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ORIGINAL ARTICLE

# Prediction of outcome in isolated methylmalonic acidurias: combined use of clinical and biochemical parameters

**F. Hörster · S. F. Garbade · T. Zwickler · H. I. Aydin · O. A. Bodamer · A. B. Burlina · A. M. Das · J. B. C. De Klerk · C. Dionisi-Vici · S. Geb · G. Gökcay · N. Guffon · E. M. Maier · E. Morava · J. H. Walter · B. Schwahn · F. A. Wijburg · M. Lindner · S. Grünewald · M. R. Baumgartner · S. Kölker**

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**Summary** *Objectives* Isolated methylmalonic acidurias (MMAurias) are caused by deficiency of methylmalonyl-CoA mutase or by defects in the synthesis of its cofactor 5′-deoxyadenosylcobalamin. The aim of this study was to evaluate which parameters best predicted the long-term outcome. *Methods* Standardized questionnaires were sent to 20 European metabolic centres asking for age at diagnosis, birth decade, diagnostic work-up, cobalamin responsiveness, enzymatic subgroup ($mut^0$, $mut^-$, cblA, cblB) and different aspects of long-term outcome. *Results* 273 patients were included. Neonatal onset of the disease was associated with increased mortality rate, high frequency of developmental delay, and severe handicap. Cobalamin non-responsive patients with neonatal onset born in the 1970s and 1980s had a particularly poor outcome. A more favourable outcome was found in patients with late onset of symptoms, especially when cobalamin responsive or classified as $mut^-$. Prevention of neonatal crises in pre-symptomatically diagnosed newborns was identified as a protective factor concerning handicap. Chronic renal failure manifested earlier in $mut^0$ patients than in other enzymatic subgroups. *Conclusion* Outcome in MMAurias is best predicted by the enzymatic subgroup, cobalamin responsiveness, age at onset and birth decade. The prognosis is still unfavourable in patients with neonatal metabolic crises and non-responsiveness to cobalamin, in particular $mut^0$ patients.

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Competing interests: None declared

*References to electronic databases*: Methylmalonic aciduria, cblA type: OMIM 251100. Methylmalonic aciduria, cblB type: OMIM 251110. Methylmalonic aciduria and homocystinuria, cblC type: OMIM 277400. Methylmalonic aciduria and homocystinuria, cblD type: OMIM 277410. Methylmalonic aciduria, cblF type, OMIM 277380. Complete defect of methylmalonyl-CoA-mutase activity, $mut^0$: OMIM 251000. Partial defect of methylmalonyl-CoA-mutase activity, $mut^-$: OMIM 251000. Methylmalonyl-CoA mutase: EC 5.4.99.2.

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F. Hörster (✉) · S. F. Garbade · T. Zwickler · M. Lindner · S. Kölker
Department of General Pediatrics,
Division of Inborn Metabolic Diseases,
University Children's Hospital Heidelberg,
Im Neuenheimer Feld 430,
D-69120 Heidelberg, Germany
e-mail: friederike.hoerster@med.uni-heidelberg.de

H. I. Aydin
Department of Metabolism, Children's Hospital,
Hacettepe University Ankara,
Ankara, Turkey

O. A. Bodamer
Department of Paediatrics, Allgemeines Krankenhaus,
Vienna, Austria

A. B. Burlina
Department of Paediatrics, Division of Metabolic Disorders,
University Hospital Padova,
Padova, Italy

A. M. Das
Department of Paediatrics II,
Medizinische Hochschule Hannover,
Hannover, Germany

**Abbreviations**

| | |
|---|---|
| cblA | methylmalonic aciduria cblA type |
| cblB | methylmalonic aciduria cblB type |
| cblC | methylmalonic aciduria and homocystinuria, cblC type |
| cblD | methylmalonic aciduria and homocystinuria, cblD type |
| cblF | methylmalonic aciduria cblF type |
| CRF | chronic renal failure |
| GFR | glomerular filtration rate |
| LRT | likelihood ratio test |
| MCM | methylmalonyl-CoA mutase |
| MMA | methylmalonic acid |
| MMAurias | methylmalonic acidurias |
| $mut^0$ | complete defect of methylmalonyl-CoA-mutase activity |
| $mut^-$ | partial defect of methylmalonyl-CoA-mutase activity |

## Introduction

Methylmalonic acidurias (MMAurias) are a heterogeneous group of inborn errors of metabolism characterized biochemically by the accumulation of methylmalonic acid (MMA) in body fluids and tissues. They are caused by a defect of the mitochondrial enzyme methylmalonyl-CoA mutase (MCM, EC 5.4.99.2) or by one of the known defects in the uptake, transport, or synthesis of 5′-deoxyadenosylcobalamin, the cofactor of MCM (Coelho et al 2008; Deodato et al 2006; Fenton et al 2001). Some of these defects affect both the metabolism of 5′-deoxyadenosylcobalamin and that of methylcobalamin, resulting in *combined* MMAuria and homocystinuria (cblC, cblD variant 1, cblF). MMAurias solely affecting MCM activity ($mut^0$, $mut^-$, cblA, cblB and cblD variant 2) are termed *isolated* MMAurias. The deficiencies of MCM caused by mutations in the apomutase locus are further subdivided into defects without ($mut^0$) and with residual activity ($mut^-$). The mitochondrial, dimeric enzyme MCM catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, thus linking the final catabolic pathways of L-isoleucine, L-valine, L-methionine, L-threonine, odd-chain fatty acids, and cholesterol side-chains to the tricarboxylic acid cycle.

The clinical presentation of affected patients is variable. The majority of patients present during the newborn period or infancy with life-threatening acute metabolic crises resulting in multi-organ failure or even death if untreated. These crises are often precipitated by conditions that are likely to induce catabolic stress. Severe combined ketoacidosis and lactic acidosis, hypoglycaemia or hyperglycaemia, neutropenia, hyperglycinaemia, and hyperammonaemia are the most common laboratory findings. In a subgroup of patients, chronic progressive disease, psychomotor retardation and failure to thrive are the leading symptoms.

The first study on the natural history of MMAuria demonstrated differences in the disease course and

J. B. C. De Klerk
Sophia Children's Hospital, Erasmus Medical Centre, Rotterdam, The Netherlands

C. Dionisi-Vici
Division of Metabolism, Bambino Gesù Children's Hospital, Rome, Italy

S. Geb
University Children's Hospital I, Frankfurt, Germany

G. Gökcay
Department of Nutrition and Metabolism, Istanbul University Medical Faculty Children's Hospital, Istanbul, Turkey

N. Guffon
Centre de Référence des Maladies Héréditaires du Métabolisme, Hôpital Femme Mère Enfant, Lyon, France

E. M. Maier
Dr. von Hauner Children's Hospital, Munich, Germany

E. Morava
Radboud University Medical Centre Nijmegen, Nijmegen, The Netherlands

J. H. Walter
Willink Unit, Royal Manchester Children's Hospital, Manchester, UK

B. Schwahn
Department of General Pediatrics, University Children's Hospital, Düsseldorf, Germany

F. A. Wijburg
Department of Pediatrics, Academic Medical Centre, University Hospital, Amsterdam, The Netherlands

S. Grünewald
Metabolic Unit, Great Ormond Street Hospital, London, UK

M. R. Baumgartner
Metabolism and Molecular Paediatrics, University Children's Hospital, Zurich, Switzerland

outcome of patients with isolated MMAurias; $mut^0$ patients were most severely affected (Matsui et al 1983). This has been confirmed in further detail in subsequent studies (Baumgartner et al 1995; Hörster et al 2007).

Other studies classified patients according to the onset of clinical symptoms and cobalamin responsiveness but not on the basis of the underlying enzymatic defect (Ogier de Baulny et al 2005; Nicolaides et al 1998; Touati et al 2006; Van der Meer et al 1994). These studies have shown that survival and neurological outcome were unfavourable in patients with early onset of symptoms and in those not responding to cobalamin. Although the overall survival has improved during the last two decades, the long-term outcome remains disappointing (Hörster et al 2007; Touati et al 2006). Neurological outcome is often impaired by extrapyramidal movement disorder and developmental delay (Nicolaides et al 1998). Furthermore, chronic renal failure (CRF) is frequently found (Hörster et al 2007).

The major aim of this study was to investigate the long-term outcome in patients with isolated MMAurias focusing on the identification of parameters that best predict survival and the manifestation of long-term neurological and renal complications.

## Methods

### Questionnaire

Standardized questionnaires were sent to European metabolic centres. The questionnaire asked for date of birth, sex, details of clinical history (complications during pregnancy, peri- and postnatal complications), age and symptoms at first manifestation, confirmation of diagnosis and classification of subgroups (enzyme analysis, mutation analysis, cobalamin responsiveness), pharmacotherapy (cobalamin, carnitine and intestinal decontamination with antibiotics), and dietary treatment (protein restriction, amino acid supplements, tube feeding). The overall and neurological outcome were assessed by survival, age at death, cause of death, anthropometric parameters (body weight, height, head circumference), developmental delay, degree of handicap, movement disorders, seizures, metabolic stroke, and the use of a wheelchair. The age at onset of chronic renal failure (i.e., GFR below 60 ml/min per 1.73 $m^2$ according to the European definition) was recorded. In addition to neurological and renal complications, the questionnaire asked for details of feeding difficulties, pancreatitis, insulin-dependent diabetes mellitus, cardiomyopathy, anaemia, and neutropenia. The questionnaire is available from the corresponding author. Informed consent was obtained from patients and/or parents in the individual centres. The study was approved by the Institutional Ethical Review Board of the University of Heidelberg, Germany (033/2006). Double entries were excluded by birth date, sex, and nationality.

At the same time, using a separate questionnaire, we evaluated the standard procedures for confirmation of diagnosis, maintenance treatment, and follow-up monitoring of patients with isolated MMAuria in the European metabolic centres included in this study. The results of this study have been published by Zwickler and colleagues (2008).

As further outlined by Zwickler and colleagues, cobalamin responsiveness was defined *in vivo* by each metabolic centre, but the individual practice varied. Parenteral hydroxycobalamin was given in a dosage from 1 to 5 mg, and a decline in urinary MMA concentration by at least 30% or below an absolute concentration of 1000 mmol/mol creatinine was considered as a positive response.

### Statistical analysis

Statistical analysis was performed using R (R Development Core Team 2008). Survival rates were compared by Cox regression analysis and recursive partitioning using log-rank tests (Hothorn et al 2006) and, subsequently, were presented as Kaplan-Meier survival curves. The impact on the survival of disease onset (neonatal versus late onset), responsiveness to cobalamin, enzymatic group, mode of and age at diagnosis, and birth decade was tested.

Generalized linear models were used to analyse the frequency of severe metabolic decompensations (i.e., when patients required intensive care treatment) in cobalamin responders and non-responders within the first 3 years of life. ICU treatment reflected an acute emergency situation and thus an acute life-threatening condition that required immediate and intensive treatment including intravenous detoxification management or even haemodialysis/haemofiltration. Other putative variables that might influence the frequency of decompensations were tested in analogy. These included the disease onset (neonatal versus late onset), the enzymatic group, age at diagnosis, diagnosis while asymptomatic or diagnosis after the onset of symptoms, and birth decade. Patients who were younger than 36 months at the end of the study or who had died before that age were excluded from this particular analysis.

The frequency of developmental delay was evaluated by logistic regression analysis depending on disease onset

(neonatal versus late onset), response to cobalamin, diagnosis before or after onset of clinical symptoms, and birth decade. An analogous model was tested including enzymatic subgroup instead of cobalamin responsiveness. Patients younger than 12 months at the end of the study or who had died before that age or with major perinatal complications (asphyxia, neonatal sepsis or premature birth before the 32nd week of gestation) were excluded from this analysis.

A proportional odds model, which is also known as cumulative link model (Agresti 2002; Venables and Ripley 2002), was applied to calculate the influence of disease onset (neonatal versus late onset), response to cobalamin, age at and mode of diagnosis, and birth decade on the degree of handicap. Patients who died during the first month of life or with major perinatal complications (see above) were excluded from this analysis.

Chronic renal failure has been defined as GFR below 60 ml/min per 1.73 $m^2$ according to the European definition. Statistical evaluation was performed using an accelerated failure time model with Weibull distribution (Venables and Ripley 2002). This analysis takes into account that CRF develops in a variable timespan before it is diagnosed, i.e. data are left-censored. The influence of birth decade and enzymatic subgroup or cobalamin responsiveness on the manifestation of CRF was tested in all patients who were at least 2 years old at the end of the study.

The effects of dietary treatment (protein restriction, amino acids supplements, tube-feeding) and pharmacotherapy (intestinal decontamination by antibiotics) concerning survival, developmental delay, and CRF have been tested in $mut^0$ patients, the largest and clinically most homogeneous subgroup of study patients. The effect of carnitine supplementation could not be tested, because virtually all study patients received carnitine.

## Results

### Study population

In total 273 patients (123 female, 150 male) with confirmed diagnosis of isolated MMAurias from 17 metabolic centres in 8 European countries were included in the study (a table showing details of patients' distribution is available online as Supplementary Table S1). The median age of patients was 6.5 years (range: 3 days to 43.7 years).

Patients were divided into four birth cohorts by decade, i.e. 1960–79 (cohort I, *n*=13), 1980–89 (cohort II, *n*=56), 1990–99 (cohort III, *n*=100), and 2000–2007 (cohort IV, *n*=104). The cut-off date for data entry of study patients was 30 November 2007.

Of the 273 patients, 94 were classified as $mut^0$, 30 as $mut^-$, and 43 as cblA/B (Table 1). Further differentiation of cblA/B has been performed in only 10 patients (cblA *n*=7, cblB *n*=3). Evaluation of cobalamin responsiveness identified 51 cobalamin responders and 182 non-responders. For 40 patients, results of cobalamin responsiveness have not been reported (Table 1). Mutational analysis has been performed in only 23 patients and revealed homozygous mutations in 9 patients associated with $mut^0$ phenotype in 8 patients and cblA in one.

According to the transnational design of the study, the ethnic backgrounds of study patients were heterogeneous. We included patients originating from Turkey (*n*=95), United Kingdom (*n*=29), Italy (*n*=27), unspecified Asian countries (*n*=27), Germany (*n*=17), Pakistan (*n*=15), the Netherlands (*n*=11), France (*n*=9), Saudi Arabia (*n*=8), Morocco (*n*=7), Bangladesh (*n*=6), Algeria (*n*=4), Caribbean islands (*n*=4), Switzerland (*n*=2), and Lebanon (*n*=2). Single patients were included from Albania, Austria, Greece, Iran, Iraq, Portugal, Russia, and the USA. In 2 patients, the ethnic origin was unknown. The frequency of known consanguinity in these families was high (49%). Eighty-nine patients had affected siblings (33%).

### Disease course and survival

Median age at onset of symptoms was 2 weeks (range: first week of life to 4.5 years; outliers at 7, 12 and 20 years) and 131 patients developed symptoms during the first 4 weeks of life (neonatal onset). The most frequent clinical presentation raising suspicion of MMAurias and initiating diagnostic work-up was an acute metabolic crisis (68%, *n*=166). Vomiting and

**Table 1** Cobalamin (Cbl) responsiveness in enzymatic subgroups

| Cbl responsiveness | Total number of patients | $mut^0$ | $mut^-$ | cblA/B[a] |
|---|---|---|---|---|
| Yes | 51 | 0 | 5 | 25 |
| No | 182 | 93 | 23 | 12 |

[a] Differentiation between cblA and cblB was not available in the majority of patients.

poor feeding (13%, *n*=33), developmental delay (6%, *n*=14) and failure to thrive (9%, *n*=23), and other causes (4%, *n*=9, such as seizures, hypothermia, polyneuropathy and metabolic stroke) were also reported but less frequently. In 80% of patients (*n*=239) the diagnosis was made by selective screening after the onset of symptoms, whereas only 34 patients were diagnosed pre-symptomatically by newborn screening (*n*=9) or by screening of high-risk families with a previously identified sibling (*n*=25). The median age at diagnosis was 3 weeks (range: prenatally-4.5 years; outliers at 7, 13 and 14 years) in all patients and 1 week (range: prenatal diagnosis to 1.5 years) in the group of patients who were diagnosed before the onset of symptoms and 7 weeks (range: 1 week to 4.5 years) in the group who were diagnosed after onset of symptoms.

Eighty-one patients (29.7%) died at a median age of 2.13 years (range: 1 day to 33.9 years). In 62 patients the cause of death was documented. Ninety per cent of patients died during or shortly after an acute metabolic crisis (*n*=56 patients). Cardiomyopathy (*n*=1), epileptic state (*n*=1), and sepsis (*n*=4) were less frequently mentioned as a cause of death. Survival was strongly influenced by the age at first onset of symptoms, by the birth decade, and by cobalamin responsiveness. The most favourable combination was late onset of symptoms and cobalamin responsiveness (*n*=33). All these patients survived until the end of this study. In contrast, poor survival was found in patients from the 1970s and 1980s birth cohort who suffered a neonatal metabolic crisis (Fig. 1). Survival was also different between $mut^0$, $mut^-$ and cblA/B patients (Fig. 2). However, this effect was only found if the disease onset type was omitted from the model calculation, since the latter variable had the strongest effect on survival.

In analogy to survival, cobalamin responders presented less frequently with acute metabolic crises than non-responders ($\chi^2[1]=8.87$, $p=0.003$; Fig. 3). Enzymatic subgroups had no significant effect on the frequency of metabolic crises ($\chi^2[2]=0.84$; $p=0.656$).

## Neurological outcome

Neurological disease is a common finding in patients with MMAurias and 220 patients were included for statistical analysis. Developmental delay was present in

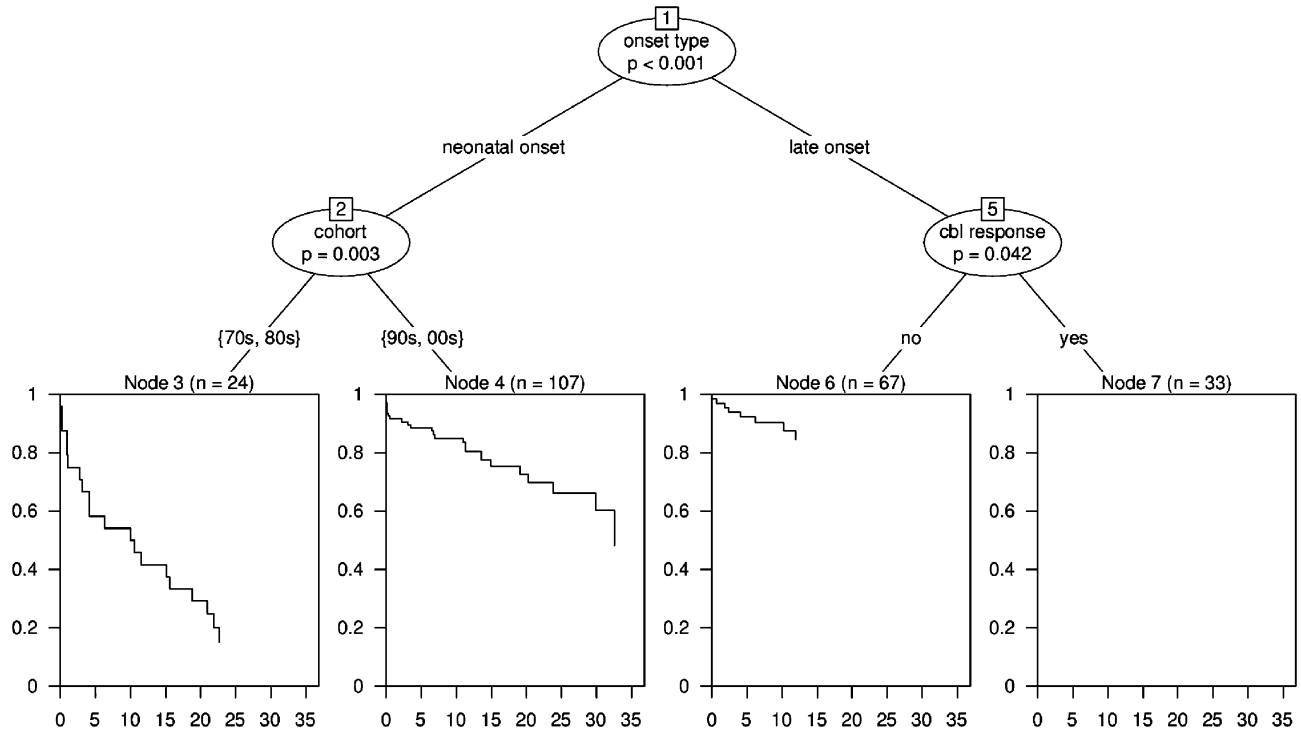


**Fig. 1** Survival in MMAurias. Survival is strongly influenced by age at first onset of symptoms, the birth decade and cobalamin responsiveness, and is most favourable in cobalamin-responsive patients with late onset of symptoms. In contrast, poor survival is found in patients from the 1970s and 1980s birth cohort who have suffered a neonatal metabolic crisis. Survival is presented as Kaplan-Meier estimates in the different groups, which have been distinguished from each other by recursive partitioning method published by Hothorn et al (2006). All cobalamin-responsive patients with late-onset disease (node 7) survived; therefore the Kaplan-Meier estimates are 1. Abscissae, age (years); ordinate, survival rate

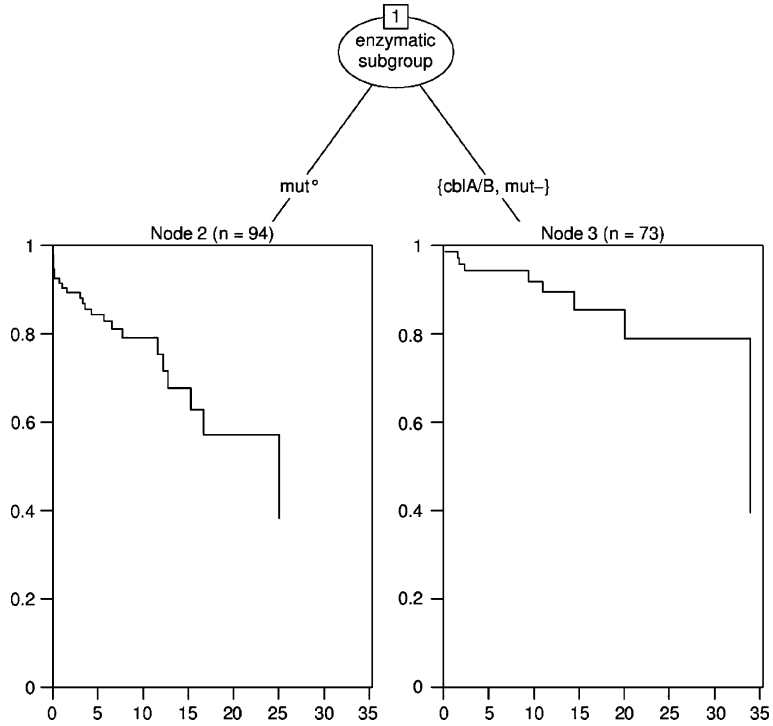


**Fig. 2** Survival in patients from enzymatically defined groups. Survival is different between $mut^0$, $mut^-$, and cblA/B patients. $Mut^0$ patients have the highest mortality ($p$=0.008). Survival rates are shown as Kaplan-Meier estimates, which have been distinguished from each other by recursive partitioning method published by Hothorn et al (2006). Abscissae, age (years); ordinate, survival rate

65% of patients ($n$=143), resulting in motor handicap of different degrees in 54% ($n$=121) (Table 2). Seizures were present in 21 patients, 11 (53%) of whom were not responsive to cobalamin. Metabolic stroke was reported in 38 patients. Of these, 30 (79%) were unresponsive to cobalamin. Details on schooling were reported for 111 patients: 42% of cobalamin non-responders and 48% of cobalamin responders attended regular school. Twenty-seven patients had been tested with different standardized IQ tests at different ages. All results of these IQ tests confirmed the subjective estimate of the reporting physician.

Cobalamin non-responders with neonatal onset of symptoms had the highest risk for developmental delay, whereas cobalamin-responsive patients with a late-onset of symptoms showed the lowest frequency of developmental delay. Patients with either neonatal onset of symptoms or non-responsiveness to cobalamin had an intermediate risk level for developmental delay (Fig. 4). In pre-symptomatically diagnosed patients developmental delay was significantly reduced (LRT [2]=11.58, $p$=0.03). The frequency and severity of handicap was also reduced in pre-symptomatically diagnosed children ($\chi^2$[1]=4.66, $p$=0.031). In analogy, cobalamin responders were less frequently disabled than non-responders ($\chi^2$[1]=6.26, $p$=0.012) (Table 2). The degree of motor handicap was significantly

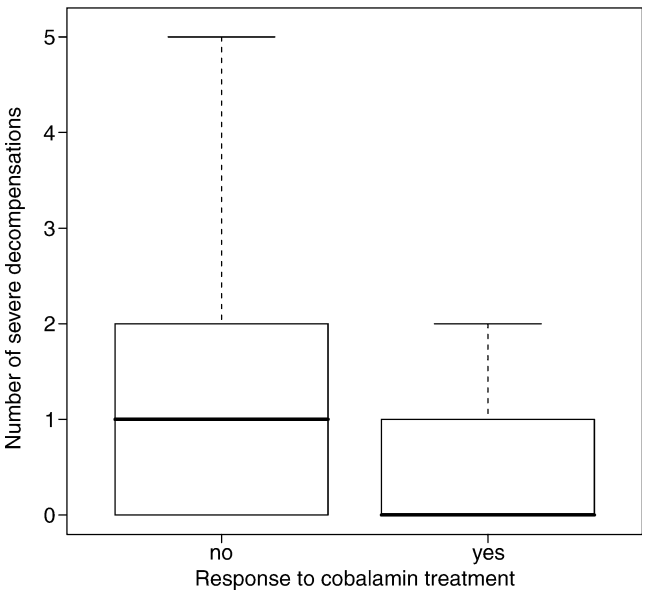


**Fig. 3** Frequency of severe metabolic decompensation. The frequency of severe decompensations (defined as metabolic decompensation requiring medical treatment in an ICU setting) in cobalamin responders ($n$=45) and non-responders ($n$=115) is presented as box plots. Cobalamin non-responders have significantly more severe metabolic decompensations ($\chi^2$[1]=8.87, $p$=0.003). Six outliers (responders $n$=1; non-responders $n$=5 have been omitted. The bold bar of the box represents the median, the margins of the box represent the interquartile range (25th to 75th centiles) and the whisker corresponds to 1.5 times deviation from 25th and 75th centiles, respectively

**Table 2** Degree of motor handicap: effect of cobalamin (Cbl) responsiveness and early diagnosis. Degree of handicap was given as individual estimate by the attending physician

| Degree of handicap | Cbl non-responders | | Cbl responders | |
|---|---|---|---|---|
| | Presymptomatic[a] | Symptomatic[b] | Presymptomatic[a] | Symptomatic[b] |
| None | 5 | 31 | 3 | 20 |
| Mild | 10 | 38 | 0 | 10 |
| Moderate | 1 | 21 | 0 | 5 |
| Severe | 1 | 28 | 0 | 7 |

[a] Diagnosis was made and treatment was started before the onset of symptoms.
[b] Diagnosis was made and treatment was started after the onset of symptoms.

different among enzymatic subgroups ($\chi^2$[2]=8.69, $p$=0.013): incidence of handicap was higher in mut$^0$ patients in comparison with other enzymatic subgroups. The protective effect of diagnosis before the onset of symptoms was not significantly different in this subgroup but showed a tendency ($\chi^2$[1]=3.43, $p$=0.064).

## Renal outcome

CRF was reported in 28% of patients and occurred at a median age of 7.5 years (range: 2–33 years). The incidence of CRF was lower in cobalamin-responsive (16%) than in cobalamin-non-responsive patients (36%) (Fig. 5), and high in mut$^0$ patients (55%), but only the differences between age cohorts were statistically significant (LRT=16.88, $p$<0.001).

## Anthropometric parameters

Weight below 3rd centile was reported in 60 patients; 28 of them (47%) were cobalamin non-responsive. Retarded growth (length below 3rd centile) was reported in 64 patients; 55 of them were cobalamin non-responsive (86%). Microcephaly (head circumference below 3rd centile) was reported in 33 patients; 28 of them were cobalamin non-responsive (85%). None of the differences were statistically significant (data not shown).

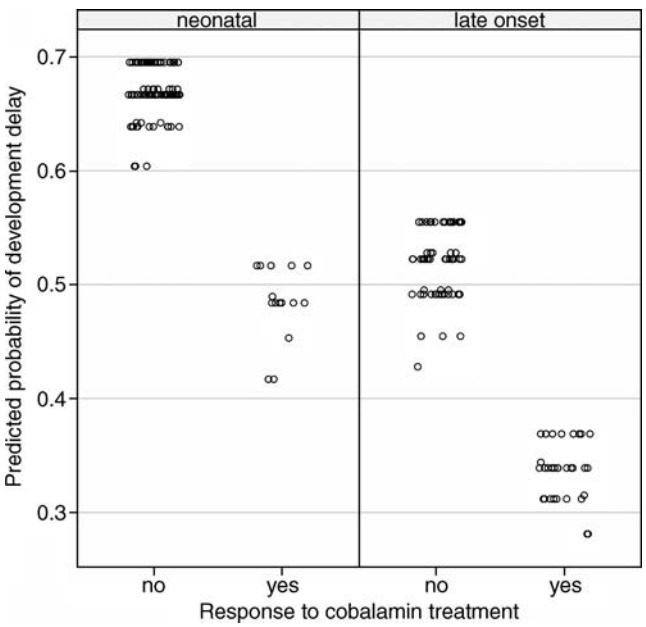


**Fig. 4** Predicted probability for developmental delay. The probability for developmental delay ($n$=206 patients) is best predicted by the disease onset ('neonatal' and 'late onset') and response to cobalamin ('yes' and 'no')

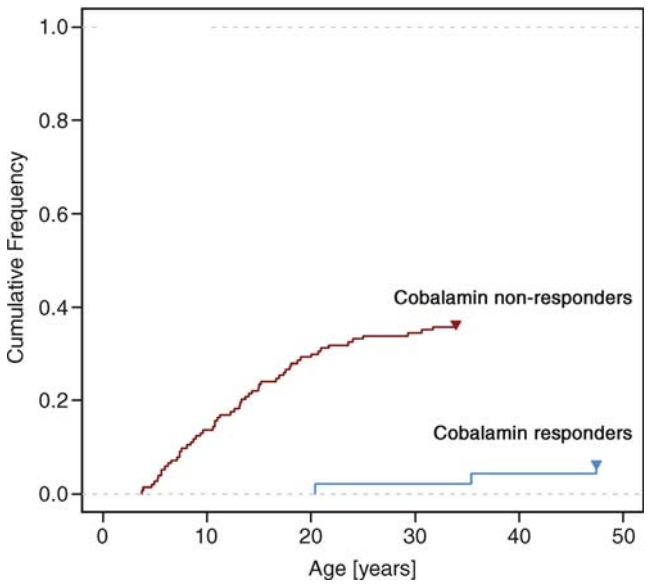


**Fig. 5** Chronic renal failure in cobalamin responders and non-responders. Manifestation of chronic renal failure in cobalamin responders ($n$=3) and non-responders ($n$=58) is shown as Kaplan-Meier analysis. Owing to the small size of the group of cobalamin responders, the differences between the groups were not statistical significant

### Pancreatitis

Pancreatitis was reported in 15 patients and was complicated by insulin-dependent diabetes mellitus in 3 patients. All three patients had frequent metabolic decompensations and died early. One patient had insulin-dependent diabetes mellitus without preceding pancreatitis.

### Cardiomyopathy

Cardiomyopathy was reported in only 4 patients (at the ages of 3, 11, and 15 years; in one patient, the age at onset of cardiomyopathy was not reported).

### Pharmacotherapy and dietary treatment

Treatment differed considerably among patients and centres.

### Cobalamin-responsive patients

The majority (84%) of cobalamin-responsive patients received hydroxocobalamin, whereas a minor subgroup of patients received cyanocobalamin (8%) or none (8%). Half of these patients (53%) received a protein-restricted diet but were only rarely treated with amino acid supplements (8%). Eighteen per cent of patients required different forms of tube feeding. Carnitine was supplemented orally in 82% of patients. Intermittent intestinal decontamination with antibiotics (metronidazole, colistin) was rarely used (8%).

### Cobalamin non-responsive patients

Dietary treatment was equally distributed between protein restriction alone (53%) and protein restriction in combination with amino acids supplements (47%). Half of patients (50%) required tube feeding. Carnitine supplementation was reported in 93% of patients. Intestinal decontamination was applied to 17% (on-off) or 23% (continuously) of patients. All patients who survived neonatal crisis or showed a late onset of symptoms received long-term oral carnitine supplementation.

Since protocols for dietary treatment and pharmacotherapy vary significantly in the contributing European metabolic centres and are used in different combinations (Zwickler et al 2008), statistical analysis demonstrated no significant effect for any of these single therapeutic interventions. The effect of carnitine supplementation could not be evaluated since nearly all patients received oral carnitine supplementation. Even in $mut^0$ patients, the most homogenous patient group, no significant effects of specific therapeutic strategies on survival, anthropometrics, neurological, and renal outcome were found.

## Discussion

This study demonstrates that the long-term outcome in isolated MMAurias is best predicted by cobalamin responsiveness, enzymatic subgroup, age at onset of first symptoms, and the birth decade. A combination of neonatal onset, cobalamin non-responsiveness (in particular $mut^0$ disease), and 1970s and 1980s birth cohorts was associated with the poorest outcome.

Previous studies have shown that the enzymatic classification is a valuable tool for the assessment of outcome parameters in these patients (Hörster et al 2007; Matsui et al 1983); however, most clinical studies have focused on the onset of symptoms and cobalamin responsiveness to classify the patients and to predict the outcome (Nicolaides et al 1998; Van der Meer et al. 1994). Our study shows the importance of both strategies for predicting the outcome in a large number of patients. It emphasizes the fact, that cobalamin responsiveness is an important predictor for the outcome and should be tested carefully in each patient to avoid misclassification of patients with a mild response. A recently published study evaluating the current diagnostic strategies in patients with isolated MMAurias has clearly shown that there is a need for standardized protocols to test cobalamin responsiveness (Zwickler et al 2008). Such a standardized test to identify patients who benefit from cobalamin has been proposed recently (Fowler et al 2008). Unfortunately, *in vitro* cobalamin responsiveness does not reliably predict *in vivo* responsiveness (Baumgartner et al 1982).

In the present study cohort, enzymatic classification was performed less often and was less detailed than in a previous prospective follow-up study (Hörster et al 2007). In particular, cblA/B patients were usually not differentiated. This is an important confounder of the present study, since cblB patients have a less favourable outcome than cblA patients (Hörster et al 2007). This may explain why the enzymatic subgroup was statistically less important in predicting the outcome than cobalamin responsiveness and age at onset of first symptoms. At present, more than 100 disease-causing mutations in the human *MUT* gene have been reported (Aquaviva et al 2005; Lempp et al 2006; Worgan et al 2006). The majority of patients have private mutations or are compound heterozygous and thus genotype-phenotype correlations remain difficult to investigate.

The increased survival rate from the older to the younger birth cohorts, in particular in cobalamin non-responsive patients, most likely reflects an improvement of emergency treatment strategies, neonatal care, rapid diagnostic work-up, and increasing awareness of paediatricians about inborn errors of metabolism.

For some inborn errors of metabolism it has been demonstrated that newborn screening followed by early start of treatment has considerably improved the outcome of affected individuals (Dionisi-Vici et al 2006; Kölker et al 2007; Wilcken et al 2003). It remains to be elucidated whether this is also true for MMAurias. At the time of our study, newborn screening for MMAurias had been available for only a short time in Germany. Since a considerable number of patients (in particular $mut^0$) present clinically before a positive screening result is available, it has been doubted whether newborn screening for MMAurias would be beneficial for all (Leonard et al 2003). In addition, screening for propionylcarnitine has a poor specificity, resulting in a high false-positive rate. Therefore, combining propionylcarnitine with acylcarnitine ratios as second tier strategy has been suggested to improve the specificity of newborn screening for MMAurias (Lindner et al 2008). Our study, however, speaks in favour of newborn screening of MMAurias, since in this study pre-symptomatically diagnosed patients were less frequently and less severely handicapped than affected individuals who were diagnosed after the onset of symptoms. Nevertheless this finding must be interpreted carefully. Since the manifestation of acute metabolic crises in the newborn period significantly influences the outcome, it seems likely that patients with late onset of symptoms would benefit most from newborn screening.

The spectrum of neurological complications observed in our study is in accordance with findings in a previous study in a cohort of cobalamin non-responders with an early onset of symptoms (Nicolaides et al 1998) and also a better neurological outcome in $mut^-$ patients has been shown previously (Shevell et al 1993). In the present study, we demonstrate that a minority of cobalamin non-responsive patients remain neurologically unaffected. It is unknown whether this finding reflects beneficial effects of therapy or variation of the natural history of the disease.

Chronic renal failure has been described as an important long-term complication of MMAurias (Hörster et al 2007). In the present study, 28% of patients developed chronic renal failure, which is lower than in a previous study reporting CRF in 43% of patients (Hörster et al 2007). This apparent discrepancy may be best explained by the different age distribution in both studies: in the present study the median age of patients was 6.5 years, whereas the median age was 18 years in the previous study. In older patients, CRF is found in all subgroups, which stresses the importance of close follow-up of renal function in all MMA patients. In this context it is important to note that CRF is diagnosed by different methods with varying sensitivity in different metabolic centres and the follow-up needs to be standardized.

The overall effect of therapeutic interventions remains unclear in severely affected patients. Neither dietary treatment with or without amino acid supplements, nor application of tube feeding, nor intestinal decontamination with antibiotics was able to influence different aspects of the outcome significantly in a relatively homogenous group of $mut^0$ patients. This reflects the lack of standardized therapy protocols (Zwickler et al 2008) and underlines the need for a prospective study with clearly defined algorithms for diagnostic work-up and protocols for maintenance and emergency treatment, and follow-up monitoring.

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