**

Clinical course

Onset

The age of onset ranged from 10 to 25 years old with the median age of 12 years. A total of 31 patients (54.4%) presented symptoms between the ages of 10 to 12 years. Sixteen patients (28.1%) showed symptoms between 13 to 15 years old. Four patients (7.0%) displayed symptoms aged 16 to 18 years. Six patients (10.5%) presented with symptoms aged 18 to 25 years.

Precipitating factors

Eight cases (14.0%) presented with symptoms after infections. The initial diagnosis was pneumonia or encephalitis. Eight patients (14.0%) presented with neuropsychiatric symptoms, such as psychotic behavioral disorders and movement disorders, after a sports training program before middle school or college.

Symptoms

A total of 46 patients (80.7%) mainly presented with neuropsychiatric diseases, and 33 patients (28.1%) had movement disorders. Thirteen patients (22.8%) presented with progressive spastic paralysis. A total of 21 patients (36.8%) had psychotic behavioral disorders, such as speaking nonsense words, hallucination, uninterested, etc. 19 patients showed symptoms of mental regression, such as memory loss and inverse learning.

Eleven patients (19.3%) mainly presented with cardiovascular diseases, and six patients (10.5%) with pulmonary hypertension. Cardiomyopathy was found in two (3.5%) patients. Two patients (3.5%) had arrhythmia.

Six patients (10.5%) presented with proteinuria. One patient developed renal insufficiency.

Seventeen patients (29.8%) presented with complications from other symptoms. Eight (14.0%) patients had anemia, four (7.0%) patients had anorexia, four (7.0%) patients were obese. One (1.8%) patient had liver damage (Table 1).

Misdiagnosis

Retrospectively, all patients experienced misdiagnosis and mistreatment between two months to 3 years until they were correctly diagnosed. The initial diagnosis was as follows: peripheral neuropathy (12, 21.1%), depression (10, 17.5%), schizophrenia (10, 17.5%), encephalitis (8, 14.0%), primary pulmonary hypertension (5, 8.8%), and epilepsy (4, 7.0%). Eight cases (14.0%) were misdiagnosed as encephalitis and exhibited symptoms that included mental regression (3, 37.5%), depression (4, 50.0%), or behavioral abnormalities (5, 62.5%). Six patients (10.5%) showed proteinuria and/or hematuria during routine urine tests that had previously been diagnosed with nephritis.

Biochemical findings

All patients revealed abnormal blood amino acid and acyl-carnitine profiles before treatment. Their plasma tHCY (126.32–213.8 μmol/L; normal control <15.0 μmol/L) were significantly increased. Elevated blood propionyl-carnitine (5.4 - 14.7 μmol/L, normal control <5.0 μmol/L) and the ratios of propionyl-carnitine/acetyl-carnitine (0.58 - 0.97, normal control <0.5) and propionyl-carnitine/free carnitine (0.3 - 0.74, normal control <0.25) were observed. Patients had decreased free carnitine in blood (5.55 - 14.51 μmol/L, normal control 15.0 - 60.0 μmol/L). A decrease in blood methionine (5.4–9.5 μmol/L, normal control 12.0 to 50.0 μmol/L) was noticed in 40 patients. Urine methylmalonic acid was elevated (53.1 - 1787.0 mmol/mol creatinine, normal control 0.2 to 3.6 mmol/mol creatinine). These biochemical findings supported a combined MMA and homocystinuria diagnosis. In addition, deceased serum 25-OH-vitamin D was observed in 15 patients (26.3%).

Genetic features

Twenty-three reported mutations were detected in 57 patients (Table 2). c.482G>A was the most common mutation and was identified in 37 (64.9%) patients. 34 patients with neuropsychiatric diseases were found to have at least one allele mutated in c.482G>A, and among which, two cases were c.482G>A homozygotes. Within 13 patients who presented with spastic paraplegia as a main manifestation, ten had c.482G>A mutants (76.9%). Five patients presented with both psychotic disorders and spastic paraplegia. Eight cases had c.482G>A and c.658_660delAAG compound heterozygotic mutations. Seven patients displayed c.482G>A and c.609G>A compound heterozygotic mutations. Five cases had c.482G>A and c.567dupT compound heterozygotic mutations. c.609G>A was the second most common mutation observed in the patients and was found in 16 (26.3%) cases. Ten cases had a c.609G>A mutation and presented with neuropsychiatric diseases. Six patients displayed c.80A>G and c.609G>A compound heterozygotic mutations and had both neuropsychiatric symptoms and pulmonary hypertension.

Table 2
MMACHC mutations in 57 cblC-deficient patients with the onset around puberty.

***MMACHC* mutations**	**Phenotypes**									
	Neuropsychiatric diseases		**Cardiovascular diseases**		**Kidney diseases**		**Others**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
c.482G>A	34	59.6	2	3.5	0	0	1	1.8	37	64.9
c.609G>A	10	17.5	3	5.3	3	5.3	0	0	16	28.1
c.658_660delAAG	8	14	0	0	0	0	0	0	8	14
c.567dupT	7	12.3	0	0	0	0	0	0	7	12.3
c.80A>G	0	0	6	10.5	0	0	0	0	6	10.5
c.394C>T	3	5.3	0	0	0	0	2	3.5	5	8.8
c.615C>A	2	3.5	1	1.8	0	0	2	3.5	5	8.8
c.444_445delTG	1	1.8	0	0	1	1.8	0	0	2	3.5
c.365A>T	1	1.8	1	1.8	0	0	0	0	2	3.5
c.452A>G	1	1.8	0	0	0	0	0	0	1	1.8
c.311G>A	1	1.8	0	0	0	0	0	0	1	1.8
c.487_489del	1	1.8	0	0	0	0	0	0	1	1.8
c.656_658delAGA	1	1.8	0	0	0	0	0	0	1	1.8
c.427C>T	1	1.8	0	0	0	0	0	0	1	1.8
c.467G>A	1	1.8	0	0	0	0	0	0	1	1.8
c.637G>T	1	1.8	0	0	0	0	0	0	1	1.8
c.565C>T	1	1.8	0	0	0	0	0	0	1	1.8
c.347T>C	1	1.8	0	0	0	0	0	0	1	1.8
c.746_748delCCCG	1	1.8	0	0	0	0	0	0	1	1.8
c.315C>G	1	1.8	0	0	0	0	1	1.8	2	3.5
c.217C>T	1	1.8	0	0	0	0	2	3.5	3	5.3
c.6000G>A	1	1.8	0	0	0	0	0	0	1	1.8
c.626dup	0	0	1	1.8	0	0	1	1.8	2	3.5

Treatment and follow-up

All of the 57 patients were treated with cobalamin intramuscular injection (1 mg, two to three times a week), supplemented with L-carnitine (1 to 2 g/d, p.o.), oral betaine (3 to 6 g/d), and normal diet. All patients were clinically improved after the metabolic treatment. Follow-ups were scheduled at three, six, and 12 months. Currently, patients are 12 to 32 years old. Among the 33 patients that showed movement disorders during the acute phase of the disease, 30 have recovered. Three patients showed unstable walking but have improved. All 30 patients with mental regression or psychotic problems have recovered and returned to school. 12 patients were over 18 years old. Nine of them have successfully graduated from college and work well. Two female patients have gotten married and had healthy babies[13].

Discussion

Puberty is a special time for human beings, for it is critical for growth and sexual development. However, it is also a critical stage for the late-onset inherited metabolic disorders. cblC deficiency is the most common defect in the intracellular cobalamin metabolism pathway, characterized by variable and non-specific symptoms [1–3]. In patients with cblC deficiency, age of onset ranges from prenatal to adult and clinical manifestations vary from mild to life-threatening. In our previous studies in 1,003 patients with MMA, 707 cases (70.5%) were combined with homocystinemia and 567 cases (80.2%) were early-onset (before the age of one year old). A total of 51 patients (7.2%) had late-onset (after the age of 4 years old) and showed significant differences in phenotypes and outcomes [14].

There are a variety of phenotypes in cblC deficiency. Most phenotypes are early-onset, usually affecting the nervous system, and mainly presenting with developmental delay, epilepsy, lethargy, and hypotonia. In addition, the disease may be complicated with multiple organ damages, such as visual impairment, kidney damages, hematological abnormalities, cardiovascular diseases, etc. However, currently, more late-onset combined MMA with homocystinuria patients have been diagnosed. Some cases have been reported in adolescence or adulthood [15–16].

In the present study, 57 previously healthy school children presented with varied diseases during adolescence. The onset was often induced by select triggers. Patients usually presented with movement disorders or psychotic symptoms after having infections or fatigue. Since these symptoms are non-specific, patients were misdiagnosed with schizophrenia, depression, autoimmune encephalitis, or neuromuscular diseases. tHCY, blood amino acids, acyl-carnitines, and genetic analysis are keys for the final diagnosis. After the metabolic treatment by cobalamin, L-carnitine, and betaine, the psychotic symptoms and movement impairments of the patients improved gradually.

A recently published China Mental Health Survey showed that depressive disorders was in a highly prevalence on adolescence [17]. The researchers generated a curves for age-at-onset of depressive disorders, showing that the age of onset was about 14 years old regardless of significantly differed probability of developing any depressive disorder across each age groups in the survey [17]. In the present

study, 46 patients (80.7%) sought relief from neuropsychiatric symptoms and 33 patients presented with movement disorders. In addition, 21 patients had psychotic behavior disorders, such as spoken nonsense words, experienced hallucinations, showed indifferent, etc. A total of 19 patients showed symptoms of mental regression, as parents complained of sudden onsets of inability to learn. Moreover, six patients presented with pulmonary hypertension, with most complaints being intolerance to activities or fainting after participating in sport activities. Proteinuria and anemia were observed in some cases. All findings were correlated with previously published literature [18–20].

Genetic analysis is crucial for making a definite diagnosis. In the present study, the most common mutation of the patients with adolescence-onset cblC deficiency was c.482G>A, followed by c.609G>A. c.609G>A was the most common variant found in Chinese patients with early onset cblC deficiency [21–22]. The c.658_660delAAG mutation was the third and the c.567dupT mutation was the fourth most common mutations. The mutations observed in our study have been noted in previous published reports [21–23]. In patients with c.482G>A or c.609G>A mutations, we identified neuropsychiatric diseases. In 13 patients with spastic paraplegia, ten (76.9%) had a c.482G>A mutation. This finding suggests that the c.482G>A mutation may be the most common mutation in late-onset patients with neuropsychiatric presentation [6]. Six patients with c.80A>G and c.609G>A compound heterozygotic mutations displayed both neuropsychiatric symptoms and pulmonary hypertension. These results suggested that if patients had a c.80A>G mutation, diseases in the cardiovascular system should be considered. This finding has been shown in previous reports [24].

In the present study, 57 patients were considered healthy before disease occurred. In most cases, the acute phase of the disease was seen in metabolic disturbances. There may be an increase observed in blood propionylcarnitine (dozens of folds) and tHCY (more than hundred-fold) compared with the normal range. Additionally, patients had combined hyperammonemia during the acute phase. Ammonia is toxic to the neural system, which could lead to neuropsychiatric symptoms [25]. Persistent hyperhomocysteinemia could damage the cardiovascular and neural systems, which may lead to movement disorders, kidney problems, or cardiopulmonary diseases [25–27]. Therefore, physicians should consider high doses of cobalamin and betamine to treat patients in the acute phase to reduce the blood level of tHCY and correct the metabolic status [4, 7]. Patients gradually regained the ability to learn and walk as blood metabolites decreased [25, 28]. In this study, the problems related to the cardiovascular and pulmonary systems of the patients were reversed. Following years of follow-up, patients gradually went back to school and lived normal lives. Some patients have jobs. Two female patients have married and produced healthy babies [13]. These results show that if treated correctly, patients with cblC deficiency can live normal lives [7, 28–29].

cblC deficiency is a treatable disease. In our study, we analyzed the clinical course, biochemical features, genetic findings, and the outcomes of 57 adolescence-onset patients with combined MMA and homocystinuria caused by cblC deficiency. Patients presented with nonspecific clinical features that could easily be misdiagnosed. Using biochemical analysis and genetic analysis, physicians could

determine the final diagnosis. After metabolic treatment, most patients could fully recover and live a normal life. These results indicated that differential diagnosis of inherited metabolic disorders should be considered for adolescent patients with neuropsychiatric diseases and multiple organ damage.

Abbreviations

MMA = methylmalonic aciduria; cblC = cobalamin C; LC-MS/MS = liquid chromatography-tandem mass spectrometry; GC/MS = Gas chromatography and mass spectrometry; tHCY = total plasma homocysteine; MRI = magnetic resonance imaging; CT = computerized tomography; MMACHC = cytoplasmic chaperone protein methylmalonic aciduria and homocystinuria

Declarations

Ethics statement

Our study was approved by the hospital's Institutional Ethics Committee and was performed in accordance with the Declaration of Helsinki. Written informed consents were obtained from the patients for collection of samples and publication of medical data.

Consent for publication

All the patients and/or their guardians who participated in the study have signed the consent for publication.

Availability of data and materials

All data generated or analysed during this study are included in this published article are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZC wrote the original draft of the mauscripts; HD, RH, YL, XL, HY, JQ, FW, HX, HZ, LK, DL and YL collected the clinical data and followed-up the treatment; YJ, ML and JS performed the metabolic assays; Yao Zhang and Yanling Yang designed the study supervized the clinical works. All authors read and approved the final manuscript.

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