

Delineating the clinical spectrum of isolated methylmalonic acidurias: *cblA* and *mut*

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Abstract

Introduction: Long-term outcome is postulated to be different in isolated methylmalonic aciduria caused by mutations in the *MMAA* gene (*cblA* type) compared with methylmalonyl-CoA mutase deficiency (*mut*), but case definition was previously difficult.

Method: Cross-sectional analysis of data from the European Registry and Network for Intoxication type Metabolic Diseases (Chafea no. December 1, 2010).

Results: Data from 28 *cblA* and 95 *mut* patients in most cases confirmed by mutation analysis (including 4 new mutations for *cblA* and 19 new mutations for *mut*). Metabolic crisis is the predominant symptom leading to diagnosis in both groups. Biochemical disturbances during the first crisis were similar in both groups, as well as the age at diagnosis. *Z* scores of body height and body weight were similar in both groups at birth, but were significantly lower in the *mut* group at the time of last visit. Glomerular filtration rate was significantly higher in *cblA*; and as a consequence, chronic renal failure and related complications were significantly less frequent and renal function could be preserved even in older patients. Neurological complications were predominantly found in the *mut* subgroup. Methylmalonic acidemia (MMA) levels in urine and plasma were significantly lower in *cblA*. 27/28 *cblA* patients were reported to

List of Abbreviations: AAM, amino acid mix; Ado-Cbl, adenosylcobalamin; BW, body weight; CRF, chronic renal failure; ECG, electrocardiogram; E-IMD, European Registry and Network for Intoxication type Metabolic Diseases; EU, European Union; GFR, glomerular filtration rate; MDRD, modification of diet in renal disease; MMA, methylmalonic acidemia; NG, nasogastric tube; OH-Cbl, hydroxocobalamin; PEG, percutaneous endoscopic gastrostomy.

[†]Additional contributors from European Registry and Network for Intoxication type Metabolic Diseases (E-IMD) are listed in Appendix section and are recognized in PubMed.

Friederike Hörster and Ali Tunç Tuncel contributed equally to this study.

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be responsive to cobalamin, only 86% of *cbLA* patients were treated with *i.m.* hydroxocobalamin. In total, 73% of *cbLA* and 98% of *mut* patients followed a calculated diet with amino acid supplements in 27% (*cbLA*) and 69% (*mut*). During the study interval, six patients from the *mut* group died, while all *cbLA* patients survived.

Conclusion: Although similar at first, *cbLA* patients respond to hydroxocobalamin treatment, subsequently show significantly lower levels of MMA and a milder course than *mut* patients.

KEYWORDS

anthropometrics, chronic renal failure, dietary treatment, methylmalonic acidemia, movement disorder, vitamin B12/hydroxocobalamin

1 | INTRODUCTION

Isolated methylmalonic acidemias (MMA) comprise a group of diseases characterized by elevated concentrations of methylmalonic acid in blood, urine, and other body fluids. These disorders of propionate catabolism are inherited in an autosomal recessive manner and represent defects of L-methylmalonyl-CoA mutase (MMUT, EC 5.4.99.2), the synthesis of its cofactor 5'-deoxyadenosylcobalamin, in rare cases deficiency of the methylmalonyl-CoA epimerase (MCEE, EC 5.1.99.1) or of the succinate-CoA ligase (SUCLG1, EC 6.2.1.4; SUCLA2, EC 6.2.1.5). This study deals with two distinct forms of MMA: the complete deficiency of L-methylmalonyl-CoA mutase (*mut*, OMIM 251100) and the deficiency of its accessory protein methylmalonic acid type A protein (MMAA) (*cbLA*, OMIM 251100). Already the first case series on natural history of MMA postulated major differences in outcome between these two disease groups.¹ Exact classification based on propionate incorporation studies in cultured skin fibroblasts remained difficult, making case definition for clinical studies less clear.^{2,3} The genetic basis of *mut* was unraveled in 1990,⁴ but it was only in 2002, that the gene responsible for *cbLA* was localized to chromosome 4q31.21.⁵ This gene, called *MMAA*, encodes a G-protein belonging to the G3E family of P-loop GTPases, which is responsible for proper incorporation of adenosylcobalamin into MUT.^{6,7}

In this study, the clinical picture of MMA due to *cbLA* is compared to that of the deficiency of methylmalonyl-CoA mutase activity (*mut*), focusing on biochemical findings, molecular genetic data, disease onset and major endpoints of clinical outcome: survival, growth, renal disease and neurological complications and reports details on their medical and dietary treatment.

SYNOPSIS

CbLA patients treated with hydroxocobalamin show significantly lower levels of methylmalonic acid and have a milder clinical course than *mut* patients.

2 | PATIENTS AND METHODS

2.1 | European Registry and Network for Intoxication type Metabolic Diseases

The European Registry and Network for Intoxication type Metabolic Diseases (E-IMD, European Agency for Health and Consumers No. 2010 12 01) received funding from the European Union (EU) in the framework of the health program 2008 to 2013. A detailed description of the registry (<https://www.eimd-registry.org>) has been published previously.⁸⁻¹⁰

The study was approved by the ethic committee of the coordinating center (University Hospital Heidelberg, Application No. S-525/2010) and consecutively by those of all contributing metabolic centers. Written assent and parental consent was obtained for all study patients before enrolment and baseline visit in countries where this was needed by law. Patients with unconfirmed suspicion of an organic acid disorder, and with unrelated serious comorbidities and patients who died before January 2011, 01 (starting date of E-IMD) were excluded. For this particular analysis data have been pulled from the database in August 2016. For classification of *cbLA* and *mut* patients' diagnostic details (mutation analysis and

enzyme studies) were reviewed by experts with long-standing experience in the field and only patients with at least two pathogenic variants or diagnostic enzyme studies have been included.

2.2 | Study population

One hundred twenty-three patients (56 female, 67 male) with a confirmed diagnosis of isolated methylmalonic aciduria from 17 countries and 26 centers were included in this study. Details on age and sex of patients are shown in Table 1 and both were equally distributed among both subgroups. Study patients and their origins were heterogeneous. Most of them came from European countries, that is, 27 patients were from France, 16 from Germany, 10 from Spain, 8 patients each from Great Britain and Italy, 7 patients from Croatia, 6 patients each from the Russian Federation and Denmark, 5 from the Czech Republic, 4 from Serbia, 3 patients each from Austria and the Netherlands, 2 patients each from Belgium and Switzerland. Single patients came from Portugal and Romania. Other patients came from non-European countries, that is, 9 patients came from Taiwan, 3 from the United States and single patients each from Japan and the island of Reunion.

Patients were classified as *cblA* or *mut* by enzyme studies in cultured fibroblasts, as formerly described,^{2,11} through mutation analysis, or both. Four patients have been classified as *mut*, leaning on the genetic testing of an affected family member (eg, sibling). *mut*⁰ and *mut*⁻ are categories defined by enzyme studies, but enzyme studies were not available for all patients. In order to compare extreme phenotypes, patients with complete deficiency of methylmalonyl-CoA mutase (enzyme studies or mutation analysis) have been preferentially chosen.

Body weight (BW), body length, and head circumference at birth were compared by using *z* values corresponding to gestational age as proposed by the Royal College of Pediatrics and Child Health Growth Chart Expert Group¹² and for the other visits reference values according to Cole et al¹³ were used, which are suitable until the age of 22 years. Glomerular filtration rate

(GFR) has been estimated by using the Schwartz formula¹⁴ up to the age of 18, afterward modification of diet in renal disease (MDRD) has been applied. Chronic renal failure (CRF) was defined according to the European definition as GFR < 60 mL/min/1.73 m².

2.3 | Statistical analysis

All statistical computation was done with *R*, a language for statistical computing and graphics (<https://www.r-project.org>). A numeric response variable between two groups was compared with a *t* test with Welch correction. A multiple linear regression was used to analyze the impact of the predictor variables *age* and *cblA* or *mut* on the response variable GFR. Count data from contingency tables were analyzed with a log-linear model. *P* values reported were two-sided. *P* ≤ .05 was considered statistically significant.

3 | RESULTS

3.1 | Classification of patients and variant analysis

Of the 123 patients, 28 were classified as *cblA* and 95 as *mut*. Tables 2 and 3 show the details of diagnosis. In the *cblA* group pathogenic variants c.433C>T (p.Arg145*) and c.592_595delACTG (p.Thr198Serfs*6) were most frequently found. In the *mut* group the pathogenic variant c.655A>T (p.Asn219Tyr) was the most frequent. In this study we identified four new pathogenic variants which lead to the *cblA* phenotype: one nonsense variant c.1098G>A (p.Trp366*) and one missense variant c.589A>G (p.Met197Val) and two deletions c.662_664delCAA (p.Thr221del), c.593_596delCTGA (p.Thr198Serfs*6). In the *mut*-group we identified 19 novel pathogenic variants (Table 4). Cobalamin responsiveness, as defined by the physician in charge, was reported in 27 of the 28 *cblA* patients and unexpectedly, also in a single patient with *mut*. In one *cblA* patient cobalamin responsiveness was not reported. Methylmalonic acid in urine and plasma at the time of last visit were significantly lower in *cblA* patients (Figure 1; urine: *t* [55.6] = -6.6, *P* < .001, *t* test; plasma: *t* [36.9] = -4.6, *P* < .001, *t* test).

3.2 | Medical and dietary treatment

Hydroxocobalamin supplementation was provided to 15 *cblA* patients, while 6 patients were treated by

TABLE 1 Study population: distribution among *cblA* and *mut*: age distribution between groups was not significantly different (*t* [33.9] = 1.63, *P* = .11, *t* test)

	n	Sex female: male	Mean age (years)
<i>cblA</i>	28	11:17	11.9
<i>mut</i>	95	45:50	8.7
Total	123	56:67	9.4

TABLE 2 Details on diagnosis of *cbfA* patients: propionate fixation (pmol/16 h/mg protein) and mutase activity measurements (pmol/min/mg protein) were performed in fibroblasts and expressed as percent of control activity (n = 28)

Nr.	Nucleotide change Mutation 1 (maternal)	Protein change Mutation 1 (maternal)	Origin Mother	Propionate fixation		Mutase activity	
				+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl
1	c.592_595delACTG	p.Thr198Serfs*6	Germany	56	19	182	27
	c.592_595delACTG	p.Thr198Serfs*6	Germany				
2	c.586C>T	p.Arg196*	Germany	38	10	48	30
	c.586C>T	p.Arg196*	Turkey				
3	c.586C>T	p.Arg196*	Germany	44	10	152	68
	c.1196_1197delGGinsTT	p.Gly399Val	Austria				
4	c.387C>A	p.Tyr129*	Portugal	-	-	-	-
	c.387C>A	p.Tyr129*	Portugal				
5	c.387C>A	p.Tyr129*	Portugal	-	-	-	-
	c.387C>A	p.Tyr129*	Portugal				
6	c.433C>T ^a	p.Arg145*	Germany	-	-	-	-
	c.551dupC ^a	p.Cys184Trpfs*3	Germany				
7	c.433C>T	p.Arg145*	N/A	45	8	100	57
	c.433C>T	p.Arg145*	N/A				
8	c.298_312delTGTTT AGCAGAGGCC	p.Cys100_ Ala104del	N/A	-	-	-	-
	c.298_312delTGTTT AGCAGAGGCC	p.Cys100_ Ala104del	N/A				
9	N/A	N/A	Netherlands	9	4	50	10
	N/A	N/A	Netherlands				
10	c.586C>T	p.Arg196*	Croatia	40	9	16	2
	c.592_595delACTG	p.Thr198Serfs*6	Croatia				
11	c.592_595delACTG	p.Thr198Serfs*6	Croatia	45	13	-	-
	c.592_595delACTG	p.Thr198Serfs*6	Croatia				
12	c.283C>T ^a	p.Gln95*	Denmark	-	-	-	-
	c.970-2A>T ^a	Splice mutation	Denmark				

TABLE 2 (Continued)

Nr.	Nucleotide change Mutation 1 (maternal)	Protein change Mutation 1 (maternal)	Origin Mother	Mutation reference/comment	Propionate fixation		Mutase activity	
					+OH-Cbl	−OH-Cbl	+Ado-Cbl	−Ado-Cbl
13	N/A	N/A	N/A	Diagnosis due to family history	-	-	-	-
	N/A	N/A	N/A					
14	c.433C>T	p.Arg145*	Denmark	Lerner-Ellis et al ¹⁷	-	-	-	-
	c.433C>T	p.Arg145*	Denmark					
15	c.433C>T	p.Arg145*	Pakistan	Lerner-Ellis et al ¹⁷	-	-	-	-
	c.433C>T	p.Arg145*	Pakistan					
16	c.433C>T	p.Arg145*	Pakistan	Lerner-Ellis et al ¹⁷	-	-	-	-
	c.433C>T	p.Arg145*	Pakistan					
17	c.662_664delCAA	p.Thr221del	Tunisia	Novel pathologic variant	40	-	5	-
	c.662_664delCAA	p.Thr221del	Tunisia					
18	N/A	N/A	Italy		74	19	29	27
	N/A	N/A	Italy					
19	c.593_596delCTGA	p.Thr198Serfs*6	Serbia	Novel pathologic variant – This variant leads to the same protein change as the c.592_595delACTG (Plessl et al ¹⁵)	-	-	-	-
	c.593_596delCTGA	p.Thr198Serfs*6	Serbia					
20	c.592_595delACTG	p.Thr198Serfs*6	Slovakia	Plessl et al ¹⁵	-	-	-	-
	c.592_595delACTG	p.Thr198Serfs*6	Slovakia					
21	c.592_595delACTG	p.Thr198Serfs*6	Slovakia	Plessl et al ¹⁵	-	-	-	-
	c.592_595delACTG	p.Thr198Serfs*6	Slovakia					
22	N/A	N/A	Belgium		44	38	100	20
	N/A	N/A	Belgium					
23	c.742C>T	p.Gln248*	Cambodia	Plessl et al ¹⁵	-	-	-	-
	c.742C>T	p.Gln248*	Cambodia					
24	c.551dupG	p.Cys184Trpfs*3	Czech Republic	Plessl et al ¹⁵	-	-	-	-
	c.551dupG	p.Cys184Trpfs*3	Czech Republic					
25	c.772G>A	p.Asp258Asn	India	Plessl et al ¹⁵	-	-	-	-
	c.772G>A	p.Asp258Asn	India					

(Continues)

TABLE 2 (Continued)

Nr.	Nucleotide change		Protein change		Origin		Mutation reference/comment	Propionate fixation	Propionate fixation	Mutase activity	Mutase activity
	Mutation 1 (maternal)	Mutation 2 (paternal)	Mutation 1 (maternal)	Mutation 2 (paternal)	Mother	Father					
26	c.365T>C	c.365T>C	p.Leu122Pro	p.Leu122Pro	Taiwan	Taiwan	Lin et al ¹⁸	-	-	-	-
27	c.742C>T	c.1098G>A	p.Gln248*	p.Trp366*	N/A	N/A	Plessl et al ¹⁵ Novel pathologic variant	-	-	-	-
28	c.433C>T ^a	c.589A>G ^a	p.Arg145*	p.Met197Val	France	France	Lerner-Ellis et al ¹⁷ Novel pathologic variant	-	-	-	-

Abbreviations: Ado-Cbl, adenosylcobalamin; OH-Cbl, hydroxocobalamin.

The bold entries represent the novel pathogenic variants in the *cblA* and *mut*-groups found in this study.

^aOrigin of inheritance unknown.

cyanocobalamin only and 1 patient was treated by both. For the other six *cblA* patients, no information was provided regarding cobalamin supplementation, although five of them are reported to be responsive. Hydroxocobalamin was mostly given to *cblA* patients by intramuscular injection (n = 12/14 [86%]), only in single cases orally [n = 1/14 [7%]] or by subcutaneous injection (n = 1/14 [7%]). In the *mut* group, 12 patients were supplemented with hydroxocobalamin, 5 by intramuscular injection and 7 received oral hydroxocobalamin.

L-Carnitine supplementation was provided to both groups: the *cblA* group was given a median/mean dosage of 52.4/52.7 mg/kg BW/d (min. 11.5-max. 102.6), while the *mut* group was provided a significant higher median/mean dosage of 70.9/77.7 mg/kg BW/d (min. 19.3-max. 200.0) ($t[43.7] = -4.1, P < .001, t$ test).

Information on dietary treatment was available for 26 *cblA* and 89 *mut* patients: all *mut* patients were on some kind of specialized diet, whereas 19.2% (n = 5/26) of *cblA* patients were not. 73.1% (n = 19/26) of *cblA* compared to 97.8% (n = 87/89) of *mut* patients followed a calculated diet, while 7.7% of *cblA* (n = 2/26) and 2.2% *mut* (n = 2/89) only avoided food with high protein content. Amino acid supplements were added to the dietary treatment for 27% of *cblA* (n = 7/26) and 69% of *mut* patients (n = 61/88).

3.3 | Initial presentation

Overall, 90% of *mut* (n = 81/90) patients and 78% of *cblA* (n = 21/27) patients have been classified as “symptomatic” by their physicians. Metabolic crisis was the leading manifestation in 76% (n = 16/21) of *cblA* and 96% (n = 77/80) of *mut* patients ($P < .05$, log-linear model). An initial crisis in the neonatal period was reported in 43% (n = 9/21) of *cblA* and 60% (n = 48/80) of *mut* patients. 33% (n = 7/21) of *cblA* and 36% (n = 29/80) of *mut* patients had their initial crisis beyond the neonatal age, whereas 24% (n = 5/21) of *cblA* and 4% (n = 3/80) of *mut* patients had no metabolic crisis at all. Details on the diagnostic process and laboratory values during first crisis are summarized in Table 5: Laboratory values were similar in both groups. The median age at first symptoms was higher in the *cblA* group (24.5 days) than in the *mut* group (5 days), although the difference was not statistically significant ($W = 915, P = .13$, Wilcoxon rank sum test). Neither the time of diagnosis ($t[34.8] = 1.2, P = .237, t$ test) nor the diagnostic delay (defined as the time between the age at first symptoms and the age at diagnosis in days) was significantly different in *mut*

TABLE 3 Details on diagnosis of *mut* patients: propionate fixation (pmol/min/mg protein) and mutase activity measurements (pmol/min/mg protein) were performed in fibroblasts and expressed as percent of control activity (n = 93)

Nr.	Nucleotide change		Protein change		Origin		Propionate fixation		Mutase activity	
	Mutation 1 (maternal)	Mutation 2 (paternal)	Mutation 1 (maternal)	Mutation 2 (paternal)	Mother	Father	+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl
1	c.607G>A ^a	c.1105C>T ^a	p.Gly203Arg	p.Arg369Cys	Germany	Germany	2	3	2	15
2	c.862T>C ^a	c.1157A>G ^a	p.Ser288Pro	p.His386Arg	Germany	Germany	-	-	-	-
3	c.1280G>A ^a	c.1677-1G>A ^a	p.Gly427Asp	Splice mutation	N/A	Germany	-	-	-	-
4	c.1106G>A	c.1106G>A	p.Arg369His	p.Arg369His	Russian Fed.	Russian Fed.	-	-	-	-
5	c.1489G>T	c.2193-2196dupTGCC	p.Glu497*	p.Val733Cysfs*6	Russian Fed.	Russian Fed.	7	6	13	100
6	c.607G>A	c.607G>A	p.Gly203Arg	p.Gly203Arg	Germany	Germany	4	4	6	14
7	c.850G>A ^a	c.1073T>C ^a	p.Gly284Arg	p.Leu358Pro	Russian Fed.	Russian Fed.	-	-	-	-
8	c.882C>T	c.862C>T	p.Leu328Phe	p.Leu328Phe	Turkey	Turkey	-	-	-	-
9	c.643G>A ^a	c.2179C>T ^a	p.Gly215Ser	p.Arg727*	Russian Fed.	Azerbaijan	-	-	-	-
10	c.607G>A ^a	c.1399C>T ^a	p.Gly203Arg	p.Arg467*	Kazakhstan	Kazakhstan	-	-	-	-

(Continues)

TABLE 3 (Continued)

Nr.	Nucleotide change		Protein change		Origin	Reference/comment	Propionate fixation		Mutase activity	
	Mutation 1 (maternal)	Mutation 2 (paternal)	Mutation 1 (maternal)	Mutation 2 (paternal)			+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl
11	c.2T>C ^a		p.MetI?		Russian Fed.	Forny et al ¹⁹	-	-	-	-
	c.850G>A ^a		p.Gly284Arg		Russian Fed.					
12	c.655A>T		p.Asn219Tyr		Russian Fed.	Merinero et al ²¹ and Forny et al ¹⁹	-	-	-	-
	c.655A>T		p.Asn219Tyr							
	c.1073T>C		p.Leu358Pro		Russian Fed.					
	c.1073T>C		p.Leu358Pro							
13	c.1677-1G>A		Splice mutation		China	Liu et al ²²	15	15	4	22
	c.224delA		p.Lys75Argfs*14		China	Novel pathologic variant				
14	c.655A>T ^{ad}		p.Asn219Tyr		Morocco	Forny et al ¹⁹	-	-	-	-
	c.904G>C^a		p.Ala302Pro		Morocco	Novel pathologic variant				
15	c.198delG^a		p.Ile67Serfs*3		France	Worgan et al ²⁰	-	-	-	-
	c.1332+1delG ^a		Splice mutation		France	Novel pathologic variant				
16	c.1106G>A		p.Arg369His		N/A	Forny et al ¹⁹	13	14	0	0
	c.1106G>A		p.Arg369His		N/A					
17	c.689C>G		p.Thr230Arg		N/A	Forny et al ¹⁹	5	5	6	74
	c.1991C>T		p.Ala664Val		N/A	Novel pathologic variant				
18	c.927G>A ^a		p.Trp309*		Great Britain	Forny et al ¹⁹	-	-	-	-
	c.983T>C ^a		p.Leu328Pro		Spain					
19	c.1844C>T		p.Pro615Leu		Italy	Forny et al ¹⁹	-	-	-	-
	c.1844C>T		p.Pro615Leu		Italy					
20	N/A		N/A		Italy	Family history	-	-	-	-
	N/A		N/A		Italy					
21	c.630delA		p.Glu211Argfs*12		Italy	Forny et al ¹⁹	-	-	-	-
	c.330T>G		p.Tyr110*		Italy					
22	c.1853T>C		p.Leu618Pro		Italy	Forny et al ¹⁹	-	-	-	-

TABLE 3 (Continued)

Nr.	Nucleotide change		Protein change		Origin	Reference/comment	Propionate fixation		Mutase activity	
	Mutation 1 (maternal)	Mutation 2 (paternal)	Mutation 1 (maternal)	Mutation 2 (paternal)			Propionate fixation	Mutase activity	Propionate fixation	Mutase activity
					Father	Reference/comment	+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl
	c.1844C>T		p.Pro615Leu		Italy					
23	c.2194_2197del4/ ins5TGGAA		p.Ala732Trpfs*6		Italy	Forny et al ¹⁹	-	-	-	-
	c.655A>T		p.Asn219Tyr		Italy					
24	c.655A>T ^a		p.Asn219Tyr		Italy	Forny et al ¹⁹	-	-	-	-
	c.1106G>A ^a		p.Arg369His		Italy					
25	c.330T>G		p.Tyr110*		Italy	Forny et al ¹⁹	-	-	-	-
	c.330T>G		p.Tyr110*		Italy					
26	N/A		N/A		Netherlands		11	8	-	-
	N/A		N/A		Netherlands					
27	c.654A>C		p.Gln218His		Netherlands	Forny et al ¹⁹	-	-	-	-
	c.654A>C		p.Gln218His		Netherlands					
28	c.129G>A		p.Trp43*		Croatia	Forny et al ¹⁹	10	9	1	27
	c.129G>A		p.Trp43*		Croatia					
29	c.129G>A		p.Trp43*		Croatia	Forny et al ¹⁹	-	-	-	-
	c.129G>A		p.Trp43*		Croatia					
30	N/A		N/A		Croatia		13	14	3	7
	N/A		N/A		Croatia					
31	c.1658del		p.Val553Glyfs*17		Croatia	Forny et al ¹⁹	-	-	-	-
	c.1658del		p.Val553Glyfs*17		Croatia					
32	N/A		N/A		Croatia		12	11	2	7
	N/A		N/A		Croatia					
33	c.-39-1G>A		Splice mutation		Iraq	Forny et al ¹⁹	5	5	11	62
	c.-39-1G>A		Splice mutation		Iraq					
34	c.1957-2A>G		Splice mutation		Denmark	Worgan et al ²⁰	14	13	0	-
	c.1957-2A>G		Splice mutation		Denmark					
	c.2089_2101del ^a		p.Ile697Valfs*4							

(Continues)

TABLE 3 (Continued)

Nr.	Nucleotide change	Protein change	Origin	Reference/comment	Propionate fixation		Mutase activity	
					+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl
	Mutation 1 (maternal)	Mutation 1 (maternal)	Mother					
	Mutation 2 (paternal)	Mutation 2 (paternal)	Father					
35	c.1531C>G	p.Arg511Gly	Pakistan	Novel pathogenic variant—the nucleotide change “c.1531C>T” with the protein change “p.Arg511*” has been published previously by Forny et al ¹⁹	16	10	1	16
	c.1531C>G	p.Arg511Gly	Pakistan					
36	c.1420C>T	p.Arg474*	France	Forny et al ¹⁹	-	-	-	-
	c.1531C>T	p.Arg511*	France					
37	c.731A>T	p.Asp244Val	France	Novel pathologic variant	5	-	0	-
	c.731A>T	p.Asp244Val	France					
38	c.607C>A	p.Gly203Arg	France	Forny et al ¹⁹	-	-	-	-
	c.322C>T	p.Arg108Cys	France					
39	c.481C>A	p.Gly161Arg	France	Mendez et al ²³ and Forny et al ¹⁹	-	-	-	-
	c.671_678dupAATTTATG	p.Val227Asnfs*16	France					
40	N/A	N/A	Turkey		12	12	5	0
	N/A	N/A	Turkey					
41	c.1897_1900dupGTTA	p.Ile634Serfs*8	France	Novel pathologic variant	-	-	-	-
	c.1897_1900dupGTTA	p.Ile634Serfs*8	France					
42	c.1531C>T ^a	p.Arg511*	France	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-
	c.1924G>C ^a	p.Gly642Arg	France					
43	c.572C>A	p.Ala191Glu	Haiti	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-
	c.1867G>A	p.Gly623Arg	Haiti					
44	c.572C>A	p.Ala191Glu	Haiti	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-
	c.1867G>A	p.Gly623Arg	Haiti					
45	c.655A>T	p.Asn219Tyr	France	Forny et al ¹⁹	-	-	-	-
	c.1911delA	p.Phe638Leufs*10	France	Novel pathologic variant	-	-	-	-
46	c.655A>T ^a	p.Asn219Tyr	France	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-
	c.1149G>T ^a	p.Gln383His	France					
47	c.572C>A	p.Ala191Glu	France	Acquaviva et al ²⁴ and Forny et al ¹⁹	-	-	-	-
	c.1025C>A	p.Ser342*	France					

TABLE 3 (Continued)

Nr.	Nucleotide change	Protein change		Origin		Reference/comment	Propionate fixation		Mutase activity	Mutase activity
		Mutation 1 (maternal)	Mutation 2 (paternal)	Mother	Father		+OH-Cbl	-OH-Cbl		
48	c.769insA	p.Asn257Lysfs*6		Algeria		Novel pathologic variant	-	-	-	-
49	c.769insA	p.Asn257Lysfs*6		Algeria		Novel pathologic variant	-	-	-	-
	c.1025C>A^a	p.Ser342*		France		Acquaviva et al ²⁴ and Worgan et al ²⁰	-	-	-	-
	c.1332+1delG^a	Splice mutation		France			-	-	-	-
50	N/A	N/A		Reunion			4	4	1	-
	N/A	N/A		Reunion			-	-	-	-
51	c.655A>T	p.Asn219Tyr		France		Forny et al ¹⁹	-	-	-	-
	c.1911delA	p.Phe638Leufs*10		France		Novel pathologic variant	-	-	-	-
52	c.322C>T^a	p.Arg108Cys		N/A		Forny et al ¹⁹	-	-	-	-
	c.1531C>T^a	p.Arg511*		N/A			-	-	-	-
53	c.2053_2055dup	p.Leu685dup		Switzerland		Forny et al ¹⁹	9	9	0	0
	c.91C>T	p.Arg31*		Switzerland			-	-	-	-
54	c.91C>T^a	p.Arg31*		Switzerland		Forny et al ¹⁹	-	-	-	-
	c.2080C>T^a	p.Arg694Trp		Switzerland			-	-	-	-
55	c.1036_1038delCTT	p.Leu346del		France		Worgan et al ²⁰	-	-	-	-
	c.1036_1038delCTT	p.Leu346del		France			-	-	-	-
56	c.655A>T	p.Asn219Tyr		Rep. of Serbia		Forny et al ¹⁹	-	-	-	-
	c.1646T>C	p.Leu549Pro		Rep. of Serbia		Novel pathologic variant	-	-	-	-
57	c.1658delT	p.Val553Glyfs*17		Rep. of Serbia		Forny et al ¹⁹	-	-	-	-
	c.1690C>T	p.Glu564*		Rep. of Serbia		Novel pathologic variant	-	-	-	-
58	c.1106G>A	p.Arg369His		Rep. of Serbia		Forny et al ¹⁹	-	-	-	-
	c.1922T>C	p.Leu641Pro		Montenegro		Novel pathologic variant	-	-	-	-
59	c.1843C>A	p.Pro615Thr		Turkey		Forny et al ¹⁹	-	-	0	0

(Continues)

TABLE 3 (Continued)

Nr.	Nucleotide change	Protein change	Origin	Reference/comment	Propionate fixation		Mutase activity		
					Mutation 1 (maternal)	Mutation 2 (paternal)	+OH-Cbl	-OH-Cbl	+Ado-Cbl
	c.1843C>A	p.Pro615Thr	Austria						
60	c.655A>T	p.Asn219Tyr	Czech Rep.	Forny et al ¹⁹	-	-	-	-	-
	c.655A>T	p.Asn219Tyr	Czech Rep.						
61	c.655A>T ^a	p.Asn219Tyr	Czech Rep.	Forny et al ¹⁹	-	-	-	-	-
	N/A	N/A	Czech Rep.						
62	c.655A>T	p.Asn219Tyr	Czech Rep.	Forny et al ¹⁹	-	-	-	-	5
	c.2179C>T	p.Arg727*	Czech Rep.						
63	c.655A>T	p.Asn219Tyr	Czech Rep.	Forny et al ¹⁹	-	-	-	-	-
	c.1881T>A^a	p.His627Gln	Czech Rep.	Novel pathologic variant					
64	c.544dup	p.Met182Asnfs*29	N/A	Forny et al ¹⁹	-	-	-	-	-
	c.544dup	p.Met182Asnfs*29	N/A						
65	c.544dup	p.Met182Asnfs*29	N/A	Forny et al ¹⁹	3	4	-	-	-
	c.544dup	p.Met182Asnfs*29	N/A						
66	c.1280G>A	p.Gly427Asp	Taiwan	Worgan et al ²⁰ and Yi et al ²⁵	-	-	-	-	-
	c.1495G>A	p.Ala499Thr	Taiwan						
67	c.454C>T ^a	p.Arg152*	Taiwan	Worgan et al ²⁰	-	-	-	-	0
	c.1280G>A ^a	p.Gly427Asp	Taiwan						
68	c.1280G>A	p.Gly427Asp	Taiwan	Worgan et al ²⁰	-	-	-	-	-
	c.1280G>A	p.Gly427Asp	Taiwan						
69	c.1655C>T	p.Ala552Val	Taiwan	Forny et al ¹⁹	-	-	-	-	1
	c.862C>T	p.Leu328Phe	Taiwan						
70	c.323G>A ^a	p.Arg108His	Taiwan	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-	1
	c.1280G>A ^a	p.Gly427Asp	Taiwan						
71	c.1280G>A ^a	p.Gly427Asp	Taiwan	Worgan et al ²⁰ and Liu et al ²²	-	-	-	-	-
	c.1677-1G>A ^a	Splice mutation	Taiwan						
72	c.1147C>G	p.Gln383Glu	China	Forny et al ¹⁹	-	-	-	-	0
	c.323G>A	p.Arg108His	Taiwan	Novel pathologic variant					

TABLE 3 (Continued)

Nr.	Nucleotide change		Protein change		Origin		Propionate fixation		Propionate fixation		Mutase activity	
	Mutation 1 (maternal)	Mutation 2 (paternal)	Mutation 1 (maternal)	Mutation 2 (paternal)	Mother	Father	+OH-Cbl	-OH-Cbl	+Ado-Cbl	-Ado-Cbl	Mutase activity	Mutase activity
73	c.1885A>G	c.1885A>G	p.Arg629Gly	p.Arg629Gly	Morocco	Morocco	-	-	-	-	-	-
74	N/A	c.572C>A	p.Gln103Arg	p-Ala191Glu	Spain	Spain	-	-	-	-	-	-
75	N/A	N/A	N/A	N/A	Spain	Spain	-	-	0	0	0	0
76	c.1421G>C	c.655A>T	p.Arg474Pro	p-Asn219Tyr	Spain	Spain	-	-	-	-	-	-
77	c.655A>T	c.655A>T	p-Asn219Tyr	p-Asn219Tyr	Romania	Romania	-	-	-	-	-	-
78	c.731A>T^a	N/A	p.Asp244Val	N/A	Germany	Germany	-	-	-	-	-	-
79	c.982C>T	c.982C>T	p.Leu328Phe	p.Leu328Phe	Turkey	Germany	3	3	-	-	-	-
80	c.1560+1A>T	c.785G>A	Splice mutation	p.Ser262Asn	France	France	2	2	2	2	-	-
81	c.323G>A ^a	c.655A>T ^a	p.Arg108His	p-Asn219Tyr	France	France	-	16	1	-	-	-
82	c.983T>C	c.104delA	p.Leu328Pro	p.Gln35Argfs*17	France	France	-	20	-	-	-	-
83	c.643G>A	c.643G>A	p.Gly215Ser	p.Gly215Ser	France	France	-	-	-	-	-	-
84	c.655A>T	c.1022dupA	p-Asn219Tyr	p-Asn341Lysfs*20	France	France	-	-	-	-	-	-
85	N/A	N/A	N/A	N/A	Spain	Spain	-	-	-	-	-	-
86	c.312delC^a	p.Trp105Glyfs*75	p.Trp105Glyfs*75	N/A	Spain	Spain	-	-	0	0	0	0

(Continues)

TABLE 3 (Continued)

Nr.	Nucleotide change	Protein change	Origin	Reference/comment	Propionate fixation	Propionate fixation	Mutase activity	Mutase activity
	N/A	N/A	Spain					
87	c.257C>T ^a	p.Pro86Leu	El Salvador	Worgan et al ²⁰	-	-	-	-
	c.671_678dup8 ^a	p.Val227Asnfs*	El Salvador					
88	c.850G>T ^a	p.Gly284*	Kenya	Forny et al ¹⁹	-	-	-	-
	c.1043G>T^a	p.Arg348Ile	United States	Novel pathologic variant				
89	c.682C>T	p.Arg228*	United States	Forny et al ¹⁹	3	3	-	-
	c.1106G>A	p.Arg369His	United States					
90	c.1885A>G	p.Arg629Gly	Morocco	Perez et al ²⁶	-	-	-	0
	c.1885A>G	p.Arg629Gly	Morocco					
91	c.977G>A	p.Arg326Lys	Spain	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	0	-
	c.454C>T	p.Arg152*	Spain					
92	c.977G>A	p.Arg326Lys	Spain	Worgan et al ²⁰ and Forny et al ¹⁹	-	-	-	-
	c.454C>T	p.Arg152*	Spain					
93	c.655A>T	p.Asn219Tyr	Spain	Perez et al ²⁶ and Forny et al ¹⁹	-	2	-	0
	c.458T>A	p.Val153Asp	Spain					

Note: If a mutation has been mentioned twice, it is a homozygous mutation. In order to keep the number of references low, the most recent publication has been mentioned in the comments. The bold entries represent the novel pathogenic variants in the *cblA* and *mut*-groups found in this study.

Abbreviations: Ado-Cbl, adenosylcobalamin; OH-Cbl, hydroxocobalamin.

^aOrigin of inheritance unknown.

TABLE 4 List of novel pathogenic variants both in the *cblA* and *mut*-group found in this study

<i>cblA</i>	c.589A>G p.Met197Val	c.593_596delCTGA p.Thr198Serfs*6	c.662_664delCAA p.Thr221del	c.1098G>A p.Trp366*
<i>mut</i>	c.104delA p.Gln35Argfs*17	c.198delG p.Ile67Serfs*3	c.224delA p.Lys75Argfs*14	c.312delC p.Trp105Glyfs*75
	c.731A>T p.Asp244Val	c.769insA p.Asn257Lysfs*6	c.904G>C p.Ala302Pro	c.1043G>T p.Arg348Ile
	c.1147C>G p.Gln383Glu	c.1421G>C p.Arg474Pro	c.1531C>G p.Arg511Gly	c.1646T>C p.Leu549Pro
	c.1690G>T p.Glu564*	c.1881T>A p.His627Gln	c.1897_1900dupGTTA p.Ile634Serfs*8	c.1911delA p.Phe638Leufs*10
	c.1922T>C p.Leu641Pro	c.1991C>T p.Ala664Val	N/A p.Gln103Arg	

The bold entries represent the novel pathogenic variants in the *cblA* and *mut*-groups found in this study.

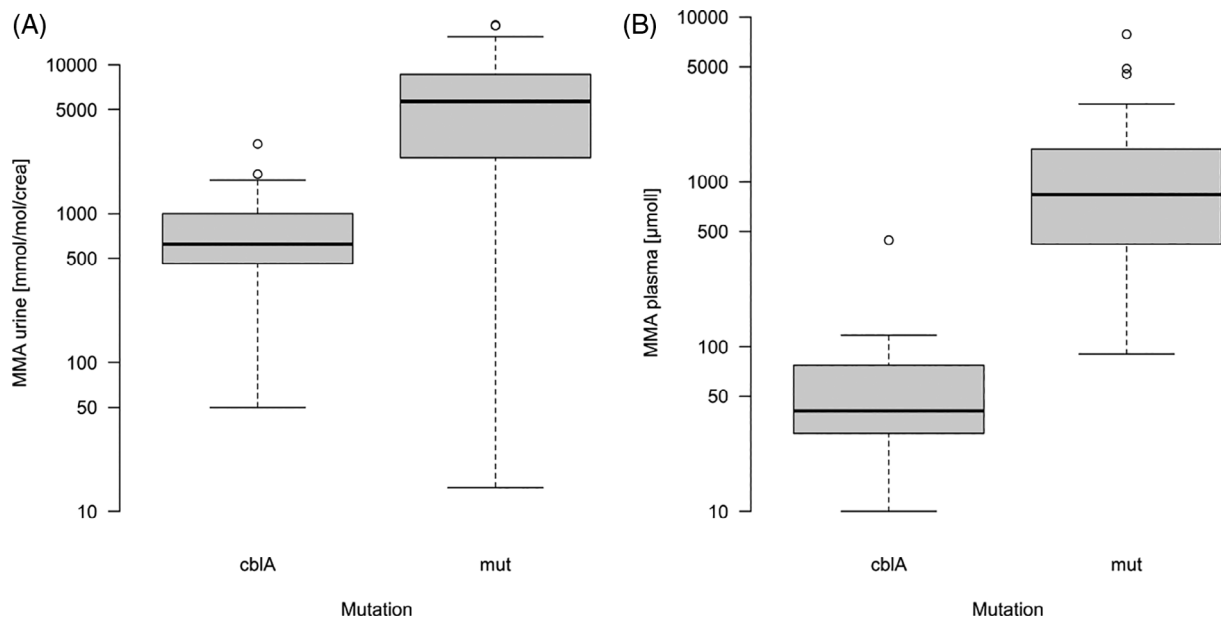


FIGURE 1 Methylmalonic acid levels in urine and plasma of the *cblA* and *mut* patients at the time of last visit. Values in the *cblA* subgroup are significantly lower than in the *mut* subgroup both in urine as well as in plasma. Patients with CRF (GFR < 60 mL/min/1.73 m²) were excluded. Urine: *cblA* (n = 16); *mut* (n = 52). Plasma: *cblA* (n = 14); *mut* (n = 37) (urine: $t[55.6] = -6.6, P < .001, t$ test; plasma: $t[36.9] = -4.6, P < .001, t$ test). CRF, chronic renal failure; GFR, glomerular filtration rate

patients in comparison to the *cblA* group ($t[17.6] = 1.0, P = .349, t$ test).

3.4 | Anthropometric development

Gestational ages were similar in both groups: *cblA* (mean 39.6 weeks \pm 1.23 [min. 37-max. 42 weeks of gestation]) and *mut* (mean 39.3 weeks \pm 1.72 [min. 32-max. 42 weeks of gestation]). Different problems during pregnancy have been reported in mothers of 4/23 *cblA* (17%) and 14/83 *mut* (17%) patients, such as gestational diabetes or arterial hypertension,

not showing any specific pattern. BW, body length, and head circumference were not significantly different between the two subgroups at birth (BW: $t[28.9] = 1.1, P = .297, t$ test; body length: $t[22.4] = 0.86, P = .400, t$ test; head circumference: $t[29.8] = 0.95, P = .349, t$ test). When compared longitudinally, head circumference showed no significant difference between the groups at the time of last visit, although a trend with 6% decrease in the *mut* group was noticeable ($t[34.1] = 1.9, P = .062, t$ test). On the other hand, BW and body length measurements at last visit were significantly lower in *mut* patients (BW: $t[38.2] = 2.0, P < .05, t$ test; body length: $t[33.5] = 3.1, P < .05, t$ test) (Figure 2).

3.5 | Renal manifestations

Information on kidney function was available for 23 *cblA* and for 69 *mut* patients. GFR, as estimated by the Schwartz formula or the MDRD equation, was significantly higher in *cblA* patients. Consequently CRF and related complications were significantly less frequent and renal function preserved even in older *cblA* patients; whereas GFR diminished significantly in *mut* patients over the course of the disease (Figure 3). The frequency of CRF was 46% ($n = 32/69$) in the *mut* group vs 9% ($n = 2/23$) in the *cblA* group. In six *cblA* patients (median age 16.5 years [min. 5-max. 34 years]) cystatin C measurements showed normal levels and cystatin C-based estimations of GFR were normal. Arterial hypertension as a common complication of CRF was present in 1 *cblA* ($n = 1/22$ [5%]) and 11 *mut*

TABLE 5 Initial metabolic crises in *cblA* and *mut* patients: time point, diagnostic process, and biochemical features

	<i>cblA</i>	<i>mut</i>
Median age at first symptoms [days]	24.5 [Q1 = 3.75; Q3 = 180]	5 [Q1 = 2; Q3 = 133]
Median age at diagnosis [days]	21 [Q1 = 5.75; Q3 = 265.5]	12 [Q1 = 4.0; Q3 = 180]
Mode of diagnosis (%)		
• Metabolic work-up of symptomatic patients (selective screening)	64.3 17.9	78.9 4.2
• High risk family screening	17.9	12.6
• Neonatal screening	0	2
• Prenatal testing	[n = 28]	[n = 93]
Mean diagnostic delay (selective screening only) [days]	91 [±274, 0:1065]	28 [±69, 0:276]
Median [Q1; Q3]		
• NH ₃ (μmol/L)	247 [155; 446]	255 [156; 497]
• pH	7.24 [7.08;	7.24 [7.10;
• BE	7,30]	7.33]
• Lactate (mmol/L) within first crisis	-18.2 [-11.1; - 24.0]	-16.2 [-11.2; -21.6]
	2.5 [2.3; 4.2]	2.4 [1.6; 3.8]

Note: Laboratory features of initial crisis are similar in both groups (NH₃: $t[58.9] = -1.58$, $P = .019$, t test; pH: $t[11.9] = -0.14$, $P = .889$, t test; BE: $t[12.1] = -0.37$, $P = .715$; lactate $t[10.9] = 0.78$, $P = .452$), as well as the time of first symptoms ($t[26.7] = 0.5$, $P = .600$, t test) and the diagnostic delay (defined as the time between the age at first symptoms and the age at diagnosis in days) ($t[17.6] = 1.0$, $P = .349$, t test).

The bold entries represent the novel pathogenic variants in the *cblA* and *mut*-groups found in this study.

patients ($n = 11/73$ [15%]). End-stage renal failure requiring hemodialysis or peritoneal dialysis was reported in 2 *cblA* and 3 *mut* patients. 5 *mut* patients ($n = 5/90$ [6%]) underwent kidney transplantation, whereas none of the *cblA* patients were transplanted during the study period.

3.6 | Gastrointestinal manifestations

Acute pancreatitis, a known complication in organic acidurias, was reported in 4 *mut* patients. Feeding difficulties leading to nasogastric tube (NG) feeding or percutaneous endoscopic gastrostomy (PEG) was more frequent in *mut* patients (PEG: $n = 23/88$ [26%], NG: $n = 12/88$ [14%]) than in *cblA* patients (PEG: $n = 1/27$ [4%], NG: $n = 3/27$ [11%]) with an odd ratio of 5.5; however, the difference in frequency was statistically not significant ($P = .28$, Fisher's exact test).

3.7 | Cardiac manifestations

Investigations of the heart were available for only a few patients: 1/5 *cblA* patients had an abnormal electrocardiogram (ECG) and 1/8 had an abnormal echocardiogram (ECG: $n = 1/5$ [20%], echocardiogram $n = 1/8$ [13%]). 4/23 *mut* patients had an abnormal ECG and 4/32 had an abnormal echocardiogram (ECG: $n = 4/23$ [17%], echocardiogram $n = 4/32$ [13%]), but further details have not been reported.

3.8 | Neurological manifestations

None of the 27 *cblA* patients were reported to have seizures, whereas 10 patients from the *mut* group were diagnosed with epilepsy ($n = 10/90$ [11%]; $\chi^2[1] = 3.3$; $P = .07$, log-linear model). One patient in the *cblA* group ($n = 1/27$ [4%]) showed movement disorders, compared to 29 patients from the *mut* group ($n = 29/89$ [30%]; $\chi^2[1] = 9.0$; $P < .05$, log-linear model). Optic atrophy was present in a single *mut* patient.

Information about schooling was available in 18 *cblA* and 49 *mut* patients. A larger percentage of patients from the *cblA* group attended regular school than the *mut* group (*cblA*: $n = 14/18$ [78%]; *mut*: $n = 24/49$ [49%]) with an odds ratio of 3.6. The statistical differences were significant at a 6% level ($P = .051$, Fisher's exact test).

3.9 | Survival

During the study interval six *mut* patients ($n = 6/95$ [6%]) died, while all *cblA* patients survived. Cause of

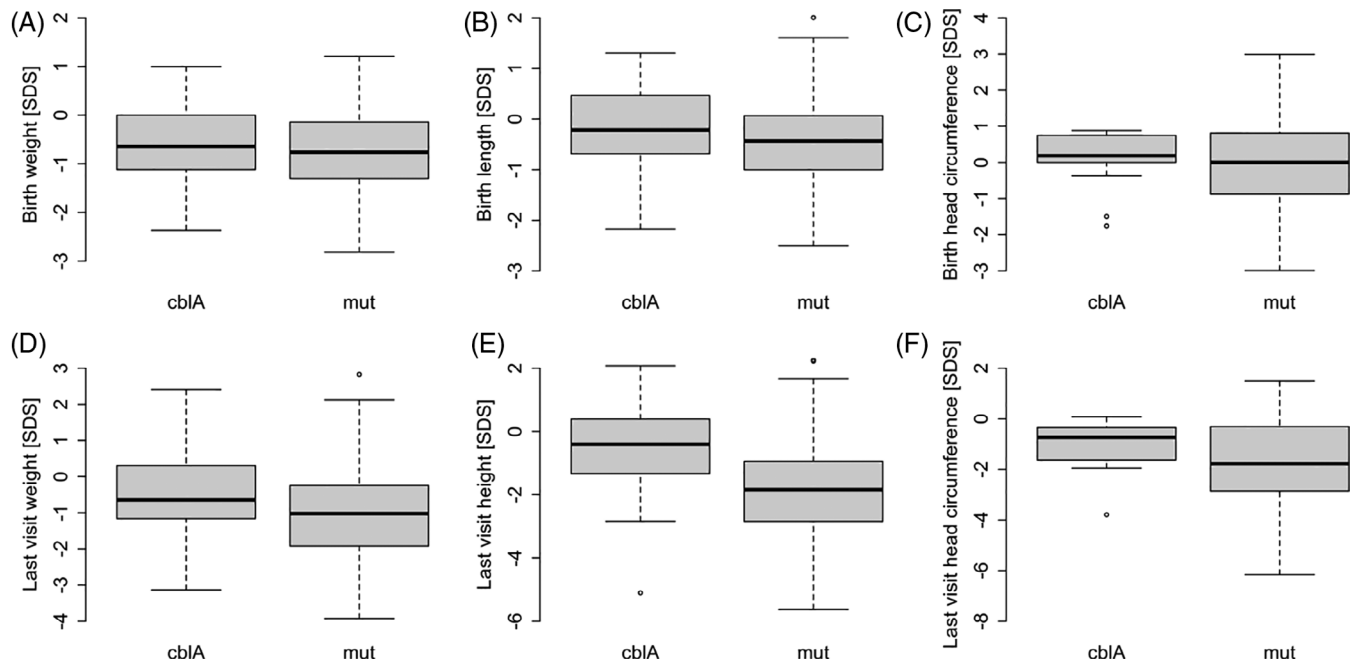


FIGURE 2 Anthropometrics in *cblA* and *mut* patients at birth and at last visit. SD scores of body weight, body length, and head circumference at birth and at last visit are shown as boxplots: All three anthropometric parameters are similar in both subgroups at birth (body weight: $t[28.9] = 1.1$, $P = .270$, t test; body length: $t[22.4] = 0.86$, $P = .400$, t test; head circumference: $t[29.8] = 0.95$, $P = .349$, t test). Body weight and body length are significantly lower in *mut* patients at last visit compared to *cblA* patients, whereas the head circumference is not significantly lower at the time of last visit (body weight: $t[38.2] = 2.0$, $P < 0.05$, t test; body length: $t[33.5] = 3.1$, $P < .05$, t test; head circumference $t[34.1] = 1.9$, $P = .062$, t test)

death was reported by the physician in charge (not proven by autopsy) to be brain edema in three patients (at the ages of 5, 6, and 20 years), one patient died at the age of 20 days, following a severe infectious disease. The remaining two patients died at the ages of 2 and 13 years. Causes of death of these patients were not reported.

4 | DISCUSSION

This study presents clinical data of 28 *cblA* and 95 *mut* patients with a definite case definition. In the *cblA* group two truncating mutations, c.433C>T (p.Arg145*) and c.592_595delACTG (p.Thr198Serfs*6), were most frequently found, which is in accordance with other studies.^{15,17} This is the largest clinical cohort of *cblA* patients published so far, and in earlier studies *cblA* patients have been difficult to differentiate from other subgroups.³ We compared the clinical picture of *cblA*-type MMA to that of deficiency of methylmalonyl-CoA mutase activity (*mut*). Presentation was by metabolic crisis in both groups and similar biochemical disturbances were found, although *cblA* patients tended to present later. This makes this group also a good candidate for newborn screening. For cobalamin non-responsive patients it has already been shown that

patients diagnosed by newborn screening have less frequent motor disorders and delay of motor milestones than patients diagnosed after developing clinical symptoms.²⁷

Anthropometrics at birth in both groups show lower z scores compared to a reference population, corresponding to earlier reports of a lower birth weight in MMA patients (without further differentiation of subgroups).⁸⁻¹⁰ While weight, length, and head circumference do not differ at birth between both groups, BW and length become significantly lower in *mut* patients over time, whereas head circumference is not significantly different between both groups at the time of last visit. There are several possible reasons for this: 40% of *mut* patients have significant feeding difficulties leading to PEG or NG-tube feeding contrasting to only 15% in the *cblA* group. A recent study by Molema et al²⁸ showed that in the E-IMD MMA patient cohort, plasma L-valine and L-isoleucine levels were very low, mainly in patients receiving amino acid mix (AAM), despite median daily natural protein intake being at the recommended daily allowance. Patient subgroups were not further subdivided in that study. In our cohort, 98% of *mut* patients follow a calculated diet with amino acid supplements in 69%. Therefore, they may also be at risk of having amino acid imbalances and these patients need careful monitoring

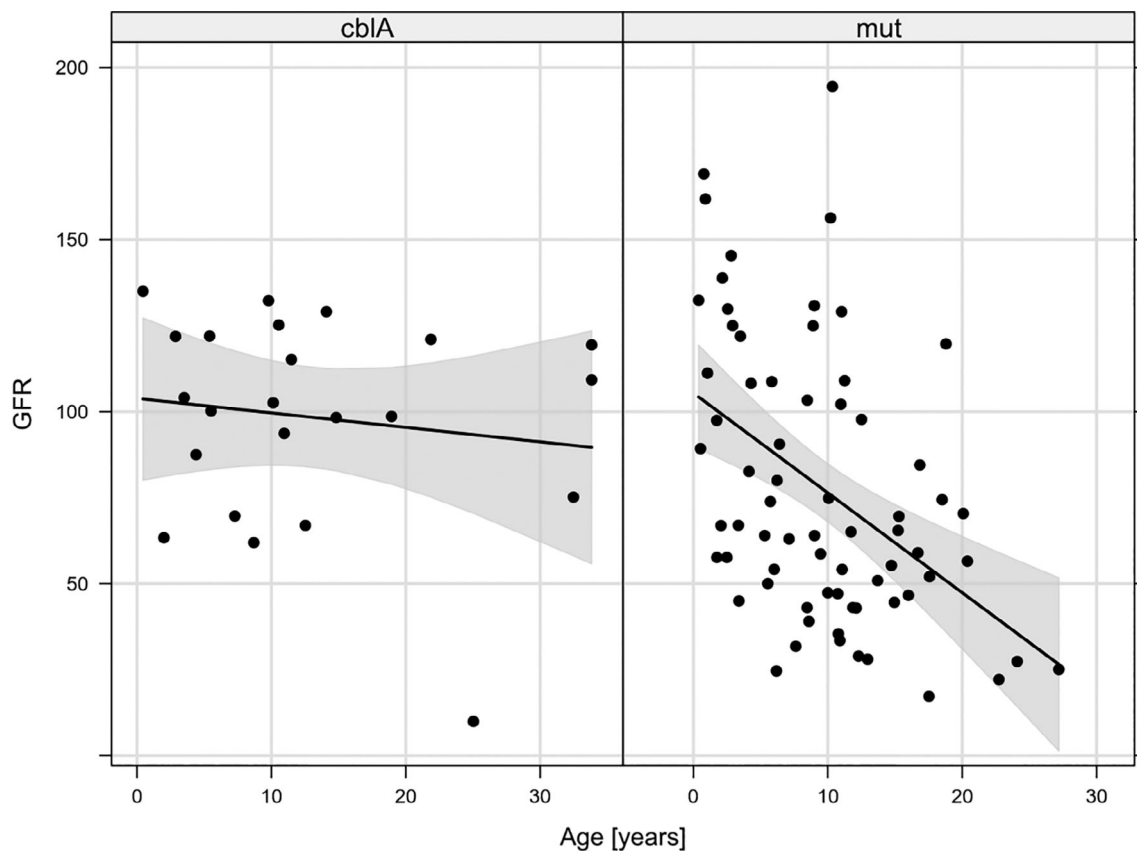


FIGURE 3 A multiple regression with response GFR and predictors mutation (*mut/cblA*) and age of individuals in years shows a significant interaction: While the GFR remains stable along life time for *cblA* patients ($n = 23$), GFR significantly decreases by nearly 3 points per year for *mut* patients ($n = 69$) (slope[*mut*] = -2.92 , $t[88] = -2.5$, $P < .02$). GFR, glomerular filtration rate

and regular adjustments of dietary treatment according to the monitoring protocol proposed in the current guideline.¹¹ This is also of importance for the fraction of *cblA* patients who follow a calculated diet and take AAM.

In our study, neurological complications such as movement disorders were significantly more frequent in the *mut* group, whereas no differences could be seen regarding seizures. Patients with organic acid disorders from the E-IMD cohort show increased frequencies of intellectual disability and behavioral/emotional problems.²⁹ Unfortunately, there is not sufficient data on formal intelligence quotient testing available in the *cblA/mut* subgroups, but if one uses regular schooling as a surrogate parameter the *cblA* patients have a better outcome. Interestingly, all four adult *cblA* patients in the study live independently. Aspects such as cognitive outcome, social development, and professional integration need to be studied in more detail in future studies.

Chronic renal failure is the most important somatic long-term complication in MMA^{3,11} and has already been demonstrated in the E-IMD cohort.⁸⁻¹⁰ In this study, only 8% of *cblA* showed CRF whereas 46% of *mut* patients did,

which is in accordance with percentages in earlier studies.

Interestingly, we were able to demonstrate that in most *cblA* patients GFR is preserved, even in older patients. The estimates of GFR by Schwarz' formula and MDRD are based on creatinine, which has to be interpreted with caution because, in pediatric patients with a low muscle mass, creatinine tends to underestimate the extent of renal damage. Therefore, cystatin C may be more reliable in this population.³⁰ Unfortunately, this parameter was not available in all participating centers. Nevertheless in six *cblA* patients cystatin C values were available, which were all normal.

The pathogenesis of chronic renal failure in MMA remains to be elucidated in detail and may be part of a multisystem involvement of altered energy metabolism via different synergistically acting mitochondrial toxins.³¹⁻³³ Concentrations of methylmalonic acid in urine and especially in plasma are important predictors of CRF.^{3,31,34} In this study methylmalonic acid in urine and plasma were significantly lower in *cblA* patients, corresponding to the higher GFR and lower incidence of CRF.

The response to cobalamin treatment has been suggested to be an important predictor of outcome³⁵ and therefore should be assessed in every MMA patient.¹¹ All *cblA* patients except one (who remained unclear) were reported to be cobalamin responsive. One major shortcoming of this study, and also an important problem in patient management, is to specifically test for cobalamin responsiveness. Although a test protocol has been proposed,² it may be difficult to apply in some patients because it requires metabolic stability, which cannot be reached when hydroxocobalamin is withdrawn. Unfortunately, the *in vitro* response to hydroxocobalamin in enzyme testing assays cannot be extrapolated to the *in vivo* situation.³⁶ Nevertheless, early and adequate hydroxocobalamin supplementation in responsive patients is crucial to postpone and even prevent chronic renal failure.³⁷ Further studies are needed to investigate the optimal dosage and target range of methylmalonic acid and if alternative use of cyanocobalamin orally is also suitable.

In conclusion *cblA* patients are responsive to hydroxocobalamin treatment and show significantly lower levels of MMA and a milder course than *mut* patients.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Friederike Hörster, Ali Tunç Tuncel, and Matthias Baumgartner: Designing, planning, and conducting the study. All authors except Tanja Plessl, Sean Froese, and Sven Garbade: Collection of patient data. Tanja Plessl, Sean Froese, and Matthias Baumgartner: Evaluation of enzyme measurement data and molecular analyses data. Sven Garbade and Florian Gleich: Statistical analysis. Friederike Hörster, Ali Tunç Tuncel, Matthias Baumgartner, and Stefan Kölker: Manuscript writing. All authors: Manuscript correction. Friederike Hörster: guarantor for this manuscript.

ETHICS STATEMENT

The study was approved by the local ethics committee at the coordinating center (University Hospital Heidelberg) and by all clinical partners. The current publication project was evaluated by the scientific board and approved by the executive board of E-IMD. All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent with regard to being included in the study was obtained from all patients or their legal guardians prior to being included in the study in countries where this was needed by law.

ANIMAL RIGHTS

This article contains no studies with animal subjects performed by any of the authors.

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REFERENCES

1. Matsui SM, Mahoney MJ, Rosenberg LE. The natural history of the inherited methylmalonic acidemias. *N Engl J Med*. 1983; 308:857-861.
2. Fowler B, Leonard JV, Baumgartner MR. Causes of and diagnostic approach to methylmalonic acidurias. *J Inher Metab Dis*. 2008;31:350-360.
3. Hörster F, Baumgartner MR, Viardot C, et al. Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (*mut0*, *mut-*, *cblA*, *cblB*). *Pediatr Res*. 2007;62:225-230.
4. Ledley FD, Jansen R, Nham SU, Fenton WA, Rosenberg LE. Mutation eliminating mitochondrial leader sequence of methylmalonyl-CoA mutase causes *mut0* methylmalonic acidemia. *Proc Natl Acad Sci USA*. 1990;87:3147-3150.
5. Dobson CM, Wai T, Leclerc D, et al. Identification of the gene responsible for the *cblA* complementation group of vitamin B12-responsive methylmalonic acidemia based on analysis of prokaryotic gene arrangements. *Proc Natl Acad Sci USA*. 2002; 99:15554-15559.
6. Padovani D, Banerjee R. A G-protein editor gates coenzyme B12 loading and is corrupted in methylmalonic aciduria. *Proc Natl Acad Sci USA*. 2009;106:21567-21572.

7. Takahashi-Iniguez T, Gonzalez-Noriega A, Michalak C, Flores ME. Human MMAA induces the release of inactive cofactor and restores methylmalonyl-CoA mutase activity through their complex formation. *Biochimie*. 2017;142:191-196.
8. Kolker S, Dobbelaere D, Haberle J, et al. Networking across borders for individuals with organic acidurias and urea cycle disorders: the E-IMD consortium. *JIMD Rep*. 2015;22:29-38.
9. Kolker S, Garcia-Cazorla A, Valayannopoulos V, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. *J Inherit Metab Dis*. 2015;38:1041-1057.
10. Kolker S, Valayannopoulos V, Burlina AB, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype. *J Inherit Metab Dis*. 2015;38:1059-1074.
11. Baumgartner MR, Horster F, Dionisi-Vici C, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. *Orphanet J Rare Dis*. 2014;9:130.
12. Cole TJ, Williams AF, Wright CM, Group RGCE. Revised birth centiles for weight, length and head circumference in the UK-WHO growth charts. *Ann Hum Biol*. 2011;38:7-11.
13. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med*. 1998;17:407-429.
14. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629-637.
15. Plessl T, Burer C, Lutz S, Yue WW, Baumgartner MR, Froese DS. Protein destabilization and loss of protein-protein interaction are fundamental mechanisms in cblA-type methylmalonic aciduria. *Hum Mutat*. 2017;38:988-1001.
16. Dempsey-Nunez L, Illson ML, Kent J, et al. High resolution melting analysis of the MMAA gene in patients with cblA and in those with undiagnosed methylmalonic aciduria. *Mol Genet Metab*. 2012;107:363-367.
17. Lerner-Ellis JP, Dobson CM, Wai T, et al. Mutations in the MMAA gene in patients with the cblA disorder of vitamin B12 metabolism. *Hum Mutat*. 2004;24:509-516.
18. Lin Y, Lin C, Lin W, Zheng Z, Han M, Fu Q. Mild clinical features of isolated methylmalonic acidemia associated with a novel variant in the MMAA gene in two Chinese siblings. *BMC Med Genet*. 2018;19:114.
19. Forny P, Schnellmann AS, Buerer C, et al. Molecular genetic characterization of 151 Mut-type methylmalonic aciduria patients and identification of 41 novel mutations in MUT. *Hum Mutat*. 2016;37:745-754.
20. Worgan LC, Niles K, Tirone JC, et al. Spectrum of mutations in mut methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. *Hum Mutat*. 2006;27:31-43.
21. Merinero B, Perez B, Perez-Cerda C, et al. Methylmalonic acidemia: examination of genotype and biochemical data in 32 patients belonging to mut, cblA or cblB complementation group. *J Inherit Metab Dis*. 2008;31:55-66.
22. Liu MY, Liu TT, Yang YL, et al. Mutation profile of the MUT gene in Chinese methylmalonic aciduria patients. *JIMD Rep*. 2012;6:55-64.
23. Mendez ST, Vela-Amieva M, Velazquez-Arellano A, Ibarra I, Flores ME. Mutation analysis of the methylmalonyl-CoA mutase gene in ten Mexican patients with methylmalonic acidemia. *Rev Invest Clin*. 2012;64:255-261.
24. Acquaviva C, Benoist JF, Pereira S, et al. Molecular basis of methylmalonyl-CoA mutase apoenzyme defect in 40 European patients affected by mut(o) and mut-forms of methylmalonic acidemia: identification of 29 novel mutations in the MUT gene. *Hum Mutat*. 2005;25:167-176.
25. Yi Q, Lv J, Tian F, Wei H, Ning Q, Luo X. Clinical characteristics and gene mutation analysis of methylmalonic aciduria. *J Huazhong Univ Sci Technol Med Sci*. 2011;31:384-389.
26. Perez B, Rincon A, Jorge-Finnigan A, et al. Pseudoexon exclusion by antisense therapy in methylmalonic aciduria (MMAuria). *Hum Mutat*. 2009;30:1676-1682.
27. Heringer J, Valayannopoulos V, Lund AM, et al. Impact of age at onset and newborn screening on outcome in organic acidurias. *J Inherit Metab Dis*. 2016;39:341-353.
28. Molema F, Gleich F, Burgard P, et al. Evaluation of dietary treatment and amino acid supplementation in organic acidurias and urea-cycle disorders: on the basis of information from a European multicenter registry. *J Inherit Metab Dis*. 2019;42:1162-1175.
29. Jamiolkowski D, Kolker S, Glahn EM, et al. Behavioural and emotional problems, intellectual impairment and health-related quality of life in patients with organic acidurias and urea cycle disorders. *J Inherit Metab Dis*. 2016;39:231-241.
30. Sharma AP, Yasin A, Garg AX, Filler G. Diagnostic accuracy of cystatin C-based eGFR equations at different GFR levels in children. *Clin J Am Soc Nephrol*. 2011;6:1599-1608.
31. Morath MA, Horster F, Sauer SW. Renal dysfunction in methylmalonic acidurias: review for the pediatric nephrologist. *Pediatr Nephrol*. 2013;28:227-235.
32. Ruppert T, Schumann A, Grone HJ, et al. Molecular and biochemical alterations in tubular epithelial cells of patients with isolated methylmalonic aciduria. *Hum Mol Genet*. 2015;24:7049-7059.
33. Tuncel AT, Ruppert T, Wang BT, et al. Maleic acid—but not structurally related Methylmalonic acid—interrupts energy metabolism by impaired calcium homeostasis. *PLoS One*. 2015;10:e0128770.
34. Kruszka PS, Manoli I, Sloan JL, Kopp JB, Venditti CP. Renal growth in isolated methylmalonic acidemia. *Genet Med*. 2013;15:990-996.
35. Horster F, Garbade SF, Zwickler T, et al. Prediction of outcome in isolated methylmalonic acidurias: combined use of clinical and biochemical parameters. *J Inherit Metab Dis*. 2009;32:630.
36. Baumgartner R, Giardini O, Cantani A, Sabetta G, Castro M. Methylmalonic acidemia due to mutase apoenzyme defect: responsive to vitamin B12 in intact fibroblasts but not in vivo. *J Inherit Metab Dis*. 1982;5:137-141.
37. Haarmann A, Mayr M, Kolker S, et al. Renal involvement in a patient with cobalamin A type (cblA) methylmalonic aciduria: a 42-year follow-up. *Mol Genet Metab*. 2013;110:472-476.

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APPENDIX

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