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ORIGINAL ARTICLE

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Phenotype, treatment practice and outcome in the cobalamin-dependent remethylation disorders and MTHFR deficiency: Data from the E-HOD registry

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Aim: To explore the clinical presentation, course, treatment and impact of early treatment in patients with remethylation disorders from the European Network and Registry for Homocystinurias and Methylation Defects (E-HOD) international web-based registry.

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Results: This review comprises 238 patients (cobalamin C defect $n = 161$; methylenetetrahydrofolate reductase deficiency $n = 50$; cobalamin G defect $n = 11$; cobalamin E defect $n = 10$; cobalamin D defect $n = 5$; and cobalamin J defect $n = 1$) from 47 centres for whom the E-HOD registry includes, as a minimum, data on medical history and enrolment visit. The duration of observation was 127 patient years. In 181 clinically diagnosed patients, the median age at presentation was 30 days (range 1 day to 42 years) and the median age at diagnosis was 3.7 months (range 3 days to 56 years). Seventy-five percent of pre-clinically diagnosed patients with cobalamin C disease became symptomatic within the first 15 days of life. Total homocysteine (tHcy), amino acids and urinary methylmalonic acid (MMA) were the most frequently assessed disease markers; confirmatory diagnostics were mainly molecular genetic studies. Remethylation disorders are multisystem diseases dominated by neurological and eye disease and failure to thrive. In this cohort, mortality, thromboembolic, psychiatric and renal disease were rarer than reported elsewhere. Early treatment correlates with lower overall morbidity but is less effective in preventing eye disease and cognitive impairment. The wide variation in treatment hampers the evaluation of particular therapeutic modalities.

Conclusion: Treatment improves the clinical course of remethylation disorders and reduces morbidity, especially if started early, but neurocognitive and eye symptoms are less responsive. Current treatment is highly variable. This study has the inevitable limitations of a retrospective, registry-based design.

1 | INTRODUCTION

The metabolism of homocysteine (Hcy) involves multiple pathways. Several inherited disorders within this complex machinery have been discovered. Hcy is formed from the amino acid methionine (Met). Hcy is either converted into cysteine via the transulphuration pathway or remethylated to Met. The central enzyme in the transulphuration pathway is cystathionine beta synthase, which is defective in classical homocystinuria (beyond the scope of this study). Remethylation of Hcy to Met is performed by the enzymes methionine synthase (MS, defective in cblG disease) and methionine synthase reductase (MSR, defective in cblE disease). Deficiency of the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) leads to impaired provision of 5-methylenetetrahydrofolate, resulting in decreased MS capacity.[1,2] Remethylation also depends on the cofactor methylcobalamin, which requires a unique set of intracellular cobalamin (Cbl) transport and processing mechanisms. In the cblD-HCY type of remethylation disorder, the cobalamin supply to MS is disturbed.[2,3] In all remethylation disorders, Hcy is elevated, while Met may be low or normal.[1,2,4]

In the combined remethylation disorders cblC, cblD-HCYMMA, cblF and cblJ, there is defective synthesis of both methylcobalamin and adenosylcobalamin; the latter is the cofactor for the intramitochondrial enzyme methylmalonyl-CoA mutase. For these diseases, elevated methylmalonic acid (MMA), propionylcarnitine and methylcitrate are additional biochemical markers.[4–6]

This study reports on data from an international registry, which is part of the European Network and Registry for Homocystinurias and Methylation Defects (E-HOD) project. From February 2013, the E-HOD project facilitated the collaboration of experts and clinically active centres, the development of clinical guidelines and the establishment of a disease registry. The E-HOD registry has expanded from an initially European to a worldwide registry for these rare inborn errors of metabolism and presently contains data from more than 600 patients.

This study is mainly descriptive and aims to:

1. Describe the phenotype of the remethylation disorders
2. Compare clinical and biochemical parameters between groups of patients cross-sectionally and longitudinally
3. Evaluate the impact of early diagnosis and treatment on outcome
4. Evaluate the concordance of treatment practice with treatment guidelines
5. Propose a core data set to characterise the diseases

2 | SUBJECTS AND METHODS

2.1 | Subjects

A search of the E-HOD registry database for patients with remethylation defects (cblE, cblG, cblD HCY, MTHFR deficiency) or combined remethylation defects (cblC, cblD HCYMMA, cblF, cblJ) retrieved 253 patients from 51 E-HOD centres. After the exclusion of 15 cases from further analyses due to lack of essential data, 238 patients were included from 47 centres with a basic data set encompassing medical history and at least enrolment visit data documented in the E-HOD registry.

2.2 | The E-HOD registry database

The E-HOD registry invites physicians to enter patient data (patients' informed consent provided). The E-HOD registry utilises the modular IT structure of the previously established E-IMD registry for urea cycle disorders and organic acidurias.[7,8] On enrolment, sociodemographic data, family and individual medical history, biochemical hallmarks at disease presentation, age at and mode of diagnosis, start of treatment, treatment modalities and genetic data are entered.

Data on the phenotype at disease presentation encompass:

- Symptoms by organ system (structured list; option to add unlisted symptoms):
- Neurologic disease (developmental delay, abnormal head size, seizures, movement disorder, myelopathy, muscle tonus, brain malformations)
- Feeding problems
- Psychiatric disease
- Thromboembolic events (stroke, deep venous thrombosis, pulmonary embolism)
- Haematologic abnormalities (neutropenia, thrombocytopenia, anaemia)
- Hepatic disease (hepatomegaly, acute liver failure, fibrosis)
- Renal disease (atypical haemolytic uraemic syndrome, chronic renal failure)
- Cardiac disease (cardiomyopathy, pulmonary hypertension)
- Ophthalmologic disease (nystagmus, others)
- Skeletal abnormalities
- Metabolic crisis

On enrolment and at every regular follow-up visit (data entry at least once a year), physical and neurological examination, biochemical data, treatment modalities, imaging studies, neuropsychological tests and quality of life assessment are entered using standardised forms. Emergency visits are documented separately.

The "physical and neurological exams form" for regular follow-up visits documents:

- Acute neurological deterioration, change in major symptoms, feeding problems and thromboembolic events since last visit
- Physical exam of general state of health and by organ system (skin, heart and vascular system, liver, lung, eyes, renal disease, hearing, skeleton) (structured list; option to add unlisted symptoms)

The registry includes data from 47 centres from countries and regions with different health systems, standards of care and funding policies. This may explain some of the heterogeneity of data sets. Data on brain imaging, neuropsychological testing and quality of life assessment were fragmentary and had to be excluded from further analyses.

2.3 | Definition of patient groups

This manuscript focuses on the analysis of cross-sectional and long-term data for the following groups of patients:

- Study population ($n = 238$)
- Patients grouped according to disease (e.g. patients with the cblC defect, MTHFR deficiency)
- Pre-clinically diagnosed patients (newborn screening, family or prenatal testing)
- Clinically diagnosed patients (ascertained on clinical grounds)
- Patients with early disease onset (<12 months at disease presentation[6])
- Patients with late disease onset (> 12 months at disease presentation[6])
- cblC sample subdivided according to quartiles of tHcy concentration on enrolment

2.4 | Time points

Data related to three time points were analysed cross-sectionally and longitudinally: disease presentation (onset of first symptoms, pre-treatment), enrolment visit and last regular follow-up.

2.5 | Statistical methods

For the small groups of patients with cblJ, cblD, cblE and cblG defects, summaries of core variables of individual cases are presented in Supplementary Tables S1–S3.

Data from the study population ($n = 238$), the cblC ($n = 161$) and MTHFR ($n = 50$) patients, as well as from preclinically ($n = 47$) versus clinically ($n = 113$) diagnosed patients with cblC disease were subjected to statistical analyses. Information on the mode of diagnosis was missing for one patient. Continuous variables are presented as mean ± standard deviation (SD) or, in smaller samples or when outliers were present, as median with quartiles. If clinically informative, ranges are provided. Continuous variables are compared between enrolment and last regular visit using the Wilcoxon signed rank test. The Sign test was used when differences were not appropriate for the evaluation of changes because of skew distributions and for ordinal variables. Groups of patients were compared using the Mann-Whitney test. Binary data were compared between enrolment and last regular visit using the McNemar test and between groups of patients using the Chi-square or Fisher's exact test. Correlations between doses of drugs and biochemical outcome parameters were assessed using Spearman rank correlations. All statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). Due to the large number of statistical tests in the analysis, only P-values ≤0.01 were considered statistically significant.

3 | RESULTS

3.1 | Description of the study population ($n = 238$): history and genetic data

Patients were born between gestational weeks 34 and 42 in the years from 1954 to 2016; 50% of the patients were born after 2005. The mean age on enrolment was 12.5 years (median 8.4; range 0.1-60.7 years). Seventy-six percent of patients were the only affected individual in their family; in 19%, 3% and 0.4% of cases, respectively, one, two or three family members suffered from the same disease.

The mean gestational week was 39th (median 40th). The overall prematurity rate (birth before gestational week 37) of 7% (cblC defect 7.6%; MTHFR deficiency 9.1%) did not exceed the average rate of preterm births in European populations.[9] Prematurity was not associated with specific genotypes.

Information on head circumference (HC) and weight at birth was available for 119 and 170 individuals, respectively. In 5% of cases, birth HC and in 14% of cases, birth weight was below the third percentile matched for gestational age and sex (World Health Organization [WHO] Child growth standards, http://www.who.int/childgrowth/standards), without preference for any of the disorders.

The male-to female ratio was 1.4 for the cblC defect and 0.85 for the MTHFR deficiency sample ($P = 0.049$ and $P = 0.572$; not significant). For the cblD, cblE, cblG and cblJ disease groups, samples were too small for meaningful conclusions on gender distribution. Figure 1 shows the distribution of birth years, the cumulative number of patients identified by newborn screening (NBS), diagnoses and age at enrolment visit. Core medical history data by disease are presented in Table 1.

Information on age at disease presentation was available for 152 clinically diagnosed patients and ranged from immediately after birth to 42 years of age (median 30 days); 75% of the patients had first symptoms within their first seven months of life. The median age at diagnosis was 3.7 months (range 3 days to 56 years). The median diagnostic delay, defined as the time between first symptoms and diagnosis, was 24 days; 75% of diagnoses were made within 4.6 months; the maximum diagnostic delay was 17.4 years (Table 1).

Measurement of tHcy, plasma amino acid profiles and urinary organic acids, namely MMA, were the most widely used identifiers for the disorders; acylcarnitines played a less prominent role. For confirmation, molecular genetic testing has clearly superseded enzymatic testing in recent years.

3.1.1 | Genetic data

Genetic data were available for 117 individuals with the cblC defect: 52 patients were homozygous for c.271dupA, 39 patients carried c.271dupA combined with other mutations and 26 patients had other genotypes (Supplementary Table S4). The subgroups were not significantly different for age and clinical pattern at disease presentation.

The frequency of the c.271dupA allele ranges from 0.0008 to 0.00015% in exome databases. The frequency of 143 of 234 (61%) mutant alleles in the cblC patients included in this study was higher than that reported by[10] (40%) and[6] (49%), but concordant with the Italian-Portuguese cohort reported by[11] (55%) and the Italian-Spanish-Portuguese cohort reported by[12] (85%). Information on a possible effect of enriching for consanguinity was unavailable.

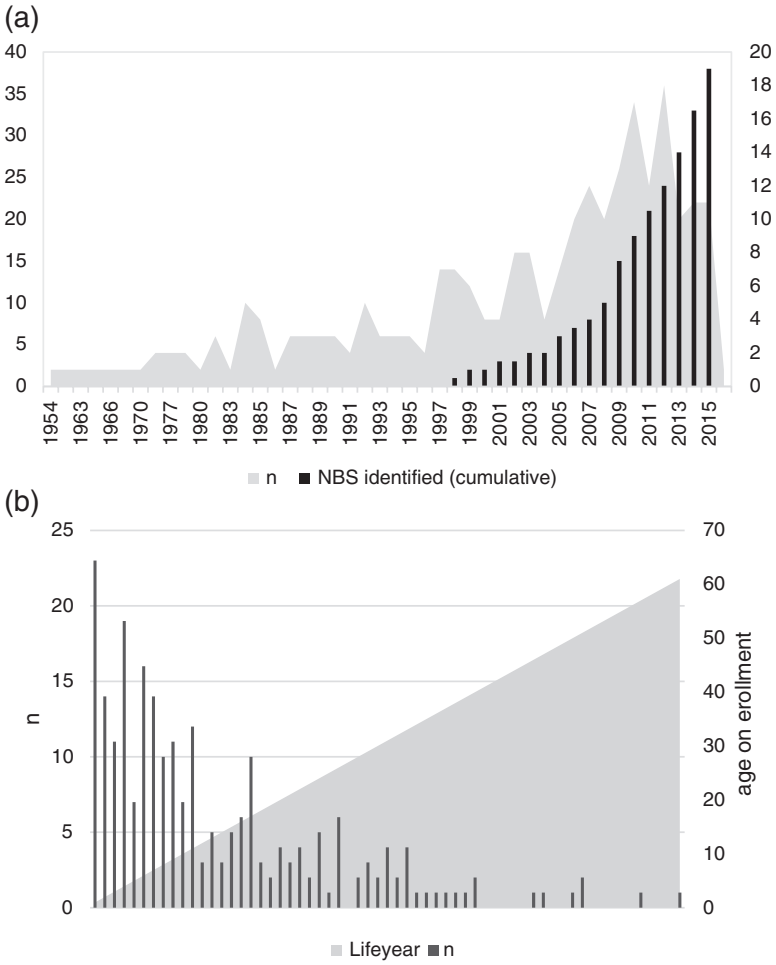
Genetic data were available for 23 patients with MTHFR deficiency; most of them carried private mutations and genotype-phenotype correlations were not observed (Supplementary Table S5).

3.2 | Cross-sectional analyses

3.2.1 | Disease presentation in the cblE, cblG, cblD and cblJ defects

The study cohort included ten patients with cblE, 11 with cblG, five with cblD and one patient with cblJ disease. The main clinical, biochemical and treatment information on these patients are summarised in Supplementary Tables S1–S3.

Disease presentations in cblE disease encompassed mainly anaemia ($n = 10$), specified as macrocytic in seven patients, developmental delay ($n = 5$), feeding problems



§= preclinical diagnosis; #= clinical diagnosis; *= undefined mode of diagnosis

FIGURE 1 (A) Study cohort ($n = 238$) by birth year, the cumulative number of patients identified by newborn screening (NBS) per year is indicated; (B) age on enrolment; (C) distribution of diagnoses (mode of diagnosis indicated)

($n = 5$) and hypotonia ($n = 4$), multisystem involvement with hepatosplenomegaly, cardiac or renal failure ($n = 3$), eye disease ($n = 2$), seizures ($n = 1$) and neutropenia ($n = 1$). Patients were treated mostly with betaine, folinic or folic acid and OH-Cbl with good biochemical response (Supplementary Table S1).

TABLE 1 Core medical history data ($n = 238$)

	cblC ($n = 161$)	MTHFR ($n = 50$)	cblG ($n = 11$)	cblE ($n = 10$)	cblD ($n = 5$)	cblJ ($n = 1$)	All
Gender							
Male	93	23	6	3	3	1	129
Female	68	27	5	7	2	0	109
Ethnic background							
White	136	38	10	10	5	1	200
Asian	9	8					17
Mixed	7		1				8
Black	3	1					4
Unknown	6	3					9
Pre-clinically diagnosed							
○ NBS	35	3					38
○ High-risk family screening	5	3	1				9
○ Prenatal	7	1					8
Clinically diagnosed	113	43	10	10	5	1	181
Unknown	1						1
Age at first symptoms in pre-clinically diagnosed patients	$n = 23$[a]	$n = 5$[a]					
P25	3 d						
P50 (median)	7 d	17[b]					
P75	15 d						
Age at first symptoms in clinically diagnosed patients	$n = 93$	$n = 38$	$n = 8$	$n = 8$	$n = 4$	$n = 1$, 35 d	152
P25	8 d	28 d	1.9 mo	7 d	2 mo		11 d
P50 (median)	21 d	3 mo	2.3 mo	1 mo	1 yr		1 mo
P75	3.9 mo	4 y	6.2 mo	3 mo	7.9 y		6.8 mo
Age at diagnosis[c]	$n = 106$	$n = 42$	$n = 10$	$n = 10$	$n = 4$	$n = 1$, 90 d	173
P25	28 d	35 d	3.2 mo	34 d	3 mo		29 d
P50 (median)	2.7 m	5.5 m	6.5 mo	4.4 mo	5.3 y		112 d
P75	14 mo	9.8 y	4.4 y	3 y	16.8 y		4 y
Diagnostic delay[c]	$n = 92$	$n = 38$	$n = 8$	$n = 8$	$n = 4$	$n = 1$	151
P25	0 d	0 d	31 d	1 d	7 d	55 d	0 d
P50 (median)	18 d	21 d	48 d	31 d	34 d		24 d
P75	2.7 mo	10 mo	3.1 y	3.5 mo	13 y		4.6 mo
Laboratory tests							
Homocysteine	139	47	11	8	4	1	210
Amino acid analysis	128	38	9	8	4		187
Organic acids	118	7	6	2	3		136
Acylcarnitines	79	3	1	1	1		85
Enzymatic results reported	13	8	7	3	1	1	33
Mutations reported	117	23	7	8	3	1	159
tHcy µmol/L before treatment[d]							
Mean ± SD	131 ± 69	182 ± 91	128 ± 43	119 ± 43	($n = 2$) 155[b]	127[b]	141 ± 73

TABLE 1 (Continued)

	cblC ($n = 161$)	MTHFR ($n = 50$)	cblG ($n = 11$)	cblE ($n = 10$)	cblD ($n = 5$)	cblJ ($n = 1$)	All
P25	65	140	76	83			83
P50 (median)	123	179	131	109			135
P75	197	219	176	163			196

d = days; mo = months; y = year(s); NBS = newborn screening; SD = standard deviation

[a]Nineteen of 47 pre-clinically diagnosed patients with the cblC defect were symptomatic in the first month of life; 4 patients between months 2 and 5. Five of 7 pre-clinically diagnosed patients with MTHFR deficiency were symptomatic in the first month of life

[b]Samples too small for calculation of SD or quartiles

[c]Only clinically diagnosed patients

[d]Met and MMA concentrations at disease presentation: not documented in the registry

Disease presentations in cblG disease encompassed developmental delay ($n = 10$), feeding problems ($n = 8$), anaemia ($n = 7$), specified as macrocytic in five and microcytic in two patients, seizures and hypotonia (both $n = 6$), eye disease ($n = 3$) and neutropenia ($n = 2$). Patients were treated mostly with betaine, folinic or folic acid and OH-Cbl with good biochemical response (Supplementary Table S2).

The five patients with cblD disease presented mainly with developmental delay and seizures, and the cblJ patient presented with hypotonia and respiratory failure. Patients were treated with betaine, folic acid and OH-Cbl with good biochemical response (Supplementary Table S3).

3.2.2 | Disease presentation in the cblC defect and MTHFR deficiency

For the patients with the cblC defect [$n = 161$; $n = 93$ (58%) males] and MTHFR deficiency ($n = 50$; $n = 23$ [46%] males), the disease presentation data are depicted in Figure 2. Generally, both diseases primarily affect the central nervous system with developmental delay, muscular hypotonia and sometimes seizures. Failure to thrive/feeding difficulties are common (46% in MTHFR deficiency and 59% in the cblC defect). The characteristic anaemia in the cblC defect as well as eye symptoms, renal disease and thromboembolism were present in 15-28% of cases. The frequently reported apnoea[13] was encountered in only two patients (4%) with MTHFR deficiency.

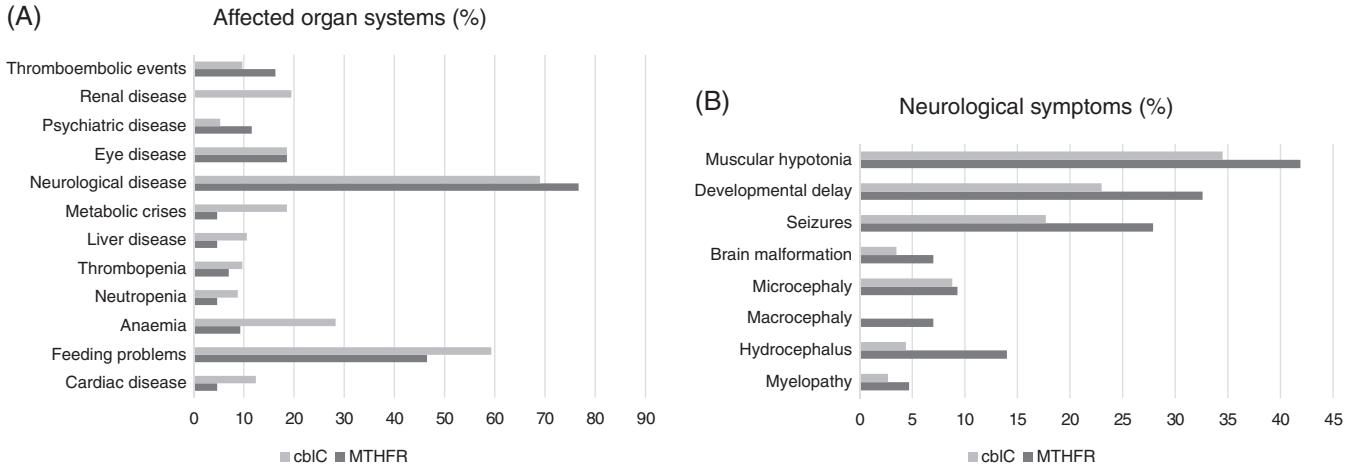
Ophthalmological problems in the cblC defect encompassed nystagmus, significant visual impairment/blindness, retinopathy and macular/optic nerve atrophy. Ophthalmological symptoms in MTHFR were multifaceted, with retinal haemorrhage, nystagmus, unspecified visual impairment and strabismus, myopia, atrophy of the iris and optical atrophy. Of 26 metabolic crises in cblC patients at disease presentation, 21 were specified as metabolic acidosis, two as hyperammonaemia; three courses remained unspecified. Metabolic crises in MTHFR patients were rare and less severe, specified as acidosis ($n = 1$) and sudden decrease of Met and increase of tHcy ($n = 2$). Thromboembolism or stroke occurred in 10% of cblC and 16% of MTHFR patients; renal disease was present in 20% of cblC cases, in the majority specified as haemolytic uraemic syndrome (HUS). The spectrum of cardiac disease in cblC disease (12%) encompassed predominantly cardiac malformations and cardiomyopathy; in MTHFR deficiency, cardiac involvement is rare and less severe. Liver affections are rare and generally mild in both diseases (Figure 2).

Clinically identified patients with the cblC defect presented with first symptoms significantly earlier compared to patients with MTHFR deficiency ($P < 0.001$; Table 1). Diagnostic delay was not significantly different. Total Hcy was significantly higher in patients with MTHFR deficiency compared to cblC patients at disease presentation, enrolment visit and at last follow-up (all $P < 0.001$) (Figure 3).

3.2.3 | Disease presentation in early- and late-onset cblC and MTHFR patients

Information on age at disease presentation was available for 114 patients with cblC disease and 44 patients with MTHFR deficiency. Eighty-nine percent ($n = 101$) of patients with cblC disease presented as early and 11% ($n = 13$) as late onset; 68% ($n = 30$) of patients with MTHFR deficiency presented as early and 32% ($n = 14$) as late onset.

Clinical patterns differ between early- and late-onset disease in both disorders (Figure 4). However, statistical significance was reached only for thromboembolic events, psychiatric disease (both $P < 0.001$) and renal disease ($P = 0.004$, cblC only), which were all more frequent in late-onset disease. There was myelopathy at presentation in two late-onset MTHFR deficiency (14%) and two late-onset cblC (15%) patients, but in only one (1%) early-onset cblC patient (numbers too small for statistical analysis). Cardiac disease is mainly seen in early-onset disease and seems very rare in MTHFR deficiency. In the cblC defect, five patients each presented with cardiac malformation and cardiomyopathy. No statistically significant difference between early- and



(C)

	clbC	MTHFR
Thromboembolic events	**n=11**	**n=7**
- Stroke	n=3	n=1
○ *with sagittal sinus thrombosis;*	*n=1*	*n=1*
○ *with haemolytic uraemic syndrome (HUS)*	*n=1*	
- Pulmonary embolism	n=2	n=1
- Deep venous thrombosis	n=3	n=2
- Other thromboembolic events associated with HUS	n=3	-
- Isolated sagittal sinus thrombosis	-	n=1
- Infarct of anterior spinal artery	-	n=1
- Retinal artery thrombosis	-	n=1
Renal disease	**n=22**	**n=0**
- HUS	n=17	-
○ *with acute renal failure*	*n=1*	-
○ *n=1 with chronic renal failure*	*n=1*	-
○ *n=2 with proteinuria / nephrotic syndrome*	*n=2*	-
- Neurogenic bladder	n=1	-
- Chronic renal failure	n=2	-
- Erythrocyturia	n=1	-
Liver disease	**n=12**	**n=2**
- Hepatomegaly	n=9	-
○ *with cholestasis*	*n=1*	-
- Liver synthesis impaired /coagulopathy	n=2	
- Indirect hyperbilirubinaemia	n=1	n=1
- Transaminases elevated	n=1	-
- Excretion of phenolic acids in urine	-	n=1
Cardiac disease	**n=11**	**n=2**
○ Cardiomyopathy	n=5	
○ *Specified as hypertrophic*	*n=2*	
○ *Unspecified*	*n=3*	
○ Cardiac malformation	n=5	n=1
○ *Patent foramen ovale*	*n=3*	
○ *Atrial septum defect*	*n=2*	
○ *Unspecified*		n=1
○ Cardiac arrest / heart failure / insufficiency	n=1/ n=1/ -	-/- / n=1
Arterial hypertension	**n=3**	

FIGURE 2 (A) Affected organ systems in clinically diagnosed patients with the cblC defect ($n = 113$) and MTHFR deficiency ($n = 43$) at disease presentation, (B) neurological symptoms in detail and (C) details on other affected organ systems

late-onset patients were observed for tHcy and MMA (cblC patients only).

For 78 patients with early-onset cblC, disease information on genotype was available. Forty-two patients (54%) were homozygous for c.271dupA, 22 (28%) patients were compound heterozygous for c.271dupA and another mutation, and the remaining patients had other genotypes (Supplementary Table S4). For eight patients with late-onset cblC disease, information on genotype was available. Some mutations (c.82-1G > A, c.276G > T, c.565C > A, c.388

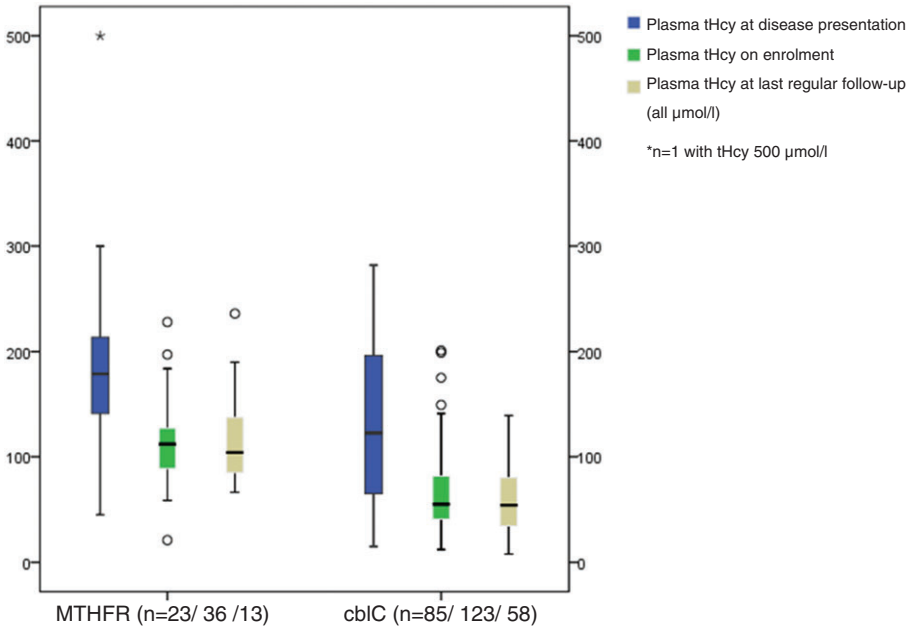


FIGURE 3 Plasma tHcy in patients with the cblC defect and MTHFR deficiency at disease presentation, on enrolment and at last regular follow-up

T > C) are known to be associated with late-onset disease, while others (c.347 T > C, c.389A > G, c.566G > A) have been observed in late-onset disease but also in patients with missing information on age at onset. These results are concordant with the observations of others.[10,12,14]

Genetic data were available for 23 patients with MTHFR deficiency; most of them carried private mutations. Genotype-phenotype correlations were not observed (Supplementary Table S5).

4 | LONGITUDINAL STUDY

4.1 | Observational period

Seventy-six patients with the cblC defect and 27 patients with MTHFR deficiency had at least one regular follow-up. The length of the observational period was 127 patient years for the study cohort, 95 years for patients with the cblC defect, 31 years for patients with MTHFR deficiency and one year for the cblJ patient. For cblE, cblG and cblD patients, only enrolment visits were documented.

4.2 | Fatal disease course and emergency visits

A fatal disease course occurred in one patient with MTHFR deficiency. One or more emergency visits after enrolment were documented for seven patients with the cblC defect (0.07/patient year) and four patients with MTHFR deficiency (0.13/patient year) and one cblE patient, suggesting a disease course generally free from acute deterioration events in the majority of patients.

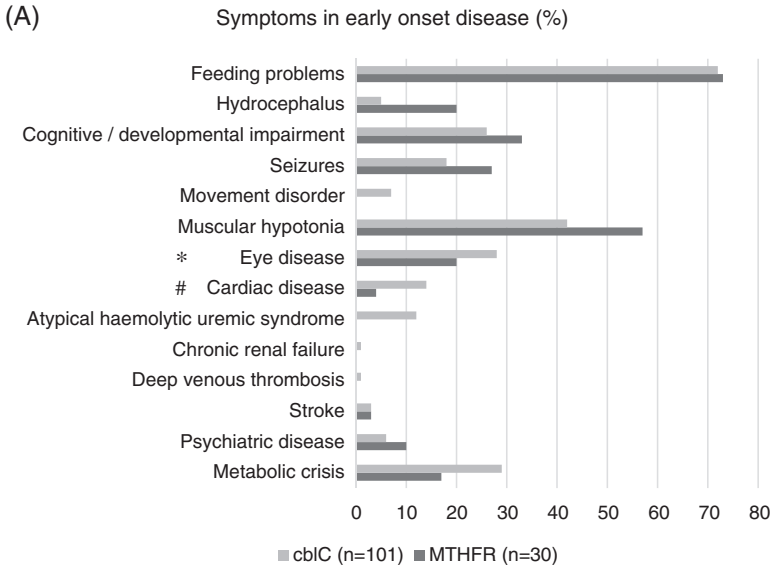
4.3 | Disease course in cblC disease and MTHFR deficiency

4.3.1 | From disease presentation to enrolment visit: the effect of treatment initiation

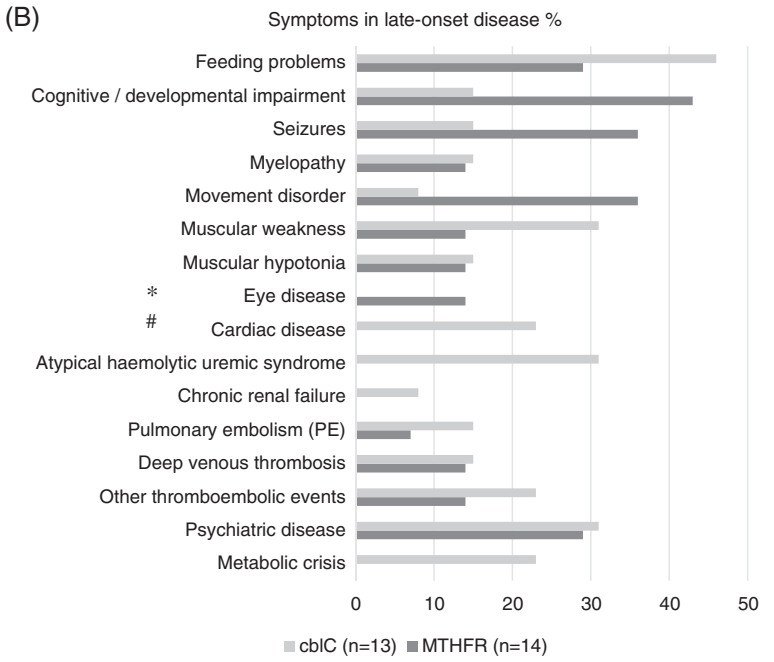
Between disease presentation and enrolment visit, frequencies of feeding difficulties (MTHFR, cblC: $P < 0.001$) and neurological incidents (MTHFR, cblC: $P < 0.001$) were significantly reduced. In contrast, eye disease and behavioural problems became more frequent in cblC disease (both $P < 0.001$). Behavioural problems increased less prominently in MTHFR disease ($P = 0.021$). Frequencies of other main organ involvements or symptoms including thromboembolic events and renal disease were generally low and did not change significantly between disease presentation and enrolment visit. Total Hcy concentrations were significantly higher at disease presentation compared to the values on treatment at enrolment (MTHFR: $P = 0.006$; cblC: $P < 0.001$). Data on MMA and Met concentrations were not documented at disease presentation.

4.3.2 | From enrolment visit to last regular follow-up: long-term effects of treatment

Between enrolment and last regular visit, in the vast majority of patients, "general state and overall well-being" (cblC: 88%; MTHFR: 80%) and "major disease symptoms" (cblC 81%; MTHFR: 72%) were considered stable or even improved over time. The frequency of feeding difficulties remained stable. Acute neurological incidents ($P = 0.008$) were less frequently observed in cblC patients. Frequencies



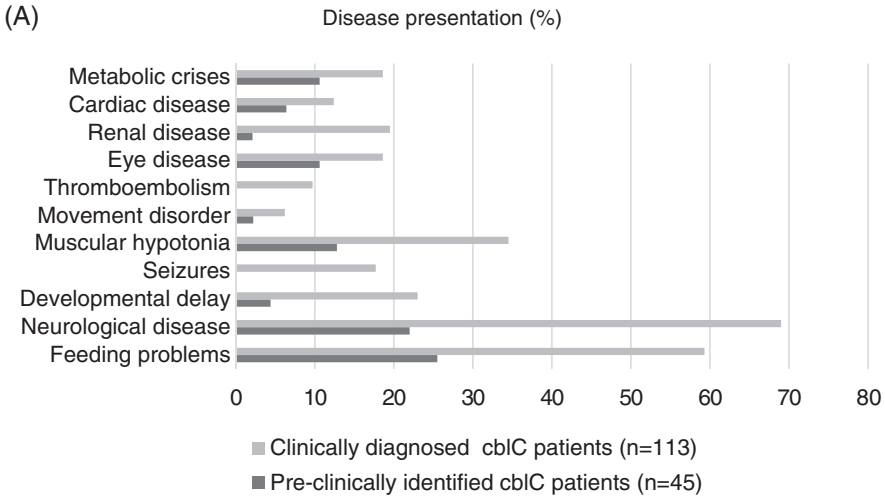
*MTHFR: strabismus (n=2); retinal haemorrhage (n=1); visual impairment and saccadic movements (n=1); myopia (n=1); nystagmus (n=1)
*cblC: Nystagmus (n=13); strabismus (n=5); visual impairment / retinopathy / maculopathy (n=12)
#MTHFR: cardiopulmonary insufficiency (n=1)
#cblC: cardiac malformation (n=5) specified as patent foramen ovale (n=3) and atrial septum defect (n=2); cardiomyopathy (n=5): specified as hypertrophic (n=2) and unspecified (n=2); high blood pressure (n=2); cardiac hypertrophy (n=1), cardiopulmonary arrest (n=1)

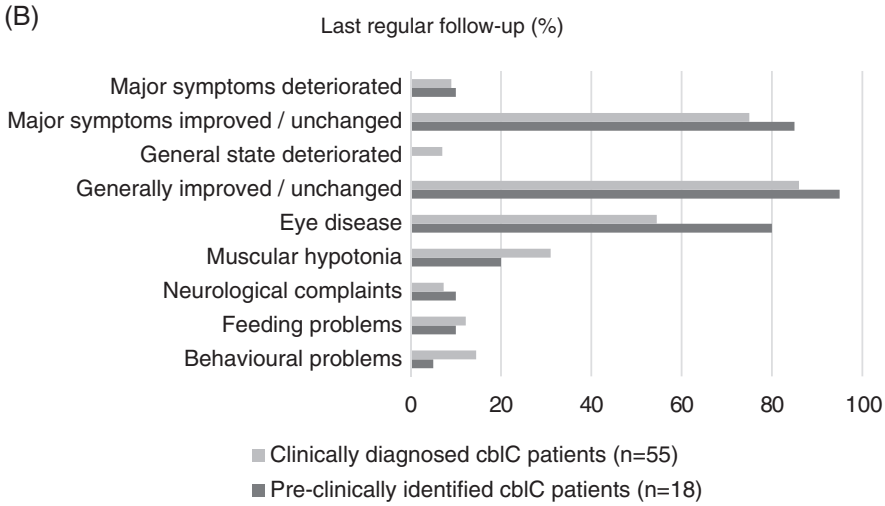


*MTHFR: visual impairment (n=1); bilateral optic atrophy (n=1)
*cblC: -
#MTHFR: -
#cblC: cardiac failure (n=1); high blood pressure and mild mitral valve insufficiency (n=1)

FIGURE 4 Comparison of disease presentation in patients with the cblC defect and MTHFR deficiency with: (A) early-onset disease (<12 months) and (B) late-onset disease (>12 months) (all modes of diagnosis included) *MTHFR: strabismus ($n = 2$); retinal hemorrhage ($n = 1$); visual impairment and saccadic movements ($n = 1$); myopia ($n = 1$); nystagmus ($n = 1$) *cblC: Nystagmus ($n = 13$); strabismus ($n = 5$); visual impairment/retinopathy/maculopathy ($n = 12$) #MTHFR: cardiopulmonary insufficiency ($n = 1$) #cblC: cardiac malformation ($n = 5$) specified as patent foramen ovale ($n = 3$) and atrial septum defect ($n = 2$); cardiomyopathy ($n = 5$): specified as hypertrophic ($n = 2$) and unspecified ($n = 2$); high blood pressure ($n = 2$); cardiac hypertrophy ($n = 1$), cardiopulmonary arrest ($n = 1$)

of all other main organ involvements remained unchanged between enrolment and last regular visit. MMA (cblC only) concentrations and Met concentrations (both diseases) were stable on treatment. Met was generally normal in treated patients; the median urinary MMA was ameliorated but still elevated at 103 mmol/mol creatinine (see Figure 5C). Total Hcy concentrations were not significantly different between enrolment and last regular follow-up.





(C)

Last regular follow-up	tHcy µmol/L	Met µmol/L	MMA (plasma) µmol/L	MMA (urine) mmol/mol crea
Pre-clinically identified				
N	15	12	4	2
Median	55	23	6	-
Range	16-139	10-40	4.4-24	-
Clinically identified				
N	42	32	13	16
Median	52	24	7	103
Range	8-113	9-53	1-88	4-2546

FIGURE 5 Pre-clinically vs clinically identified patients with the cblC defect: (A) clinical symptoms at disease presentation and (B) last regular follow-up; **c** biochemical parameters at last regular follow-up

4.4 | Disease course in early-onset compared to late-onset cblC disease and MTHFR deficiency

4.4.1 | From enrolment visit to last regular follow-up

In early-onset disease, at least one regular follow-up visit was documented for 52 patients with the cblC defect and 13 patients with MTHFR deficiency. In the early-onset cblC cohort ($n = 52$), general state and well-being and major disease symptoms were considered stable or improved at last regular follow-up compared to enrolment in 89% ($n = 46$) and 81% ($n = 42$) of patients, respectively. The main persisting symptoms were ophthalmological or oculomotor ($n = 36$; 69%), specified as nystagmus ($n = 26$) and/or maculopathy ($n = 14$), muscular hypotonia ($n = 19$; 37%), feeding problems ($n = 8$; 15%), movement disorders ($n = 8$; 15%) and behavioural problems ($n = 7$; 14%).

In patients with early-onset MTHFR deficiency ($n = 13$), general state and well-being and major disease symptoms were considered improved or unchanged from enrolment to

last regular follow-up in 10 patients (77%), while deterioration was perceived in a single individual. The main persisting symptoms were nystagmus ($n = 2$), unspecified eye disease ($n = 1$), seizures ($n = 3$), feeding difficulties ($n = 2$) and hypotonia ($n = 1$).

Seven patients with late-onset cblC defect had at least one regular follow-up visit. General status and well-being was considered normal in six patients and abnormal in one patient, and major disease symptoms had improved or remained unchanged in six of seven individuals. Behavioural problems, muscular hypotonia and seizures were each present in a single patient. No eye disease, feeding difficulties, acute neurological or thromboembolic incidents were reported.

The pattern is quite similar for the nine patients with late-onset MTHFR deficiency with at least one regular follow-up visit. General status and well-being was considered normal in six and abnormal in two individuals. Major disease symptoms had improved or remained unchanged in six individuals and deteriorated in one individual. Nystagmus persisted in two individuals, unspecified eye disease in one patient, behavioural problems and seizures in two patients each, while feeding difficulties, acute neurological incidents and thromboembolism were not observed at follow-up.

We did not observe statistically differences of tHcy and MMA (cblC patients only) between early- and late-onset patients at last regular follow-up. This finding is probably biased due to the small size of the late-onset cohorts and the wide distribution of the parameters.

4.5 | Best responders to treatment (cblC disease only)

In the cblC sample, we looked for differences between "best biochemical responders to treatment", defined as tHcy concentrations on enrolment in the lowest quartile (≤ 25th percentile = 40.9 μmol/L) and patients with tHcy values in the higher quartiles: 25th-50th percentile: 55 μmol/L; 50th-75th percentile: 82 μmol/L; > 75th percentile: > 82 μmol/L.

We compared all quartiles as well as extreme groups (quartiles 1 and 4). The tHcy quartile at enrolment was not significantly related to symptoms, age or tHcy quartile at disease presentation, nor to the genotype, gender, clinical or pre-clinical diagnosis or treatment modalities. Older patients had a significantly higher frequency of tHcy values >75th percentile ($P < 0.001$) on enrolment and at last regular follow-up; this confirms the well-known association between age and tHcy.[15] Corresponding analyses were not performed for MTHFR due to the smaller sample size.

4.6 | Comparison of pre-clinically and clinically diagnosed cblC and MTHFR patients

Delay to diagnosis did not correlate with outcome parameters, in cblC disease, MTHFR deficiency or in the complete study cohort.

Of the pre-clinically diagnosed cblC cases ($n = 47$), 35 had been identified by NBS, five by high-risk family and seven by prenatal screening. Two patients identified by high-risk family screening were detected late (14 months/8.7 years) and, thus, excluded from further analyses of pre-clinically diagnosed cases ($n = 45$). Despite early detection, 23 patients (49%) were considered symptomatic. Seventy-five percent of the pre-clinically diagnosed patients became symptomatic within the first 15 days of life (Table 1), with predominantly neurological symptoms combined with renal disease and metabolic crises.

However, comparison of pre-clinically and clinically diagnosed individuals revealed that all clinical symptoms were more frequent in the clinically diagnosed individuals ($n = 113$) (Figure 5). For neurological disease ($P = 0.005$), this difference reached significance. For renal disease ($P = 0.068$) and all other organ manifestations, as well as tHcy levels, no statistically significant differences were detectable.

Likewise, we compared core clinical data at last regular visit between clinically ($n = 55$) or pre-clinically ($n = 18$) diagnosed patients with the cblC defect. No statistically significant differences were found between pre-clinically and clinically diagnosed patients for any major disease symptom or biochemical marker at last regular follow-up.

Of the pre-clinically diagnosed patients with the cblC defect, 43% were homozygous and 12.5% heterozygous for c.271dupA; the c.328_331delAACC deletion was present in 13% of cases (two homozygous, two heterozygous). In the clinically diagnosed group, 41% were homozygous and 41% were heterozygous for c.271dupA. In the latter, no predominant second mutation was present.

Seven patients with MTHFR deficiency had been identified pre-clinically either by NBS ($n = 3$), prenatal testing ($n = 1$) or high-risk family screening ($n = 3$; two younger than one week old and one symptomatic patient aged 24 years at diagnosis were excluded from further analyses). Despite early detection, four of six patients were considered clinically symptomatic early in life (median 17 days) with feeding difficulties and neurological symptoms; one patient even had a metabolic crisis. Due to the small sample size, we conducted no further statistical analyses.

4.7 | Modalities of treatment and outcome

Treatment was highly heterogeneous with respect to dosages (Supplementary Table S6) and combinations of drugs

(Tables 2 and 3). No significant correlations were present between treatment modalities (betaine, OH-Cbl, folic/folinic acid, carnitine) and biochemical (tHcy, Met; MMA in cblC disease) or any clinical outcome parameter in either MTHFR or cblC disease.

On enrolment, 127 patients with cblC disease (79%) were treated with parenteral OH-Cbl (mean 0.13; median 0.07 mg/kg/d) and 126 (78%) with oral betaine (mean 163; median 160 mg/kg/d). Carnitine was given to 30 (19%) patients (mean 51; median 39 mg/kg/d). Additional treatments for cblC disease were folic acid (n = 58 [36%]; mean 0.4, median 0.2 mg/kg/d) and folinic acid (n = 45 [28%]; mean 0.6, median 0.3 mg/kg/d). One patient each received N-acetylcysteine, creatine monohydrate, acetylsalicylic acid, ascorbic acid and riboflavin.

On enrolment, 41 (82%) of the patients with MTHFR deficiency were treated with oral betaine (mean 165; median 124 mg/kg/d). The 17 patients (34%) with MTHFR deficiency treated with parenteral OH-Cbl received between one and seven intramuscular injections/week. The mean dosages for folic acid (n = 18; 36%) and folinic acid (n = 18; 36%; data on dosage available for n = 14) were 0.15 and 1.05 mg/kg/d (medians 0.08 and 0.8 mg/kg/d, respectively). Thirteen patients (26%) received oral riboflavin (mean 1.4, median 0.49 mg/kg/d), five patients (10%) acetylsalicylic acid and a single patient 5-methyltetrahydrofolate. In both

TABLE 2 Combinations of the four most frequently used treatment modalities betaine, OH-Cbl, folate/folinic acid and carnitine and dietary treatment in 161 patients with the cblC defect and 50 patients with MTHFR deficiency (dose ranges in Supplementary Table S6)

cblC defect	**n**	**%**
OH-Cbl & betaine	15	9.3
OH-Cbl &betaine & folic/folinic & carnitine	57	35
OH-Cbl &betaine & folic/folinic	20	12
OH-Cbl & betaine & carnitine	17	11
OH-Cbl	8	5
OH-Cbl & folic/folinic	6	4
OH-Cbl & folic/folinic & carnitine	3	2
OH-Cbl & carnitine	1	0.6
Betaine	5	3
Betaine & folic/folinic	5	3
Betaine & carnitine	1	0.6
Betaine & folic/folinic & carnitine	6	4
Folic/folinic	1	0.6
Folic/folinic & carnitine	1	0.6
Carnitine	1	0.6
None of these substances	14	9
Dietary treatment	***n***	***%***
Low-protein/low-methionine diet*	20	12.4
Low-protein diet & special foods*#	15	9.3
Low-protein diet & special foods*# & methionine	2	1.2
Low-protein diet & valine*	4	2.4
Low-protein diet & methionine*	1	0.6
Low-protein diet & methionine & valine*	1	0.6
Methionine supplementation only	11	6.8
Normal-protein diet without supplementation	107	66.4

White: No drugs as recommended in guidelines
Light grey: One drug with proven effect ± other drugs
Grey: Two drugs with proven effect ± other drugs
Dark grey: Drugs with proven effect only
*Guidelines recommend against the use of low-protein/low-methionine diet ± amino acid blends
#Valine and methionine-free, low isoleucine and threonine amino acid blends

TABLE 3 Combinations of the four most frequently used treatment modalities betaine, OH-Cbl, folate/folinic acid and carnitine and dietary treatment in 161 patients with the cblC defect and on enrolment (dose ranges in Supplementary Table S6)

MTHFR deficiency	n	%
Betaine & OH-Cbl & folic/folinic	14	28
Betaine & folic/folinic	13	26
Betaine	8	16
Betaine & OH-Cbl & folic/folinic & carnitine	2	4
Betaine & OH-Cbl	2	4
Betaine & folic/folinic & carnitine	2	4
OH-Cbl	1	2
Folic/folinic	3	6
None of these substances	5	10

White: No drugs as recommended in guidelines
Grey: Drug with proven effect ± other drugs
Dark grey: Drug with proven effect only

groups, some individuals were treated with oral cyanocobalamin ($n = 8$ cblC; $n = 9$ MTHFR) and pyridoxine ($n = 16$ cblC; $n = 12$ MTHFR).

Forty-six individuals [43 of 161 (27.9%) cblC and 3 of 50 (6%) MTHFR deficiency patients] adhered to a special dietary treatment with natural protein intake from 0.3 to 1.5 g/kg/d (mean 1.19, median 1.1 g/kg/d; $n = 43$). Supplementary mean methionine intake was 22 mg/kg/d (median 20 mg/kg/d); synthetic amino acids were used by 18 patients (mean 1.0 g/kg/d, median 1.2 g/kg/d).

Of the 43 patients with the cblC defect treated with diet, 34 were followed by 16 European and nine by three US centres. Seventeen patients received amino acid blends (valine and methionine-free, low in isoleucine and threonine). Fifteen patients were supplemented with methionine and a single centre supplemented valine. Some centres treated all of their patients (documented in the registry) with diet, while other centres treated only selected patients.

At the last regular follow-up, 78% of patients with the cblC defect ($n = 77$) were treated with OH-Cbl, 70% with betaine, 61% with carnitine, 46% with folic and 16% with folinic acid; 33% followed a special diet. Eighty percent of patients with MTHFR deficiency ($n = 25$) were treated with betaine, 40% with OH-Cbl, 28% with folinic and 12% with folic acid; a single patient had dietary treatment.

5 | DISCUSSION

Analyses of registry data can never replace a prospective clinical trial and this study has several limitations. Subjects were enrolled at random ages and disease durations. Furthermore, the duration of observation varied, as well as the completeness and quality of the entered data, because all collaborating centres handled data collection according to local standards and resources. Moreover, any investigation of the impact of treatment on outcome based on registry data is inevitably limited, especially in a "young" registry with a short follow-up period. Due to these limitations, we have taken a most conservative statistical approach in analyzing the data. Conversely, these data are valuable because they represent the largest and most systematically documented cohorts of patients with the cblC defect and MTHFR deficiency; they also shed some more light on the very rare inborn errors of intracellular cobalamin metabolism, the cblD, cblE, cblG and cblJ defects. For the latter, we have presented an overview of clinical, treatment and outcome parameters for each disorder.

The most widely used tests used to identify the diseases are tHcy and MMA in urine. Acylcarnitines, namely C3 or the recently suggested marker C17, which have their place in NBS,[16] are rarely used in the clinical search for (combined) remethylation disorders. Molecular genetic studies have recently become the first-line approach to confirm the diagnosis.

The incidences of prematurity in the study cohort and the subgroups of cblC and MTHFR patients were comparable to the general population.[9,17] Prenatal damage may, however, be responsible for the microcephaly (5%) or intra-uterine growth retardation (14%) seen in a minority of patients.

In this population, the cblC defect is the most frequent remethylation disorder. Since some European countries have recently introduced NBS, increasing numbers of identified patients can be expected.[18]

For cblC disease, severe organ manifestations such as thromboembolic events, cardiac or small vessel involvement (e.g. atypical HUS, pulmonary hypertension) were

rarer in this sample than previously reported.[6,19,20] High mortality has been reported in patients with severe renal disease (up to 44%,[21]) or pulmonary hypertension.[22] Deceased patients (especially those dying young) are less likely to be included in a registry like the E-HOD that does not have active ascertainment of deceased patients. Due to this potential bias, the very low mortality and incidence of pulmonary hypertension and acute renal disease in our sample must be interpreted with caution. Nevertheless, there is no doubt that there has been an enormous improvement in the survival of patients with the cblC defect over the last two decades. Earlier series reported a disease-related mortality of 26% ($n = 50$)[20] or 11% ($n = 88$).[6] Mortality in MTHFR deficiency has not formally been investigated but several fatal courses have been reported[23] and the trend may be similar.

Early diagnosis by pre-clinical screening may be one reason for this observation. Early diagnosis by means of NBS is associated with decreased mortality and less severe organ damage.[24,25] Furthermore, the diagnostic delay for clinically presenting cases was relatively short in our sample. The widespread availability of tHcy measurement and the growing familiarity with MMA as a parameter in acquired vitamin B12 deficiency[26] probably contributed to this, as well as the increased awareness for rare diseases in general and remethylation disorders in particular. Selection bias due to the predominant recruitment by large metabolic centres may also be relevant.

Our data on disease presentation confirm that most patients become symptomatic very early in life. A novel observation is that cblC patients present significantly earlier than MTHFR patients (median 21 days vs. 3 months). Although the samples of individuals with the cblD, cblE or cblG defect are small, they suggest that the onset of first symptoms in these disorders is slightly later than in the cblC defect. This pattern may help clinicians to suspect the correct remethylation defect before confirmation analyses are available.

At presentation, the clinical fingerprint of the early-onset remethylation disorders is clearly a multisystem disease dominated by severe neurological symptoms and failure to thrive. These findings corroborate the phenotype described by others.[6,13,20] Anaemia, neurocognitive impairment, feeding difficulties and eye disease are predominant features of the clinically indistinguishable cblE and cblG defect and in the very rare cblD and cblJ defects.

For late-onset remethylation disorders, we confirm the additional hallmarks of thromboembolic, psychiatric and renal disease.[5,19] Cardiac disease was not frequent in this study, being limited to a small number of mostly early-onset cblC patients. The cardiac malformations in five patients indicate prenatal damage.

Pre-clinical diagnosis was associated with significantly fewer neurological incidents (e.g. seizures). Due to the low mortality in the registry cases, we cannot draw conclusions on the impact of pre-clinical diagnosis on mortality, but the pattern of manifestations suggests less severe, life-threatening disease in pre-clinically diagnosed individuals. When investigating the outcome at last regular follow-up, however, we could not demonstrate an improved outcome in patients identified pre-clinically compared to the clinically diagnosed group, nor a significant correlation between the delay in diagnosis and any outcome parameters. We believe that the small numbers of screened patients and the absence of long-term follow-up data in the registry may partly explain this finding.

Treatment corrects Met, decreases tHcy and MMA (cblC) concentrations and reduces the severity of clinical symptoms and incidence of complications such as neurological incidents and feeding difficulties. Following treatment, the general health and major disease symptoms are stable or even improve in the vast majority of patients, and life-threatening organ damage is rare. The low number of metabolic crises and emergency visits further support the impression of a stable course over time.

Although we can thus conclude that treatment in general is beneficial, we cannot make statistically sound comments on the effect of single drugs or treatment regimens, due to the heterogeneity of the treatments used. It is striking that treatment practice often differs from the evidence-based recommendations in the recently published guidelines for remethylation disorders.[4]

In the cblC defect, the variety of treatment approaches is broad. Fewer than 70% of patients receive both OH-Cbl and betaine; for these two drugs, the clinical evidence of efficacy is sound. In most cases, OH-Cbl and betaine are combined with medications of unproven benefit. About 20% of the patients omit either OH-Cbl or betaine and treatment in 11% of cblC patients follows other schemes, including cyanocobalamin in some cases, which is known to be ineffective. In MTHFR deficiency, more than 80% of patients receive betaine, mostly combined with add-on medications; 18% follow a regime without betaine, the drug of choice for this disease.[23] It is noteworthy that on enrolment 34% and at last regular visit 40% of 25 MTHFR patients were treated with parenteral OH-Cbl, which has no proven benefit in this disease but causes considerable discomfort.[27] In addition, folic acid, which should be avoided in this disease,[4] was given to 36% of patients with MTHFR deficiency on enrolment.

As first reported by Manoli et al.,[28] our data show that 19 of 47 European and US centres apply dietary treatment despite convincing evidence against this approach. Ahrens-Nicklas et al.[24] reported that the intake of medical foods improved metabolic control but resulted in perturbations of

essential amino acids ratios and lower z-scores for head circumference in their cblC cohort. A protein-reduced diet would be expected to decrease Met and, consequently, methylation capacity even further. Therefore, the treatment guidelines recommend against a protein-restricted diet in remethylation disorders.[4] Protein-reduced diets are known to be burdensome for patients and families[29] and did not lead to any improvement in tHcy or clinical outcome parameters in this study.

Our observation that 43 patients were treated with protein restriction with or without additional supplementation of amino acid products requires further exploration given the results of Manoli et al.[28] and Ahrens-Nicklas et al..[24] While our study could neither identify nor confirm the unintended lowering of branched chain amino acids or methionine, this effect may well have been missed given the non-experimental, retrospective design of this study. Additionally, the expected lowering effect of a methionine-reduced diet on Met may have been compensated by the co-administration of betaine and/or OH-Cbl, which increase Met.

Based on OH-cobalamin dose escalation studies, it has been suggested that vitamin B12 plasma concentrations correlate with clinical outcome and tHcy concentration and should, therefore, be a biochemical target and regular follow-up parameter in cblC disease.[30,31] However, this seems not (yet) to be regular practice. Plasma vitamin B12 levels were documented scarcely and mostly only once. Further prospective studies will be necessary to show whether OH-Cbl dose-escalating schemes aiming at maximum dosages and blood vitamin B12 concentrations[31–33] are superior to titration to individual patients' optimal tHcy levels,[27] and also to identify optimal betaine requirements.[34] For MTHFR deficiency, the meta-analysis conducted by Diekman et al.[23] elegantly showed that betaine in a dosage of >100 mg/kg/d is an effective treatment, but prospective studies are still warranted to substantiate this finding.[23] A practice consensus on evidence-based treatment would be a promising approach to develop an optimal regime and to unravel effects of treatment on predefined outcome parameters.

To learn more about the pathophysiological role of tHcy levels in cblC disease, we compared "best" (tHcy under treatment in the lowest quartile) to "poorest" (tHcy in the highest quartile) biochemical responders and could not identify any relation between tHcy levels and treatment, genotype, severity of disease before treatment or delay to diagnosis. In addition, levels of tHcy at disease presentation do not determine levels on treatment nor have they a statistically significant impact on outcome. It remains uncertain which agents cause brain and eye disease. Our data and those provided by others suggest that tHcy may not be the only major pathogenic factor.[35,36] This is supported by the absence of "remethylation disease-typical" eye disease in classical homocystinuria, a disease with comparably elevated levels of tHcy.[37]

When started, a registry is always exploratory. As well as the established symptoms and novel findings, it is interesting to note which data sets remain empty, indicating that abnormalities are absent or rarely assessed in a specific disease. This first analysis of the registry data may help to extract a core set of descriptors encompassing main disease manifestations. These core variables should be as pragmatic as possible to guide physicians through individual patient follow-up in different settings, to provide data for further characterisation of the disease and to define meaningful outcome parameters for interventions. In every patient with a remethylation disorder, we recommend documenting feeding behaviour, weight gain, muscle tone, cognitive development, seizures, psychiatric symptoms, myelopathy, renal and cardiac disease, thromboembolic events and eye disease on a regular basis. The specific biochemical parameters tHcy, Met and MMA (in combined disorders) should be regularly assessed, as well as parameters indicating thromboembolic events, functional renal impairment or bone marrow involvement.

Visual deterioration is a major, well-known finding in remethylation disorders. Interestingly, in this sample, patients with MTHFR deficiency developed ophthalmological disease more frequently and at a younger age than expected. Nystagmus, reflecting oculomotor abnormalities as well as pigmentary retinopathy, macular disease and optic atrophy, either isolated or combined, are characteristically seen in early-onset remethylation disorders[35,38–40] but generally not in late-onset disease.[41] Weisfeld-Adams et al.[42] proposed regular fundus photography, electroretinogram, visual field test and ocular coherence tomography in patients with cblC disease. Clinically, oculomotor disturbances (nystagmus), optic atrophy, pigmentary retinopathy and maculopathy should be documented.

Brain magnetic resonance imaging (MRI) data were sparse in the registry and have, therefore, not been analysed. Published evidence[6,43–45] names brain atrophy, white matter abnormalities, hydrocephalus and basal ganglia lesions as main features. For prognosis or treatment evaluation, additional quantitative measures, especially from volumetric and diffusion studies, may be helpful.[46]

Although cognitive and developmental impairment is an early, frequent and, unfortunately, treatment-resistant complication,[6,47,48] the registry included only fragmentary quantitative data on cognitive abilities, which seldom seem to be formally tested. To get more precise insight into the extent and pattern of cognitive impairment and to establish outcome parameters for new treatment approaches, regular, reliable testing using standardised, age-appropriate instruments would be of great value. The same is true for health-

related quality of life as well as the burden of disease and treatment, which are the most meaningful outcome parameters for patient care and research.[29]

In conclusion, the registry teaches us that the diagnosis of remethylation disorders is quite well established. It confirms that the disease pattern is dominated by neurological disease, cognitive and behavioural impairments and eye disease. Thromboembolic events, cardiac, renal or other small vessel disease may be severe complications. Treatment is generally effective in preventing complications other than eye and brain disease; it stabilises or improves clinical and biochemical features, but treatment protocols vary considerably; they should be standardised and investigated prospectively to identify optimal strategies. A core set of clinical and biochemical variables should be agreed on to describe and follow the clinical course, to monitor treatment and to generate a homogeneous body of evidence for further studies and recommendations.

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Compliance with ethical standards

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