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GUIDELINE OPEN ACCESS

First Revision of the Guidelines for the Diagnosis and Management of Remethylation Disorders

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ABSTRACT

This guideline summarizes diagnostic and therapeutic approaches based on a systematic literature review and evidence evaluation using the GRADE methodology. Given the limited high-quality data, expert consensus was additionally obtained through a modified Delphi process. Remethylation disorders are rare inherited conditions that disrupt the methionine–homocysteine cycle and consecutively impair essential methylation dependent metabolic pathways. Remethylation disorders are caused by defects in the cobalamin or folate metabolism. The disorders typically result in elevated homocysteine and often low methionine; combined cobalamin-related defects also affect mitochondrial methylmalonic acid clearance. The cblC-*MMACHC* defect is the most common cobalamin-related remethylation disorder. Early-onset patients usually present with severe neurological and eye symptoms. Late-onset cases show variable symptoms (e.g., psychiatric, renal, thromboembolic events). Plasma total homocysteine, methionine, methylmalonic acid, serum vitamin B12 (and folates) should be assessed in suspected cases. Early detection through newborn screening is associated with improved clinical outcomes. Betaine as first-line therapy for methylenetetrahydrofolate reductase deficiency and parenteral hydroxocobalamin for cobalamin-related defects have reduced mortality and morbidity. Total homocysteine, methionine (and methylmalonic acid) should be kept as close to normal values as achievable. Emerging evidence suggests that early use of high-dose hydroxocobalamin (> 0.35 mg/kg/day) may improve neurocognitive impairment and may ameliorate eye disease in severe cobalamin-related defects. A major limitation in current practice is the lack of availability of high concentration hydroxocobalamin formulations for parenteral administration.

Abbreviations: aHUS, atypical haemolytic uraemic syndrome; cbl, cobalamin; cRD, combined remethylation disorders; DBS, dried blood spot; DLD, diffuse lung disease; E-HOD, European network and registry for homocystinurias and methylation defects; GRADE, Grading of Recommendations Assessment, Development and Evaluation; Hcy, homocysteine; holoTC, holotranscobalamin; ILD, interstitial lung disease; iRD, isolated remethylation disorders; LC–MS/MS, Liquid Chromatography—Tandem Mass Spectrometry; Met, methionine; MMA, methylmalonic acid; MS, methionine synthase; MS/MS, tandem mass spectrometry; MTHF, 5-methyltetrahydrofolate; MTHFD1, methylenetetrahydrofolate dehydrogenase 1; MTHFR, methylenetetrahydrofolate reductase; NBS, newborn screening; OH-Cbl, hydroxocobalamin; PAH, pulmonary arterial hypertension; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RD, remethylation disorders; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; TC, transcobalamin; tHcy, total homocysteine; TMA, thrombotic microangiopathy.

For affiliations refer to page 31.

1 | Introduction

This guideline revision process was initiated within the frame of the "European network and registry for homocystinurias and methylation defects" (E-HOD) project, an international consortium of metabolic centres (https://www.e-hod.org) with the aim to update the original guidelines [1] and to include recently discovered remethylation disorders (RD).

In isolated RD (iRD), the remethylation of homocysteine (Hcy) to methionine (Met) is perturbed. Very basic physiological processes and reactions such as DNA methylation or myelin synthesis depend on methyl groups provided by the remethylation pathway. Combined RD (cRD) is a disorder in which a build up of methylmalonic acid (MMA) is also observed [2, 3].

Since the 2017 guidelines were published, new RD have been identified, and the spectrum of clinical presentations and genotypes of well-known RD has been described in more detail and in new populations. These descriptions supplement the earlier data published in the 2017 guidelines, which mainly referred to patients from Europe and the Americas. Additionally, the number of cases identified presymptomatically by newborn screening (NBS) from dried blood spots (DBS) has increased and allows stratification according to the mode of diagnosis. Furthermore, new treatment approaches challenge the initial recommendations.

This manuscript substantiates the recommendations and expert opinions based on the underlying literature. If a quick, pragmatic overview is needed in everyday clinical practice, the following sections should be consulted:

- Figure 2 overview of the metabolic pathways involved in remethylation.
- Table 2 signs and symptoms in individuals with remethylation defects.
- Table 3 overview of clinical conditions, causative genes/conditions, and biomarkers for RD and important differential diagnoses.
- Figure 3 diagnostic pathway and management of the patient with a suspected remethylation disorder.
- Chapter 5.5 for guidance on newborn screening flow with first- and second-tier markers
- Chapters 5.6 and 6. (including Table 4) for recommendations on standard and high-dose OH-Cbl treatment

2 | Methods

Twenty-six panellists were invited due to their expertise in biochemistry, laboratory diagnosis, genetics, clinical and psychosocial patient management and/or research activities related to RD. Adequate heterogeneity of the panel in terms of gender, geographic distribution, and representation of important stakeholder groups was considered, and a patient representative was involved in all working steps.

In the first hybrid meeting, the populations, interventions and comparators of interest were defined, and the outcomes were graded according to their importance by the panel (Table 1).

The article selection process was guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [14] approach. Identification, screening, exclusion, and inclusion of studies are documented in Figure 1.

Articles were searched for by entering the following search query into the PubMed, Embase, and Cochrane scientific article databases and by manually entering the same key terms separately. Applied filters were Human Species and English Language, the research timeframe spanned from January 2015 to August 2023. [(cblC deficiency) OR (cblC defect) OR (cblC) OR (cbl-C) OR (cobalamin-C)] OR [(remethylation defects) OR (remethylation disorders)] OR [(severe MTHFR) OR (severe MTHFR deficiency) OR (severe methylenetetrahydrofolate reductase) OR (severe methylene-tetrahydrofolate reductase)] OR (cblD deficiency) OR (cblD defect) OR (cblD) OR (cbl-D) OR (cobalamin-D) OR (cblE deficiency) OR (cblE defects) OR (cblE) OR (cobalamin-E) OR (cblF deficiency) OR (cblF defects) OR (cblF) OR (cobalamin-F) OR (cblG deficiency) OR (cblG defects) OR (cblG) OR (cbl-G) OR (cobalamin-G) OR (cblJ deficiency) OR (cblJ defects) OR (cblJ) OR (cbl-J) OR (cobalamin-J) OR (PRDX1 AND cobalamin) OR (HCFC1 AND cobalamin) OR (THAP11 AND cobalamin) OR (methylmalonic aciduria homocystinuria) OR (methylmalonic aciduria homocysteinemia) OR (methylmalonic acidemia homocysteinemia) OR (cblc type methylmalonic acidemia).

A total of 1741 records were identified through combined database searches, and 493 through manual searches, resulting in 2234 records. After removing duplicates, 1217 records were screened by reviewing the abstracts. Of these, 988 were excluded because they focused on other conditions, were conference abstracts, or did not contain relevant information, 229 records remained for the final analysis, and 143 were finally included in the final text. To this number, an additional 164 studies considered relevant by the panelists were added. Of these, 134 have been published before the selected reference period and, in most cases, had already been considered for the drafting of the original guidelines; six were published after the reference timeframe and were added during the working process due to their substantial scientific relevance and meaningful impact on the decision-making process.

Since only very limited numbers of publications (case reports and small case series) were available on the recently introduced high-dose hydroxocobalamin (OH-Cbl) treatment in patients with cblC-*MMACHC*, a survey was sent out to physicians via the E-HOD and informal professional networks on their practice and experience with this therapeutic approach. The survey data were pooled, statistically analysed, and included in the evaluation of this new treatment intervention. Similarly, a survey was developed and distributed to collect unpublished information on clinical course and treatment in the ultra-rare diseases cblE-*MTRR* and cblG-*MTR*.

Working groups focused on specific outcomes in defined populations (e.g., eye symptoms in infants with the cblC-*MMACHC* defect) and prepared text drafts that were merged to a first draft

TABLE 1 | Overview of the populations of interest, interventions, and the critical outcomes.

Populations	Interventions/comparators	Outcomes considered critical for decision making (scores between 7 and 9 on a scale from, 1 = least important to 9 = most important)
1. Patients with the cblC-*MMACHC* defect and other cRD 2. Patients with iRD 3. Patients with MTHFR deficiency	• OH-Cbl standard treatment according to the definition in the first guidelines version • OH-Cbl high-dose treatment in cRD • Betaine treatment for MTHFR deficiency • Early versus late treatment: all disorders	– Mortality/metabolic crises – Eye disease/loss of visual acuity – Neurocognitive development – Neuropsychiatric symptoms including epilepsy/seizures – Microangiopathy, haematology, thromboembolism – Renal disease – Cardiovascular disease – Quality of life

by CDV, MH and GO. The elaboration of the recommendations, and first assessment of the quality of the evidence was accomplished by moderated, consensus-oriented face-to-face discussions according to the "Grading of Recommendations Assessment, Development and Evaluation" (GRADE) working group approach (http://www.gradeworkinggroup.org/; [15–18]).

The wording of the recommendation was refined by written assessments by the panellists using the following set of questions:

- Are you confident that the benefits of following this recommendation outweigh the harms and burdens?
- Is the evidence supporting the recommendation of high, moderate, or low quality? Please consider risk of bias, study design, directness and consistency of results, magnitude of effect, dose–response gradient, and publication bias.
- Are you confident that the recommendation meets typical values and preferences of the target population of patients, parents, and metabolic experts?
- Are the resources spent worth the expected net benefit?
- How do you rate the strength of this recommendation: weak, strong or very strong?
- How do you rate the quality of the evidence for this recommendation?
- Do you have suggestions for rephrasing?

Case reports, case series or any other type of observational studies were by principle considered low quality evidence and only upgraded to moderate quality if an agreement could be reached that the results were highly consistent, or a dose-effect gradient was present.

To offer readers more guidance, expert advice statements were developed using a modified Delphi method. First versions of expert advice statements were drafted during the face-to-face meeting and submitted to the working groups with a request for feedback. Statements were adapted as required and, following an iterative approach, sent to the panellists to collect their agreement/disagreement and suggestions for rephrasing. Controversial statements were revised iteratively until no further suggestions were submitted and at least consensus was achieved (strong consensus was reached with >95% consensus with 75%–95% agreement) [19, 20].

The clinical presentation, biochemical characteristics, and treatment outcomes of very recently discovered RD were summarized descriptively.

3 | Introduction to Remethylation Disorders

It is important to note that although the diseases discussed in this guideline share the feature of remethylation disruption, they represent different entities.

In iRD, only the remethylation of Hcy to Met is impaired either by reduced or absent activity of the enzyme methionine synthase (cblG-*MTR* defect) or the enzyme methionine synthase reductase (cblE-*MTRR*), or by insufficient synthesis of the cofactor methylcobalamin (cblD-*MMADHC* subtype cblD-Hcy) or by deficiency of the substrate methyltetrahydrofolate (MTHF) in 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency.

The cblC-*MMACHC* defect as well as the rarer *PRDX1*, cblX-*HCFC1, THAP11, ZNF143* defects, and cblD-*MMADHC*, cblF-*LMBRD1*, cblJ-*ABCD4* are associated not only with increased Hcy and low to normal Met, but also with increased MMA due to genetic disorders of cbl intracellular processing affecting both the synthesis of methyl- and adenosylcobalamin, the cofactors for methionine synthase (MS) and methylmalonyl-CoA mutase, respectively (Figure 2). CblC-*MMACHC* is by far the most prevalent cRD.

3.1 | Overview of the Clinical Presentations of Combined and Isolated Remethylation Disorders

The phenotypes associated with RD have been extensively described in the original guidelines [1]. Here we summarize the key clinical signs and symptoms, reporting their frequency

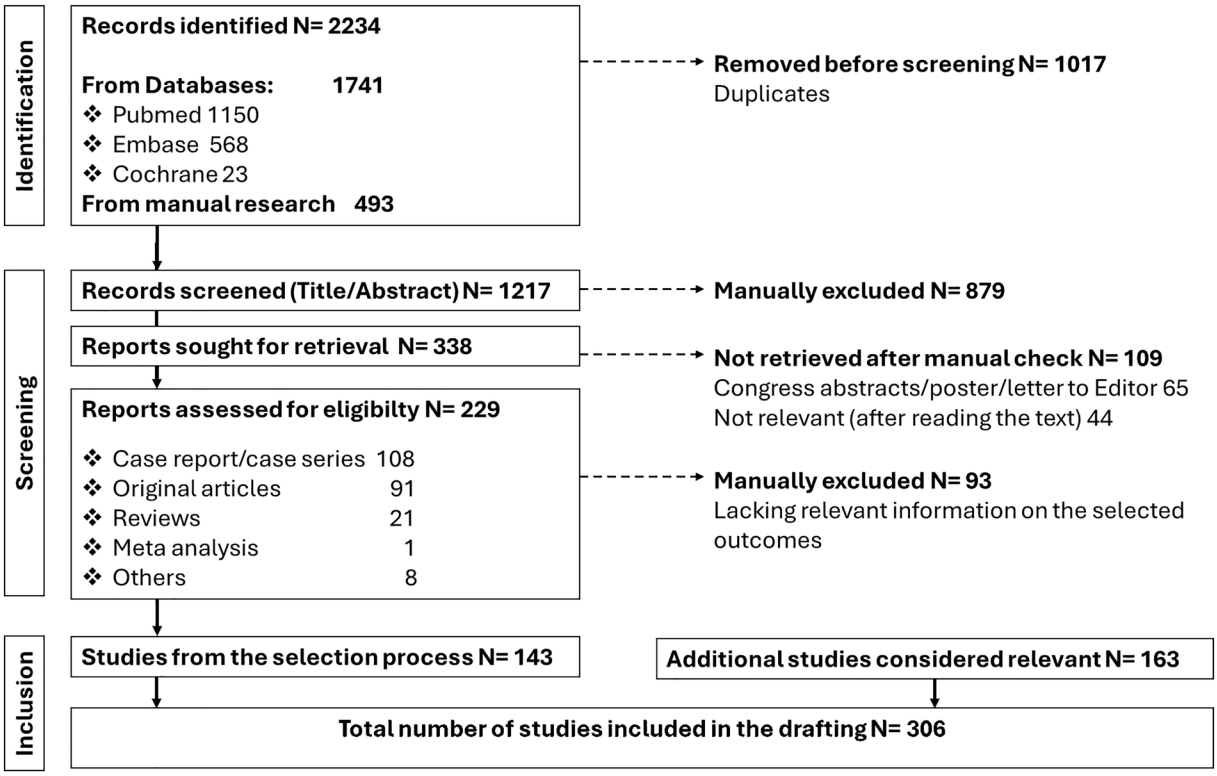

FIGURE 1 | The PRISMA flow diagram.

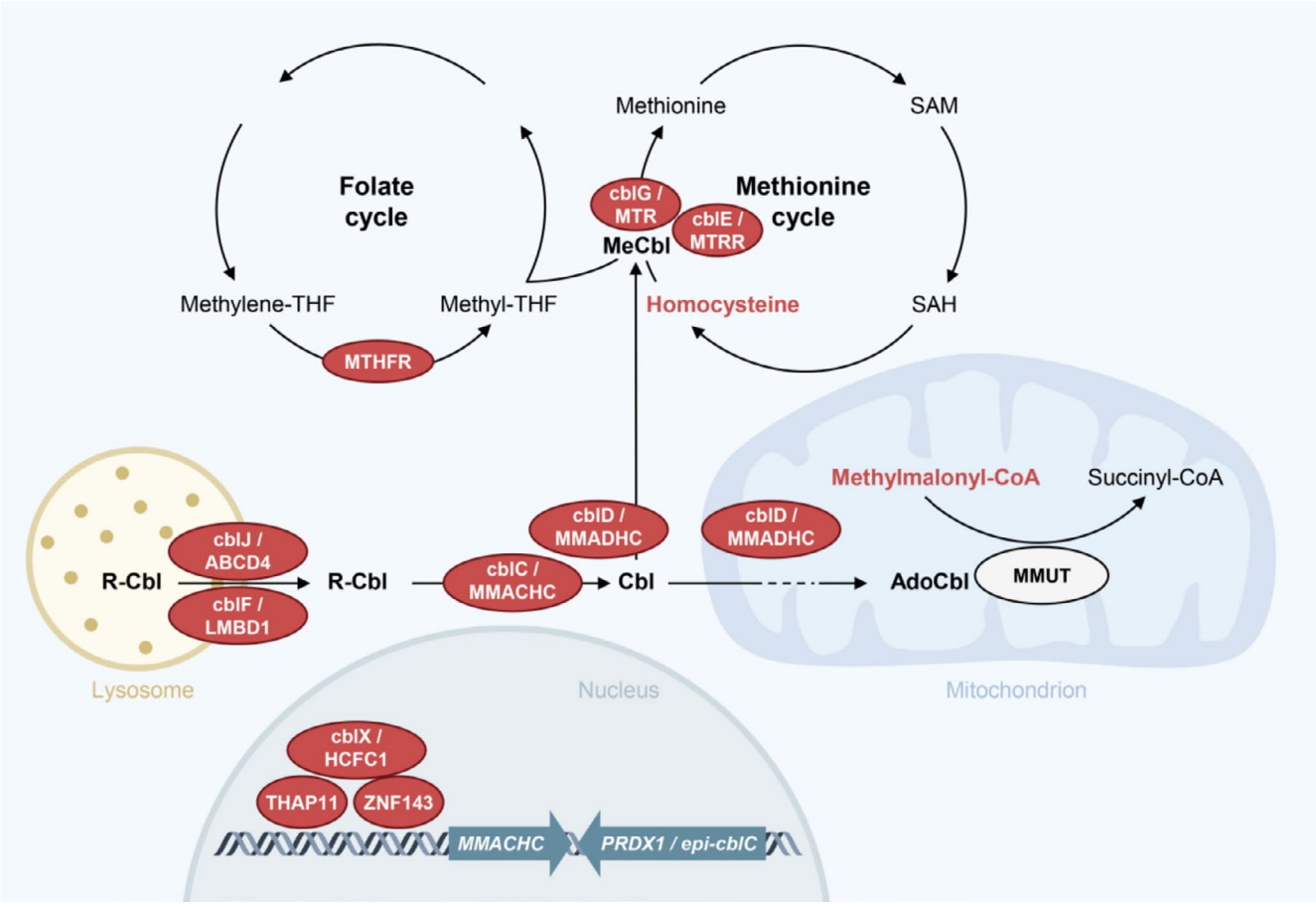

FIGURE 2 | Overview of the metabolic pathways involved in remethylation.

TABLE 2 | Signs and symptoms in individuals with remethylation defects[c].

		cblC-*MMACHC*									MTHFR deficiency	
		Early onset	Late onset	Epi-cbl (*PRDX1*)	cblX-*HCFC1* *THAP11* *ZNF143*	cblD-*MMADHC* subtype MMA-Hcy	cblF-*LMBRD1* cblJ-*ABCD4*	cblD-*MMADHC* subtype Hcy	cblE-*MTRR*	cblG-*MTR*	Early onset	Late onset
Failure to thrive	Small for gestational age	+		+	+++	+	+++			+	(+)	
	Feeding problems	+++		+++	+++	+	+++		+++	++++	+++	
Blood and bone marrow	Metabolic acidosis	+		+/++					(+)	(+)		
	Hyperammonemia	(+)		+		(+)						
	Megaloblastic anemia	++		++	+	++	+++	+++	++++	++++	++	
	Neutropenia or pancytopenia	++		++	+		+++		++	++	++	
Nervous system	Microcephaly	++			++++		++	++++	++	+	++	
	Hyporeactivity/lethargy	++++		++++	+++	(+)			+++	+++	+++	
	Apnoea										++	
	Seizures	+++	+++[a]	+++	++++[b]	+	++	++	+++	++++	+++	++++
	Hydrocephalus	++							++	++	++	
	Cerebral atrophy[c]	++++				++++			+++	++++	++++	+++
	White matter changes	++++	++	++++					+++	+++	++++	++++
	Basal ganglia involvement	(+)							+			
	Hypotonia	++++		+++	++++	+++	+++	+++	++++	++++	++++	
	Hypertonia/dystonia	++	++	++	+++			++	++	++	+++	
	Developmental delay	++++		+++	++++	+++	++++	++++	++++	++++	++++	
	Cognitive disorders/decline	++++	+++/−			(+)	++		++++	+++	+++	+++/++++
	Behavioural disorders	+++	+++		+++	(+)				(+)	++	+++
	Psychosis	+++	+++			(+)						++++
	Myelopathy/spastic paraplegia		++							+	+	++++
	Peripheral Neuropathy		++						(+)	(+)		++++

(Continues)

TABLE 2 | (Continued)

		cblC-*MMACHC*									MTHFR deficiency	
		Early onset	Late onset	Epi-cbl (*PRDX1*)	cblX-*HCFC1* *THAP11* *ZNF143*	cblD-*MMADHC* subtype MMA-Hcy	cblF-*LMBRD1* cblJ-*ABCD4*	cblD-*MMADHC* subtype Hcy	cblE-*MTRR*	cblG-*MTR*	Early onset	Late onset
Eye	Maculopathy	++++	(+)	++++	(+)	++	(+)	++	++	(+)		(+)
	Optic nerve atrophy	++	+							(+)		+
	Nystagmus	+++				(+)			(+)	(+)		
	Other ocular abnormalities	+	+	+	(+)	+	+	++	++	++	+	+++
Skeletal system	Congenital malformations				++++		(+)					
	Mild facial dysmorphism	+++		++	++++		+			(+)		
	Skeletal deformity	+++		++	+	+	++			(+)	++	
Kidneys	Atypical HUS	+++	++						(+)	+		
	Tubulointerstitial nephropathy	+					(+)			(+)		
Cardio-pulmonary	Cardiac malformation	++		++			+++					
	Cardiomyopathy	++		++								
	Diffuse lung disease	+	+									
	Pulmonary hypertension	(+)	+				(+)					
Vascular events	Thrombosis		+						(+)	(+)		++
	Cerebrovascular events									(+)		++
Gastrointestinal system	Liver steatosis	(+)					++					
	Hypercholesterolaemia	(+)										
Skin	Cheilitis/stomatitis/ gastritis					(+)	++			(+)		
	Skin abnormalities (hyperpigmentation)					(+)	++				+	

Note: Signs and system frequency: ++++: > 50% of cases; +++: 25%–50% of cases; ++: 10%–25% of cases; +: < 10% of cases; (+) single cases reports suggest a possible correlation with the disease. Most of the clinical manifestations described refer to patients diagnosed on a clinical basis (in contrast to patients diagnosed e.g., by newborn screening). The frequencies shown in the table are derived from case series, collated case reports and meta-analysis cited in the present work, reflecting an extension and further elaboration of the knowledge provided in previous guidelines [1, 4]. The true prevalence of organ involvements also depends on the extent of technical investigations, particularly for milder forms that may escape clinical detection. For very rare RD (e.g., *THAP11, ZNF143*, cblF-*LMBRD1*, cblJ-*ABCD4*, and cblD-*MMADHC subtype Hcy*), data may be incomplete due to the small number of reported cases.

[a]Usually only in acute phase and responsive to treatment.

[b]Severe epileptic encephalopathy.

[c]Slowly progressive.

TABLE 3 | Overview of clinical conditions, causative genes/conditions, and biomarkers for remethylation disorders and important differential diagnoses.

			Typical reference ranges and cut-offs of biomarkers in plasma or sera of healthy individuals[a]					
Traditional disease name	**Gene OMIM**	**Disease OMIM**	**tHcy (5–15 μmol/L) [5]**	**MMA (< 271–350 nmol/L) [6]**	**Met (18–50 μmol/L) [7]**	**Total vitamin B_{12} (> 150 pmol/L–203 pg/mL) [6, 8, 9]**	**Folate (> 4 ng/mL) [9]**	**Other**
Remethylation defects								
cblC	*MMACHC* 609831	277400	↑	↑	↓ to nl	nl	nl	Cystathionine ↑ to nl
epi-cblC	*PRDX1* 176763 *MMACHC* 609 831	277400	↑	↑		nl	nl	
cblD-MMA/HC	*MMADHC* 611935	277410	↑	↑		nl	nl	
cblD-HC	*MMADHC* 611935	620952	↑	nl		nl	nl	
cblF	*LMBRD1* 612 25	277380	↑	↑		↓ to nl	nl	
cblJ	*ABCD4* 603214	614857	↑	↑		↓ to nl	nl	
cblE	*MTRR* 602568	236270	↑	nl		nl	nl	MCV ↑, Cystathionine ↑ to nl
cblG	*MTR* 156570	250940	↑	nl		nl	nl	MCV ↑, Cystathionine ↑ to nl
MTHFR deficiency[b]	*MTHFR* 607093	236250	↑	nl		nl	↓ to nl	MCV nl, Cystathionine ↑ to nl
MTHFD1 deficiency	*MTHFD1* 172460	617780	↑ to nl	nl		nl	nl	MCV↑, combined immunodeficiency
Vitamin B12 absorption, transport and related defects								
Nutritional vitamin B12 deficiency or malabsorption			↑	↑	↓ to nl	↓	nl	
Transcobalamin (TC) deficiency	*TCN2* 613441	275350	↑	↑	↓ to nl	↓ to nl	nl	

(Continues)

TABLE 3 | (Continued)

Traditional disease name	Gene OMIM	Disease OMIM	Typical reference ranges and cut-offs of biomarkers in plasma or sera of healthy individuals[a] tHcy (5–15 μmol/L) [5]	MMA (< 271–350 nmol/L) [6]	Met (18–50 μmol/L) [7]	Total vitamin B_{12} (> 150 pmol/L–203 pg/mL) [6, 8, 9]	Folate (> 4 ng/mL) [9]	Other
Intrinsic factor deficiency	*CBLIF* *609342*	261000	↑	↑	↓ to nl	↓	nl	
Cubilin deficiency or Najman-Imerslund-Gräsbeck syndrome	*CUBN* *602997*	261100	↑	↑	↓ to nl	↓	nl	
Amnionless deficiency or Najman-Imerslund-Gräsbeck syndrome	*AMN* *605799*	618882	↑	↑	↓ to nl	↓	nl	
Haptocorrin deficiency	*TCN1* *189905*	193090	nl	nl	nl	↓	nl	holoTC nl
Transcobalamin receptor defect	*CD320* *606475*	613646	↑	↑	↓ to nl	↓ to nl	nl	
Folate absorption, transport and related defects								
Nutritional folate deficiency or malabsorption			↑	nl	↓ to nl	nl	↓	MCV ↑
Hereditary folate malabsorption	*SLC46A1* *611672*	229050	↑ to nl	nl	↓ to nl	nl	↓	MCV ↑, sarcosine and cystathionine ↑, orotic acid in urine and FIGLU ↑
Folate receptor alpha deficiency	*FOLR1* *136430*	613068	↑ to nl	nl	N/A	nl	nl	CSF 5-methyltetrahydrofolate ↓, MCV nl
DHFR deficiency	*DHFR* *126060*	613839	nl	nl	N/A	nl	nl	CSF 5-methyltetrahydrofolate ↓, MCV ↑
Formiminoglutamic aciduria	*FTCD* *606806*	229100	↑	nl	↓ to nl	nl	↑ to nl	

(Continues)

TABLE 3 | (Continued)

Traditional disease name	Gene OMIM	Disease OMIM	Typical reference ranges and cut-offs of biomarkers in plasma or sera of healthy individuals[a]					
			tHcy (5–15 μmol/L) [5]	MMA (< 271–350 nmol/L) [6]	Met (18–50 μmol/L) [7]	Total vitamin B_{12} (> 150 pmol/L–203 pg/mL) [6, 8, 9]	Folate (> 4 ng/mL) [9]	Other
MTHFS deficiency	*MTHFS* 604197	618367	↑	nl	N/A	nl	↓ 5MTHF, ↑ 5FTHF	CSF 5-methyltetrahydrofolate ↓ to nl CSF 5FTHF ↑
Reduced folate carrier deficiency	*SLC19A1* 600424	601775	↑	nl	↓ to nl	nl	nl	MCV ↑, immunodeficiency
Other defects								
CBS deficiency	*CBS* 613381	236200	↑	nl	↑ to nl	nl	nl	Cystathionine ↓ to nl
HCFC1 (cblX)	*HCFC1* 300019	309541	↑ to nl	↑ to nl	↓ to nl	nl	nl	
ZNF143 deficiency	*ZNF143* 603433	N/A	↑ to nl	↑ to nl	↓ to nl	nl	nl	
Ronin deficiency	*THAP11* 609119	620940	↑ to nl	↑ to nl	↓ to nl	nl	nl	

Abbreviations: CSF, cerebral spinal fluid; MCV, mean corpuscular volume; N/A, not available; nl, normal; tHcy, total homocysteine.
[a]Reference ranges and cut-offs can vary across diagnostic laboratories due to differences in instrumentation and methods of analysis.
[b]Testing for common MTHFR polymorphisms such as the thermolabile variant c.665C>T p.(Ala222Val), previously known as c.677C>T is not indicated as they do not cause severe MTHFR.

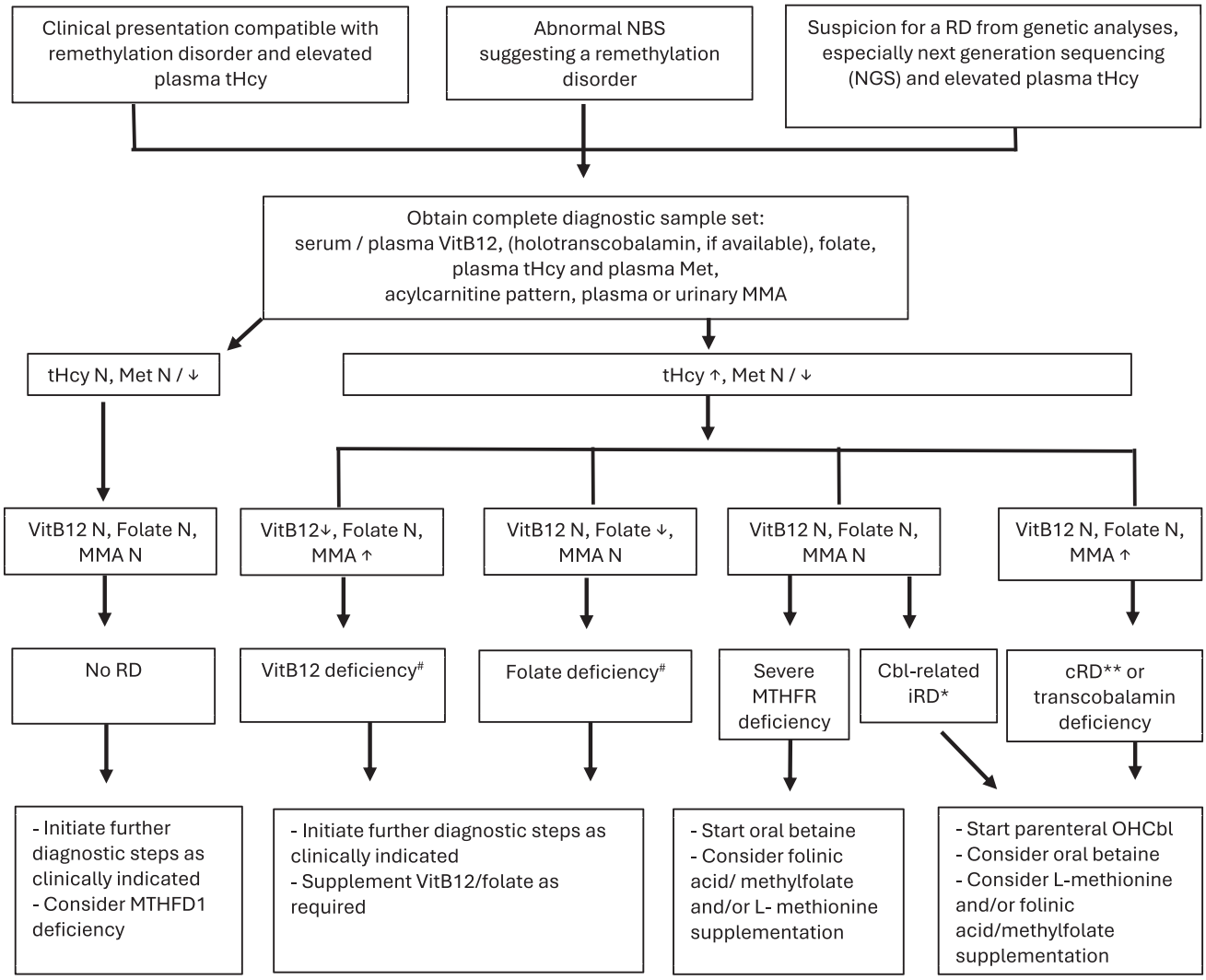

FIGURE 3 | Diagnostic pathway and management of the patient with a suspected remethylation disorder (all biochemical results refer to pre-treatment samples). #Includes supply, absorption, and transport defects. Consider that folate and/or vitamin B12 deficiency can occur in coincidence with cRD and iRD. *cblG-*MTR*, cblE-*MTRR*, cblD-*MMADHC* subtype cblD-Hcy. **cblC-*MMACHC*; cblD-*MMADHC* subtype cblD-MMA/Hcy; cblF-*LMBRD1*; cblJ-*ABCD4*, epi-cblC (*PRDX1*), cblX-*HCFC1, ZNF143, THAP11*.

across disorders and updating the data based on recent literature (Table 2).

3.1.1 | The cblC-*MMACHC* Defect

The cblC-*MMACHC* defect is the most prevalent RD. Before the introduction of NBS for RD, two main phenotypes (or forms), based on the age at disease onset, have been used for classification: early-onset (below the age of 1 year) and late-onset (all other) [21]. Through NBS identified cases, it has become obvious that RD has a phenotypic spectrum ranging from severe, usually early-onset cases with a high risk for rapidly progressive eye disease and cognitive impairment, to less severe, usually later-onset cases that carry a significantly lower risk for maculopathy and cognitive decline but present with heterogeneous, often more treatment-responsive clinical symptoms.

Neurological symptoms such as muscular hypotonia, developmental delay, cognitive impairment, seizures, and white matter impairment [22] as well as feeding difficulties, behavioural and psychiatric problems, microangiopathy manifesting as atypical haemolytic uraemic syndrome (aHUS) or pulmonary hypertension are frequent clinical features. Anaemia, neutropenia, and thromboembolic events may occur, and some early-onset patients may have hydrocephalus, cerebral atrophy or structural cardiac abnormalities. Eye disease with loss of visual acuity, bull's eye maculopathy, and retinopathy manifesting early in life is particularly frequent in early-onset patients carrying the common c.271dup p.(Arg91fs) variant in homozygosity [1, 21]. Hydrocephalus was also observed in association with early-onset variants, in particular the c.271dup p.(Arg91fs) variant in homozygosity in Europe and the c.609G>A p.(Trp203Ter) variant in homozygosity in China [23].

Overall, in late onset cases, a good response to therapy and even complete restoration were observed when treatment was initiated before irreversible damage occurred [24–26].

Remarkably, cblC-*MMACHC* is a relatively common inherited metabolic disorder in Europe, with incidences such as 1:32.271

TABLE 4 | Overview of treatments and supplements for cblC-*MMACHC*, cblE-*MTRR*, cblG-*MTR*, *and MTHFR* deficiency.

	cblC-*MMACHC*	cblE-*MTRR* and cblG-*MTR*	MTHFR deficiency
Parenteral OH-Cbl	Daily high dose OH-Cbl IM/SC Start at ≥0.35 mg/kg/day after NBS/early diagnosis in infants homozygous for the c.271dup variant or other genotypes associated with maculopathy/cognitive impairment and maintain this dose at least to the age of approximately 2 years[c]. In older children, the minimum biochemically effective[d] OH-Cbl dose and frequency of administration should be individually titrated and balanced with the burden of injections. A dose of 0.3 mg/kg/day is frequently applied/targeted[c]. cblC-*MMACHC* cases without eye disease/cognitive impairment can respond well to lower doses and the minimum biochemically effective[d] OH-Cbl dose and frequency of administration should be individually titrated and balanced with the burden of injections[c]. CN-Cbl is contraindicated in cblC-*MMACHC*	Start with a minimum dose of 0.3 mg/kg/day OH-Cbl IM/SC[a] The minimum effective OH-Cbl dose and frequency of administration should be individually titrated[b–d] Compounds other than OH-Cbl (e.g., CN-Cbl) cannot be recommended as their efficacy has not been proven in sufficient numbers of cases	Unnecessary with intact enteral Cbl resorption.
Oral Cbl preparations	No efficacy	No efficacy	To avoid Cbl deficiency and to optimize tHcy[c].
Oral betaine	100–250[f] mg/kg/day (2–3 doses/day) to further improve tHcy and Met under optimized treatment with OH-Cbl [10]	100–250[f] mg/kg/day (2–3 doses/day) to further improve tHcy and Met under optimized treatment with OH-Cbl	100–250[f] mg/kg/day in children; 6–9 g/day in adolescents/adults (2–3 doses/day). A maximum dosage is defined by the drug manufacturer.
Oral folates	To avoid folate deficiency and to optimize tHcy[c]. 400 μg daily or 5 mg once or twice weekly are frequently applied doses[c,e]. If available, oral folinic acid (or methylfolate) is preferred over folate to avoid the probability of folate depletion/formate starvation [11, 12]	To avoid folate deficiency and to optimize tHcy[c]. 400 μg daily or 5 mg once or twice weekly are frequently applied doses[c,e]. If available, oral folinic acid (or methylfolate) is preferred over folate to avoid the probability of folate depletion/formate starvation[c].	Folic acid is to be avoided. If available, methylfolate can be used, otherwise folinic acid [11, 12]. Doses of 45 mg/day in children; 75 mg/day in older patients are frequently applied[c].
L-carnitine	50–100 mg/kg/day to acutely lower MMA (oral/IV) Supplement if free carnitine is low [13].	—	—
Oral methionine	Supplement (2–3 doses/day) if Met cannot be maintained safely within normal ranges under optimized treatment with OH-Cbl/betaine.	Supplement (2–3 doses/day) if Met cannot be maintained safely within normal ranges under optimized treatment with OH-Cbl/betaine.	Supplement (2–3 doses/day) if Met cannot be maintained safely within normal ranges under optimized treatment with betaine.

Note: Treatment options are reported in gray and white boxes, supplements are reported in blue boxes.
[a]IV also possible if not prohibited by drug manufacturers.
[b]Insufficient evidence for a general superiority of high-dose treatment but high-dose treatment can be considered in cases with limited response since it has been effective in single cases.
[c]Expert opinion.
[d]To the individual lowest possible tHcy value.
[e]The range of age-dependent recommended dietary allowances is 65–400 μg of folate from food and half of that if given as supplements.
[f]Betaine dose of 250 mg/kg/day lowered tHcy significantly more than 100 mg/kg/day [10].

in Italy, where it is one of the most frequent inborn errors of metabolism [27]. The disease is also relatively common in Asia, and studies on NBS in China report an incidence of 1:3.920 [28] to 1:11.730 live births [29]. In Chinese populations, the distribution and frequency of the *MMACHC* variants are different from European and North American populations [30]. The c.609G>A p.(Trp203Ter) nonsense variant, the 3-bp deletion c.658_660del p.(Lys220del), the c.482G>A p.(Arg161Gln) missense variant, the c.567dup p.(Ile190Tyrfs) frameshift variant, the c.217C>T p.(Arg73Ter) missense variant, and the c.80A>G p.(Gln27Arg) missense variant are the most frequent variants. Many cases in these cohorts show an attenuated phenotype with neurological symptoms such as limb weakness (50%) or gait instability (42%), often associated with spinal cord atrophy, pyramidal signs, cognitive impairment, epilepsy, peripheral neuropathy, optic neuropathy, and psychiatric symptoms (46%) [31] cardiovascular and renal symptoms, and pulmonary hypertension [32, 33] as presenting signs. Pulmonary hypertension and renal disease were often associated with compound heterozygosity for the c.80A>G p.(Gln27Arg) variant [34–38].

3.1.2 | Rare Combined and Isolated Remethylation Disorders

The clinical presentation of the much rarer cRD cblD-*MMADHC*, cblF-*LMBRD1*, cblJ-*ABCD4*, and the iRD cblD-*MMADHC* subtype cblD-Hcy, cblE-*MTRR*, cblG-*MTR*, and severe MTHFR deficiency have been described in the original guidelines. While they may present clinically indistinguishable from the cblC-*MMACHC* defect, MTHFR deficiency usually has no renal and less eye involvement than the cbl-related RD [1, 30].

3.1.3 | Recently Identified Remethylation Disorders

Besides pathological variants in target genes of inborn errors of the cbl metabolism, causal mechanisms of unresolved cases may also include variants located in genes coding for transcription factors regulating expression of the *MMACHC* gene. This has been described in cblC-type cases due to altered *MMACHC* expression evidenced by RNA-seq and due to variants in *HCFC1, THAP11*, and *ZNF143* transcription factors [39, 40]. Another category of mechanisms is gene silencing by secondary epimutations (epi-cblC), caused by a mutation in *PRDX1*, a gene adjacent to *MMACHC* [41].

3.1.3.1 | Epi-cblC Disease. Epi-cblC disease is characterised by a primary cis-acting genetic variant in the *PRDX1* gene (c.515-1G<T or c.515-2A>T) adjacent to *MMACHC*. Both these variants lead to aberrant antisense transcription of the *MMACHC* gene resulting in secondary hypermethylation of the promoter region and silencing of *MMACHC* [41]. Most patients with epi-cblC disease are compound heterozygotes for an epimutation and a pathogenic variant in the *MMACHC* gene in trans [41–45].

The Epi-cblC phenotype may partially overlap with the early-onset cblC-*MMACHC* phenotype. Patients may present with hypotonia, failure to thrive, megaloblastic anaemia, developmental delay, and visual impairment. Skeletal deformities, metabolic acidosis, hyperammonemia, and recurrent severe infections are more frequently observed in Epi-cblC than in cblC-*MMACHC* [4, 41–44]. Prenatal damage is reflected by intrauterine growth restriction.

A patient homozygous for Epi-cblC variants has been reported with anaemia, thrombocytopenia, recurrent severe infections, metabolic acidosis, maculopathy, and psychomotor delay, later evolving into an autism spectrum disorder [42].

Compound heterozygosity for a *PRDX1* variant and a *MMACHC* variant results in a clinical phenotype primarily determined by the severity of the *MMACHC* variant [42]. The incidence of epileptic seizures with five of 11 cases [42] is comparable to the cblC-*MMACHC* defect, where the incidence amounts to over 50% of cases [1]. Elevated tHcy levels accompanied by MMA were reported in all patients so far. A single patient had low arginine, ornithine, glutamine, and alanine and increased glycine and citrulline blood levels [4].

3.1.3.2 | The *HCFC1, THAP11,* and *ZNF143* Defects. Host Cell Factor C1 (*HCFC1*; Xq28) regulates *MMACHC* transcription in interaction with the THAP Domain Containing 11 (*THAP11*; 16q22.1) and Zinc Finger Protein 143 (ZNF143; 11p15.4) [46–48]. Individuals carrying variants in these genes are thought to have decreased MMACHC-mRNA expression during embryogenesis, leading to a *MMACHC*-cblC-like disorder with prenatal onset [46, 48]. *HCFC1* deficiency, termed cblX disease, is inherited in an X-linked manner, whereas *THAP11* and *ZNF143* defects follow autosomal inheritance. Pathogenic variants in *HCFC1* associated with the classical CblX phenotype cluster within the N-terminal Kelch repeat domain, which mediates critical protein interactions for transcriptional regulation of Cbl metabolism genes [48]. These Kelch-domain variants are associated with a severe neurological phenotype characterized by microcephaly, brain malformation especially of the midline structures and pharmacoresistant epilepsy suggesting pathogenic mechanisms beyond *MMACHC* deficiency [48–50]. By contrast, variants located outside the Kelch domain have been associated with broader and more heterogeneous neurodevelopmental phenotypes, without hyperhomocysteinemia or methylmalonic acidemia, making genotype–phenotype interpretation more challenging, and highlighting the importance of family history and genetic counselling. In these cases, patients may present with only mild intellectual disability or learning difficulties, mostly normal brain magnetic resonance imaging (MRI) and no bull's eye maculopathy commonly seen in cblC-*MMACHC* disease [51].

3.1.3.3 | Methylenetetrahydrofolate Dehydrogenase 1 Deficiency (MTHFD1). MTHFD1 is a trifunctional protein (*MTHFD1*; 14q23.3) not only involved in the remethylation of Hcy to Met by providing the methyl group donor 5-methyltetrahydrofolate but also in other pathways such as purine neosynthesis. The clinical presentation of MTHFD1 deficiency has some overlaps with other RD (megaloblastic anaemia, microangiopathy and atypical haemolytic uraemic syndrome, retinopathy) but also includes autoimmune disease and severe immunodeficiency [52]. Hyperhomocysteinaemia was observed in some, but not in all patients [53]. Since only very few patients

with heterogeneous disease symptoms and response to treatment with folic acid combined with cobalamin have been described, no recommendations for the treatment of patients can be derived.

4 | Recent Pathophysiological Insights

In patient cells and animal models, impaired MS activity leads to the development of endoplasmatic reticulum stress and decreased expression of cytosolic sirtuin-1 (SIRT1) and mitochondrial SIRT3-5 through altered splicing and nucleo-cytoplasmic shuttling of mRNAs and RNA binding proteins, with subsequently altered expression of genes involved in energy metabolism, neuroplasticity, and brain development, including in the optic area [54–57]. The impaired synthesis of S-adenosylmethionine (SAM) and sirtuin activities alters mitochondrial energy metabolism through inactivation of nuclear receptors (PPAR-α, ER-α, ERR-α, and HNF-4α)/coactivators (PGC-1α) [54, 58, 59]. The accumulation of organic acids may also trigger ultrastructural mitochondrial alterations in brain, liver, kidney, and heart through mechanisms that include PINK1/Parkin–mediated mitophagy and post-translational modifications [60–64].

Untargeted urine metabolomic analysis comparing samples from patients with cblC-*MMACHC* with methylmalonic and propionic aciduria reveals selective excretion of thioproline and oxidized vitamin E derivatives in cblC-*MMACHC*, indicating increased oxidative stress. Moreover, elevated urinary levels of neurosteroids with patterns like those observed in neurodegenerative and ocular diseases were detected in cblC-*MMACHC* cases. This may suggest a potential link with the characteristic neurological and visual manifestations of cblC-*MMACHC* [65].

5 | Parameters for the Valid and Timely Laboratory Diagnosis of Remethylation Disorders

Patients with a suspected RD require immediate biochemical investigation to guide further testing and inform therapeutic interventions before starting therapy (to not delay treatment, freezing pre-treatment samples for later investigations is an option). The method of choice to confirm the diagnosis of RD is molecular genetic analysis. Enzymatic studies can be helpful if molecular genetic testing is unavailable or inconclusive.

5.1 | Biochemical Parameters and Most Important Differential Diagnoses

The differential diagnosis of vitamin B12–related disorders requires distinguishing between defects of intracellular metabolism and those affecting absorption or transport. Intracellular metabolic defects typically impair the enzymatic conversion of cobalamin within the cell, whereas disorders of absorption or transport result in insufficient cellular delivery of cobalamin, leading to intracellular deficiency. Among transport defects, transcobalamin (TC) deficiency is a rare, autosomal recessive disorder characterized by early-onset pancytopenia, gastrointestinal symptoms, growth retardation, and potentially severe neurological complications. TC deficiency is well responsive to parenteral vitamin B12 treatment. Biochemically, it is characterized by low holoTC, whereas intracellular defects usually have adequate holoTC.

Untreated patients with RD exhibit tHcy levels usually $>100\,\mu mol/L$ [66–69]; but tHcy <100 or even $<50\,\mu mol/L$ has been observed [69].

Routinely, tHcy is measured by immunoassays or chromatographic methods. Chromatographic methods are more specific than immunoassays and are usually coupled with fluorescence detection or tandem mass spectrometry (MS/MS) for the simultaneous determination of other compounds of interest, such as Met or cystathionine [71]. There is no influence of the type of collection tube used (EDTA, heparinised, citrate, serum, gel separator tubes). However, blood samples with anticoagulants should be centrifuged within 1 h or kept in an ice/water bath until centrifugation (< 6 h). After removal of blood cells, tHcy is stable for several weeks at 4°C and for several years after freezing at −18°C [72]. Free homocystine in plasma or urine measured by conventional ion-exchange chromatography of amino acids is not the method of choice since it may remain undetectable or very low even in the presence of significantly elevated tHcy in plasma [73, 74] if a fast protein precipitation is not carried out [75, 76].

Since hyperhomocysteinaemia is also a hallmark of cystathionine beta synthase (CBS) deficiency and associated with vitamin B12 or folate deficiencies, common MTHFR polymorphisms, severe vitamin B_6 deficiency, renal failure or hypothyroidism, further parameters must be assessed (Table 3, Figure 3).

Plasma Met, and if available cystathionine (using sensitive Liquid Chromatography–Tandem Mass Spectrometry, LC–MS/MS methods) and the Met/cystathionine ratio should be determined [77] since plasma Met and Met/cystathionine ratio are low or normal in RD while they are usually elevated in CBS deficiency [78, 79].

MMA is elevated in serum and/or urine in cRD and functional vitamin B12 deficiency of other causes [73, 80] and should be determined promptly. In patients with elevated tHcy and MMA, simultaneous investigation of serum total vitamin B12, plasma holotranscobalamin (holoTC) if available, and serum or erythrocyte folates should be performed to exclude insufficient vitamin saturation. In newborns detected by NBS and breastfed infants, the maternal vitamin B12 status should also be assessed. Low holoTC may also indicate transcobalamin (TC) deficiency, a disorder that can be confirmed by molecular genetic investigation [81].

If the shipment of urine or blood samples is difficult, an experienced laboratory should be consulted for advice on investigating selected parameters from dried blood spots (DBS). Treatment should not be delayed awaiting confirmation of the exact defect.

Figure 3 illustrates the recommended steps for diagnostic workup of a patient with a suspected RD.

Recommendation 1: we strongly recommend that investigations in patients with a suspected RD should start with the measurement of total homocysteine in plasma (or serum, if plasma is not available).

Quality of the evidence: high.

Recommendation 2: we recommend the blood sample for tHcy to be centrifuged within an hour and kept at +4° or frozen until analysis. Immunoassays, chromatographic assays, or LC–MS/MS methods are suitable for tHcy measurement.

Quality of the evidence: moderate.

Recommendation 3: we recommend against measuring free homocystine instead of total homocysteine.

Quality of the evidence: moderate.

Recommendation 4: we strongly recommend that in the case of total homocysteine above the age-adjusted reference interval, plasma samples for determination of methionine, folate, vitamin B12 and, if available, holoTC; plasma samples for determination of MMA are obtained before treatment is started.

Quality of the evidence: high.

Expert advice 1: Determination of renal function parameters (creatinine and/or cystatin C) is recommended, particularly in adult patients (Strong consensus; 96%).

If plasma MMA analysis is unavailable, MMA may be assessed in urine.

5.2 | Optional Biomarkers

Adherence to treatment with betaine can be monitored by quantitative determination of plasma betaine, dimethylglycine and sarcosine, if available [82]. The determination of plasma SAM and S-adenosylhomocysteine (SAH)—if available—can be utilized to assess methionine cycle status, particularly in patients who fail to maintain adequate plasma methionine [83, 84].

5.3 | Molecular Genetic Analysis

Molecular genetic analysis is the method of choice to confirm the diagnosis of RD. Since many of these disorders share overlapping clinical and biochemical features and may involve multiple genes, the most effective approach is to employ next generation sequencing techniques. Independent confirmation of variants in the index case and determination of the phase of variants in their parents or other relatives are strongly recommended.

If resources are restricted and clinical and biochemical parameters characteristic for a cRD, it may be a pragmatic approach to look first for the cblC-*MMACHC* defect and extend the molecular genetic analysis only if these analyses prove negative.

In iRD, genetic testing for MTFHR deficiency should be interpreted with caution since there are numerous polymorphisms in this gene, including the most investigated thermolabile variant c.665C>T p.(Ala222Val); previously known as c.677C>T, which are not responsible for severe RD and do not need to be tested under this differential diagnosis [85]. For *MTHFR* variants, prediction of functional consequences may be derived from a variant effect map of all amino acid substitutions in the MTHFR enzyme [86].

Enzyme assays are helpful when only one pathogenic variant is found in a disease with autosomal recessive inheritance and to characterise the functional consequences of new variants and variants of unknown significance. Direct enzyme assays in cultured skin fibroblasts are only available for MTHFR [87] and MTHFD1 [52]. Indirect assays measuring the integrity of several enzymes in the same pathway are useful for RD investigations [88] (Information on laboratories offering these analyses can be obtained from the authors).

Recommendation 5: we strongly recommend diagnostic confirmation of a RD by molecular genetic analysis in qualified laboratories. In case molecular genetic analyses are inconclusive, we recommend enzymatic testing.

Quality of the evidence: high.

5.4 | Prenatal Diagnosis

Molecular genetic diagnosis is the most advisable method for prenatal testing provided the causative variants in the index case and carrier status in the parents have been identified. If the disease-causing variants are known, preimplantation diagnosis is also feasible. Molecular genetic testing can be performed from chorionic villi or amniotic fluid samples [89].

Preconception/antenatal expanded carrier screening panels including *MMACHC* and other cRD or iRD genes are being offered [90, 91] and proof of concept studies have successfully diagnosed infants with common variants in *MMACHC* by non-invasive prenatal testing using sequencing of circulating cell-free fetal DNA in the blood of the pregnant mother [92–96].

In rare cases where molecular genetic prenatal testing is not available, metabolite concentrations (tHcy and MMA) in cell-free amniotic fluid, enzyme activity of MTHFR, methionine synthase or methionine synthase reductase in cultured amniotic cells, and incorporation of propionate and methyltetrahydrofolate can be considered [73, 97]. Indirect enzyme assays in chorion biopsy should be avoided [89, 97].

Recommendation 6: if prenatal or preimplantation diagnosis is considered, we recommend performing molecular genetic analysis of chorionic villi or amniotic fluid samples. This should be performed after pathogenic variants in the index case and carrier status in the parents have been identified.

Quality of the evidence: moderate.

5.5 | Feasibility of NBS for Isolated and Combined Remethylation Disorders

The specificity of C3 and/or C3/C2 for detecting RD is generally low [98–100] but the positive predictive value is substantially

increased by MMA (4% to 100%) and tHcy (11% to 36%) as second tier biomarkers [98–101]. However, the sensitivity of C3 and C3/C2 for mild/late-onset forms is unknown. Heptadecanoylcarnitine (C17) may have an even higher predictive value for perturbations of MMA metabolism including combined RD and may thus be a promising primary biomarker [102]. Combined multiple-tier strategies are today considered the best working practice to achieve the most benefit out of NBS programs for cRD [103–105]. It is of note that neonatal metabolic disturbances due to maternal vitamin B12 deficiency may also be detected. Furthermore, asymptomatic women have been diagnosed with an RD [36] after biochemical abnormalities in their newborn baby were found on NBS [106].

Recommendation 7: we strongly recommend obtaining plasma from both child and mother for determination of B12 status before treatment is started in cases identified by NBS to assess maternal nutritional vitamin B12 deficiency and/or genetic defects in the B12 absorption/transport system.

Quality of the evidence: high.

Recommendation 8: we recommend use of C3 acylcarnitine and/or the C3/C2 ratio and/or C17 acylcarnitine as primary markers to screen for the cblC-*MMACHC* defect and to perform second tier testing using tHcy and MMA to improve specificity and differentiate the defect from other disorders.

Quality of the evidence: moderate.

Neonatal screening for the iRD cblD-*MMADHC* subtype cblD-Hcy, cblE-*MTRR*, and cblG-*MTR*, and for MTHFR deficiency appears to be feasible by detecting decreased Met and/or methionine-to-phenylalanine ratio [107]. The second-tier marker tHcy clearly differentiates patients from controls [99, 108]. Only recently has tHcy been used as a primary biomarker [109] in DBS.

Several countries have explored and demonstrated the potential of genomic newborn screening. This approach can identify conditions missed by traditional NBS, including RD or MTHFR deficiency by analyzing exome or genome data for a predefined set of conditions [110–115].

5.6 | Monitoring of Therapy

Biochemical treatment response to parenteral OH-Cbl can be expected within days. MMA concentrations usually improve significantly and may even normalize. Monitoring of RD should include plasma tHcy and plasma Met. Plasma Met should be kept safely in the normal range. The heterogeneity of molecular cause, age and clinical presentation, treatment modality, follow-up time and response to treatment in RDs precludes a recommended target concentration of tHcy applicable to all patients.

Retrospective cohort studies of cRD and iRD suggest that treatment may lower plasma tHcy from > 100 μmol/L at diagnosis to 20–80 μmol/L [21, 33, 76, 82, 116–119]. Regarding MMA, a single study with 12 cblC-*MMACHC* patients reported pre-treatment plasma MMA median 51 μmol/L (16–480) and post-treatment 6 μmol/L (0.5–38) [117]. Reports on urinary MMA have shown late onset cblC-*MMACHC* patients with pre-treatment median 94 (5.5–85 mmol/mol creat) and post-treatment median 6 (0–80 mmol/mol creat) [33]. Late-onset cblC-*MMACHC* patients exhibited better metabolic control and response to treatment compared to the early-onset forms of the disorder [120]. Determination of circulating levels of free carnitine is useful to detect deficiency.

In MTHFR deficiency, median plasma tHcy at diagnosis is typically 170–200 μmol/L (range 30–353), with post-treatment concentrations lowered by approximately 40% (median plasma tHcy 70–110 μmol/L, range 16–328) [121–123].

Surveillance of other relevant parameters (e.g., renal function) should be guided by the patient's phenotype.

Recommendation 9: we recommend reducing plasma tHcy to the lowest achievable concentration on an individual basis, and maintaining plasma Met concentration safely within age-adjusted reference ranges.

Quality of evidence: moderate.

Expert advice 2: tHcy values below 100 μmol/L are considered safe for patients with CBS deficiency [75]. In daily practice, individual titration of tHcy to the lowest achievable value for patients with RD is considered useful by the panel (Consensus; 92%).

6 | Introduction to the Recommendations on Treatment

6.1 | Standard OH-Cbl Treatment Versus High-Dose Treatment for Remethylation Disorders

For decades, the mainstay of treatment for cRD and the Cbl-associated iRD cblE-*MTRR*, cblG-*MTR*, cblD-*MMADHC* subtype cblD-Hcy has been parenteral OH-Cbl with a starting dose of 1 mg/day (approximately 0.3 mg/kg/day in a newborn, referred to as standard dose) and later adjustment according to the patient's biochemical (tHcy, Met) and clinical response [1]. The effects of OH-Cbl treatment will in the following be discussed with a special focus on the recent literature suggesting the superiority of high-dose (> 0.35 mg/kg/day) OH-Cbl treatment regarding neurocognitive outcome and eye disease in cblC-*MMACHC* disease.

For the rarer cRD and iRD variants, there is no evidence that high-dose therapy is more effective than standard therapy. In clinical practice, treatment of the rarer RD usually follows the treatment for the cblC-*MMACHC* defect. There is no concrete evidence that this is the best approach.

For all other outcomes discussed in this guideline there is currently no evidence supporting a superiority of high-dose OH-Cbl treatment and the effects of treatments discussed and presented in these guidelines refer to standard treatment.

Unfortunately, both high-dose and "standard" treatment are applied with great variability. These circumstances make it impossible to give exact, evidence-based dosage recommendations. This applies to all the following recommendations for OH-Cbl treatment.

Access to high concentration OH-Cbl other than 1 or 5 mg/mL is limited and lacks regulatory approval worldwide. An approved preparation (Cyanokit) is available for the treatment of acute cyanide poisoning that contains OH-Cbl in 5 g vials that are reconstituted with 200 mL of normal saline to yield a final concentration of 25 mg/mL and administered intravenously over 15 min. Off-label use of this product to treat patients with RD would lead to a substantial proportion of the reconstituted product being discarded, with associated cost considerations. Some compounding pharmacies have prepared concentrations up to 25–50 mg/mL, but source materials, excipients, and preservatives vary. OH-Cbl is generally well-tolerated and hypersensitivity reactions have only rarely been reported [124]. However, safety data for high-dose, long-term treatment are currently unavailable. These should be obtained in future through careful monitoring.

It should be noted that NBS may occasionally identify individuals with cblC–MMACHC defect by detecting slightly elevated C3-carnitine and tHcy levels from dried blood spots, while plasma tHcy values remain within the normal range. In these individuals, genetic analysis may reveal late-onset variants associated with adult-onset disease, such as the c.440G>C variant [125] (Table 8), and they may remain asymptomatic for years (authors' personal observation). An adapted therapeutic strategy for such cases has yet to be defined.

6.2 | Betaine

Betaine has often been effectively used to further lower tHcy and to maintain Met levels safely within normal ranges in iRD and cRD.

Recommendation 10: we recommend adding oral betaine (100–250 mg/kg/day) to optimize OH-Cbl therapy in cRD and iRD to further improve tHcy and Met levels.

Quality of the evidence: moderate.

For severe MTHFR deficiency, betaine is the treatment of choice.

Quality of the evidence: high.

6.3 | Additional Substances

Due to their variable application and a lack of data on their impact on defined outcomes, the clinical effects of folate, folinic acid, methylfolate, L-carnitine, and Met cannot be estimated. These substances are therefore considered supplements that should be individually considered and supplemented to prevent (or treat) substrate deficiencies. Carnitine depletion may impair propionyl group detoxification and mitochondrial energy metabolism [13].

7 | Protein Restriction in Remethylation Disorders

Met-free formulas and protein restriction lead to low Met plasma levels and should be avoided in cblC-*MMACHC* [1, 117, 126, 127] as well as in other RD due to their shared pathophysiological mechanisms.

Recommendation 11: we strongly recommend against dietary protein restriction in cblC-*MMACHC* disease and other RD.

Quality of the evidence: high.

8 | Background and Recommendations on Important Outcomes for cblC-*MMACHC* Disease

The overwhelming majority of data for cRD comes from patients with cblC-MMACHC disease. Therefore, all the following recommendations apply primarily to cblC-MMACHC. If a recommendation or expert opinion also applies to the other, extremely rare cRDs, this is made clear in the text.

8.1 | CblC-*MMACHC*, Disease-Related Mortality

In a recent meta-analysis including 824 patients with inherited disorders of vitamin B12 metabolism without restriction to RD, pulmonary hypertension (OR 7.08%, 95% CI 2.6–19.29) and age below 1 year were associated with an increased risk of death (OR 2.84, 95% CI 1.58–5.12) [4].

A prospective 6-year cohort study from Beijing including children with the biochemical phenotype of "methylmalonic acidaemia with homocystinuria" without genetic confirmation of the underlying defect and with only very general information on treatment reported significant mortality (18/45) and confirms newborn onset as the most important risk factor for death (OR 6.86, 95 CI % 2.24–20.98). Predominant findings in non-survivors were renal and multi-organ failure, and higher values of MMA before treatment [128].

Mortality, mainly due to pulmonary hypertension and thrombotic microangiopathy (TMA) and consecutive cardiopulmonary failure, was high (44%) in 36 cblC-*MMACHC* patients with renal disease. Mortality was higher in early (57%) compared to late onset (35%) cases. Treatment with OH-Cbl (without information on dosages) was associated with clinical improvement ($n = 17/31$) or stabilisation ($n = 2/31$). Eight of 31 treated patients (26%) and four of the five untreated patients (80%) died [129]. Gupta et al. 2021 confirmed the high mortality in cblC-*MMACHC* cases with pulmonary hypertension and renal involvement without OH-Cbl treatment [130].

Patients with disease onset after the first year of life exhibited a mortality of only 6% (12/199 cases) but data on treatment and causes of death were not precisely documented [131].

In 85 late onset cblC-*MMACHC* patients from a single centre in China, all patients survived until a median age of 15 years (4.6–37.2 years; mean follow-up 4.9 years, range 0.5–13.4 years)

on treatment with individually adjusted doses of OH-Cbl, oral L-carnitine, betaine, and folic acid [33].

Recommendation 12: parenteral treatment with OH-Cbl reduces mortality and we strongly recommend initiating it in all patients with cblC-*MMACHC* and other cRD without delay.

Quality of the evidence: high.

8.2 | CblC-*MMACHC*, Neurocognitive Development

Cognitive impairment is a prevailing manifestation of CblC-*MMACHC* and other cRD [1, 21, 30]. The cognitive profile is highly heterogeneous, ranging from attentional instability affecting learning capabilities to profound cognitive impairment [132, 133].

In patients with the cblC-*MMACHC* defect, global developmental delay and cognitive dysfunction are more frequently observed (50%–75%) in early-onset forms, particularly when the diagnosis is made based on clinical presentation [21, 101, 134, 135]. The implementation of large-scale NBS programs has made early diagnosis possible, resulting in improved survival through the prevention of severe major organ complications [30, 103, 136] as well as an improvement of global cognitive outcome [28]. However, in patients diagnosed by NBS, treatment with OH-Cbl at doses of approximately 1 mg/day (corresponding to 0.3 mg/kg/day in newborns) [1] has not been sufficient to fully prevent cognitive and adaptive impairments [22, 28, 117, 137]. Subsequent studies have shown that better neurocognitive outcomes are associated with continuous treatment at doses exceeding 0.35 mg/kg/day (Table 4). Whether there is any added benefit for exceedingly high dosages (between 2 and 7.3 mg/kg/day), as suggested by some authors [118, 119, 138, 139] requires further study.

To further investigate cognitive outcomes of standard versus higher dose OH-cbl, we shared a survey with the E-HOD and guidelines group members. Thirty unpublished cblC-*MMACHC* patients treated with a high dose (0.35–3.5 mg/kg/day) regimen were collected, including 16 patients homozygous for c.271dup p.(Arg91fs), for which statistical analyses were conducted (Table 5). Pre-symptomatically diagnosed patients treated with higher dose OH-Cbl within the first 2 months of age showed better cognitive outcomes. There was a significant negative correlation between the patient's age at the beginning of treatment and IQ/DQ values (Spearman $r = -0.557$, $p = 0.025$), indicating that earlier treatment results in better developmental/cognitive outcomes. No significant correlation was found between OH-Cbl dose and IQ/DQ values.

Since the highest concentration of OH-Cbl solutions presently available in many countries is 5 mg/mL, maintaining a long-term dose ≥0.35 mg/kg/day must be expected to become increasingly burdensome in terms of injection frequency and volume when children grow and gain weight. Daily parenteral injections– especially of larger volumes– of OH-Cbl are painful and affect the quality of life [141]. Thus, they become a major challenge for patients and their families.

In contrast to early-onset patients in whom cognitive impairment develops and/or persists despite treatment, later-onset patients' cognitive dysfunction, such as memory issues, confusion, reduced calculation or verbal and conversational capacities, is in more than 40% of cases part of the acute/subacute symptoms at onset [142], and reverts upon treatment with standard-dose OH-Cbl [142–145].

Recommendation 13: to improve neurocognitive outcome we suggest daily parenteral high-dose OH-Cbl treatment (≥0.35 mg/kg/day) from immediately after diagnosis in both clinically diagnosed early-onset and in NBS identified patients with cblC-*MMACHC*. The dose should be selected so that the lowest individual tHcy can be achieved and Met can be safely maintained within the normal range with a patient-tolerated number/volume of injections.

There is no data supporting the superiority of high-dose over standard OH-Cbl treatment for patients with late-onset disease.

Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Quality of the evidence: low.

Expert advice 3: To improve neurocognitive outcome the panel suggests maintaining high-dose OH-Cbl treatment during the first 2–3 years of life in cblC-*MMACHC* cases in whom the clinical course and/or the genotype indicates a course with high risk for neurocognitive impairment or when a clear genotype–phenotype correlation cannot be established. (Consensus; 92%).

Expert advice 4: If high-dose treatment has been initiated and incoming genetic, biochemical, and clinical data suggest an attenuated disease course, decreasing the OH-Cbl dose below 0.35 mg/kg/day by reducing the frequency of injections first should be attempted while preserving the best possible individual biochemical profile. (Strong consensus; 100%).

Expert advice 5: Genetic testing should be initiated as soon as possible with an urgent processing request, as the results may influence the further therapeutic approach. (Strong consensus; 96%).

Expert advice 6: The panel recommends considering cblC-*MMACHC* (or other cRD) in patients of any age with unexplained neurocognitive impairment or decline, especially in the context of accompanying neurological abnormalities or multiorgan disease. (Consensus; 92%).

Expert advice 7: Standardized neuropsychological evaluation using validated assessment tools should be used for the screening and diagnosis of cognitive impairment in cblC-*MMACHC* and patients with other cRD. (Strong consensus; 100%).

8.3 | CblC-*MMACHC*, Eye Disease

Visual defects such as maculopathy, peripheral retinal degeneration, strabismus, nystagmus, and optic atrophy are described in all cRD. In the cblC-*MMACHC* defect, macular abnormalities with subtle pigmentary changes, classic bull's eye maculopathy, and macular atrophy or pseudo-coloboma in advanced stages

TABLE 5 | Studies reporting on high OH-Cbl regimens in early-onset cblC-*MMACHC* and epi-cblC patients.

References	Pt	Age at dose escalation start (years)	Genotype	Baseline OH-Cbl dosage (mg/kg/day)	Max OH-Cbl dosage (mg/kg/day)	tHcy at baseline (μmol/L)	tHcy at max OH-Cbl (μmol/L)	Cognitive outcome or IQ/DQ	Age at last evaluation (years/month)
Carrillo-Carrasco et al. (2009) [66]	I	13	271dup/271dup	0.0075	0.35	112	50	Long term memory	NA
Matos IV et al. (2013) [140]	I	14	271dup/271dup	0.04	0.14	90	63.8	Slight/ unchanged	16 years
	II	11	271dup/615C>G	0.1	0.22	53.6	44.7		12.5 years
	III	7	271dup/271dup	0.02	0.10	43.2	45.8	Unchanged	8.5 years
	IV	6	271dup/271dup	0.02	0.15	36.1	34.1	Unchanged	8.5 years
	V	5	271dup/271dup	0.2	0.35	29.5	28.7	Unchanged	7 years
								Slight/ unchanged	
Kacpura et al. (2022) [138]	I	13 days	271dup/271dup	—	2.0	57	10.3	Normal	18 m
Scalais et al. (2019–2023) [118, 119]	I	0.8	271dup/271dup	1.2	5	15	14.1	NA	6 years
	II	0.5	271dup/271dup	1.5	4.7	14	14.3	77	6 years
	III	0.1	271dup/271dup	1.3	6.8	46	15.9	82	5 years
	IV	0.15	271dup/435-436del	4.8	7.3	26	12.2	81	2.5 years
	V	5.9	271dup/394C>T	3.5	6.2	34	12.1	50 estimated	NA
Sloan et al. (2024) [139]	I–VI	0–1	271dup/271dup	NA	0.4–2.7	NA	15.2–31.1	78–105 (VABS)	6 months–8.5 years
Olivieri et al. (2024) [141]	I	0.5	394C>T/PRDX1	0.3	0.9	29	10	100	44 months
	II	0.3	271dup/271dup	0.19	0.83	50	23.8	100	36 months
	III	0.16	271dup/271dup	0.3	0.47	32	15.6	98	42 months
	IV	0.25	271dup/271dup	0.25	0.45	41	23.7	75	32 months
	V	10 days	271dup/271dup	—	0.35	188	27	88	24 months

are observed. The underlying pathophysiological mechanisms remain poorly understood [146–149]. The onset is early in life and sometimes even prenatally with a tendency to worsen over time [63, 150–154].

First changes can be observed using spectral domain optical coherence tomography (SD-OCT) before the first year of life in 80% of cblC-*MMACHC* patients [63, 118, 149, 153, 154], when electroretinography (ERG) may still be normal [63, 153].

Nystagmus and strabismus are described in 76% and 31% of early-onset patients, respectively (Table 3). Optic atrophy is more frequent in early-onset cases (64%) compared to late-onset patients (36%) [63].

From the E-HOD registry (until 2019), 29 of the 161 (18%) cblC-*MMACHC* patients presented with visual problems. This prevalence increased to around 28% when considering only early-onset forms [30]. A higher incidence of ocular problems, 84% and 93% in 137 and 53 cases, respectively [63, 150], confirms the marked prevalence and severe macular involvement in early-onset forms. In late-onset forms, ocular problems are rare [26, 155, 156] (Table 6).

Larger case series with a significant number of cblC-*MMACHC* patients are reported in Table 7. These studies not only confirm the higher prevalence of eye disease in early-onset forms but also suggest an even greater prevalence among patients with the homozygous c.271dup p.(Arg91fs) genotype, in whom maculopathy was observed in 79.3% of cases, compared to 70.5% in other genotypes associated with early-onset disease and 11% in late-onset cases. Estimating prevalence in other cRD is challenging due to the small number of reported patients [30, 63].

Early treatment with parenteral OH-Cbl at standard doses does not prevent the progression of ophthalmological manifestations

TABLE 6 | Ocular findings in early and late-onset cblC-*MMACHC* patients reported in case series (more than 10 patients) published between 2015 and 2024.

References	*N* Pts		*N* early-onset		*N* late-onset
			c.271dup/271dup	Other genotypes	
Aleman (2015) [150]	53		10	37	6
		Maculopathy	9/9 tested	24/33 tested	—
		Nystagmus	4/4 tested	27/34 tested	1
		Strabismus	Not reported	Not reported	Not reported
Bonafede (2015) [151]	11		3	6	2
		Maculopathy	2	5	
		Nystagmus	3	5	
		Strabismus	Not reported	Not reported	
Brooks (2016) [152]	25		14	7	4
		Maculopathy	14	4	—
		Nystagmus	9	7	—
		Strabismus	8	5	—
Bacci (2017) [153]	11		5	5	1
		Maculopathy	5	4	—
		Nystagmus	3	2	—
		Strabismus	3	2	—
Matmat (2022) [63]	137		47	74	14
		Maculopathy	31	54	6
		Nystagmus	Not reported	Not reported	2
		Strabismus	Not reported	Not reported	1
Kalantari (2022) [26]	45		0	0	45
		Maculopathy			2
		Nystagmus			—
		Strabismus			—
		Optic atrophy			6
Olivieri (2024) [141]	20		13	7	0
		Maculopathy	12	5	
		Nystagmus	Not reported	Not reported	
		Strabismus	Not reported	Not reported	

TABLE 7 | High-dose OH-cbl and cognitive outcome in cblC-*MMACHC* patients: data from the E-HOD members' survey.

N Pts	Mode of diagnosis	Age at the start of high OH-cbl dose treatment (months)	Cognitive outcome	Age at follow up (months)	Plasma tHcy (μM/l) under high-dose OH-Cbl	Mean (range), OH-Cbl (mg/kg/day)
8	NBS/FS	≤2	4 normal	6–60	19–31 ($n=2/4$)	1.3 (0.4–2.0)
			3 borderline	21–64	12–18 ($n=2/3$)	
			1 mild ID	144	20–30	
3	NBS	≥3	1 borderline	60	20–32	1.1 (0.37–2.0)
			2 mild ID	35 and 84	24–30	
5	Clinical	≥3	2 borderline	79 and 103	9 and NA	1.4 (0.37–3.5)
			1 mild ID	14	49	
			2 moderate ID	108 and 105	20 and NA	

Abbreviations: FS, familial screening; ID, intellectual disability; NA, not assessed; NBS, newborn screening.

and early visual loss [1, 63, 101, 117, 140, 141, 151–153, 157–162]. However, recent literature reports that early initiated high-dose OH-Cbl treatment may have a protective effect against severe forms of maculopathy in patients homozygous for the cblC-*MMACHC* c.271dup p.(Arg91fs) variant. Five out of six patients, who received between 0.4 and 2.7 mg/kg/day OH-Cbl since the first month of life, two prenatally, showed no evidence of maculopathy at follow-up after more than 1 year [139]. Five patients ($n=4$ homozygous for c.271dup p.(Arg91fs)), treated with OH-Cbl at 6.5 ± 3.3 mg/kg/day from between 1 and 5 months, had mild to moderate nystagmus and perifoveal irregularity, but no bull's eye maculopathy at the age of 3.5 to 7.3 years [118, 119]. A homozygous c.271dup p.(Arg91fs) patient diagnosed by NBS and treated with 2 mg/kg/day of OH-Cbl from day 13 of life had a normal eye exam at the age of 19 months [138].

A survey among E-HOD members identified that 8 of the 16 unpublished homozygous c.271dup p.(Arg91fs) cases treated with high dose OH-Cbl started at ages ranging from the prenatal period to 5 years old, developed maculopathy. A significant dose-effect gradient could not be established (X^2: 0.0847, $p=0.771$).

In utero treatment with OH-Cbl has been described in anecdotal cases with mixed outcomes. A dose of 10 mg three times per week, starting from the 15th–20th week of gestation, followed by 1 mg/day after delivery with later escalation to 10 mg twice per week, resulted in mild ophthalmological findings and a normal cognitive profile in a girl in contrast to her severely affected sibling who presented symptomatically on day 20 of life [163]. However, previously described cases report that despite ensuring good metabolic control and achieving major psychomotor developmental milestones, prenatal and postnatal treatment could not prevent nystagmus and macular remodelling [161]. High-dose treatment is not expected to reverse established maculopathy in older individuals [154].

Recommendation 14: to improve the eye phenotype we suggest daily parenteral high-dose OH-Cbl treatment (≥0.35 mg/kg/day) from immediately after diagnosis in both clinically diagnosed early-onset and NBS identified patients with cblC-*MMACHC*. The dose should be selected so that the lowest individual tHcy can be achieved and Met can be safely maintained within the normal range with a patient-tolerated number/volume of injections. Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Maculopathy is rare and less severe in patients with late-onset disease. There is no data supporting the superiority of high-dose over standard OH-Cbl treatment for this group.

Quality of the evidence: low.

Expert advice 8: To improve the eye phenotype the panel suggests maintaining high-dose OH-Cbl treatment during the first 2–3 years of life in cblC-*MMACHC* cases in whom the clinical course and/or the genotype indicates a high risk for maculopathy or when a clear genotype–phenotype correlation cannot be established. (Strong consensus; 100%).

Expert advice 9: If high-dose treatment has been initiated and incoming genetic, biochemical, and clinical data suggest an attenuated disease course, decreasing the OH-Cbl dose below 0.35 mg/kg/day by reducing the frequency of injections should be attempted while preserving the best possible individual biochemical profile. (Strong consensus; 96%).

Expert advice 10: Genetic testing should be initiated as soon as possible with an urgent processing request, as the results may influence the further therapeutic approach. (Consensus; 88%).

Expert advice 11: The panel recommends timely testing for cblC-*MMACHC* (or other cRD) in patients of any age with unexplained maculopathy, especially in the context of accompanying neurological abnormalities or multiorgan disease. (Strong consensus; 100%).

Expert advice 12: Standardized ophthalmology evaluations should be initiated in the neonatal period for patients diagnosed through NBS or presenting with an early disease onset.

Standardized ophthalmological and orthoptic evaluations during follow-up should be planned for patients diagnosed with maculopathy, strabismus, and/or nystagmus. (Strong consensus; 100%).

Expert advice 13: For macular examination, the gold standard is the DS-OCT. If it is not available, the exclusion of maculopathy should be obtained at least by a fundus examination and, if available, by ERG. The macular examination should be repeated periodically (e.g., every 6–12 months) to assess potential progression, especially in the first years of life. The assessment of the optic nerve should consist of a neurophysiological evaluation with VEP. Repeat assessments should be done as clinically indicated. (Strong consensus; 100%).

8.4 | CblC-*MMACHC*, Epileptic Seizures and Epilepsy

Seizure prevalence in cRD as inferred from published case series and the E-HOD registry ranges between 16% and 50% [21, 22, 30, 117, 164]. In early-onset forms, the overall epilepsy prevalence is 25%–50% [1, 22, 164], with focal seizures and infantile spasms accounting for 70%–80% of the cases and status epilepticus for about 20%–30% [165]. In late-onset forms, the prevalence is estimated at approximately 25%–30%, and seizure semiology is heterogeneous, with a predominance of focal seizures [123, 166]. Seizure prevalence is higher in clinically diagnosed patients and usually occurs at disease onset [101]. This is attributed to a lower epileptogenic threshold linked to the neurotoxic effect of high tHcy [167, 168] and methylmalonic acid [169].

Although a strict genotype–phenotype correlation is lacking, certain *MMACHC* variants are more consistently associated with epilepsy (Table 8). In early-onset cblC–*MMACHC* patients, the c.271dup p.(Arg91fs) variant is associated with neonatal-onset seizures as part of a multisystemic presentation when present in homozygosity or in compound heterozygosity with other severe variants [101]. The homozygous c.609G>A p.(Trp203Ter) variant—prevalent in the Chinese population—is generally linked to early-onset disease with epileptic seizures and hydrocephalus [23, 170, 171]. The c.394C>T p.(Arg132Ter) variant, reported in both early- and, more commonly, late-onset cases [101, 171, 172] has been associated with infantile spasms [173], focal or generalized seizures [101] and/or movement disorders such as tremor and dyskinesia [174]. In late-onset phenotypes, the *c*.482G>A p.(Arg161Gln) variant—prevalent in the Chinese patients—is reported with seizures in about 26% of cases, alongside psychiatric symptoms, peripheral neuropathy, and pyramidal signs [26, 31, 32, 123, 155, 171, 175].

Treatment with antiepileptic drugs can be added to OH-Cbl therapy as clinically warranted without disease-specific limitations. Although valproate can theoretically lead to a derangement in mitochondrial metabolic balance, its use has been reported without significant side effects and with a good response for certain types of epileptic seizures in cblC-*MMACHC* patients [25, 176, 177].

Response to treatment is variable. Early-onset patients with epilepsy on OH-Cbl treatment more often require additional, long-term antiepileptic treatment with one or more combination drugs [165, 176]. In late-onset cases with epileptic seizures at disease onset, seizures mostly respond well to standard disease-specific therapy [25, 31, 33, 176].

Recommendation 15: we suggest treating seizures in cblC-*MMACHC* with parenteral OH-Cbl and antiepileptic drugs, as clinically required. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment. Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Quality of the evidence: low.

Expert advice 14: The panel recommends timely testing for cblC-*MMACHC* (or other cRD) in patients of any age with unexplained seizures, especially in the context of accompanying neurological abnormalities or multiorgan disease. (Consensus; 92%).

Expert advice 15: An EEG should be performed at the time of a cblC-*MMACHC* (or other cRD) diagnosis in patients with neurological signs. (Consensus; 88%).

Expert advice 16: There is no clinical evidence that certain antiepileptic drugs should not be used in patients with cblC-*MMACHC* or other cRD, but preclinical data suggest that other drugs should be favoured over valproate [177]. (Strong consensus; 100%).

8.5 | CblC-*MMACHC*, Psychiatric and Behavioural Problems

Data derived from case reports, case series, and the E-HOD registry suggest that the prevalence of psychiatric disorders in cRD is around 5%–25% [30, 116]. In cblC-*MMACHC* patients, neuropsychiatric disorders are reported in 5%–40% of cases in Europe [21, 30, 142] and about 46% in Chinese late onset case series [171].

In early-onset forms, chronic behavioural disorders such as autism spectrum disorders, attention-deficit/hyperactivity disorder (ADHD), attentional instability [101], irritability, sleep disturbance, and hetero- or self-aggression [135] frequently accompany psychomotor delay. These symptoms typically emerge around the peripubertal age [101, 135]. Long-term treatment with risperidone, aripiprazole, or other antipsychotics has been reported as beneficial [135, 178].

In late-onset forms, acute psychosis or transient behavioural changes are reported, mostly at disease presentation. The prevalence is 24%–40% in European and American cases [21, 142], and 68.2% in Chinese cases [33], respectively. In Europe, America, and South Asia, the age at onset ranges from 6–10 to 42–45 years [135, 142] with a peak in adolescence or adulthood, but presentation during childhood (mean age 7 years, range 6–9 years) has also been reported [179–182]. Similarly, in Chinese cases the average age of onset is during school age or adolescence [33, 171]. A review of the literature from 2015 to 2023 on the cblC-*MMACHC*

TABLE 8 | Common *MMACHC* variants in the cblC-*MMACHC* defect: type, clinical onset, and population distribution.

cDNA	Protein	Type	Residual function	Typical onset*	Main ethnic distribution (% of affected alleles)	Phenotype summary
c.271dup	p.Arg91Lysfs*14	Frameshift → premature stop	None	Early	Very common in Caucasian/ Mediterranean populations (~40%–55%); rare in Chinese population (~0.8%)	Multisystem disease, hydrocephalus, developmental delay, seizures, maculopathy (~79%), aHUS
c.394C>T	p.Arg132*	Nonsense → premature stop	Partial	Late/variable	Observed worldwide (Chinese population ~2.3%)	Seizures and movement disorders, psychiatric symptoms, neuropathy. Also, early onset epileptic encephalopathy
c.389A>G	p.Tyr130Cys	Missense	Partial	Late	Observed worldwide	aHUS, psychiatric symptoms, variable severity
c.440G>C	p.Arg147Pro	Missense	Partial	Late (adult)	Very common in Caucasian/ mediterranean populations (frequently found by NBS)	Psychiatric symptoms, PAH
c.80A>G	p.Gln27Arg	Missense	Partial	Late (rarely early)	Chinese populations (~6%); also reported worldwide	Renal TMA, DLD/ILD and PAH
c.658_660del	p.Lys220del	In-frame deletion	Partial	Late	Chinese populations (~10%–14%)	Psychiatric symptoms, Heart failure
c.484G>T	p.Gly162Cys	Missense	Partial	Late	Nord European countries	Isolated PAH
c.464G>A	p.Gly155Glu	Missense	Partial	Late	Nord European countries	aHUS, PAH
c.276G>T	p.Glu92Asp	Splite-site mutation	Partial	Late	Nord European countries (~17%)	Renal TMA, PAH
c.565C>A	p.Pro189Thr	Missense	Partial	Late	Nord European countries (~11%)	Renal TMA, PAH
c.609G>A	p.Trp203*	Nonsense → premature stop	None	Early	Very common in Chinese/East Asian populations (~35%–49%)	Multisistem disease, hydrocephalus (15%) epileptic seirures
c.482G>A	p.Arg161Gln	Missense	Partial	Late	Chinese/East Asian populations (~6%–57%); Hispanic, also reported worldwide	Psychiatric symptoms, neuropathy, spastic paraplegia, epileptic seizures, also mild phenotype
c.567dup	p.Ile190Tyrfs*13	Frameshift → premature stop	None	Early	Chinese population (~5%–6%), occasionally reported worldwide	Multisystem disease, developmental delay, seizures, maculopathy
c.217C>T	p.Arg73*	Nonsense → premature stop	None	Early	Reported in Chinese cohorts (~1.4%)	Multisystem disease, developmental delay, seizures, maculopathy

Note: *The clinical presentation depends on the combined functional impact of both MMACHC variants present in each patient: alleles associated with a complete loss of function will cause the respective clinical phenotype if present in homozygous state or with a similarly severe allele.
Abbreviations: aHUS, atypical hemolytic uremic syndrome (hemolytic anemia, uremia, and thrombocytopenia); DLD, diffuse lung disease; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; TMA, thrombotic microangiopathy (microvascular thrombosis with thrombocytopenia, hemolytic anemia, and red blood cell fragmentation).

defect has allowed for the identification of the following neuropsychiatric symptoms: psychosis (30.6%), visual and auditory hallucinations (16.5%), depression (16.5%), emotional instability (12.9%), troubled interpersonal relations (11.8%), irritability (10.6%), sleeping problems (10.6%), eating disorders (10.6%), introversion and withdrawal (8.2%), use of nonsensical, obscene, or perseverative speech (7.1%) and hetero- and self-aggression (5.9%). Most patients showed an immediate and sustained positive response of their psychiatric symptoms with standard OH-Cbl treatment [26, 145, 151, 178, 182–184]. Nevertheless, impairments in daily functioning to varying degrees have been reported in a follow-up study (median 4.9 years; range 0.5 to 13.4 years) [33]. Multivariate logistic regression analysis revealed that the time from onset to diagnosis is an independent risk factor affecting prognosis, highlighting the importance of early diagnosis and timely treatment (Table 8).

Recommendation 16: we suggest treating psychiatric and behavioural problems in cblC-*MMACHC* with parenteral OH-Cbl and psychiatric drugs, as clinically required. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment. Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Quality of the evidence: low.

Expert advice 17: The panel recommends considering cblC-*MMACHC* and other cRD in the differential diagnosis of unexplained acute psychoses/behavioural changes. (Strong consensus; 100%).

Expert advice 18: Standardized neuropsychological and neuropsychiatric evaluations using validated assessment tools should be applied for the screening and diagnosis of behavioural and psychiatric symptoms in paediatric and adult patients with cblC-*MMACHC* or other cRD. (Strong consensus; 100%).

Expert advice 19: There is no evidence that certain antipsychotic drugs should not be used in patients with cblC-*MMACHC* and other cRD. (Strong consensus; 100%).

8.6 | CblC-*MMACHC*, Renal Disease

Renal manifestations have been reported in 58/824 patients with disorders of cbl transport and processing (not restricted to RD), of which 37/824 presented with aHUS [4]. Chronic kidney disease was reported in 37/509 before the age of 1 year, in 12/133 between the age of 1 and 14 years, and in 7/74 after the age of 14 years. Acute kidney failure or aHUS occurred in 21/209 before 1 year of age, in 14/133 in 1 to 14-year-olds, and in only 2/74 above the age of 14 years [4].

Renal manifestations of cblC-*MMACHC* disease include thrombotic microangiopathy (TMA), aHUS, or proteinuria and nephrotic syndrome. Renal biopsies have revealed fibrin thrombi in glomerular capillaries, endothelial thickening, and detachment of the glomerular basal membrane as well as mesangial deposits of IgA or IgM, but not of complement component 3 or IgG [185, 186]. Renal TMA can present at any age, but it mostly manifests during the neonatal and infantile period [21, 187]. Renal TMA has so far not been reported in cblD-*MMADHC*, cblF-*LMBRD1*, and cblJ-*ABCD4* disease.

Of a sample of 19 patients with early onset cblC-*MMACHC* disease and renal TMA, 10 patients manifested in the neonatal period; seven patients died. Homozygosity for the c.271dup p.(Arg91fs) variant was observed in most infants [186].

A comprehensive review of 192 patients with cblC-*MMACHC* disease with disease onset after the first 12 months of life reported 31 patients presenting with renal TMA or aHUS, and four with unspecified kidney disease [131]. Twelve of the 31 patients with renal TMA also had pulmonary TMA, sometimes manifesting years after renal TMA. The age at manifestation of renal disease ranged between 1.5 and 26 years (median 8.0, mean 9.87, SD 7.02) [131].

The pathogenesis of microangiopathy and aHUS in patients with RD is not clear. A possible contribution of abnormalities in the complement system has been suggested [186]. However, most patients that have been treated with steroids, plasma exchange or suppressors of the complement system such as eculizumab prior to the diagnosis of a RD have shown only temporary or no response to this treatment [188, 189].

Treatment with parenteral OH-Cbl and oral betaine resulted in clinical improvement in most of the 12 surviving early-onset patients with TMA/aHUS presented by Karava [186]. This is further supported by a case series from Liu [190] presenting seven children with a different genetic background (variants c.80A>G p.(Gln27Arg), c.609G>A p.(Trp203Ter) and c.658_660del p.(Lys220del)) and by the case report (homozygous for c.484G>T p.(Gly162Trp) from Adrovic [191]). Early treatment with (very variable) doses of vitamin B12 and betaine was the most important predictor of a good outcome [186, 190–192]. Based on the review by Arhip 2024, 24 late onset patients with renal TMA could be identified for whom long-term data was available [131]. Initial OH-Cbl doses varied between 1 and 5 mg/day. Resolution of aHUS/TMA but residual damage with chronic renal failure, hypertension, and proteinuria was reported in 20/24 cases treated with OH-Cbl, betaine and carnitine [24, 37, 66, 185, 189, 193, 195-202], while renal transplant was required in one case [167]. Two patients died despite OH-Cbl treatment [193, 202]. Complete resolution under treatment with parenteral OH-Cbl, folic acid, betaine, and carnitine was reported in 2/24 cases [203, 204].

A superior effect of higher doses of OH-Cbl on recovery from aHUS was observed in a single case study [66].

Recommendation 17: we suggest treating renal TMA/aHUS in cblC-*MMACHC* patients with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no sufficient evidence of the superiority of high-dose OH-Cbl treatment. Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Quality of the evidence: low.

Expert advice 20: The panel recommends timely testing for cblC-*MMACHC* (or other cRD) in patients of any age with

atypical HUS/renal TMA or unexplained acute renal dysfunction. (Strong consensus; 100%).

8.7 | CblC-*MMACHC*, Cardiopulmonary Disease

The cardiopulmonary involvement in cRD includes structural cardiac defects with or without heart failure, pulmonary arterial hypertension (PAH), and diffuse (DLD) or interstitial lung disease (ILD). The incidence of structural cardiac defects in patients with the cblC-*MMACHC* defect has long been underestimated [127, 205, 206]. Recent advances in cardiac screening revealed that approximately 50% of patients present structural heart defects, including left ventricular hypertrabeculation with or without non-compaction, secundum atrial septal defect, and dysplastic pulmonary valve [207, 208]. Rarely, coronary artery ectasia has been reported [34]. The commonly described septal abnormalities and left ventricular non-compaction trabeculation can be associated with enhanced risks of systemic embolization and stroke. Septal irregularities may cause paradoxical embolization, while left ventricular non-compaction trabeculation can lead to thrombus formation within the muscle trabeculae crypts, especially in the presence of hyperhomocysteinemia [207].

DLD/ILD due to microangiopathy has been observed with or without PAH in patients with the cblC-MMACHC defect [37, 38, 198, 209]. This clinical phenotype has been described in Asian countries, with a strong association with the c.80A>G p.(Gln27Arg) variant, mainly in late-onset cases presenting in childhood, [37, 38, 210] although some early-onset cases with this presenting feature have also been described [211].

Along with cardiological support therapy, Liu et al. report the use of cyanocobalamin in some of these cases at an unspecified dosage (except for a single patient who received 0.5 mg/day), showing clinical and biochemical benefit [37]. The generalisability of this treatment approach seems questionable because an impaired cblC-*MMACHC* protein could most probably not process cyanocobalamin effectively [212].

Heart failure mostly occurs in early onset cblC-*MMACHC* patients early during the disease, but can also appear later, especially in patients with a non-compacted myocardium [186, 208, 210]. These patients generally respond well to specific cardiac therapy, alongside cblC-*MMACHC*-specific treatment [36, 210], although one case has been reported requiring left ventricular assist device implantation and later a heart transplant [208].

In late-onset patients, heart failure is less frequent, and development is often associated with PAH [130, 210, 213]. Case studies from China showed approximately 10% of late onset cblC-*MMACHC* patients with PAH had a poor prognosis if not diagnosed and treated early [32, 198, 214]. Renal microangiopathy is also frequent in these patients [129, 130, 196, 213]. The *MMACHC* variants c.276G>T p.(Glu92Asp), c.464G>A p.(Gly155Glu), and c.565C>A p.(Arg189Ser) are frequently linked to PAH and renal thrombotic microangiopathy [129, 130, 196].

Recommendation 18: we suggest treating cardiovascular disease in cblC-*MMACHC* with parenteral OH-Cbl and cardiological therapy, as clinically required. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence to support the superiority of high-dose OH-Cbl treatment. Due to the pathophysiological analogies, the panel recommends that this approach also be considered for patients with other cRD.

Quality of the evidence: low.

Expert advice 21: The panel recommends timely testing for cblC-*MMACHC* (or other cRD) in patients of any age presenting with left ventricular hypertrabeculation with or without non-compaction cardiomyopathy and/or unexplained PAH or DLD/ILD. (Strong consensus; 96%).

Expert advice 22: Cardiological screening with echocardiography should be performed at the time of diagnosis of a cRD to detect/exclude structural cardiac abnormalities. Follow-up of cardiological investigations should be performed as clinically indicated. (Strong consensus; 100%).

8.8 | CblC-*MMACHC*, Thromboembolic Events

Thromboembolic events are a significant cause of morbidity and mortality in patients with cRD [1]. The higher frequency of vascular incidents described in the cblC-*MMACHC* defect may be due to its higher prevalence compared to other cRD. Reports of thrombotic events in other cRD are anecdotal [215].

The description of diffuse vascular lesions with proliferative fibrous intimal plaques and focal necrosis of the arterial wall dates to the first reported case of cblC-*MMACHC*, which contributed to the development of the homocysteine theory of arteriosclerosis [1, 217].

Thromboembolic events in the cblC-*MMACHC* defect are described almost exclusively in adults with the late-onset form of the disease [26]. The reported vascular events include primarily deep vein thrombosis and/or recurrent thrombosis [26, 31, 32, 166, 184] sometimes complicated by massive and even fatal pulmonary embolism [129, 130, 210].

Whether specific tHcy concentrations are associated with vascular complications is not entirely clear. While the reported cases consistently show homocysteine levels > 150 μmol/L, these values are often not strictly correlated with the exact time at which the vascular incident occurs.

Acute vascular issues resolve with targeted anticoagulant therapies, and patients under standard cRD treatment seem not to experience new thromboembolic events [32]. Although evidence suggests that hyperhomocysteinaemia promotes atherothrombosis, at least in part through platelet activation, the effect of antiplatelet agents in hyperhomocysteinaemia remains uncertain. The clinical safety and utility of combination low-dose aspirin and folate remain to be confirmed [217].

Recommendation 19: we suggest treating thromboembolic events in cblC-*MMACHC* with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There

is no evidence of the superiority of high-dose OH-Cbl treatment. The panel advises considering this approach also in patients with other cRD.

Quality of the evidence: low.

Expert advice 23: The panel recommends timely testing for cblC-*MMACHC* (or other cRD) in patients of any age presenting with unexplained thromboembolic events. (Strong consensus; 100%).

9 | The Cobalamin-Related Isolated Remethylation Disorders: cblD-*MMADHC* Subtype cblD-Hcy, cblE-*MTRR*, cblG-*MTR*

Disease course and outcome in the very rare Cbl-related iRD generally share many features with cRD and are mostly treated according to recommendations for cRD. However, it remains unclear whether this approach provides the best available treatment options.

Due to the rarity of the diseases, there are not many publications available. To broaden the basis for recommendations, we collected 30 (23 cblG-*MTR* and 7 cblE-*MTRR*) additional clinical case descriptions via a survey shared within E-HOD and through professional networks. The data did not allow for statistical workup and were analysed descriptively. In the survey cases, treatment doses covered broad ranges and dosing intervals.

9.1 | Cobalamin-Related Isolated Remethylation Disorders, Disease-Related Mortality

Deceased patients with iRD have been reported in the literature, but the current data do not allow for a recommendation on the prevention of disease-related mortality.

9.2 | Cobalamin-Related Isolated Remethylation Disorders, Neurocognitive Development

For cbl-related iRD, feeding problems, anemia, muscular hypotonia, seizures, cognitive impairment, microcephaly, brain atrophy and white matter changes as well as behavioural problems and psychiatric symptoms are the characteristic findings [116, 218–220]. Hydrocephalus has rarely been reported [116]. Of the survey cases, 64% had some degree of cognitive impairment and treatment was considered to at least prevent progression in 61% of cases. Median age at treatment start was very variable with 7 (range 0.25–360) months for all, 6 (range 0.25–360) months for cblE-*MTRR*, and 8 (range 0.5–216) months for cblG-*MTR* patients. Therefore, the survey cannot add information to the suggestion of a more favourable neurodevelopmental outcome in cblG-*MTR* and cblE-*MTRR* disease if treated early [221, 222].

Recommendation 20: we suggest treating neurocognitive impairment in patients with iRD with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment.

Quality of the evidence: low.

Expert advice 24: The panel recommends considering an iRD in patients of any age with unexplained neurocognitive impairment or decline, especially in the context of accompanying neurological abnormalities or multiorgan disease. (Strong consensus; 100%).

Expert advice 25: Standardized neuropsychological evaluation using validated assessment tools should be conducted at diagnosis and during regular monitoring of cognitive function in iRD patients. (Strong consensus; 100%).

9.3 | Cobalamin-Related Isolated Remethylation Disorders, Eye Disease

Cbl-related iRD eye disease is mainly comprised of retinopathy; however, nystagmus, strabismus, or unspecific visual impairment are frequently observed, and none of these symptoms seem to respond well to treatment [63, 116, 218]. Two cblG-*MTR* and two cblE-*MTRR* patients had severely affected ERG despite normal fundus and retina exam. Macular changes were characterised by bull's eye maculopathy and in advanced stages by macular atrophy and chorioretinal lesions referred to as pseudo-coloboma or coloboma-like macular lesions [63]. From the 30 patients in the survey, 13% had mild and 23% moderate eye disease. Treatment was considered to have at least prevented progression in 43% of cases. There is insufficient data to allow for any dose-related recommendation.

Recommendation 21: we suggest treating ocular involvement in patients with iRD with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment.

Quality of the evidence: low.

Expert advice 26: The panel recommends timely testing for a Cbl-related iRD in patients of any age with unexplained visual impairment, especially in the context of accompanying neurological abnormalities. (Strong consensus 100%).

9.4 | Cobalamin-Related Isolated Remethylation Disorders, Epileptic Seizures and Epilepsy

Epilepsy occurs predominantly in severe, early onset forms of the cblG-*MTR* defect. The types of seizures vary, ranging from neonatal seizure, epileptic spasms, complex partial seizures, atypical absences to generalized seizures [223, 224]. Response to antiepileptic therapy is variable, as is the response to OH-Cbl treatment [223, 225]. From the 30 patients in the survey, 23% had epilepsy/seizures. Treatment was considered to have at least prevented progression in 76% of cases. There is insufficient data to allow for any dose-related recommendation.

Recommendation 22: we suggest treating seizures in patients with iRD with parenteral OH-Cbl and antiepileptic drugs as clinically required. Oral betaine can potentially be added to

optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment.

Quality of the evidence: low.

Expert advice 27: An EEG should be performed at the time of an iRD diagnosis in patients with neurological signs. (Consensus; 88%).

Expert advice 28: There is no clinical evidence that certain antiepileptic drugs should not be used in patients with iRD, but preclinical data suggest that other drugs should be favoured over valproate [177]. (Consensus; 92%).

9.5 | Cobalamin-Related Isolated Remethylation Disorders, Psychiatric Symptoms

From the 30 patients in the survey, 28% had psychiatric symptoms. Treatment was considered to have at least prevented progression in 40% of cases.

A recommendation cannot be given.

Expert advice 29: Standardized neuropsychological and neuropsychiatric evaluations using validated assessment tools should be applied for the screening and diagnosis of behavioural and psychiatric symptoms in paediatric and adult iRD patients. (Strong consensus; 100%).

Expert advice 30: The panel recommends considering iRD in the differential diagnosis of unexplained acute psychoses/behavioural changes. (Strong consensus; 96%).

Expert advice 31: There is no evidence that certain antipsychotic drugs should not be used in patients with iRD. (Strong consensus; 100%).

9.6 | Cobalamin-Related Isolated Remethylation Disorders, Renal Disease/Microangiopathy

Atypical HUS and glomerulopathy have been reported in single patients with cblE-*MTRR* and cblG-*MTR* disease from the neonatal phase to adolescence [188, 226–231].

The mode of presentation and renal histology findings overlap with cblC-*MMACHC* disease. A case study reported minimal improvement under betaine (240 mg/kg per day) and 1 mg/day OH-Cbl in an 8-month-old child. Regression of acute TMA and partial recovery of kidney function was observed when OH-Cbl was increased to 10 mg/day [228]. The trajectory of other case reports suggests that patients may develop chronic renal problems [188, 226, 227]. Renal disease may occur in combination with neurocognitive impairments [215, 227].

Recommendation 23: we suggest treating renal TMA/aHUs in patients with iRD with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment.

Quality of the evidence: low.

Expert advice 32: The panel recommends timely testing for Cbl-related iRD in patients of any age with atypical HUS/renal TMA or unexplained acute renal dysfunction or pulmonary hypertension (Strong consensus; 100%).

9.7 | Cobalamin-Related Isolated Remethylation Disorders, Cardiopulmonary Disease

PHA with respiratory failure resolved after treatment in a single cblG-*MTR* patient with aHUS/TMA [228]. Subacute respiratory failure as part of a general neurological deterioration has been described in cblG-*MTR* disease. Treatment restored respiratory function in weeks to months [232, 233]. Central myelopathy and peripheral neuropathy were proposed as possible mechanisms. Structural heart disease and cardiomyopathy are very rare in iRD. These data were supported by the survey: a single patient presented with hypertonia, another with microangiopathy and cardiac hypertrophy.

A recommendation cannot be given.

9.8 | Cobalamin-Related Isolated Remethylation Disorders, Thromboembolism

Thromboembolism may lead to severe clinical pathology but has been only rarely reported in Cbl-related iRD [116].

A recommendation cannot be given.

Expert advice 33: The panel recommends timely testing for a Cbl-related iRD in patients of any age presenting with unexplained thromboembolic events. (Strong consensus; 100%).

9.9 | Cobalamin-Related Isolated Remethylation Disorders, Haematological Problems

Macrocytic anaemia is a typical manifestation of the cblG-MTR and cblE-MTRR defects, but not of severe MTHFR deficiency. Methionine synthase is the only enzyme in mammals that uses 5-MTHF as a substrate. Tetrahydrofolate is in the process regenerated for the active folate pool, which supplies one-carbon units for nucleotide synthesis. A severe deficiency of methionine synthase leads to an accumulation of 5-MTHF at the expense of methylenetetrahydrofolate and tetrahydrofolate. The resulting disruption of nucleic acid synthesis causes apoptosis in rapidly dividing cells, leading to megaloblastic anemia [234]. By contrast, in severe MTHFR deficiency the formation of 5-MTHF is reduced, but the folate pool needed for DNA synthesis is preserved and anaemia is usually absent [220, 235].

iRD patients may present with isolated megaloblastic bone marrow failure and no further neurocognitive nor other impairments. Mostly, haematological problems respond well to OH-Cbl treatment [236]. Forty percent of survey cases had haematological problems, mainly (macrocytic) anaemia and occasionally

neutropenia. Haematological problems responded very well to treatment in 85% of cases.

Recommendation 24: we suggest treating haematological problems in patients with iRD with parenteral OH-Cbl. Oral betaine can potentially be added to optimize tHcy and Met levels. There is no evidence of the superiority of high-dose OH-Cbl treatment.

Quality of the evidence: low.

Expert advice 34: The panel recommends timely testing for Cbl-related iRD in patients of any age with unexplained (macrocytic) anaemia and/or unexplained neutropenia or pancytopenia, especially in the context of multiorgan disease. (Strong consensus; 96%).

10 | Severe MTHFR Deficiency

10.1 | Severe MTHFR Deficiency, Mortality

Early-onset disease is associated with a high risk of early death [121], whereas no or very low mortality has been reported in patients presenting in adolescence or adulthood [122, 123]. Recent experience suggests that early initiated betaine treatment reduces mortality in patients with severe MTHFR deficiency [123, 237].

Recommendation 25: we recommend early treatment with betaine as it reduces mortality in severe MTHFR deficiency.

Quality of the evidence: moderate.

10.2 | Severe MTHFR Deficiency, Neurocognitive Development

Severe neurological signs and cognitive decline characterize the natural course of MTHFR deficiency in paediatric patients, while neurological manifestations such as cognitive disorders, gait impairment, epilepsy, psychiatric symptoms, polyneuropathy, and visual deficits are the main symptoms of adult onset MTHFR deficiency [122, 238].

Aggravation of intellectual disability has been observed during the disease course in late-treated patients [122]. However, presymptomatic diagnosis and subsequent therapeutic management with betaine is associated with a better long-term neurodevelopmental outcome in MTHFR deficiency [123].

In a series of 13 adult-onset patients, cognitive and gait difficulties were associated with prevalent posterior white matter abnormalities on brain MRI. Notably, psychiatric symptoms (57%) and seizures (71%) were highly prevalent. On betaine treatment, most patients ($n = 9$) experienced a clinical improvement of the initial acute symptoms such as confusion, psychiatric decompensation, walking deterioration, and seizures. Most chronic symptoms, such as mild cognitive impairment, stabilized with treatment ($n = 4$) [122].

Recommendation 26: we recommend early treatment with betaine as it significantly improves clinical outcome and potentially prevents neurological deterioration in MTHFR deficiency if started before the onset of symptoms.

Quality of the evidence: moderate.

Expert advice 35: The panel recommends considering MTHFR deficiency in patients of any age with unexplained neurocognitive impairment or decline, especially in the context of accompanying neurological abnormalities or multiorgan disease. (Consensus; 92%).

Expert advice 36: Standardized neuropsychological evaluation using validated assessment tools should be applied for the screening and diagnosis of cognitive impairment in MTHFR patients. (Strong consensus; 100%).

10.3 | Severe MTHFR Deficiency, Eye Disease

In a retrospective multicentre cohort study, 48% of symptomatic, early-onset MTHFR deficiency patients developed a range of ocular complications (strabismus, nystagmus, optic nerve atrophy, uncoordinated eye movements, visual impairment) not ameliorated by treatment [123]. Up to 43% of patients with late-onset MTHFR disease exhibited ophthalmological complications [122]. Acute visual loss, strabismus, optic atrophy, retinopathy, secondary cataract, and lens dislocation may be presenting symptoms or appear during the disease course [122, 239]. A late-onset adult case demonstrated full vision recovery, while two cousins of the index patient showed only partial improvement under therapy [240].

A recommendation cannot be given.

Expert advice 37: Ophthalmological examination in MTHFR deficiency should be performed as clinically indicated. Whether MTHFR deficiency is associated with eye disease that responds to betaine or can be prevented by treatment is unclear. (Strong consensus; 100%).

10.4 | Severe MTHFR Deficiency, Epileptic Seizures and Epilepsy

Epilepsy is observed in most (70%) late onset MTHFR patients either at onset, at diagnosis or during the disease course. While betaine treatment is considered to stabilize and sometimes improve epilepsy, more specific data are not available [122]. However, Yverneau showed that presymptomatic diagnosis and subsequent therapeutic management with betaine in MTHFR deficiency is not only associated with improved long-term neurodevelopmental outcome but also with epilepsy prevention [123].

A recommendation cannot be given.

Expert advice 38: An EEG should be performed at the time of diagnosis with severe MTHFR deficiency in patients with neurological signs. (Consensus; 88%).

10.5 | Severe MTHFR Deficiency, Psychiatric Symptoms

Psychiatric/behavioural problems may be present at diagnosis; the proportion affected increases with age and seems not to be influenced by treatment [30, 121, 122, 136, 241]. The effectiveness of betaine and folates (folic or folinic acid, methylfolate) on psychiatric and behavioural symptoms has not been systematically studied. However, psychotic symptoms and affective disorders have been reported to be reversible with treatment using betaine (and B-vitamins) in MTHFR deficiency [242, 243], [authors' own observation].

A recommendation cannot be given.

Expert advice 39: Every patient with severe MTHFR deficiency should be treated with betaine and folinate or methylfolate, regardless of the presence of psychiatric symptoms.

Expert advice 40: Standardized neuropsychological and neuropsychiatric evaluations using validated assessment tools should be used for the screening and diagnosis of behavioural and psychiatric symptoms in paediatric and adult MTHFR patients. (Strong consensus; 100%).

Expert advice 41: The panel recommends considering MTHFR deficiency in the differential diagnosis of unexplained acute psychoses/behavioural changes. (Strong consensus; 96%).

Expert advice 42: There is no evidence that certain antipsychotic drugs should not be used in patients with MTHFR deficiency. (Strong consensus; 100%).

10.6 | Severe MTHFR Deficiency Deficiency, Renal Disease

MTHFR is not associated with renal disease.

10.7 | Severe MTHFR Deficiency, Cardiopulmonary Disease

Subacute respiratory failure as part of a general neurological deterioration has been described in MTHFR case reports. Treatment restored respiratory function within weeks to months [232, 233]. Central myelopathy and peripheral neuropathy were proposed as possible mechanisms. Structural heart disease or cardiomyopathy are very rare in MTHFR deficiency.

A recommendation cannot be given.

10.8 | Severe MTHFR Deficiency, Thromboembolism

In MTHFR deficiency, arterial and venous thrombosis are generally rare in childhood but occur in 18%–28% of adults [30, 122]. Recurrence risk seems to be low under treatment.

Recommendation 27: We suggest treating patients with MTHFR deficiency and thromboembolism with betaine.

Quality of evidence: low.

Expert advice 43: The panel recommends timely testing for MTHFR deficiency by tHcy in patients of any age presenting with unexplained thromboembolic events. (Strong consensus; 96%).

11 | The Impact of NBS on Outcomes in Combined and Cobalamin-Related Isolated Remethylation Disorders and Severe MTHFR Deficiency

NBS for the cblC-*MMACHC* defect should be considered since survival and prevention of severe complications such as HUS, hydrocephalus and haematological abnormalities in early-onset patients can be improved by early treatment [136, 244]. While the impact of early standard treatment on neurocognitive development and eye disease is limited [22, 101, 245], recent data suggest that early high dose treatment > 0.35 mg/kg/day may be more effective in alleviating or even preventing cognitive and eye impairment [118, 139].

Early diagnosis could be advantageous for late-onset cblC-*MMACHC* patients since cognitive and psychiatric problems, as well as renal function, myelopathy and axonal neuropathy, can be resolved or improved by treatment [1, 104, 105, 131, 142].

Recently, a 17-month-old male with cblJ-*ABCD4* deficiency detected by NBS has been described. With early detection and initiation of treatment, this patient remained asymptomatic with normal growth parameters and neurodevelopmental function [246]. In general, the present knowledge allows no conclusions concerning the clinical benefit of early treatment for the cblD-*MMADHC* subtype, cblF-*LMBRD1*, and cblJ-*ABCD4* defects.

Recommendation 28: we recommend NBS for cRD to allow for early treatment in patients as it improves survival and may prevent severe organ complications including aHUS and hydrocephalus in the cblC-*MMACHC* defect.

Quality of the evidence: moderate.

Recommendation 29: we suggest that NBS may be favourable by allowing for early high dose OH-Cbl treatment which may be more effective to treat neurocognitive problems and ocular involvement than standard treatment.

Quality of the evidence: low.

The use of NBS for detection of Cbl-associated iRD appears worthy of consideration. In the cblE-*MTRR* and cblG-*MTR* defects, macrocytic anaemia [70, 116, 247], and neurocognitive performance [223, 246, 247] often respond to treatment [248]. Eye disease seems often not to be responsive to standard treatment [116]. Early detection by NBS and timely treatment improved short-term outcomes of two reported asymptomatic patients with cblE-*MTRR* and cblG-*MTR* defects [249].

Recommendation 30: we suggest considering NBS for iRD since it allows for early treatment which may result in a more favourable clinical course.

Quality of the evidence: low.

Early detection by NBS and timely treatment improved short-term outcomes of two reported symptomatic patients with severe MTHFR deficiency [249]. Early betaine treatment has a clear positive impact on outcome in severe MTHFR deficiency [123, 237].

Recommendation 31: we recommend NBS for MTHFR deficiency to allow for early treatment with betaine. Presymptomatic betaine treatment improves survival and prevents severe neurological impairment.

Quality of the evidence: moderate.

12 | The Impact of Prenatal Treatment of an Affected Fetus

In utero treatment of a fetus affected by a cRD may result in positive clinical outcome and normal development after birth [163, 250, 251]. The earlier maternal OH-Cbl treatment is initiated (between the 15th–17th week of gestation), and the higher the OH-Cbl dose administered (30 mg/week IM [163] or 5 mg twice a week IM plus oral administration on the remaining 5 days [251]) to the mother, the better the clinical outcome of the child [251]. Normal development has been documented at 11 years of age, although with some ophthalmic abnormalities [163]. In the case of an affected fetus not treated in utero, the baby developed hydrocephalus, metabolic acidosis, and multiorgan failure within the first month of life [252]. Amniotic fluid metabolites and non-invasive prenatal testing can be used for diagnosis and monitoring of biochemical markers [95, 253–255], which may be useful for titrating the OH-Cbl dose.

Recommendation 32: we suggest the treatment of a fetus with a cRD with high doses of OH-Cbl early in pregnancy as it has improved clinical outcome and prevented neurological deterioration in single affected fetuses.

Quality of the evidence: low.

13 | Overview of Treatment Recommendations for Remethylation Disorders

Here we provide an overview of the treatment-related experience for cblC-*MMACHC*, cblE-*MTRR*, cblG-*MTR*, and MTHFR deficiency. Other cRD/iRD are usually treated accordingly but it is unknown whether this is the optimal approach as the evidence quality is very low.

Parenteral therapy with OH-Cbl is the cornerstone of treatment for cbl-related cRD and iRD. However, daily intramuscular injections are painful and can make adherence to therapy difficult. In most cases only volumes up to 2 mL can be administered in a single injection.

Metabolic teams should explore individual patient preferences: while some patients favour IM injection in deeper muscle compartments, which are less densely supplied with pain receptors, others find slow administration of the drug to be most important. Although subcutaneous administration appears to reduce injection pain, some discomfort– such as burning sensation related to the drug itself- has been reported (authors' personal observation).

Few publications have explored the safety and efficacy of alternative routes of administration for OH-Cbl, such as a continuous subcutaneous infusion via a pump or daily injections via a subcutaneous catheter. The use of such devices has proven safe and effective in reducing Hcy and/or MMA [256, 257].

14 | Management of Anaesthesia in Remethylation Disorders

Nitrous oxide (N_2O) is a known inhibitor of methionine synthase and aggravates impaired remethylation. A fatal adverse event was reported in a patient with MTHFR deficiency anaesthetised with N_2O [258, 259].

Recommendation 33: we strongly recommend against the use of nitrous oxide in patients with any type of RD.

Quality of the evidence: high.

In seven patients with cblC-*MMACHC* and one with cblG-*MTR* defect, propofol was safe and effective as an induction and maintenance agent for elective short procedures in metabolically and haemodynamically stable patients [260].

A recommendation on the most favourable approach for general anaesthesia in RD patients cannot be given.

15 | Health-Related Quality of Life in Remethylation Disorders

HrQoL has so far not greatly been addressed in patients with RD [30]. Only a single report described a patient with a late-onset mild form of cblD-*MMADHC* disease and an impaired quality of life as measured by the PedsQL [261]. The treatment of a Cbl-related RD involves the frequent and lifelong administration of parenteral OH-Cbl, which may be a particularly distressing and painful experience, especially for children [256].

A recent patient/caregiver survey including 24 patients with RD and 110 patients with classical homocystinuria showed that nearly 50% of respondents considered the treatment of the metabolic disease to be only partially satisfactory, with a relevant psychological impact on the family's well-being [262]. Some families encountered problems related to accessibility of medication, financial burdens, or complex administrative and logistic processes. Very few families received support from a psychologist following the diagnosis or were informed about support groups [262]. Individuals with RD often experience visual deterioration and blindness, and it is known that children with visual impairments show significantly lower HrQoL scores than healthy age-matched controls [263].

16 | Expert Considerations on Standardized Testing of Neurodevelopment and Behavioural/Psychiatric Symptoms

Neuropsychological test results have mainly been reported for early onset cblC-*MMACHC* patients. Their developmental performance characteristically shows a decline over time that is mainly attributed to the increasing gap between their stable skills and the growing demands of the tests. Progressive visual impairment and attention deficits may hinder the acquisition of new skills [264].

CblC-*MMACHC* patients often show a characteristic pattern of strengths and weaknesses with pronounced deficits in sustained attention and executive functions and more favourable results in verbal expression, comprehension, and social interaction [22, 265].

Cognitive profile, attention, memory, as well as executive, adaptive, and behavioral functions should be assessed longitudinally using standardized neuropsychological tests [265]. Testing should ideally be performed in a familiar setting by a tester who has built a relationship with the patient, is familiar with the phenotype and with materials specifically adapted for visual impairment (e.g., enlarged dimensions, high-contrast colors, extension of timed tools). Only few studies provide detailed information about the tools used for neuropsychological evaluations in cblC-*MMACHC* patients [22, 132, 265].

To facilitate standardization, we provide an expert opinion without claim to completeness and with the limitation that none of these tests have specifically been adapted for patients with visual impairment. It is suggested to use a comprehensive test battery including cognitive and adaptive assessment, and an exploration of neuropsychological and visuoperceptive functions.

- Development/intelligence: the Mental Developmental Griffith's Scales-III Ed. (MDGS-III) [266, 267]; the Wechsler Preschool and Primary Scales of Intelligence-Third Edition WPPSI-III [268], the Wechsler Intelligence Scale for children- 4th ed. (WISC-IV) [269], and the Wechsler Adult Intelligence Scales- 4th Ed. (WAIS) [270]. The Leiter International Performance Scale, third edition (Leiter-3), can be used for nonverbal patients [271].
- Specific neuropsychological domains, social and emotional processing: individual tests, test groups, or the entire battery of the NEPSY-II [272]. Montreal Cognitive Assessment (MoCA) [273]; Mini-Mental State Examination (MMSE) [274].
- Executive functions: the BRIEF questionnaires [275–277]; Trail Making Test (TMT) [278]; Wisconsin Card Sorting Test (WCST) [279].
- Visuoperceptual functions (central and peripheral): the Visual-Motor Integration (VMI) test [280] the Rey-Osterrieth Complex Figure Test (RCFT) [281].
- Adaptive behavior abilities based on semi-structured interviews and questionnaires with parents/caregivers: Vineland Adaptive Behavior Scale-VABS [282]; the Adaptive behavior assessment system II edition- ABAS-II [283].

Some of the most widely used and rigorously validated measures for identifying behavioural and emotional profiles of mental disorders are:

- The Child Behaviour Checklist (CBCL) [284, 285]. Versions for caregivers are tailored to the age of the child/adolescent (1.5–5 old, 6–18 years, 11–18 years). The CBCL generates syndrome scales (e.g., anxious/depressed, withdrawn/depressed, attention problems, aggressive behaviours), broad-band scales (internalizing, externalizing, total problems), and Diagnostic and Statistical Manual for Mental Disorders *Fifth Edition* (DSM-5) oriented scales (e.g., anxiety, attention deficit, oppositional defiant problems).
- Adult Self-Report (ASR) and Adult Behaviour Checklist (ABCL). These are designed specifically to assess psychopathology in individuals aged 18 to 59 [286]. While the ASR is self-assessed, the ABCL gathers ratings from an adult closely associated with the subject. The Symptom Checklist-90–Revised (SCL-90-R) is a self-report psychological inventory designed to screen for a broad range of psychological problems and symptoms of psychopathology [287].
- The Kiddie Schedule for Affective Disorders and Schizophrenia—Present and Lifetime Version (K-SADS PL) [288] is a semi-structured interview investigating psychopathological disorders according to the Diagnostic and Statistical Manual for Mental Disorders *Fifth Edition* (DSM-5). Both the child/adolescent and the parents can be interviewed.

17 | Expert Considerations on the Monitoring of Health-Related Quality of Life (HrQoL)

Generic HrQol instruments allow for comparisons across different patient groups and with healthy populations, but meaningful clinical outcomes are often missed. Chronic-generic tools target the impact of chronic disease on everyday life. Disease-specific instruments incorporate questions specifically relevant to a particular disease and are more responsive and sensitive [289]. The complementary use of generic and disease-specific instruments has been recommended [290], but so far, specific RD-related tools do not exist.

A comprehensive understanding of the HrQoL in children with RD could be achieved by the combined use of the generic PedsQL [291], and the DISABKIDS chronic-generic tool (The DISABKIDS Group Europe tool (2005) [292, 293] which targets the impact of chronic disease on everyday life). For both tools, age-specific patient and parent-report versions are available. For adults, the SF-36 can be employed as a validated and comprehensive instrument to assess health-related quality of life, encompassing both physical and mental health dimensions [294].

To account for the impact of visual impairment, patient-reported outcome measures (PROM) designed for children with visual impairment should be used, such as the Vision-related Quality of Life questionnaire for children (VQoL_Child, 8–12 years) and young people (VQoL_Young People, 13–18 years) [295] and the Functional Vision Questionnaire

[296] for children (FVQ_C) and young people (FVQ_YP) that evaluates aspects of vision specifically important for everyday tasks. Another valuable tool for assessing the impact of visual impairment on the patient and their family is the Pediatric Eye Questionnaire (PedEyeQ; [297]), which comprises child self-report (5–11 and 12–17 years) versions, parent proxy report (0–4, 5–11, and 12–17 years) versions, as well as a parent self-report. The NEI VFQ-25 (National Eye Institute Visual Function Questionnaire—25 items) is a self-report tool designed to assess the impact of visual impairment on the quality of life in adults. It covers various domains such as vision-related function (near and distance vision), social interaction, emotional well-being, role limitations, dependency, driving ability, and ocular pain [298].

Parents of children with chronic diseases find themselves engaged in caregiving tasks, offering support during hospitalizations and medical appointments, and making decisions regarding treatment options [299, 300]. Studies focusing on the well-being of caregivers have shown their QoL to decrease, and feelings of anxiety, depression, stress, and a sense of being overwhelmed to increase [301, 302]. The stress associated with parenting can interfere with the effective management of a child's disease [303]. Valuable tools to assess these aspects are, for example, the PedsQL Family Impact Module (PedsQL-FIM) [304], the Zarit Burden interview [305] and the Parenting Stress Index (PSI-SF) [306].

Author Contributions

All authors of this work have contributed to the development of its content based on the GRADE and Delphi consensus methodologies and to the writing and revising of this manuscript. Martina Huemer (M.H.) moderated and coordinated the guideline revision process and drafted the final manuscript together with Carlo Dionisi-Vici (C.D.-V.) and Giorgia Olivieri (G.O.). The final manuscript was read and approved by all authors.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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