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Clinical diagnosis, treatment, and genetic analysis of adolescent onset holocarboxylase synthetase deficiency and cobalamin C deficiency: A case report and literature review

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ABSTRACT

Background: Holocarboxylase Synthetase Deficiency (HCSD) is an uncommon autosomal recessive genetic disorder that manifests with symptoms such as metabolic acidosis, lethargy, hypotonia, seizures, and persistent rashes, typically emerging during infancy. The HLCS gene has been identified as the source of pathogenic mutations associated with this condition. Cobalamin C (cblC) deficiency is another rare autosomal recessive disorder resulting from defects in cobalamin metabolism, attributable to mutations in the MMACHC gene. This disorder often leads to methylmalonic aciduria and homocystinuria and is classified into early-onset and late-onset types. The late-onset type is characterized by acute or chronic progressive neurological symptoms and behavioral disturbances. To date, there have been no documented cases worldwide of individuals diagnosed with both HCSD and cobalamin C deficiency.

Case presentation: This report details the case of an 11-year-and-9-month-old female patient from China who presented with symptoms including vomiting, altered consciousness, and a rash. Laboratory evaluations indicated the presence of metabolic acidosis, methylmalonic aciduria, and homocystinuria. Genetic analysis revealed mutations in the MMACHC gene: c.482G > A (p.R161Q) and c.567dup (p.I190Yfs*13). Additionally, two previously unreported mutations in the HLCS gene, c.1922G > T (p.G641V) and c.1754C > T (p.P585L), were identified. She was diagnosed with Holocarboxylase Synthetase Deficiency and Cobalamin C deficiency. The child showed significant improvement following treatment with hydroxocobalamin, betaine, and biotin.

Conclusion: This article reports a case of adolescent onset HCSD and cobalamin C deficiency. Treatment with hydroxocobalamin, betaine, and biotin is effective. Two novel mutations in the HLCS gene causative for HCSD have been reported, providing a broader foundation for mutational screening and offering insights into the diagnosis and treatment of similar disorders.

1. Introduction

Holocarboxylase Synthetase Deficiency (HCSD) is a rare autosomal recessive disease, with an incidence rate of about 1/100,000 [1]. Due to the decrease in the activity of holocarboxylase synthetase, it cannot catalyze biotin and biotin-dependent multiple carboxylases. Thus it affects the activities of multiple carboxylases, which impairs the metabolic processes of fatty acid catabolism, gluconeogenesis, and amino acid catabolism. This leads to the accumulation of abnormal metabolites in the body, such as lactic acid, 3-hydroxyisovaleric acid, 3-methylcrotonylglycine, methyl citrate, and 3-hydroxypropionic acid in the body, resulting in various non-specific clinical manifestations. This leads to a variety of non-specific clinical manifestations, including metabolic acidosis, lethargy, hypotonia, convulsions and dermatitis [2]. In the

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analysis results of urinary organic acids, the excretion levels of several metabolites, such as 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, and 3-hydroxypropionic acid, have increased. Mutations in the HLCS gene have been identified as a causative factor. The gene is located at 21q22.1 and has 14 exons, of which exons 6–14 contain all the coding sequences, and so far, at least 50 related mutations have been reported in the Human Gene Mutation Database.

Cobalamin C (cblC) deficiency is an autosomal recessive genetic disorder with inborn errors of cobalamin metabolism [3], with an estimated prevalence of 1 in 100,000 [4]. Cobalamin C (cblC) deficiency is caused by mutations in the MMACHC gene. The defect in the MMACHC gene product leads to impaired intracellular synthesis of adenosylcobalamin and methylcobalamin. Adenosylcobalamin and methylcobalamin are essential cofactors required for the conversion of homocysteine to methionine and the conversion of methylmalonic acid to succinic acid, respectively, which results in patients exhibiting methylmalonic aciduria and homocystinuria. Based on the age of onset, Cobalamin C (cblC) deficiency is classified into early-onset and late-onset types. Approximately 90 % of reported cblC deficiency patients are early-onset, which can present with various neurological symptoms, including hydrocephalus, hypotonia, cognitive impairment, and seizures [5]. Late-onset cblC deficiency is rarer and may manifest as decreased learning or work ability, impaired flexibility and memory, changes in behavior and personality, social withdrawal, speech difficulties, dementia, visual and auditory hallucinations, delirium, as well as acute confusion, drowsiness, and seizures. Due to the abnormal accumulation of toxic metabolites, it may cause damage to organs such as the nervous system, liver, kidneys, and bone marrow [6].

Currently, there are no reported cases worldwide of individuals simultaneously suffering from the two genetic metabolic disorders mentioned above. In this paper, we present a case of holocarboxylase synthetase deficiency and Cobalamin C deficiency caused by heterozygous mutation in the HLCS gene and the MMACHC gene. We also summarize the clinical and genetic characteristics.

2. Case presentation

2.1. Clinical manifestation

In July 2024, a female child aged 11 years and 9 months from China was referred to the emergency department. The child presented with vomiting and impaired consciousness without convulsive seizures. Admission findings: moderate-deep coma. Neurological examination: bilateral pupils were unequal in size, 3 mm on the left and 3.5 mm on the right, and light reflexes were absent. There was no trauma or deformity of the head. Significant increase in muscle tone in limbs and hyperreflexia in knees. Suspiciously positive Babinski sign on the right side, and negative Babinski sign on the left side. One week after admission, clusters of vesicles and papules appeared on the forehead and perioral area and increased progressively, involving the perineum and the back, presenting as dense corn-grain-sized dark red macules with a few pustules on them, with a thin wall of the vesicles and a sparsely scaly surface (Fig. 1). Convulsive episodes appeared 20 days after admission. Reviewing the recent medical history, the child experienced low mood, irritability, and auditory hallucinations five months prior to admission. Two months prior to admission, the child had abnormal mental behavior and one convulsion, and was diagnosed with depression when there was no evidence of autoimmune encephalitis. There was no previous growth or developmental delays and no neuropsychiatric symptoms. Her parents and generations before the parents, who were not consanguineous, were both alive and had no siblings. She experienced menarche three months ago.

2.2. Assistant examination

The results of auxiliary examination at the time of admission of the child were as follows. Arterial blood gas (ABG) analysis revealed severe metabolic acidosis and hyperlactatemia: pH 7.128, $PaCO_2$ 23.4 mmHg, PaO_2 122 mmHg, HCO^{3-} 8.8 mmol/L, BE -22 mmol/L, Na^+ 134 mmol/L, K^+ 3.5 mmol/L, C-reactive protein <0.8 mg/L, lactate 7.5 mmol/L,blood ammonia 16.2μmol/L, anion gap 22 mmol/L. Blood routine: erythrocyte count 4.25×10^{12}/L, mean erythrocyte volume 93.4 fl, mean erythrocyte hemoglobin content 29.4 pg, mean erythrocyte hemoglobin concentration 329 g/L. Urinalysis demonstrated ketonuria (7.8 mmol/L). Pyruvate 358 μmol/L ↑, free fatty acid 0.44mmol/L, β-hydroxybutyric acid 2.82 mmol/L ↑. The results of blood metabolism disease screening tandem-mass spectrometry suggested that the level of methionine is normal, propionylcarnitine and various acylcarnitines were mildly elevated. Urinary organic acid profile (gas chromatography-mass spectrometry) showed: lactic acid and pyruvic acid were elevated, suggesting lactic acidemia; 3-hydroxybutyric acid and acetoacetic acid were elevated, suggesting ketoacidosis; adipic acid was elevated, suggesting dicarboxyluria; 2-keto isohexanoic acid and 2-hydroxyhexanoic acid were elevated, suggesting maple syrup urine disease (MSUD); 3-hydroxyisovaleric acid and 3-methylcrotonylglycine were elevated, suggesting 3-methylcrotonyl coenzyme A carboxylase deficiency. However, the child's biochemical findings could not be explained by an isolated 3-methylcrotonyl carboxylase deficiency because the patient's lactate was elevated. Upon admission, the child's interleukin-6 (IL-6) level was measured at 217.5 pg/ml. Nerve conduction suggested bilateral peroneal nerve CMAP and F-wave deficits. Cranial MRI suggested signs of diffuse cerebellar edema with symmetrical abnormal signal in the basal ganglia bilaterally, as shown in Fig. 2. Cranial MRI 21 days prior to admission showed slightly plumped bilateral ventricles and slightly

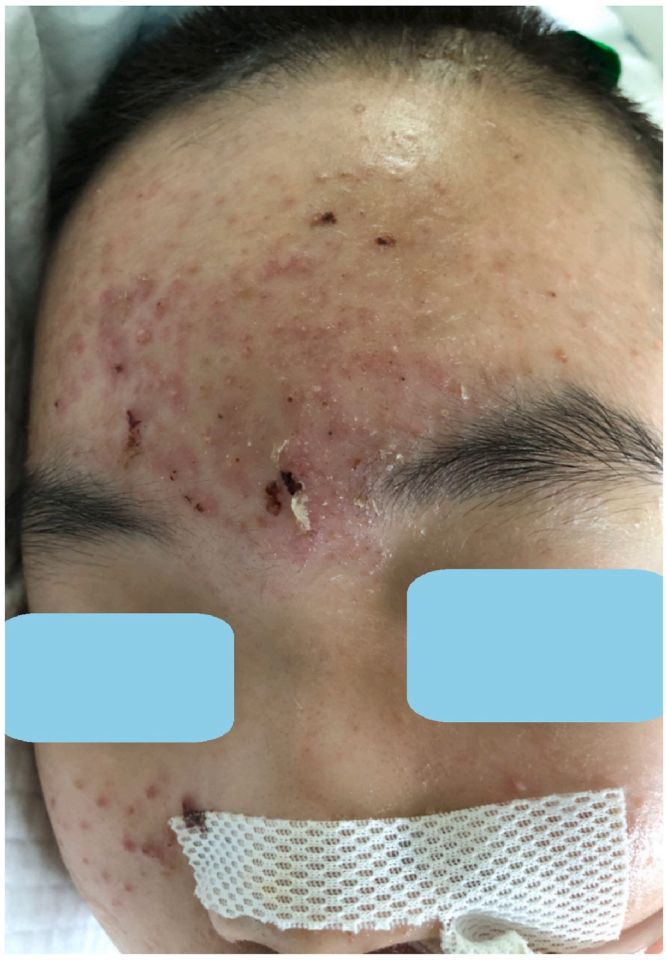

Fig. 1. Forehead rash before treatment.

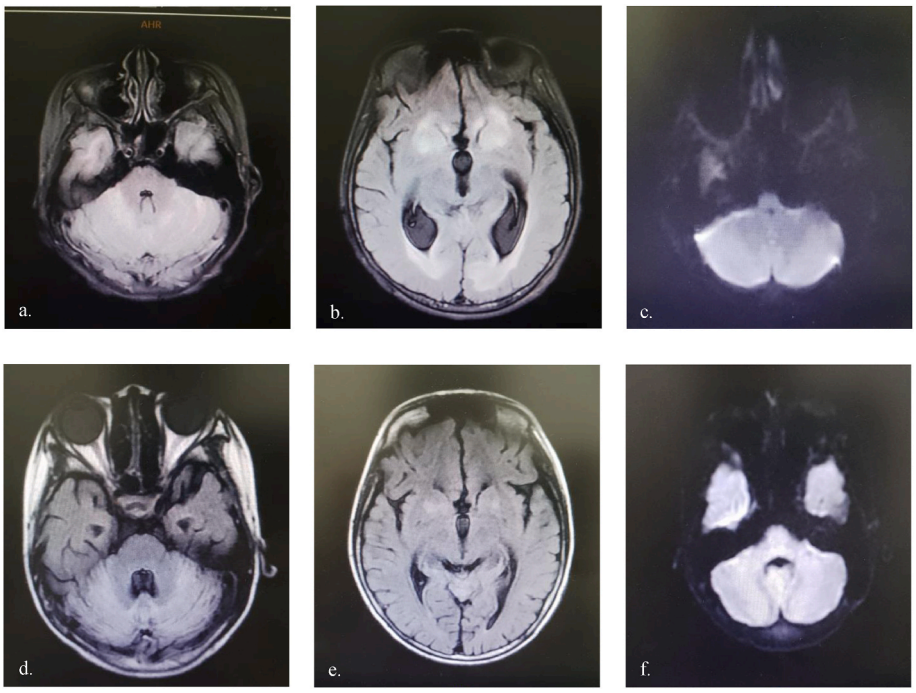

Fig. 2. At the time of admission, the child's cranial MRI showed symmetric high signal in the cerebellum (a.) and bilateral basal ganglia regions (b.) on the T2 Flair sequence, and symmetric diffusion restriction in the bilateral cerebellum on the DWI sequence (c.); after the treatment, the child's cranial MRI showed reduced high signal in the cerebellum (d.) and bilateral basal ganglia regions (e.) on T2 Flair sequences, and bilateral cerebellar symmetric diffusion restriction disappeared on DWI sequences (f.).

widened plurilateral sulci.

2.3. DNA isolation and sequence analysis

Informed consent was obtained from the child and her guardians, and the study was approved by Ethics Review Committee of Chongqing Medical University (2024-KY-0020). Peripheral blood sample was collected from the child and their parents. High-throughput sequencing, bioinformatics and clinical information analysis technology, and Sanger sequencing technology were used for the sequencing of metabolic disease-related genes, data analysis, and validation of suspected pathogenic variants (Wuhan Kangshengda Laboratory of Medical Laboratory, Wuhan, China).

The child and her parents each underwent4 loci verification, a total of 4 pairs of primers, 24 sequencing chromatograms, detailed information on the variant loci corresponding to the primers is shown in Table 1. PCR amplification steps for each reagent components were as follows: 2ul of DNA added, 1ul of each of the forward and reverse primers, 8.5ul of RNase-free ddH2O, 12.5ul of 2 × Taq Master Mix (Novocain). The amplification protocolwas pre-denaturation at 95 °C for 5min, 95 °C for 30s, 60 °C for 15s, 72 °C for 50s, 35cycle, final extension at 72 °C for 5min, and 4 °C hold; the amplified products were subjected to Sanger sequencing, and the data were analyzed using Chromas software.

2.4. Identification of gene mutation and diagnosis

DNA analysis revealed that the child had c.482G > A and c.567dup compound heterozygous mutations in exon 4 of the MMACHC gene, both of which have been reported. The former was inherited from the mother. The mutation results in a missense mutation from guanine to adenine at nucleotide 482 in the cDNA of the MMACHC gene, and a missense mutation from arginine to glutamine at amino acid 161 of its coding, which is pathogenic [7,8]. The latter was inherited from the father, and the mutation resulted in a duplication of nucleotide 567 in the cDNA of the MMACHC gene, and the 190th amino acid encoded by it was changed from isoleucine to tyrosine, and then the 12th codon was changed to a stop codon, which was a frameshift mutation and of suspected pathogenicity [9,10] (Fig. 3).

The child had a c.1922G > T heterozygous mutation in exon 7 and a c.1754C > T heterozygous mutation in exon 6 of the HLCS gene. The

Table 1
Primers corresponding to the locus information of the genetic variants of the child and the child's parents.

Sample	locus of mutation	Forward Primer	Reverse Primer
Child (peripheral blood)	MMACHC NM_015506.3:exon4:c.482G > A:p.R161Q	TCAGTGTACACTGAGTGGGAAGT	GGGCGATACGGTCAGCTC
	MMACHC NM_015506.3:exon4:c.567dup:p.I190Yfs*13	GGGATAGAGGTGCCAGAT	ACTTCCTTGAGCCTTGTT
	HLCS NM_001352514.2:exon7:c.1922G > T:p.G641V	AGTACAGACACAGAAGGCTCCT	CTCATGGCTCCACATTCCTGAA
	HLCS NM_001352514.2:exon6:c.1754C > T:p.P585L	CTGCTTGGTCTGCAGATTTTGG	AACGGAGGACTGGAGAACTACT
Father of the child (peripheral blood)	MMACHC NM_015506.3:exon4:c.482G > A:p.R161Q	TCAGTGTACACTGAGTGGGAAGT	GGGCGATACGGTCAGCTC
	MMACHC NM_015506.3:exon4:c.567dup:p.I190Yfs*13	GGGATAGAGGTGCCAGAT	ACTTCCTTGAGCCTTGTT
	HLCS NM_001352514.2:exon7:c.1922G > T:p.G641V	AGTACAGACACAGAAGGCTCCT	CTCATGGCTCCACATTCCTGAA
	HLCS NM_001352514.2:exon6:c.1754C > T:p.P585L	CTGCTTGGTCTGCAGATTTTGG	AACGGAGGACTGGAGAACTACT
Mother of the child (peripheral blood)	MMACHC NM_015506.3:exon4:c.482G > A:p.R161Q	TCAGTGTACACTGAGTGGGAAGT	GGGCGATACGGTCAGCTC
	MMACHC NM_015506.3:exon4:c.567dup:p.I190Yfs*13	GGGATAGAGGTGCCAGAT	ACTTCCTTGAGCCTTGTT
	HLCS NM_001352514.2:exon7:c.1922G > T:p.G641V	AGTACAGACACAGAAGGCTCCT	CTCATGGCTCCACATTCCTGAA
	HLCS NM_001352514.2:exon6:c.1754C > T:p.P585L	CTGCTTGGTCTGCAGATTTTGG	AACGGAGGACTGGAGAACTACT

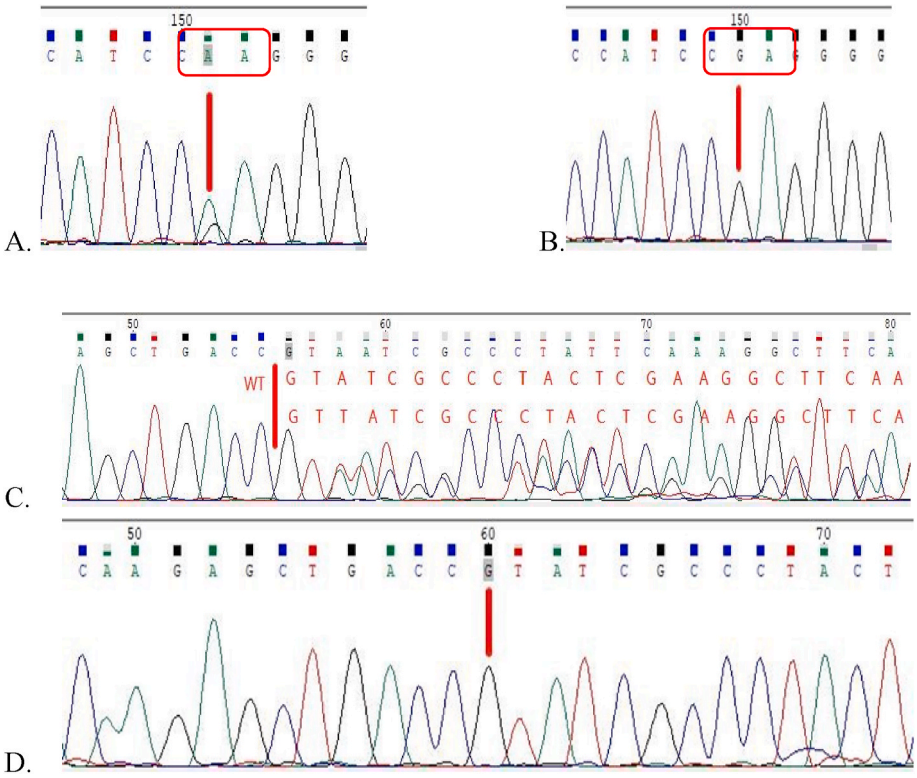

Fig. 3. A &B shows the c.482G > A mutation in the MMACHC gene, which is a nucleotide change detected in the patient 1 (A) compared to an unaffected control (B). C & D shows the c.567dup:p.I190Yfs*13 in the MMACHC gene, which is a nucleotide change detected in the patient 1 (C) compared to an unaffected control (D).

former was inherited from the father, and resulted in a missense mutation in the cDNA of the HLCS gene from guanine to thymine at nucleotide 1922, and a missense mutation in the cDNA of the HLCS gene from glycine to valine at amino acid 641. The latter was inherited from the mother and results in a missense mutation from cytosine to thymine at nucleotide 1754 and from proline to leucine at amino acid 585 of the HLCS gene cDNA. These two mutations were not reported in the ESP, Thousand Genomes, EXAC, or GnomAD databases, and were newly identified (Fig. 4).

Taken together with the signs and symptoms of the child and the genetic test report, cobalamin C deficiency can explain the methylmalonic aciduria and homocystinuria, as well as the psychiatric and neurological symptoms. Meanwhile, holocarboxylase synthetase deficiency could explain the nondiabetic ketoacidosis and lactic acidosis in the child.

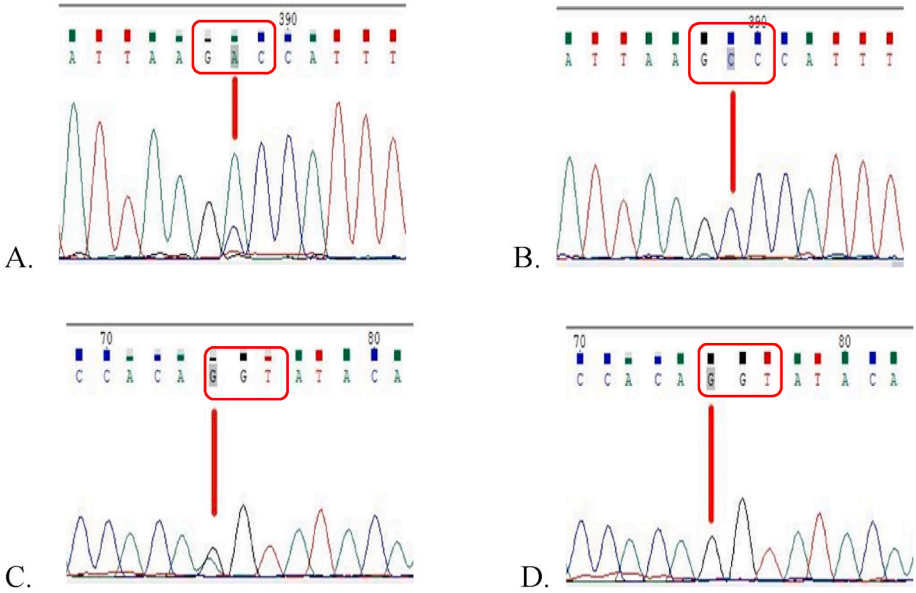

Fig. 4. A &B shows the c.1922G > T mutation in the HLCS gene, which is a nucleotide change detected in the patient 1 (A) compared to an unaffected control (B). A &B shows the c.567dup mutation in the HLCS gene, which is a nucleotide change detected in the patient 1 (C) compared to an unaffected control (D).

2.5. Treatment

After admission, emergency Omaya reservoir placement was performed. Combined with the child's clinical manifestations and auxiliary examinations, genetic metabolic diseases were highly suspected. The child was given levocarnitine (1g daily), vitamin B1 (20 mg daily in 2 divided doses), vitamin B6 (100 mg daily), vitamin B complex (2 tablets daily in 2 divided doses), and coenzyme Q10 (20 mg daily divided 2 dose). Considering that the nerve conduction results of both lower limbs of the child suggested peripheral nerve damage, and methylcobalamin tablets (1 mg daily in 2 divided doses) were added.

Subsequently, the genetic test results of the patient confirmed a diagnosis of holocarboxylase synthetase deficiency and Cobalamin C deficiency. The treatment involved Vitamin B12 1 mg daily intramuscularly for 5 days was added and then switched to every other day for 3 doses; hydroxocobalamin 10 mg daily for 5 days, and then adjusted to every other day and biotin 10 mg per day, and betaine 100 mg/kg/d were administered.

We conducted a follow-up with the pediatric patient for a duration of 123 days. On the 20th day after admission, the child had 3 grand mal convulsive seizures lasting about 2 min. After treatment with biotin, betaine, and hydroxocobalamin, the child experienced no further convulsive seizures. From the 7th day of admission to the start of biotin therapy, the child's rash increased in size and covered about 17% of the body surface area (forehead, face, perioral area, perineum, buttocks, and back). On the 39th day of admission (10th day after biotin treatment), the rash on the perineum, buttocks and back of the child subsided, and the flaking and vesiculation of the rash on the face was better than before, with the rash covering about 3 % of the body surface area. There were no new areas of rash in the subsequent course of the disease. Her consciousness gradually improved. On day 29 of admission (before receiving biotin therapy) the child Glasgow Coma Scale (GCS) was 8 and consciousness was rated as moderate-deep coma by the physician. On day 36 of admission (day 7 after biotin treatment) the child GCS was 13, and on day 79 of admission (50 days of biotin treatment and 42 days of betaine and hydroxocobalamin) consciousness turned to lucidity and the child GCS was 15. She was able to follow commands and communicate with her family members, the content of her language was clear. She was able to turn over on the bed and walk on her own. she could go up the stairs with support. The muscle strength of the left upper limb has recovered to grade 5 while the right one is grade 4+. Proximal muscle strength of the both lower limbs is proximal grade 4+ and distal grade 5. A follow-up of IL-6 decreased (Day 10): 20.63 pg/mL; urine organic acid (Day 30) analysis did not show significant metabolic abnormalities. 3-Hydroxyisovaleric acid-2 and 3-methylcrotonylglycine in urinary organic acids were significantly decreased from the previous levels. Blood metabolic disease screening (tandem mass spectrometry, Day 29) results suggest that: propionylcarnitine and multiple acylcarnitines are elevated; blood homocysteine and metabolite testing returned to normal (Table 2). Five days after receiving biotin therapy, blood ammonia decreased from 36.6 μmol/L to 19.4 μmol/L and remained normal in several subsequent consecutive reviews. Forty-two days after receiving biotin therapy, blood pyruvate decreased from 358 μmol/L to 113 μmol/L. Repeat cranial MRI revealed a reduction in the extent of diffuse cytotoxic edema in the cerebellum and a reduction in abnormal signal in the basal ganglia bilaterally (Fig. 2). The child's key therapeutic timeline is shown in Fig. 5.

Table 2
Blood homocysteine and metabolite tests.

Date/item	D29	D41	D52	D70	D123
homocysteine μmol/L	57.25↑	24.75↑	5.3	7.36	8.98
Methylmalonic acid(MMA) nmol/L	4845.69↑	994.34↑	157.7	233.25	35.06
S-Adenosylhomocysteine (SAH)nmol/L	34.65↑	39.16↑	19.37	17.39	12.25

3. Literature review and case discussion

3.1. Adolescent-onset holocarboxylase synthetase deficiency

The clinical symptoms of holocarboxylase synthetase deficiency are highly variable. Children may present with nonspecific clinical manifestations involving digestive system, skin damage, nervous system and other multi-organ systems. In this case, the child had vomiting, lactic acidosis, metabolic acidosis, mental behavioral abnormalities, convulsions, and desquamative rash, which are consistent with the clinical manifestations.

The disease often presents with acute metabolic disorders and severe metabolic acidosis during the neonatal period and early infancy, but a few children develop the disease after the age of 1 year [11], presenting with developmental delay and hypertonia [12]. Other case reports of late-onset forms of the disease, to our knowledge, have not exceeded a maximum age of 8 years [13,14].However, the age of onset in the present case was 11 years and 9 months, making her the oldest patient reported to have the first onset of the condition. The patient has no prior developmental delay or neurological or psychiatric symptoms. We speculate that this may be related to the patient's mutation points or differences in gene expression, as well as variations in compensatory mechanisms.

Biotinidase deficiency and holocarboxylase synthetase deficiency are both forms of multiple carboxylase deficiency (MCD), patients with similar clinical manifestations and urinary organic acid profiles, blood esteroyl carnitine profiles, late-onset biotinidase deficiency can be onset at all ages from early childhood to adulthood, and this child needs to be differentiated from this condition [2]. Currently, the differential diagnosis can be made by measuring biotinidase activity and conducting genetic analysis. Biotinidase activity is significantly reduced in biotinidase-deficient patients, and mutation analysis of the BTD gene can clearly identify biotinidase deficiency. Although the child did not undergo biotinidase activity measurement, gene mutation clearly identified mutations in the HLCS gene.

3.2. A novel HLCS mutation

Clinical manifestations, response to biotin therapy, and prognosis of patients with HCSD with mutations at different loci may be different [15]. R508W may be an important and relatively common disease-causing mutation in Chinese patients with MCD, associated with late phenotype [16]. Studies have shown that patients with the c.1522C > T (p.R508W) mutation significantly improved their clinical symptoms and stabilized their metabolic status during long-term follow-up when they received less than the usual dose of biotin (1.2 mg daily) [17]. However, patients with heterozygous Y663H (c.1987T > C) and Q379X (c.1135C > T) mutations required treatment with biotin at 100 mg/day before their metabolic status was gradually improved [15].Patients with L216R homozygous were treated with 1.2 g of oral biotin per day, which is much higher than any previously reported dose for the treatment of HLCS deficiency, and after treatment thrombocytopenia and metabolic acidosis improved [18].

The HLCS gene is considered to be responsive to biotin if the mutation is located in the C-terminal biotin-binding domain or its adjacent region (amino acids 448–701). In addition, if mutations in both alleles are in the C-terminal biotin-binding domain, the required biotin dose can even be as low as 1.2 mg daily. However, if the mutation occurs in the N-terminal extension, such as in the substrate-binding region (amino acids 159–314), it results in a decrease in the enzyme's affinity for the substrate and a lack of response to biotin [2,19].

The mutation loci of c.1922G > T (p.G641V) & c.1754C > T (p.P585L) have not been previously reported before, the affected amino acid positions are 641 & 558 between 448 and 701, which may be

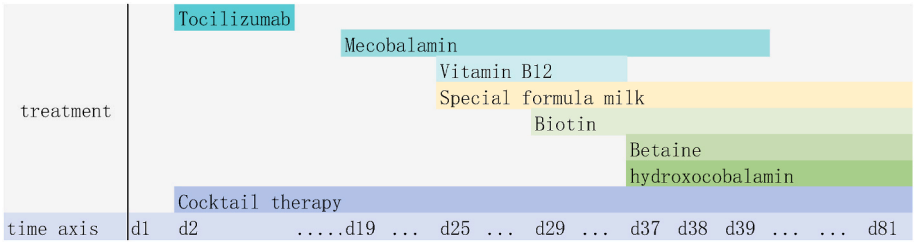

Fig. 5. Timeline of important treatments for the child. Special formula milk is free of isoleucine, valine, threonine, methionine.

responsive to biotin therapy, consequently, the child was treated with regular dose of biotin (10 mg daily), leading to improvements in clinical symptoms and urinary organic acid levels.

The prognosis of the child may be influenced by the timing of treatment. Due to the lack of specificity in clinical symptoms, the clinical identification is challenging. If the timing of treatment is delayed, it may lead to serious, irreversible neurological complications, and even death.

3.3. Clinical characterization and genetic analysis of cobalamin C deficiency

CblC deficiency is a congenital error in vitamin B12 metabolism, with an estimated incidence of 1:100,000 [4]. It can be classified into two phenotypes based on the age of onset: early-onset and late-onset. Early-onset cblC has obvious clinical symptoms, usually including muscle tone loss, drowsiness, epileptic seizures, microcephaly, hydrocephalus, developmental delay, and other multi system involvement in the first year after birth, including hematological, ocular, renal, hepatic, and cardiac symptoms. The clinical manifestations of late-onset patients vary, and symptoms often appear between the ages of 4–14 [20]. The most common clinical manifestations of late-onset cblC are encephalopathy, psychiatric symptoms, thrombotic microangiopathy, seizures, renal disease, mild to severe pulmonary hypertension with heart failure, and thrombosis [21]. The clinical presentation of the child in this case, which included acute neurologic symptoms such as convulsions and impaired consciousness, acidosis, and ketosis, combined with multi-system damage to the peripheral nerves and skin, had a great degree of conformity.

In addition, the child exhibited psychiatric and psychological abnormalities 5 months before admission. Studies have found that some patients have psychiatric and psychological abnormalities as their first manifestations [22]. So it is necessary to identify late-onset cblC in some psychiatric patients. However, early diagnosis of late-onset cblC is challenging. Three case reports of late-onset cblC in adolescents have demonstrated delays from first symptomatic presentation to diagnosis [23]. Firstly, the symptoms and metabolic changes of late-onset cobalamin C defects occur after months of life and cannot be definitively diagnosed by newborn screening. Secondly, the clinical presentations of delayed cobalamin C deficiency are variable, and this child had family and social factors as triggers prior to the onset of the disease. Adolescence is associated with a higher risk of developing mental illness and a high risk of depression due to physiological, psychological, and cognitive changes [24], and psychiatrists have limited knowledge of rare congenital errors of metabolism, making it challenging to identify them.

Hydrocephalus is one of the serious neurologic complications in cblC [25]. MRI manifestations are less specific and include periventricular white matter abnormalities, cortical atrophy, and bilateral ventricular dilatation [26]. A study reviewing 70 cases of hydrocephalus secondary to cblC showed that the most common imaging abnormalities were cerebral atrophy and bilateral ventricular dilatation [27]. In this case, the child had an insidious onset of disease, and although the exact time of hydrocephalus onset could not be traced, and there may be a diagnostic delay, a comprehensive analysis of the time of onset of symptoms, clinical manifestations, genotypic analysis, progression of the disease, and previous growth and development suggests that it is more likely that the child has a late-onset cobalamin C deficiency.

Cobalamin C deficiency is caused by mutations in the MMACHC gene [3]. The MMACHC c.567dup (p.I190Yfs*13) variant in this child may be associated with hydrocephalus. This is in contrast to previous reports that the most common pathogenic variant in patients with cblC type with hydrocephalus was MMACHC c.609G > A, followed by other variants such as c.658_660del, c.567dupT and c.217C > T [28]. The other locus c.482G > A pathogenicity variant in this child is milder and is associated with milder disease, usually late-onset, however, the child still developed severe complications due to the inability of the child to receive timely diagnosis and treatment in the early stages [29].

3.4. Pharmacotherapy and outcomes of holocarboxylase synthetase deficiency and cobalamin C deficiency

Patients with holocarboxylase synthetase deficiency are unable to synthesize carboxylase normally, resulting in abnormal function of many carboxylase enzymes. Biotin, as a coenzyme of carboxylase, is able to bind to carboxylase and restore its activity, so the main drug treatment for holocarboxylase synthetase deficiency is supplementation of biotin. The dose of biotin ranges from 10 to 100 mg/d, depending on the mutation site [12,13]. It has been reported that if both alleles are mutated in the C- terminal biotin-binding domain, the biotin dose can even be as low as 1.2 mg/day [17]. In our case, both alleles were mutated in the C- terminal biotin-binding domain, suggesting a possible response to biotin therapy, and therefore a smaller dose of 10 mg/day of biotin therapy was provided as part of the routine.

In our hospital, biotinase activity levels are not routinely measured, so we measured amino acid metabolites such as urinary organic acids, blood ammonia, and pyruvate indirectly to quantitatively assess the effect of treatment [11]. One day after treatment with biotin, 3-hydroxyisovaleric acid-2 and 3-methylcrotonylglycine in urinary organic acids decreased significantly. The therapeutic effect of biotin was satisfactory, and a case report found that the metabolic disorder was corrected within 48 h of biotin treatment [30]. Rash involving body surface area improved in the child 7–10 days after treatment with biotin.The study by Tammachote R et al. showed that the patients' rashes all improved in about 1 week, but it still took longer for complete recovery [17].

Cobalamin C deficiency is caused by mutations in the MMACHC gene, which encodes a protein that plays a central role in the intracellular metabolism of vitamin B. It is responsible for converting vitamin B that enters the cell into its two active forms: adenosylcobalamin and methylcobalamin. Adenosylcobalamin is involved in methylmalonic acid metabolism and methylcobalamin is involved in homocysteine metabolism [3]. Therefore, one of the medications used for treatment is supplementation with hydroxocobalamin, the active form of vitamin B. The optional range of therapeutic doses of hydroxocobalamin is 0.11–0.36 mg/kg/day, with patients receiving 0.23 mg/kg/day presenting the lowest homocysteine and MMA concentrations [31]. The more conventional therapeutic dose is 0.33 mg/kg/day, which can be reduced in frequency as the situation stabilizes, and parenteral OHCbl (IV, SQ, or IM) is the only form of cobalamin that has been shown to be beneficial for patients with cblC disease. Daily IM injections provide

larger amounts of the drug [3]. This regimen was followed in our child.

Betaine plays an important role in homocysteine metabolism as a methyl donor. Through the betaine-homocysteine methyltransferase (BHMT) pathway, it is possible to bypass defective vitamin B metabolism and convert homocysteine to methionine. There is also a large variable range of therapeutic doses of betaine. A dose of 250 mg/kg/d three times a day has been suggested for betaine [3]. Doses of 100–444 mg/kg/day were used in a retrospective study of 88 cases [5]. The dose of betaine in another retrospective study from China was 100–300 mg/kg/d [27]. Therefore, we decided to start with a dose of 100 mg/kg/d and adjust the dose according to the biochemical results.

In this case, blood homocysteine and methylmalonic acid decreased to normal on the 15th day after receiving hydroxocobalamin and betaine. In most cases, blood homocysteine and methylmalonic acid levels gradually returned to normal and consciousness gradually improved 2–4 weeks after receiving hydroxocobalamin and betaine [22,32,33]. One case report showed an alarming drop in blood homocysteine already 3 days after only receiving the treatment [34]. Since both holocarboxylase synthetase deficiency and cobalamin C deficiency may cause convulsive seizures, impaired consciousness, and the patient's state of consciousness is a continuous process of improvement, we believe that the improvement of the child's GCS is related to the effective treatment of both biotin, hydroxocobalamin, and betaine. Although the neurologic symptoms and biochemical indices of this child improved, similar to previous case reports of late-onset seizures, the recovery of neurologic function was incomplete [35].

Hydroxocobalamin is the active form of vitamin B. Biotin and vitamin B are both B vitamins and may have indirect interactions in cellular metabolism, but there are no reports demonstrating direct interactions between them [36]. The metabolic pathways of betaine do not directly cross over with biotin or vitamin B in the body. No interactions between the three drugs have been reported.

This study has some limitations. First, homocysteine was not included in the initial tests because methionine was at normal levels in the initial tests, and we considered mitochondrial disease by the child's clinical presentation, increased lactate, and abnormal glucose metabolism, but not cobalamin C deficiency. A proper and systematic analysis and interpretation of the organic acid results might have allowed an early differential diagnosis of HCSD and Cbl C. However, having both recessive genetic disorders at the same time is extremely rare and therefore leads to a very challenging diagnosis. Secondly, long-term follow-up of the metabolic status, growth and development of the child is required to elucidate the impacts of pharmacological interventions and to ascertain long-term prognostic outcomes.

4. Conclusion

This article presents a case study of a patient with two genetic metabolic disorders: holocarboxylase synthetase deficiency and cobalamin C deficiency, which manifested during adolescence. Additionally, it identifies two novel mutation sites in the HLCS gene. The effective treatment regimen included hydroxocobalamin, betaine, and biotin. This report establishes a broader foundation for gene mutation screening and serves as a valuable reference for the diagnosis and treatment of similar conditions.

CRediT authorship contribution statement

Ye Ren: Writing – original draft. **Hongxing Dang:** Writing – review & editing. **Yueqiang Fu:** Resources. **Chengjun Liu:** Resources. **Jing Li:** Resources. **Jinhua Cai:** Methodology.

Availability of data and materials

All data generated or analyzed during this study are included in the article, further inquiries can be directed to the corresponding author.

Ethics approval and consent to participate

This study was approved by Ethics Committee of Chongqing Medical University. This study did not involve any changes to the treatment plans of the patient. The patient was anonymized, and the ethical approval was obtained from the ethics committee of Chongqing Medical University, and informed consent was secured from the patient and her guardians.

Consent for publication

All authors agreed on the manuscript.

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Competing of interest

The authors declare no potential competing interest.

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