Original Article

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Clinical and genetic characteristics of late-onset cobalamin C deficiency: A multicenter study in northern China

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SUMMARY: Late-onset cobalamin C (cblC) deficiency, an inherited metabolic disorder, is often misdiagnosed due to its heterogeneous clinical presentation. This study aims to characterize the clinical and genetic spectrum of late-onset cblC deficiency in a large northern Chinese cohort and proposes a novel clinical classification based on initial symptoms. A retrospective, multicenter study of 156 patients diagnosed between October 2012 and December 2023 was conducted. Clinical, biochemical, neuroimaging, and genetic data were analyzed. Patients were classified into six subtypes based on predominant initial symptoms, and genotype-phenotype correlations were explored. The cohort (95 males, 61 females) had a median onset age of 16 years (range: 2–65). Common symptoms included spastic paralysis (41.0%), mental and behavioral abnormalities (36.5%), and renal damage (28.8%). Genetic analysis identified 52 *MMACHC* variants, with c.482G>A (34.3%) and c.609G>A (17.6%) being most frequent. Elevated total homocysteine (tHcy) levels correlated with mental and behavioral abnormalities, renal damage, and anemia ($p < 0.05$). The proposed clinical classification identified six subtypes, with encephalopathy-dominant and encephalomyelopathy-dominant types being most prevalent. This study highlights the clinical heterogeneity of late-onset cblC deficiency and introduces a novel symptom-based classification system to aid diagnosis and management. Elevated tHcy levels and specific *MMACHC* variants are key biomarkers for disease severity. These findings underscore the importance of early intervention to improve outcomes.

Keywords: methylmalonic academia, hyperhomocysteinemia, late onset, *MMACHC* gene, Clinical manifestation

1. Introduction

Cobalamin C (cblC) deficiency (CCD; OMIM #277400), the most common inherited disorder of intracellular cobalamin metabolism, was first described by Mudd *et al*. over five decades ago (*1*). In 2006, Lerner-Ellis *et al.* identified *MMACHC* (OMIM #609831), the causative gene located on chromosome 1p34.1, which encodes a 282-amino acid protein (*2*). Dysfunction of *MMACHC* reduces intracellular levels of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), essential cofactors for methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (EC 1.16.1.8), respectively (*3*). Key biomarkers of CCD include elevated blood total homocysteine (tHcy), an increased propionylcarnitine/acetylcarnitine (C3/C2) ratio, and elevated urinary methylmalonic acid (MMA)(*4*). CCD manifests from prenatal stages to adulthood, with clinical severity

ranging from mild to life-threatening (*5*). Disease onset is categorized as early (< 1 year) or late (> 1 year), with late-onset patients typically exhibiting milder symptoms and better prognoses (*6*).

Early-onset CCD is characterized by severe manifestations, including feeding difficulties, growth retardation, lethargy, hypotonia, hydrocephalus, and maculopathy (*7-9*). In contrast, late-onset CCD presents with mental and behavioral abnormalities, cognitive impairment, epilepsy, spastic paralysis, myelopathy, peripheral neuropathy, ataxia, hematological abnormalities, renal damage, ocular involvement, and thromboembolic events (*10*). Although rare, the CCD incidence in Shandong Province, China, is approximately 1 in 4000, significantly higher than the 1 in 272,411 reported in some southern provinces such as Jiangsu Province (*11,12*). Recent increases in late-onset diagnoses suggest a higher prevalence than previously estimated. However, few studies have explored genotype-phenotype correlations, which could help predict disease onset and severity.

This study established a multicenter clinical cohort of late-onset cblC deficiency (LCCD) patients from eight major medical centers in northern China to analyze their clinical and genetic characteristics, providing critical insights for diagnosis and prognosis in this population.

2. Patients and Methods

2.1. Study participants

We included 156 patients with LCCD from Qilu Hospital of Shandong University, Ji nan Maternity and Child Care Hospital, Peking Union Medical College Hospital, XuanWu Hospital of Capital Medical University, Peking University First Hospital, Hebei Medical University Third Hospital, Beijing Tiantan Hospital and Lanzhou University Second Hospital between October 2012 and November 2023. The inclusion criteria for patients were as follows: *i*) hyperhomocysteinemia (HHcy), tHcy > 50 μmol/L; *ii*) biallelic pathogenic *MMACHC* variations confirmed; and *iii*) age of onset > 1 year. The exclusion criteria were as follows: *i*) HHcy caused by secondary or other genetic factors, and *ii*) incomplete clinical data or a lack of genetic diagnostic evidence.

2.2. Data collection

Clinical data, including demographics, age of symptom onset and diagnosis, family history, treatment regimens, and clinical outcomes, were collected. Additional data encompassed clinical manifestations, laboratory results, neuroimaging findings, electroencephalography (EEG), electromyography (EMG), and genetic testing results. Cognitive and executive functions were assessed using the standardized Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

2.3. Biochemical examination

Blood levels of acylcarnitines (including propionylcarnitine [C3] and acetylcarnitine [C2]) and amino acids were quantified using tandem mass spectrometry (MS/MS; Applied Biosystems, API 4000, California, United States) in dried blood spot samples (*13*). The C3/C2 ratios were calculated from these measurements. Urinary organic acids were analyzed by gas chromatography–mass spectrometry (GC–MS; Shimadzu Limited, QP2010, Kyoto, Japan) (*14*). Plasma homocysteine levels were measured *via* fluorescence polarization immunoassay (*15*). Additionally, routine blood and urine analyses were performed to assess electrolyte and glucose levels, liver and renal function, and folate and vitamin B_{12} concentrations.

2.4. Genetic testing

Whole-exome sequencing (WES) was performed on all 156 patients. Genomic DNA was extracted from peripheral blood leukocytes, and next-generation sequencing (NGS) was used to identify *MMACHC* variants. All variants were assessed for pathogenicity according to the *Standards and Guidelines for the Interpretation of Sequence Variants* published by the American College of Medical Genetics and Genomics (ACMG) in 2015.

2.5. Statistical analysis

Statistical analyses were performed using SPSS (version 27.0.0.0) and GraphPad Prism. The Shapiro–Wilk test indicated a non-normal distribution of the data; consequently, continuous variables are presented as medians (interquartile ranges), and group comparisons were performed using the nonparametric Mann–Whitney *U* test. Categorical variables are expressed as absolute frequencies and percentages, with comparisons made using the chi-square test or Fisher's exact test, as appropriate. A two-tailed p value < 0.05 was considered statistically significant.

2.6. Ethical approval

This study complied with the Ethics Guidelines and was approved by the Ethics Committee of Qilu Hospital of Shandong University. All patients or their parents (for patients younger than 18 years) signed informed consent forms prior to inclusion in the study. This study was conducted in accordance with the Declaration of Helsinki.

3. Results

3.1. Study population

The demographic, biochemical, and clinical features of the cohort are summarized in Table 1. This case series included 156 patients with late-onset cobalamin

Table 1. Clinical features of patients with different types of cblC deficiency

	Total	cblC-E	cblC-M	cblC-EM	cblC-R	cblC-N	cblC-O
No.	156	57 (36.5%)	27 (17.3%)	31 (19.9%)	11 (7.1%)	10 (6.4%)	20 (12.8%)
Sex ratio (male: Female)	95:61	32:25	17:10	16:15	7:4	9:1	14:6
Age at onset, years, median (IQR)	16.0 (12.0–24.0)	16.0 (9.5–25.5)	17.0 (13.0–24.0)	17.0 (14.0–23.0)	14.0 (9.0–21.0)	20.0 (16.0–25.8)	13.5 (10.8–24.5)
Time to molecular diagnosis, months, median (IQR)	12.0 (2.0–60.0)	24.0 (2.0–66.0)	12.0 (3.0–84.0)	12.0 (2.0–72.0)	24.0 (5.0–48.0)	2.0 (1.0–66.0)	5.5 (1.0–39.0)
Biochemical features							
Total plasma homocysteine level at presentation[a] (μmol/L) (n = 156) (IQR)	118.0 (95.4–185.0)	135.0 (102.7–188.8)	100.1 (77.2–136.5)	115.2 (91.7–193.4)	176.0 (89.1–293.0)	162.6 (109.5–198.4)	99.9 (73.5–164.1)
Methylmalonic acid level at presentation[b] (μg/mg creatinine) (n = 94) (IQR)	116.0 (63.8–191.6)	121.5 (83.5–204.7)	89.8 (43.6–141.9)	109.4 (57.6–187.8)	133.3 (35.1–1772.9)	209.7 (104.5–412.7)	85.1 (26.1–171.4)
C3/C2 ratio at presentation[c] (n = 94) (IQR)	0.5 (0.4–0.7)	0.6 (0.3–0.7)	0.4 (0.3–0.6)	0.5 (0.4–0.7)	0.5 (0.4–0.6)	0.8 (0.6–1.0)	0.5 (0.4–0.8)
Clinical manifestations							
Spastic paralysis	64/156 (41.0%)	3/57 (5.3%)	27/27 (100%)	31/31 (100%)	2/11 (18.2%)	0/10 (0.0%)	1/20 (5.0%)
Mental and behavioral abnormalities	57/156 (36.5%)	32/57 (56.1%)	2/27 (7.4%)	22/31 (71.9%)	1/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Renal damage	45/156 (28.8%)	15/57 (26.3%)	4/27 (14.8%)	9/31 (29.0%)	11/11 (100.0%)	2/10 (10.0%)	4/20 (20.0%)
Anemia	42/156 (26.9%)	15/57 (26.3%)	6/27 (22.2%)	9/31 (29.0%)	6/11 (54.5%)	1/10 (10.0%)	5/20 (25.0%)
Cognitive decline	34/156 (21.8%)	19/57 (33.3%)	3/27 (11.1%)	7/31 (22.6%)	2/11 (18.2%)	2/10 (20.0%)	1/20 (5.0%)
Limb weakness	31/156 (19.4%)	10/57 (17.5%)	1/27 (3.7%)	1/31 (3.2%)	0/11 (0.0%)	10/10 (100.0%)	9/20 (45.0%)
Ataxia	28/156 (17.9%)	3/57 (5.3%)	12/27 (44.4%)	8/31 (35.8%)	1/11 (9.1%)	1/10 (10.0%)	3/20 (15.0%)
Epilepsy	26/156 (16.7%)	18/57 (31.6%)	1/27 (3.7%)	7/31 (22.6%)	0/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Mental retardation	11/156 (7.1%)	10/57 (17.5%)	0/27 (0.0%)	0/31 (0.0%)	1/11 (9.1%)	0/10 (0.0%)	0/20 (0.0%)
Thrombus	8/156 (5.1%)	5/57 (8.8%)	2/27 (7.4%)	1/31 (3.2%)	0/11 (0.0%)	0/10 (0.0%)	0/20 (0.0%)
Visual impairment	8/156 (5.1%)	5/57 (8.8%)	1/27 (3.7%)	0/31 (0.0%)	2/11 (18.2%)	0/10 (0.0%)	1/20 (5.0%)
Pulmonary hypertension	5/156 (3.2%)	0/57 (0.0%)	0/27 (0.0%)	0/31 (0.0%)	2/11 (18.2%)	0/10 (0.0%)	2/20 (10.0%)

[a]Typical reference range, 5–15 μmol/L with slight variations in some tests. [b]Typical reference range, 0.2–3.6 μg/mg creatinine with slight variations in some tests. [c]Typical reference range, 0.02–0.20 with slight variations in some tests.

C deficiency (LCCD) due to pathogenic *MMACHC* variants, representing 144 families: 95 males (60.9%) and 61 females (39.1%). Age at symptom onset ranged from 2 to 65 years (median: 16 years). Patients were stratified into three age groups: young children (< 14 years), adolescents (14–17 years), and adults (≥ 18 years) (Figure 1A).

The interval between symptom onset and diagnosis varied from months to 28 years (median: 12 months). Diagnostic delays were significantly longer in the young children group compared to the other groups (Figure 1B). Two patients died: one at age 6 from severe pulmonary hypertension and another at age 23 due to metabolic cardiomyopathy precipitated by pregnancy and infection; neither had received regular treatment. Twenty-four patients were lost to follow-up, and 12 sibling pairs were identified. All patients were from 10 provinces in northern China.

3.2. Phenotypes and clinical features

Based on comprehensive clinical evaluation, we classified 156 LCCD patients into six distinct phenotypic subgroups according to their predominant initial symptoms: (1) encephalopathy-dominant (cblC-E, n = 57, 36.5%), (2) myelopathy-dominant (cblC-M, n = 27, 17.3%), (3) encephalomyelopathy-dominant (cblC-EM, n = 31, 19.9%), (4) renal dysfunction-dominant (cblC-R, n = 11, 7.1%), (5) peripheral neuropathy-dominant (cblC-N, n = 10, 6.4%), and (6) other symptom-dominant (cblC-O, n = 20, 12.8%) (Figure 2A).

The cblC-E subtype primarily presented with neuropsychiatric features, including mental/behavioral abnormalities (36.5%), epilepsy, and cognitive decline. cblC-M patients showed spinal cord involvement manifesting as spastic paralysis, while cblC-EM represented a combination of these neurological presentations. Renal impairment defined the cblC-R subtype, and cblC-N patients exhibited peripheral nerve dysfunction with limb weakness. Patients with other predominant initial symptoms were categorized as cblC-O (Figure 2A).

Beyond initial symptoms, disease progression occurred in the overwhelming majority of CCD patients. Figure 2B shows the frequency of overall clinical manifestations in the 156 LCCD patients. The most common manifestations were spastic paralysis (41.0%), followed by mental and behavioral abnormalities (encompassing social withdrawal, abnormal behavior,

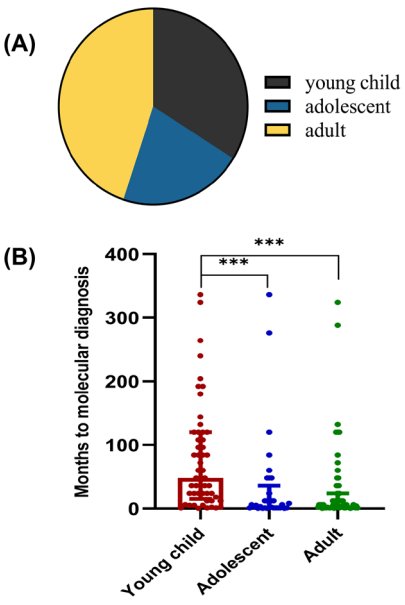

Figure 1. Patient characteristics. (A) Age stratification revealed three distinct groups: young children (< 14 years, n = 53, 34.0%), adolescents (14–17 years, n = 33, 21.2%), and adults (≥ 18 years, n = 70, 44.9%). **(B)** Diagnostic delays ranged from months to 28 years (median 12 months), with significantly prolonged intervals observed in young children versus older groups. ***p < 0.001.

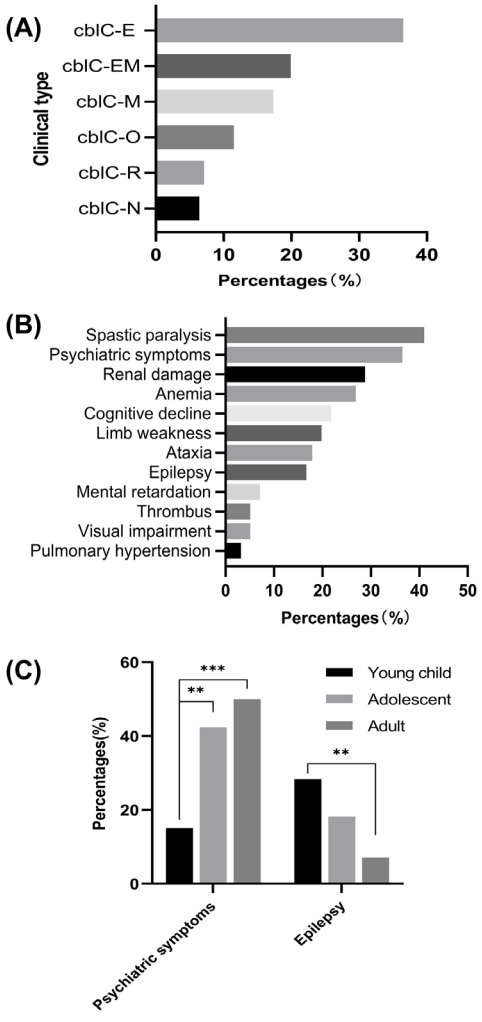

Figure 2. The phenotypes and clinical features of the patients. (A) Patients with cblC were divided into 6 types according to the symptoms at onset. **(B)** 156 patients had a total of 12 clinical signs. **(C)** Age-stratified analysis revealed significantly higher seizure frequency in children versus adults), while adults showed predominance of mental/behavioral abnormalities. **p < 0.01; ***p < 0.001.

raving, depression, and auditory hallucinations; 36.5%), renal damage (28.8%), anemia (26.9%), cognitive decline (21.8%), limb weakness (19.8%), ataxia (17.9%), epilepsy (16.7%), intellectual disability (7.1%), thrombotic events (5.1%), visual impairment (5.1%), and pulmonary hypertension (3.2%). Age-stratified analysis demonstrated higher seizure frequency in pediatric patients versus greater neuropsychiatric manifestations in adults (Figure 2C).

Patients were grouped based on the presence of spastic paralysis, mental/behavioral abnormalities, renal damage, anemia, cognitive decline, limb weakness, ataxia, or epilepsy. Factors including age of onset, diagnostic delay, and initial biochemical/metabolic data were analyzed (Table 2). Elevated tHcy levels correlated with both neuropsychiatric symptoms ($p < 0.05$) and renal impairment. Moreover, patients with limb weakness or epilepsy experienced a prolonged interval between symptom onset and diagnosis, whereas epilepsy patients had an earlier age of onset. Other clinical and biochemical features showed no significant differences. This phenotypic classification system provides a clinically meaningful framework for anticipating disease course and guiding management in LCCD patients.

3.3. Biochemical features

Homocysteine (Hcy) levels were measured in 156 patients at the first visit, and urine organic acid concentrations and plasma amino acid levels were recorded for 112 patients. Serum total homocysteine (tHcy) values were significantly increased in all patients (range: 53.0–447.7 μmol/L; median: 117.4 μmol/L). Additionally, urine methylmalonic acid (MMA) concentrations (range: 5.7–1213.9 μg/mg creatinine; normal: 0.2–3.6 μg/mg creatinine) and C3/C2 ratios (range: 0.20–2.39; normal: 0.02–0.20) were elevated in 112 patients. These biochemical findings supported a diagnosis of methylmalonic acidemia (MMA) combined with homocystinuria.

Age-stratified analysis demonstrated significantly higher tHcy levels in adults versus younger patients (*p* < 0.05; Figure 3A), whereas MMA levels, C3/C2 ratios, and other metabolic parameters showed no age-dependent variation (Figure 3A). Chi-square testing indicated significant differences in tHcy levels across groups, with lower levels observed in the cblC-M group (Figure 3B). Elevated tHcy correlated strongly with renal impairment (*p* < 0.05), neuropsychiatric manifestations, and anemia. In contrast, spastic paralysis was associated with comparatively reduced tHcy concentrations (Figure 3C).

3.4. Neuroimaging features

Brain MRI was performed on 119 patients. Results showed that 68 patients (57.1%) primarily exhibited cerebral atrophy and white matter lesions; a subset also presented with cerebral venous sinus thrombosis (Figure 4A). Spinal cord MRI was performed in 105 patients, with 31 (29.5%) exhibiting spinal cord atrophy and longitudinally extensive transverse myelitis (Figure 4B). Scoliosis was a common imaging abnormality among the patients.

3.5. Genotypes and degrees of heteroplasmy

Genetic analysis identified 52 distinct *MMACHC* variants (including 13 VUS) in 156 patients. Exon 4 harbored 57.7% (30/52) of these mutations (Figure 5A). The most frequent variant was c.482G>A, present in 107 (34.3%) alleles, followed by c.609G>A (17.6%), c.658_660del (7.1%), c.567dupT (4.8%), and c.80G>A (4.2%).

Compared to patients without the c.482G>A mutation, those with the mutation exhibited a lower incidence of epilepsy, mental retardation, visual impairment, and pulmonary hypertension, but a higher frequency of spastic paralysis and mental/behavioral abnormalities (Figure 5B). Additionally, the c.80G>A variant was consistently associated with earlier onset age, nephropathy, and pulmonary hypertension. Patients harboring c.80G>A typically presented with early onset, cblC-R subtype, renal damage, pulmonary hypertension, and mental retardation (Figure 5C).

The predominant variant combination was c.482G>A and c.609G>A, found in 29 patients (18.6%). Patients were stratified into four genotypic groups: c.482G>A alone, c.609G>A alone, both mutations, or other variants. Patients with c.482G>A had a significantly later age of onset than those with c.609G>A (Figure 5D). No significant differences in treatment response or biochemical markers (tHcy, MMA, C3/C2 ratio) were observed across the genotypic groups.

3.6. Long-term treatment and follow-up

All patients received acute-phase therapy with intramuscular hydroxocobalamin (OH-Cbl; 10 mg/day) or mecobalamin (1,000 μg/day), combined with metabolic adjuncts: L-carnitine (2 g/day), betaine (6–9 g/day), leucovorin (7.5 mg/day), and vitamin B6 (10–100 mg/day). Long-term treatment was then individualized based on patient condition.

While all patients showed initial clinical improvement — with neuropsychiatric symptoms resolving faster than motor deficits — while outcomes varied by subtype. Among the 156 patients, 24 (15.4%) were lost to follow-up, one patient died at age 6 years due to severe pulmonary hypertension, and one died at age 23 years from heart disease.

Patients with the cblC-E subtype consistently demonstrated a better prognosis than those with other subtypes; however, rigorous analysis is needed to confirm these results due to differences in treatment regimens and adherence. Treatment adherence issues

Table 2. Variable phenotypes and factors in 156 patients (symptoms in > 20 patients were analyzed)

Symptoms and signs		Cases *n*	Age at onset (years) Median (P25, P75)	Time from onset to treatment initiation (years) Median (P25, P75)	Blood tHcy (μmol/L) Median (P25, P75)	Urinary methylmalonic acid (μg/mg creatinine) Median (P25, P75)	C3/C2 Median (P25, P75)
Spastic paralysis	N	92	16.0 (10.3, 25.0)	12.0 (1.0, 48.0)	133.7 (98.2, 203.4)	121.5 (79.5, 204.7)	0.6 (0.4, 0.8)
	Y	64	17.0 (13.3, 22.8)	12.0 (3.0, 84.0)	112.5 (88.2, 158.2)	101.9 (46.2, 175.2)	0.5 (0.4, 0.7)
Z			-0.795	-1.235	-2.151	-1.411	-1.071
P			0.426	0.217	0.031	0.158	0.284
Mental and behavioral abnormalities	N	99	14.0 (10.0, 20.0)	12.0 (2.0, 72.0)	111.5 (90.0, 164.0)	111.7 (62.9, 182.5)	0.5 (0.4, 0.7)
	Y	57	18.0 (15.0, 26.0)	12.0 (2.0, 42.0)	139.0 (100.3, 233.5)	107.5 (70.1, 197.3)	0.6 (0.4, 0.7)
Z			-3.338	-0.783	-2.560	-0.237	-0.087
P			< 0.001	0.434	0.010	0.813	0.931
Renal damage	N	111	16.0 (12.0, 20.0)	12.0 (2.0, 48.0)	112.6 (88.0, 162.0)	111.7 (62.9, 185.8)	0.6 (0.4, 0.7)
	Y	45	19.0 (12.0, 26.0)	24.0 (2.0, 72.0)	146.3 (107.4, 248.2)	132.6 (79.2, 214.2)	0.5 (0.4, 0.7)
Z			-0.997	-0.909	-3.440	-0.752	-0.087
P			0.319	0.363	< 0.001	0.452	0.931
Anemia	N	114	16.0 (12.8, 24.3)	12.0 (2.0, 48.0)	115.2 (94.8, 165.6)	126.9 (66.9, 206.4)	0.5 (0.4, 0.7)
	Y	42	16.0 (12.0, 22.3)	12.0 (2.0, 51.0)	134.9 (94.1, 245.2)	102.2 (45.5, 155.8)	0.5 (0.4, 0.8)
Z			-0.540	-0.066	-2.054	-1.769	-0.907
P			0.589	0.947	0.040	0.077	0.365
Cognitive decline	N	122	16.0 (12.8, 24.0)	12.0 (2.0, 60.0)	115.5 (94.8, 180.5)	107.0 (58.1, 179.1)	0.5 (0.4, 0.7)
	Y	34	15.5 (12.0, 23.5)	7.0 (1.8, 60.0)	147.4 (94.5, 190.0)	165.7 (79.4, 236.6)	0.6 (0.3, 0.7)
Z			-0.069	-0.500	-0.766	-1.435	-0.374
P			0.945	0.617	0.444	0.151	0.709
Limb weakness	N	125	15.0 (12.0, 22.5)	12.0 (3.0, 84.0)	120.0 (96.0, 173.1)	107.0 (54.5, 172.4)	0.5 (0.4, 0.7)
	Y	31	18.0 (14.0, 26.0)	2.0 (1.0, 12.0)	109.9 (86.7, 211.4)	112.0 (77.5, 236.5)	0.6 (0.4, 0.8)
Z			-1.696	-3.042	-0.291	-1.652	-0.369
P			0.090	0.002	0.771	0.098	0.171
Ataxia	N	128	16.0 (12.0, 23.0)	12.0 (2.0,57.0)	119.0 (95.6, 184.6)	121.1 (67.0, 198.0)	0.6 (0.4, 0.7)
	Y	28	16.5 (14.0, 25.5)	6.0 (3.0, 69.0)	113.1 (82.3, 182.8)	85.0 (36.4, 147.6)	0.4 (0.3, 0.6)
Z			-0.583	-0.023	-0.455	-1.688	-2.386
P			0.560	0.982	0.649	0.091	0.017
Epilepsy	N	130	17.5 (13.0, 25.0)	11.0 (2.0, 48.0)	122.6 (96.0, 188.0)	121.5 (62.9, 198.0)	0.5 (0.4, 0.7)
	Y	26	12.5 (5.8, 17.0)	30.0 (12.0, 93.0)	106.1 (89.5, 146.9)	107.1 (66.3, 168.8)	0.6 (0.3, 0.8)
Z			-3.247	-2.326	-1.510	-0.451	-0.630
P			0.001	0.020	0.131	0.652	0.529

Notes: *n*, number; N, No; Y, Yes; *Z*, Mann–Whitney *U* Z-score; *P*, value was calculated by using a nonparametric unpaired Mann-Whitney *U* test; $P < 0.05$ was considered as statistically significant.

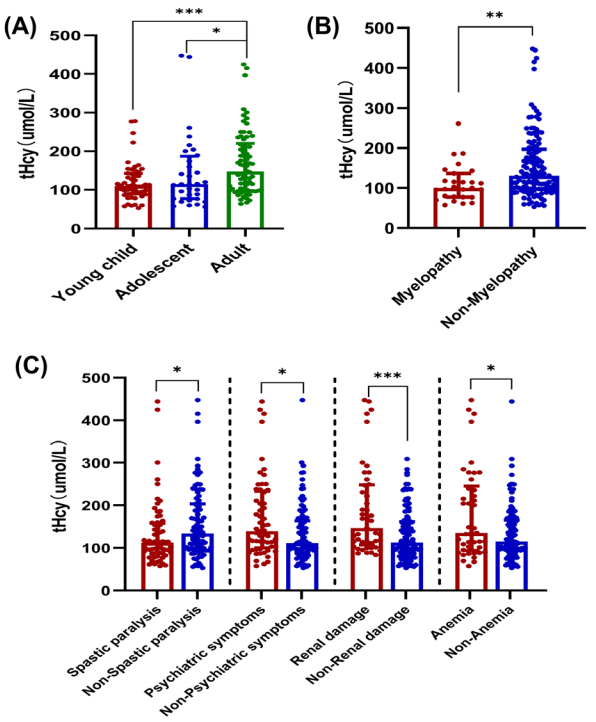

Figure 3. The biochemical features of the patients. (A) Adults demonstrated significantly elevated tHcy levels compared to younger patients. **(B)** The myelopathic subgroup (cblC-M) exhibited markedly lower tHcy concentrations versus other subtypes. **(C)** Hyperhomocysteinemia correlated strongly with renal impairment, neuropsychiatric manifestations, and anemia, while spastic paralysis associated with reduced tHcy levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$..

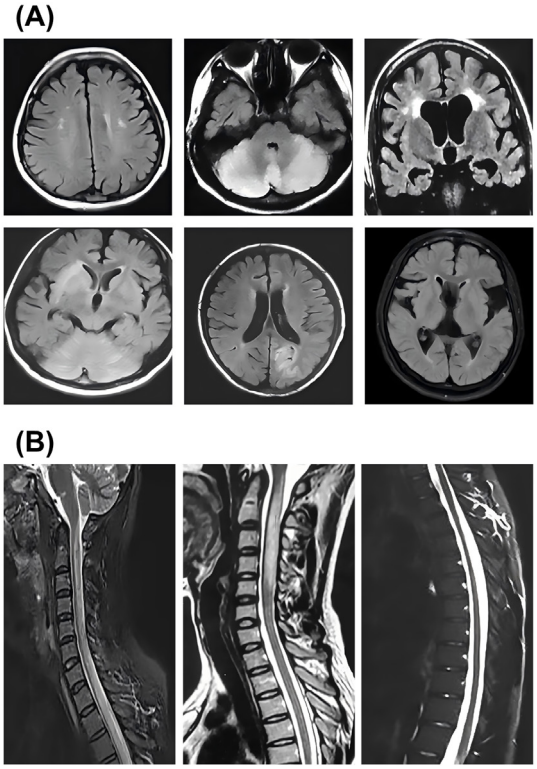

Figure 4. Neuroimaging Findings. (A) Brain MRI of some patients were characterized primarily by cerebral atrophy and white matter lesions. **(B)** Spinal cord MRI of some patients exhibited spinal cord atrophy and longitudinally extensive transverse myelitis.

affected 28% of patients, particularly with intramuscular regimens.

4. Discussion

Cobalamin C deficiency (CCD), caused by *MMACHC* mutations, is the most common form of methylmalonic acidemia (MMA) combined with homocystinuria (*16*). Late-onset cblC deficiency (LCCD) is rare and diagnostically challenging due to heterogeneous clinical manifestations (*5,17*). While approximately 90% of reported cases involve early-onset disease, fewer than 300 LCCD cases have been documented (*18,19*). Although neonatal screening based on elevated C3 and reduced methionine levels in dried blood spots has improved early detection, neurological and visual symptoms often progress despite timely intervention (*20,21*). In contrast, late-onset patients exhibit rapid biochemical and clinical improvement with prompt treatment, underscoring the critical importance of early diagnosis (*10*).

In this study, we analyzed 156 LCCD patients (61 females, 95 males) from northern China. The interval between symptom onset and diagnosis ranged from days to 28 years, with only 43.6% diagnosed within 12 months. Earlier symptom onset was associated with prolonged diagnostic delays, a known risk factor for poor outcomes (*22*). Notably, siblings with identical *MMACHC* variants exhibited variable ages of onset and prognoses, highlighting the disease's phenotypic variability.

The most common clinical manifestations in our cohort were spastic paralysis, mental/behavioral abnormalities, renal damage, and anemia. To date, most reports on LCCD are descriptive cohort studies and case reports. Currently, no consensus classification system exists for this disease, and few cohort studies have attempted a systematic or stratified analysis of its clinical presentations. In this context, the symptom-based classification system we propose represents a paradigm shift.

We classified patients into six subtypes based on initial symptoms, with encephalopathy-dominant (cblC-E) and encephalomyelopathy-dominant (cblC-EM) being most prevalent. Notably, among patients with the cblC-E subtype, those presenting with psychiatric abnormalities predominantly carried the c.482G>A mutation. The cblC-EM subtype, which is not uncommon in this disease, typically presents with concurrent encephalopathy and myelopathy in its early stages. This specific subgroup consequently exhibited a more favorable prognosis. In contrast, the myelopathy-dominant (cblC-M) subtype was associated with a potentially less favorable clinical outcome. Furthermore,

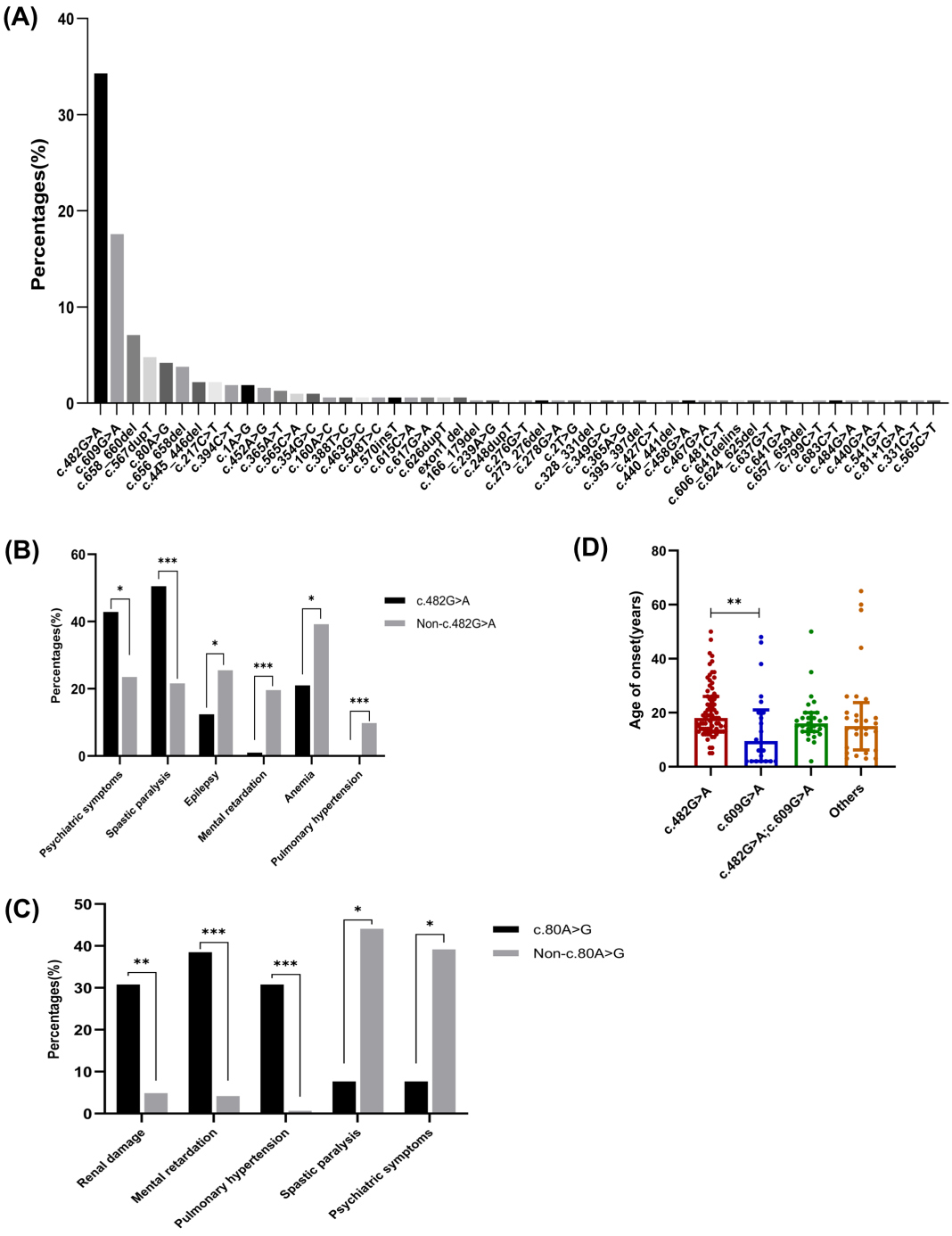

Figure 5. Genetic Characteristics and Phenotypic Correlations. (A) 52 distinct variants were identified among 156 patients. **(B)** Patients with c.482G>A mutation presented a lower incidence of epilepsy, mental retardation, anemia, visual impairment and pulmonary hypertension, along with a greater frequency of spastic paralysis, mental and behavioral abnormalities and anemia. **(C)** Patients with the c.80A>G mutation had the following characteristics: early onset age, renal damage, pulmonary hypertension and mental retardation. **(D)** The age of onset of patients with c.482G>A were significantly older than those with c.609G>A. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

the renal dysfunction-dominant (cblC-R) subtype demonstrated a higher prevalence of the c.80G>A variant and was characterized by an earlier age of onset. The peripheral neuropathy-dominant (cblC-N) subtype was the least prevalent among our cohort. Affected patients frequently presented with peripheral neuropathy, which predominantly manifested as axonal damage with limited potential for full recovery. Myelopathy—characterized by spastic paralysis, abnormal gait, and limb weakness—was exclusively observed in late-onset patients (*16,19,23*). Age-stratified analysis revealed epilepsy was more common in younger patients, while mental/behavioral abnormalities predominated in adults.

Megaloblastic anemia was the primary hematological abnormality, consistent with prior findings (*24*). Unlike early-onset patients, late-onset patients showed less

ocular involvement, with no clear evidence of retinal degeneration. However, poor visual acuity was noted in nine patients, though its direct association with CCD remains unclear. Ophthalmological evaluations should be incorporated into diagnostic protocols to clarify this relationship. CCD can present acutely or insidiously, with periods of stability interrupted by triggers such as infection, fever, strenuous exercise, or vegetarian diets (*25*). In our cohort, common triggers included pregnancy, surgery, infections, and dietary changes. Given its multisystemic involvement, CCD should be considered in patients with unexplained neuropsychiatric symptoms, metabolic crises, or multi-organ dysfunction.

Elevated plasma tHcy and urinary MMA levels remain reliable diagnostic markers. In our study, all patients exhibited significantly increased tHcy and MMA levels, supporting their utility in diagnosing CCD. Patients with high tHcy levels were more likely to exhibit renal damage and neuropsychiatric symptoms, while those with the cblC-M subtype had lower tHcy levels. Interestingly, younger patients had lower tHcy levels than adults, though no significant correlation was found between tHcy levels and clinical severity.

Neuroimaging revealed cerebral atrophy, white matter lesions, spinal cord atrophy, and chronic spinal cord injury as common findings (*26-28*). MRI findings may help assess CCD severity (*29*). Electromyography (EMG) indicated neurogenic damage in nearly half of tested patients. Electroencephalography (EEG) abnormalities were observed in 38 of 53 patients, predominantly in the cblC-E subtype. Recent studies suggest EEG may reveal early neuronal dysfunction in CCD (*30,31*), though no disease-specific EEG patterns were identified.

To date, over 100 different *MMACHC* mutations have been reported (*32*). While c.482G>A is most frequent in European CCD patients (*10*), c.609G>A predominates in Chinese patients, accounting for 55.4% of known mutations (*33,34*). In our cohort, c.482G>A was most frequent (34.3%) and associated with spastic paralysis and milder phenotypes. Other common variants included c.609G>A and c.658_660del, consistent with Chinese population reports. The c.609G>A mutation — typically linked to early-onset disease — was prevalent in our cohort, likely due to a founder effect (*35*). Patients with c.80G>A frequently presented with renal damage and pulmonary hypertension, highlighting the need for cardiovascular and renal monitoring in these cases (*36-38*). Morel *et al.* reported c.394C>T is common in South Asian and Middle Eastern populations with late-onset disease (*39*); however, only six patients with this mutation were included here. The wide clinical heterogeneity of CCD likely stems from *MMACHC* variants, though environmental factors, ethnicity, and diet may influence phenotypic expression.

The therapeutic agents for cblC include cobalamin, levocarnitine, betaine, folic acid and vitamin B. Intramuscular HoCbl is the only cobalamin form known to benefit CCD patients; cyanocobalamin (CNCbl) and methylcobalamin (MeCbl) are not recommended (*40,41*). High-dose cobalamin (approximately 0.33 mg/kg/day), L-carnitine, and betaine are used acutely to reduce tHcy and correct metabolic status (*41,42*). Maintenance therapy dosages are adjusted based on plasma tHcy and urinary MMA levels. Most LCCD patients respond well, showing significant biochemical, clinical, and neuroimaging improvement. However, delayed or non-standardized treatment can cause sequelae or death. In our cohort, cblC-E patients exhibited marked improvement in cognitive decline and mental/behavioral abnormalities. In contrast, spinal cord injury responded poorly, and treatment non-adherence was associated with symptom recurrence and adverse outcomes. Treatment response varied by genotype: patients with c.482G>A showed better metabolic control and clinical improvement with cobalamin therapy. However, poor treatment adherence was common, with many experiencing symptom exacerbation or death due to irregular medication. These findings underscore the need for rigorous follow-up to evaluate long-term outcomes and optimize strategies. Despite the discovery of CCD over 50 years ago, treatment advancements remain limited, relying heavily on clinical experience rather than robust trial evidence. Frequent intramuscular HoCbl administration poses significant practical challenges (*43*). Ongoing research into gene and cell therapies may offer future alternatives (*44,45*).

Our Study has several limitations: *i*) Retrospective data collection may have introduced incomplete datasets; *ii*) Lack of standardized treatment guidelines resulted in management variability; *iii*) Regional bias may limit generalizability, 100 of the 156 patients were recruited from one center. Despite these limitations, our multicenter analysis provides valuable insights into the clinical and genetic spectrum of LCCD, enhancing recognition and management among clinicians.

In conclusion, this multicenter study establishes the first symptom-based classification system for LCCD, identifying six distinct clinical subtypes with specific genotype-phenotype correlations. The c.482G>A variant (34.3%) is associated with spastic paralysis and a milder disease course, while c.80G>A predicts renal and pulmonary complications. Elevated tHcy levels serve as key biomarkers for neuropsychiatric and renal manifestations. Despite management strategies, diagnostic delays (median 12 months) and treatment adherence remain critical challenges. These findings enable tailored monitoring and emphasize the need for standardized management protocols to improve outcomes in this metabolically complex disorder.

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References

1. Mudd SH, Levy HL, Abeles RH, Jennedy JP, Jr. A derangement in B 12 metabolism leading to homocystinemia, cystathioninemia and methylmalonic aciduria. Biochem Biophys Res Commun. 1969; 35:121-126.
2. Lerner-Ellis JP, Tirone JC, Pawelek PD, *et al*. Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nat Genet. 2006; 38:93-100.
3. Fowler B. Genetic defects of folate and cobalamin metabolism. Eur J Pediatr. 1998; 157 Suppl 2:S60-66.
4. Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis. 2012; 35:91-102.
5. Chen Z, Dong H, Liu Y, *et al*. Late-onset cblC deficiency around puberty: A retrospective study of the clinical characteristics, diagnosis, and treatment. Orphanet J Rare Dis. 2022; 17:330.
6. Rosenblatt DS, Aspler AL, Shevell MI, Pletcher BA, Fenton WA, Seashore MR. Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). J Inherit Metab Dis. 1997; 20:528-538.
7. Huemer M, Baumgartner MR. The clinical presentation of cobalamin-related disorders: From acquired deficiencies to inborn errors of absorption and intracellular pathways. J Inherit Metab Dis. 2019; 42:686-705.
8. Akar HT, Yıldız H, Öztürk Z, Karakaya D, Sezer A, Olgaç A. Case presentation: A severe case of cobalamin c deficiency presenting with nephrotic syndrome, malignant hypertension and hemolytic anemia. BMC Nephrol. 2024; 25:217.
9. Gao CF, Wang D, Zeng LK, Tao XW. Pulmonary fungal infection in a neonate with methylmalonic acidemia: A case report. World J Clin Cases. 2023; 11:8158-8163.
10. Huemer M, Scholl-Bürgi S, Hadaya K, Kern I, Beer R, Seppi K, Fowler B, Baumgartner MR, Karall D. Three new cases of late-onset cblC defect and review of the literature illustrating when to consider inborn errors of metabolism beyond infancy. Orphanet J Rare Dis. 2014; 9:161.
11. Yang H, Li M, Zou L, Zou H, Zhao Y, Cui Y, Han J. A regionally adapted HRM-based technique to screen *MMACHC* carriers for methylmalonic acidemia with homocystinuria in Shandong province, China. Intractable Rare Dis Res. 2023; 12:29-34.
12. Han B, Cao Z, Tian L, Zou H, Yang L, Zhu W, Liu Y. Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic acidemia and homocysteinemia (cblC type) in Shandong province, China. Brain Dev. 2016; 38:491-497.
13. Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem. 2003; 49:1797-1817.
14. Kimura M, Yamamoto T, Yamaguchi S. Automated metabolic profiling and interpretation of GC/MS data for organic acidemia screening: A personal computer-based system. Tohoku J Exp Med. 1999; 188:317-334.
15. Liu Y, Liu YP, Zhang Y, *et al*. [Heterogeneous phenotypes, genotypes, treatment and prevention of 1003 patients with methylmalonic acidemia in the mainland of China]. Zhonghua Er Ke Za Zhi. 2018; 56:414-420.
16. Carrillo-Carrasco N, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. II. Complications, pathophysiology, and outcomes. J Inherit Metab Dis. 2012; 35:103-114.
17. Kalantari S, Brezzi B, Bracciama V, Barreca A, Nozza P, Vaisitti T, Amoroso A, Deaglio S, Manganaro M, Porta F, Spada M. Adult-onset CblC deficiency: A challenging diagnosis involving different adult clinical specialists. Orphanet J Rare Dis. 2022; 17:33.
18. Ailliet S, Vandenberghe R, Schiemsky T, Van Overbeke L, Demaerel P, Meersseman W, Cassiman D, Vermeersch P. A case of vitamin B12 deficiency neurological syndrome in a young adult due to late-onset cobalamin C (CblC) deficiency: A diagnostic challenge. Biochem Med (Zagreb). 2022; 32:020802.
19. Fischer S, Huemer M, Baumgartner M, *et al*. Clinical presentation and outcome in a series of 88 patients with the cblC defect. J Inherit Metab Dis. 2014; 37:831-840.
20. Ahrens-Nicklas RC, Whitaker AM, Kaplan P, Cuddapah S, Burfield J, Blair J, Brochi L, Yudkoff M, Ficicioglu C. Efficacy of early treatment in patients with cobalamin C disease identified by newborn screening: A 16-year experience. Genet Med. 2017; 19:926-935.
21. Han L, Wu S, Ye J, Qiu W, Zhang H, Gao X, Wang Y, Gong Z, Jin J, Gu X. Biochemical, molecular and outcome analysis of eight chinese asymptomatic individuals with methyl malonic acidemia detected through newborn screening. Am J Med Genet A. 2015; 167A:2300-2305.
22. Ding S, Ling S, Liang L, Qiu W, Zhang H, Chen T, Zhan X, Xu F, Gu X, Han L. Late-onset cblC defect: Clinical, biochemical and molecular analysis. Orphanet J Rare Dis. 2023; 18:306.
23. Bodamer OA, Rosenblatt DS, Appel SH, Beaudet AL. Adult-onset combined methylmalonic aciduria and homocystinuria (cblC). Neurology. 2001; 56:1113.
24. Gerth C, Morel CF, Feigenbaum A, Levin AV. Ocular phenotype in patients with methylmalonic aciduria and homocystinuria, cobalamin C type. J AAPOS. 2008; 12:591-596.
25. Liu YR, Ji YF, Wang YL, Zhang BA, Fang GY, Wang JT, Sun GF, Lu H. Clinical analysis of late-onset methylmalonic acidaemia and homocystinuria, cblC type with a neuropsychiatric presentation. J Neurol Neurosurg Psychiatry. 2015; 86:472-475.
26. Wang X, Sun W, Yang Y, Jia J, Li C. A clinical and gene analysis of late-onset combined methylmalonic aciduria and homocystinuria, cblC type, in China. J Neurol Sci. 2012; 318:155-159.
27. Radmanesh A, Zaman T, Ghanaati H, Molaei S, Robertson RL, Zamani AA. Methylmalonic acidemia: Brain imaging findings in 52 children and a review of the literature. Pediatr Radiol. 2008; 38:1054-1061.
28. Chen Q, Tang J, Zhang H, Qin L. Case report: Desquamating dermatitis, bilateral cerebellar lesions in a late-onset methylmalonic acidemia patient. Front Neurol. 2023; 14:1255128.
29. Chen T, Sui C, Lin S, Guo B, Wang Y, Yang L. Follow-up study of neuropsychological scores of infant patients with cobalamin C defects and influencing factors of cerebral magnetic resonance imaging characteristics. Front Neurosci. 2022; 16:1093850.
30. Wang X, Yang Y, Li X, Li C, Wang C. Distinct clinical, neuroimaging and genetic profiles of late-onset cobalamin C defects (cb1C): A report of 16 Chinese cases. Orphanet

J Rare Dis. 2019; 14:109.
31. Yuan Y, Ma Y, Wu Q, Huo L, Liu CF, Liu X. Clinical and electroencephalogram characteristics of methylmalonic acidemia with *MMACHC* and *MUT* gene mutations. BMC Pediatr. 2024; 24:119.
32. Liu F, Wu Y, Li Z, Wan R. Identification of *MMACHC* and *ZEB2* mutations causing coexistent cobalamin C disease and Mowat-Wilson syndrome in a 2-year-old girl. Clin Chim Acta. 2022; 533:31-39.
33. Wang F, Han L, Yang Y, Gu X, Ye J, Qiu W, Zhang H, Zhang Y, Gao X, Wang Y. Clinical, biochemical, and molecular analysis of combined methylmalonic acidemia and hyperhomocysteinemia (cblC type) in China. J Inherit Metab Dis. 2010; 33 Suppl 3:S435-442.
34. He R, Mo R, Shen M, *et al*. Variable phenotypes and outcomes associated with the *MMACHC* c.609G>A homologous mutation: Long term follow-up in a large cohort of cases. Orphanet J Rare Dis. 2020; 15:200.
35. Wang C, Li D, Cai F, Zhang X, Xu X, Liu X, Zhang C, Wang D, Liu X, Lin S, Zhang Y, Shu J. Mutation spectrum of *MMACHC* in Chinese pediatric patients with cobalamin C disease: A case series and literature review. Eur J Med Genet. 2019; 62:103713.
36. Liu J, Tang X, Zhou C, Xu H, Yang H, He R, Li H, Zhao S. Cobalamin C deficiency presenting with diffuse alveolar hemorrhage and pulmonary microangiopathy. Pediatr Pulmonol. 2020; 55:1481-1486.
37. Wen LY, Guo YK, Shi XQ. Pulmonary hypertension in late-onset Methylmalonic Aciduria and Homocystinemia: A case report. BMC Pediatr. 2020; 20:243.
38. Grangé S, Bekri S, Artaud-Macari E, Francois A, Girault C, Poitou AL, Benhamou Y, Vianey-Saban C, Benoist JF, Châtelet V, Tamion F, Guerrot D. Adult-onset renal thrombotic microangiopathy and pulmonary arterial hypertension in cobalamin C deficiency. Lancet. 2015; 386:1011-1012.
39. Morel CF, Lerner-Ellis JP, Rosenblatt DS. Combined methylmalonic aciduria and homocystinuria (cblC): Phenotype-genotype correlations and ethnic-specific observations. Mol Genet Metab. 2006; 88:315-321.
40. Froese DS, Zhang J, Healy S, Gravel RA. Mechanism of vitamin B12-responsiveness in cblC methylmalonic aciduria with homocystinuria. Mol Genet Metab. 2009; 98:338-343.
41. Huemer M, Diodato D, Schwahn B, *et al*. Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. J Inherit Metab Dis. 2017; 40:21-48.
42. Huemer M, Diodato D, Martinelli D, *et al*. Phenotype, treatment practice and outcome in the cobalamin-dependent remethylation disorders and MTHFR deficiency: Data from the E-HOD registry. J Inherit Metab Dis. 2019; 42:333-352.
43. Lotz-Havla AS, Weiß KJ, Schiergens KA, Brunet T, Kohlhase J, Regenauer-Vandewiele S, Maier EM. Subcutaneous vitamin B12 administration using a portable infusion pump in cobalamin-related remethylation disorders: A gentle and easy to use alternative to intramuscular injections. Orphanet J Rare Dis. 2021; 16:215.
44. Chern T, Achilleos A, Tong X, Hsu CW, Wong L, Poché RA. Mouse models to study the pathophysiology of combined methylmalonic acidemia and homocystinuria, cblC type. Dev Biol. 2020; 468:1-13.
45. Guan J, Li Z, Zhang H, Yang X, Ma Y, Li Y, Dong R, Gai Z, Liu Y. Generation of a Human iPSC line (SDQLCHi021-A) from a patient with methylmalonic acidemia cblC type carrying compound heterozygous mutations in MMAHC gene. Stem Cell Res. 2020; 43:101709.

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