

# Causes of and diagnostic approach to methylmalonic acidurias

B. Fowler · J. V. Leonard · M. R. Baumgartner

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**Summary** Several mutant genetic classes that cause isolated methylmalonic acidurias (MMAuria) are known based on biochemical, enzymatic and genetic complementation analysis. The  $\text{mut}^0$  and  $\text{mut}^-$  defects result from deficiency of MMCoA mutase apoenzyme which requires adenosyl-cobalamin (Ado-Cbl) as co-enzyme. The *cblA*, *cblB* and the variant 2 form of *cblD* complementation groups are linked to processes unique to Ado-Cbl synthesis. The *cblC*, *cblD* and *cblF* complementation groups are associated with defective methyl-cobalamin synthesis as well. Mutations in the genes associated with most of these defects have been described. Recently a few patients have been described with mild MMAuria associated with mutations of the MMCoA epimerase gene or with neurological symptoms due to *SUCL* mutations. A comprehensive diagnostic approach involves investigations at the level of

metabolites, genetic complementation analysis and enzymatic studies, and finally mutation analysis. MMA levels in urine range from 10–20 mmol/mol creatinine in mild disturbances of MMA metabolism to over 20 000 mmol/mol creatinine in severe MMCoA mutase deficiency, but show considerable overlap and are of limited value for differential diagnosis. The underlying defect in isolated MMAuria can be characterized in cultured skin fibroblasts using several assays, e.g. conversion of propionate to succinate, specific activity of MMCoA, cobalamin adenosyltransferase assay, cellular uptake of  $\text{CN-}^{[57\text{Co}]}$  cobalamin and its conversion to cobalamin coenzymes and complementation analysis. The reliable characterization of patients with isolated MMAuria pinpoints the correct gene for mutation analysis. Reliable classification of these patients is essential for ongoing and future prospective studies on treatment and outcome.

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*References to electronic databases:* Methylmalonic aciduria due to methylmalonyl-CoA mutase deficiency,  $\text{mut}^0$  and  $\text{mut}^-$  defects: OMIM 251000. *cblA*: OMIM 251100. *cblB*: OMIM 251110. *cblD*: OMIM 277410. Cobalamin adenosyltransferase: EC 2.5.1.17. MMCoA mutase: EC 5.4.99.2.

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B. Fowler (✉)  
Metabolic Unit, University Children's Hospital,  
Roemergasse 8, Basel, CH-4058, Switzerland  
e-mail: Brian.Fowler@unibas.ch

J. V. Leonard  
Institute of Child Health, London, UK

M. R. Baumgartner  
Division of Metabolism and Molecular Pediatrics,  
University Children's Hospital, Zürich, Switzerland

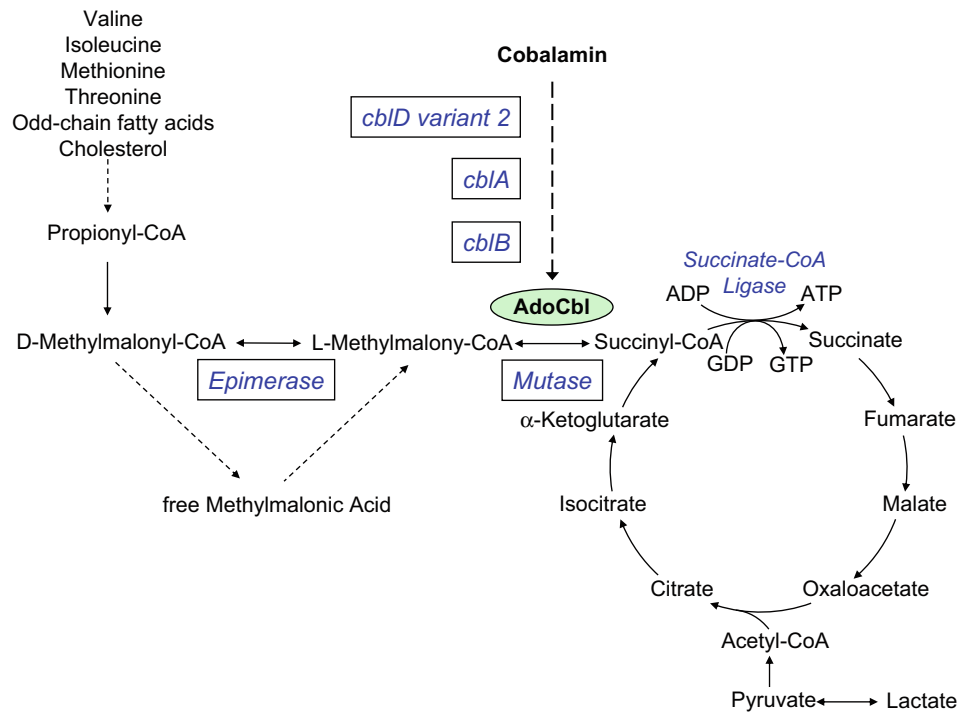
## Abbreviations

MMCoA	Methylmalonyl-CoA
MMA	methylmalonic acid
MMAuria	methylmalonic aciduria
Me-Cbl	methylcobalamin
Ado-Cbl	adenosylcobalamin

## Introduction

Methylmalonyl-CoA (MMCoA), the D-isomer, is a key metabolite in the catabolism of propionate, which is derived from the breakdown of four amino acids, pyrimidines, odd-chain fatty acids and cholesterol side-chains (Fig. 1). D-MMCoA is converted to succinyl-CoA through its L form by the successive action of

**Fig. 1** Metabolic interrelationships of methylmalonic acid. Epimerase, methylmalonyl CoA epimerase (EC:5.1.99.1); mutase, methylmalonyl-CoA mutase (EC 5.4.99.2); AdoCbl, adenosylcobalamin; Ligase, succinate-CoA ligase (EC 6.2.1.4); ADP, adenosine diphosphate; ATP, adenosine triphosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate



MMCoA racemase (epimerase) and MMCoA mutase. The mutase enzyme requires adenosylcobalamin (Ado-Cbl) as coenzyme so that the integrity of methylmalonic acid (MMA) metabolism is inextricably linked to vitamin B<sub>12</sub> (cobalamin), its adequate intake and correct uptake, transport and intracellular metabolism. Deficiency of MMCoA mutase leads to elevated levels of MMA in body fluids. In humans vitamin B<sub>12</sub> is a co-factor for one other enzyme, methionine synthase. This enzyme requires methylcobalamin (Me-Cbl) for activity. Deficiency of this enzyme results in accumulation of homocysteine and occurs in isolation or combined with MMCoA mutase deficiency in certain genetic defects of intracellular cobalamin metabolism

as well as in nutritional deficiency or disturbed uptake or transport of vitamin B<sub>12</sub> (Rosenblatt and Fenton 2001; Suormala et al 2004). In the combined defects (cblC, cblD and cblF) both MMA and homocysteine will accumulate and these defects must be borne in mind when characterizing methylmalonic aciduria.

**Genetic disorders causing methylmalonic aciduria (MMAuria)**

Several distinct mutant genetic classes, based on biochemical, enzymatic and genetic complementation analysis are known (Table 1).

**Table 1** Methylmalonic acidurias: complementation groups

Complementation group	Impaired synthesis	Primary defect	Elevated metabolite
mut <sup>0</sup> /mut <sup>-</sup>	Mutase apoenzyme	MMCoA mutase	MMA
cblA	AdoCbl	? Mitochondrial Cbl import	MMA
cblB	AdoCbl	ATP:cobalamin adenosyl-transferase	MMA
cblD-Var2	AdoCbl	? Cytosolic Cbl transport	MMA
cblC	AdoCbl, MeCbl	? Cytosolic Cbl transport	MMA, Hcy
cblD	AdoCbl, MeCbl	? Cytosolic Cbl transport	MMA, Hcy
cblF	AdoCbl, MeCbl	Lysosomal Cbl release	MMA, Hcy
cblE	MeCbl	MS-reductase	Hcy
cblG	MeCbl	Methionine synthase (MS)	Hcy
cblD-Var1	MeCbl	? Cytosolic Cbl transport	Hcy

MMCoA, methylmalonyl-coenzyme A; MMA, methylmalonic acid; Hcy, homocyst(e)ine; cbl, cobalamin; AdoCbl, adenosylcobalamin; MeCbl, methylcobalamin; MS, methionine synthase

**Table 2** Summary of mutations causing MMAuria

Enzyme/protein	Structure	Short name	Gene	Locus	No. of exons	ORF (bp)	Pathogenic mutations		References
							No. of mutations <sup>a</sup>	Common mutations <sup>b</sup>	
Methylmalonyl-CoA mutase	Homo-dimer	Mutase	<i>MUT</i>	6p21	13	2250	188	c.655A>T (p.N219Y) c.1106G>A (p.R369H) (mut <sup>0</sup> patients) c.2080C>T (p.R694W) (mut <sup>-</sup> patients) c.322C>T (p.R108C) (Hispanic patients) c.2150G>T (p.G717V) (Black patients) None	Ledley et al (1988) Worgan et al (2006) Lempp et al (2007)
Methylmalonyl-CoA epimerase	Homo-dimer	MMCoA epimerase	<i>MCEE</i>	2p13.3	3	525	3	None	Bikker et al (2006) Dobson et al (2006) Granger et al (2007)
? Mitochondrial translocation of vitamin B <sub>12</sub>	Homo-dimer	cbIA	<i>MMAA</i>	4q31.1	7	1254	28	c.433C>T (p.R145X) c.592_595delACTG	Dobson et al (2002a) Lerner-Ellis et al (2004) Korotkova and Lidstrom (2004) Banejee (2006) Merinero et al (2008)
? Protection of mutase from inactivation									
ATP-cobalamin adenosyltransferase	Homo-trimer	cbIB	<i>MMAB</i>	12q24	9	750	24	c.556C>T (p.R186W)	Dobson et al (2002b) Saridakis et al (2004)
? Channeling of vitamin B <sub>12</sub> to its cytosolic and mitochondrial targets	?	cbID	<i>MMADHC</i>	2q23.2	8	891	13	c.700C>T (p.Q234X) c.197-1G>T None	Lerner-Ellis et al (2006a) Merinero et al (2008) Coelho et al (2008) Coelho et al (2008)
? Binding and intracellular trafficking of cobalamin	?	cbIC	<i>MMACHC</i>	1p34.1	4	849	44	c.271dupA (R91KisX14) c.331C>T (R111X) c.394C>T (R132X)	Lerner-Ellis et al (2006b) Nogueira et al (2008)

<sup>a</sup> As reported in The Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk>).

<sup>b</sup> Mutations with an overall allele frequency of >10% were considered as common mutations. ORF, open reading frame; bp, base pairs.

The  $\text{mut}^0$  and  $\text{mut}^-$  defects (OMIM 251000) belong to a single genetic complementation class and result from deficiency of MMCoA mutase apoenzyme due to mutations of the *MUT* gene (Ledley et al 1988). Differentiation of these defects is based on the presence ( $\text{mut}^-$ ) or absence ( $\text{mut}^0$ ) of residual enzyme activity in cultured fibroblasts and response to vitamin  $\text{B}_{12}$  *in vitro* and *in vivo*. MMCoA mutase (MCM, EC 5.4.99.2) is a mitochondrial enzyme requiring 5'-deoxyadenosylcobalamin. The gene (*MUT*) maps to 6p21 and its full-length cDNA codes for a 750-amino-acid protein of 88.2 kDa. The gene contains 13 exons spanning more than 35 kb and so far approximately 200 mutations have been identified (Table 2). The enzyme contains an N-terminal mitochondrial leader sequence of 32 amino acids and the mature form is a dimer of identical subunits of 78.5 kDa. Each subunit contains two main functional domains, an N-terminal ( $\beta\alpha$ )<sub>8</sub> barrel (residues 88–422) with the substrate binding site and a C-terminal ( $\beta\alpha$ )<sub>5</sub> Ado-Cbl binding domain (residues 578–750). The two domains are connected by a long linking region (residues 423–577) (Fenton et al 2001).

The *cblA* (OMIM 251100) and *cblB* (OMIM 251110) complementation groups are linked to processes unique to Ado-Cbl synthesis and cause isolated MMAuria. Genes for the *cblA* (*MMAA*) and *cblB* (*MMAB*) groups have recently been described (Dobson et al 2002a, b).

The *MMAA* gene is located at 4q31.1-q31.2 and spans about 17.1 kb containing 7 exons, but exon 1 is untranslated. The full-length cDNA codes for a 418-amino-acid protein of 46.5 kDa. At least 28 mutations have been identified in 37 *cblA* patients (Table 2). The *cblA* protein contains an N-terminal mitochondrial leader sequence and cleavage site as well as Walker A and B ATP-binding motifs, a  $\text{Mg}^{2+}$ -binding site and a GTP-binding site, but its exact function is unclear. It was first thought to be responsible for translocation of cobalamin into mitochondria prior to the final steps of AdoCbl synthesis (Dobson et al 2002a). More recent evidence, however, points to a role of the protein in the assembly and stabilization of holo-MMCoA-mutase analogous to its bacterial homologue *meaB*. This enzyme forms a complex with MMCoA mutase, stimulates its activity and protects the enzyme from irreversible inactivation (Padovani and Banerjee 2006).

The *MMAB* gene for cobalamin adenosyltransferase (EC 2.5.1.17) maps to 12q24; it consists of 9 exons extending over 18.87 kb and its full-length cDNA codes for a 250-amino-acid protein (27.3 kDa) (Dobson et al 2002b). The *cblB* enzyme contains a leader sequence and signal cleavage site consistent with localization to

the mitochondrion. At least 24 mutations associated with low Ado-Cbl synthesis and mainly involving amino acids from the active site have been found in patients in the *cblB* complementation group.

Defective synthesis of both Ado-Cbl and Me-Cbl occurs in the *cblC*, *cblF* and combined form of *cblD* complementation groups as well as in nutritional deficiency or disturbed uptake or transport of vitamin  $\text{B}_{12}$  (for review see Whitehead 2006).

The gene associated with the *cblC* defect is *MMACHC* which maps to chromosome 1p34.1; it comprises 5 exons and its full length cDNA contains 846 base pairs corresponding to a protein of 282 amino acids with a molecular weight of 31.7 kDa (Lerner-Ellis et al. 2006b). The protein appears to locate to the cytosol and homology with bacterial cobalamin transport proteins suggests a role in intracellular channelling of Cbl (Table 2).

The *cblD* complementation group (OMIM 277410) was first associated with combined MMAuria and homocystinuria, but it is now known that two additional variant forms of this mutant class exist with either isolated homocystinuria (*cblD*-variant 1) or isolated MMAuria (*cblD*-variant 2) (Coelho et al 2008; Suormala et al 2004).

The previously described single patient (Watkins et al. 2000) who was thought to belong to a new complementation group (*cblH* Watkins et al 2000) also belongs to the *cblD*-variant 2 mutant class.

The *cblF* defect is caused by defective release of Cbl from lysosomes (Suormala et al 2004) and its gene has not yet been identified.

Recently a few patients have been described with mild MMAuria associated with a mutation of the MMCoA epimerase gene (*MCEE*), but these patients had no consistent clinical phenotype, as outlined below.

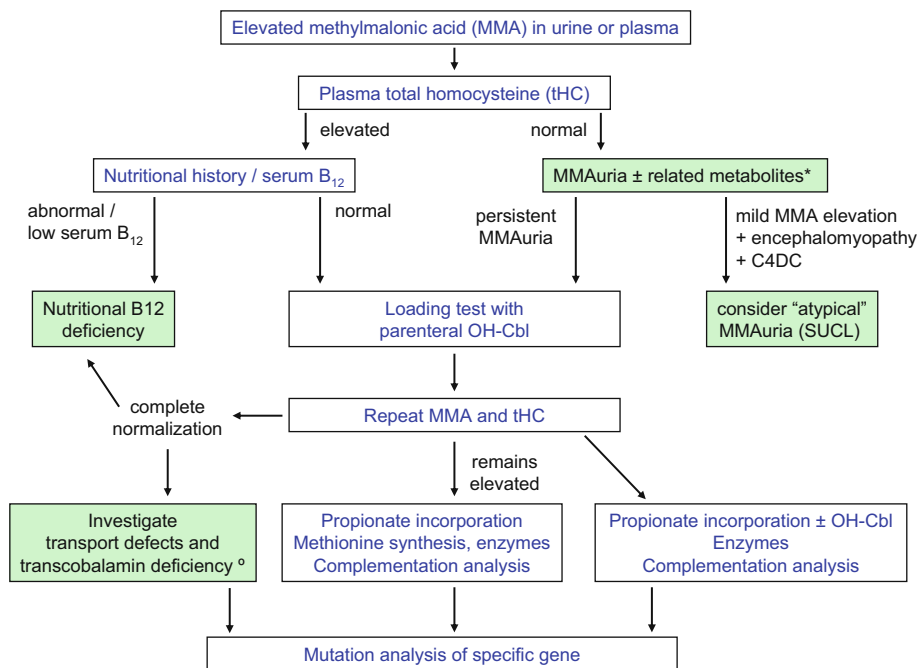
## Diagnostic approach

A comprehensive diagnostic approach involves investigations at the level of metabolites, genetic complementation analysis and enzymatic studies, and finally mutation analysis (Fig. 2).

## Metabolites

The measurement of MMA and related metabolites such as 3-hydroxypropionate and methylcitrate in urine provides a reliable first-line diagnostic approach in patients suspected to have a disorder of MMA metabolism.

**Fig. 2** Diagnostic flowchart.  
 \*Related metabolites such as methylcitrate, 3-hydroxypropionate, lactate and propionylglycine.  
 °For further details see Whitehead (2006). C4DC, succinyl- or methylmalonylcarnitine



In normal subjects MMA concentrations are age-dependent with median values (P95 limits) of 1.1 (4.8) mmol/mol creatinine below 30 days, 5.2 (49) mmol/mol creatinine at 1–6 months, and 0.8 (6.5) mmol/mol creatinine at 6–12 months of age (Boulat et al 2003).

Elevated levels range from the order of 10–20 mmol/mol creatinine in mild disturbances of MMA metabolism to over 20 000 mmol/mol creatinine in severe MMCoA mutase deficiency (Fig. 3) and can be accompanied by increased levels of a range of related

metabolites such as methylcitrate, 3-hydroxypropionate, lactate and propionylglycine.

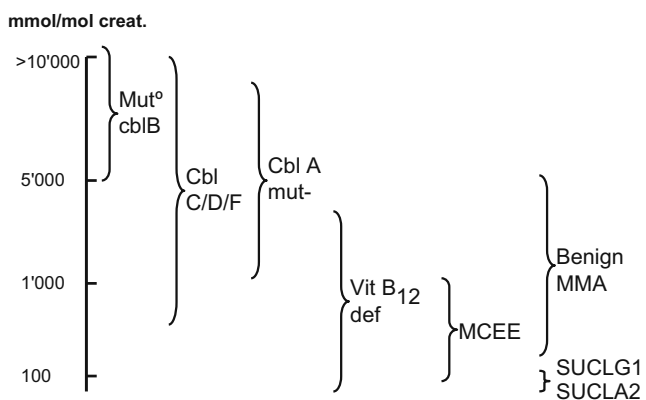
Since several possible causes of elevated MMA are known (Table 3), each must be carefully considered, taking into account the clinical presentation and nutritional state of the patient (Fig. 2).

Although concentrations of MMA are generally higher in the genetic disorders than in absorption and transport defects or nutritional vitamin B<sub>12</sub> deficiency, there is considerable overlap. Thus the values themselves are of limited value in the differential diagnosis because of this overlap (see Fig. 3).

In addition, several factors such as protein intake, the catabolic state and associated metabolic decompensation, renal function as well as vitamin B<sub>12</sub> status can greatly influence MMA levels, especially in urine. Also there appears to be wide variation in MMA levels independent of the changes in these factors. Furthermore, analytical variation as shown by results within the ERNDIM EQA scheme for organic acids can contribute to apparent variation in individual subjects (<http://www.erndim.unibas.ch>). This variation makes comparison of concentrations obtained in different laboratories of limited value. Analytical improvements such as the use of GC-MS stable-isotope dilution methods and participation of laboratories in external QA will improve analytical variation.

Use of MMA levels in plasma (normal <0.27 μmol/L) may well be a more reliable approach (Chandler et al 2007), but more evidence is needed.

CSF measurements of MMA (control values 0.19–0.67 μmol/L) are generally not necessary for diagnostic



**Fig. 3** Urinary MMA levels in various types of disorders. The levels shown are approximations based on ranges of values reported in the literature. Note the arbitrary nonlinear scale. Complementation groups, mut<sup>0</sup>, mut<sup>-</sup>, cblA, cblB, cblC, cblD, cblF. MCEE, methylmalonyl-CoA epimerase deficiency; MMA, methylmalonic acid; SUCLG1, succinate-CoA ligase, alpha subunit; SUCLA2, succinate-CoA ligase, ADP-forming beta subunit

**Table 3** Causes of elevated MMA in plasma and urine**Non-genetic**

## Nutritional deficiency

- Subjects on strict vegetarian diet/vegans
- (Breastfed) babies of vegan mothers or mothers with unrecognized disturbed vitamin B<sub>12</sub> metabolism
- Reduced intestinal absorption (elderly persons)

**Genetic disorders**

## Disorders of MMA-CoA conversion

- Methylmalonyl-CoA mutase
- Methylmalonyl-CoA epimerase (racemase)

## Disorders of absorption and transport of cobalamin

- Hereditary intrinsic factor deficiency
- Defective transport of Cbl by enterocytes (Imerslund–Gräsbeck syndrome)
- Haptocorrin (R Binder) deficiency
- Transcobalamin (TC) Deficiency

## Disorders of intracellular utilization of Cbl

- Combined deficiencies of AdoCbl and MeCbl
- AdoCbl deficiency

## Atypical MMAurias associated with encephalomyopathy

- SUCLG1, succinate-CoA ligase, alpha subunit
- SUCLA2, succinate-CoA ligase, ADP-forming beta subunit

purposes although this may be useful in the investigation of atypical forms, especially in those with mainly neurological involvement (Mayatepek et al 1996).

Increasingly, measurement of acyl carnitine esters in dried blood spots is being applied to diagnosis of the MMAurias, in particular in extended newborn screening programmes (Schulze et al 2003; Wilcken et al 2003). Note, however, that MMAuria is not included in all such schemes as, for example, in Germany (discontinued in 2005).

It is likely that in future, as more and more laboratories undertake metabolic screening and also newborn screening by acylcarnitine measurements, a more common marker of MMA metabolism will be C<sub>4</sub>-dicarboxylic acylcarnitine (C4DC) but this can be either methylmalonyl- or succinylcarnitine.

Plasma total homocysteine measurement (Fowler and Jakobs 1998) is essential in the differential diagnosis of elevated MMA levels of all degree to exclude combined forms of disorders of intracellular cobalamin metabolism as well as transport defects and nutritional causes (Fig. 2).

**Testing of the response to vitamin B<sub>12</sub>**

Defects of the translocation and intracellular synthesis of the active cofactors of vitamin B<sub>12</sub> often respond to pharmacological doses of the vitamin with clinical and biochemical improvement. CblC and cblD defects are

routinely treated with hydroxocobalamin but not all patients with defects of synthesis of adenosylcobalamin (cblA and cblB) will respond. While most patients with cblA will improve, only about one-third of cblB patients will do so (Matsui et al 1983). This response was first noted in a patient reported by Rosenberg and his colleagues (Rosenberg et al 1968) and since then many responsive patients have been identified. Some patients in the mut complementation group will also respond, particularly mut<sup>-</sup> (Hörster et al 2007). Mut<sup>0</sup> patients have also been reported to have responded to parenteral vitamin B<sub>12</sub> (Matsui et al 1983), but a genuine response is rare.

The difficulty in interpreting previous reports is that the testing with vitamin B<sub>12</sub> has never been standardized and what constitutes a response never clearly defined. In a survey of European centres there was a very marked variation in practice (Zwicker et al 2008). Vitamin B<sub>12</sub> was given as hydroxocobalamin intramuscularly or intravenously or as cyanocobalamin intramuscularly. The response was assessed by changes in plasma or urine methylmalonate or in blood spot propionylcarnitine. One to three specimens were collected before giving the vitamin and 1–7 specimens afterwards. The response was judged by a fall in metabolite concentration of between 10% and 90% or by a fall in methylmalonate to a value below 1000 mmol/mol creatinine. Clearly, using some of these criteria makes it doubtful that there was a genuine response and such variation in practice makes it impossible to make any comparisons.

A standardized test is proposed. The purpose of this test is to identify those patients who genuinely benefit from vitamin B<sub>12</sub>. It is important to remember that no child should be given unnecessary or ineffective treatment, particularly by injection.

Parallel to this test the enzymology including *in vitro* response to adenosylcobalamin and if possible the complementation group should be determined (see below). The vitamin B<sub>12</sub> test should not be delayed by waiting for the results of these tests.

1. The patient should be clinically stable on the same treatment for at least one month. The protein and energy intake should be specified.
2. If the patient is already receiving cobalamin, this should be stopped for at least one month before the test. If the patient appears to deteriorate, restart vitamin B<sub>12</sub> and defer the test. Note: As a general rule patients with MMA excretion greater than 10 000 mmol/mol creatinine and those who are clinically unstable rarely respond to vitamin B<sub>12</sub>.
3. Baseline urine collections. At least three specimens should be collected on different days. Plasma

- concentrations may be used only if a sensitive assay (stable-isotope dilution assay) is available.
4. Give hydroxocobalamin 1 mg intramuscularly on three consecutive days.
  5. After the cobalamin injection, collect urine (or plasma) specimens on alternate days for 10 days.
  6. The urine or plasma samples should be analysed in the same run in a laboratory participating in a recognized quality control scheme for methylmalonic acid using GCMS.
  7. A decrease of the mean urine or plasma MMA concentrations of more than 50% should be regarded as indicative of a beneficial response.

### Enzymatic and complementation analysis

Once nutritional causes have been excluded, the complexity of causes of inherited isolated MMAuria necessitates characterization of the underlying defect in cultured skin fibroblasts using several assays.

The overall conversion of propionate to succinate is determined by the incorporation of label from [ $^{14}\text{C}$ ]propionate into cell proteins (Willard et al 1976) (Fig. 4). Specific activity of MMCoA mutase can be measured using a radioactive substrate in the presence and absence of Ado-Cbl (Baumgartner 1983) (Fig. 5).

Distinction of isolated MMAurias into  $\text{mut}^0$  or  $\text{mut}^-$  is based on the somewhat arbitrary biochemical

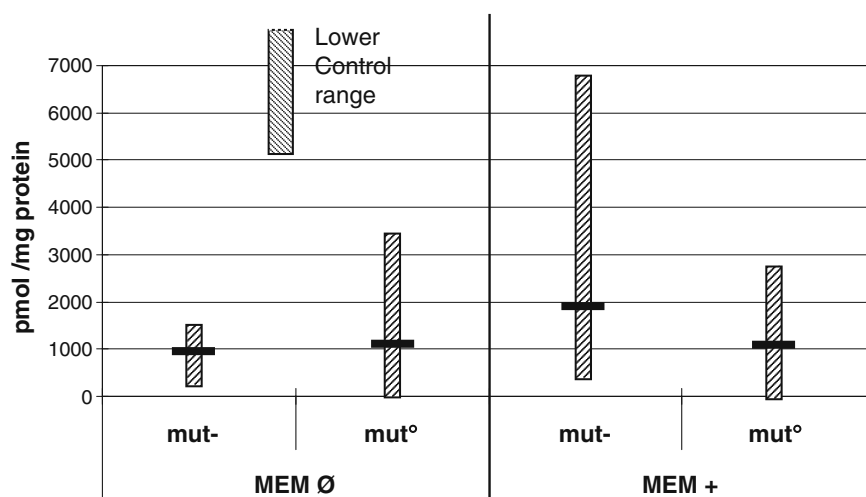
assessment of *in vitro* response to hydroxocobalamin.  $\text{Mut}^0$  is defined as very low mutase activity together with a ratio of propionate incorporation with or without supplemented hydroxocobalamin lower than 1.5 which is the upper limit found in control cell lines.  $\text{Mut}^-$  is defined as low to moderate residual mutase activity and a ratio of  $>1.5$  although it must be borne in mind that in our series some cell lines showed borderline values of 1.2–1.7 and assays needed to be repeated several times to enable definite classification (Lempp et al 2007).

Cobalamin adenosyltransferase can be measured by monitoring the conversion of  $\text{OH-}[^{57}\text{Co}]$ cobalamin to Ado-Cbl. The reaction must be performed in the dark and under hydrogen to provide reducing conditions (Fenton and Rosenberg 1981).

The cellular uptake of  $\text{CN-}[^{57}\text{Co}]$ cobalamin and its conversion to cobalamin coenzymes can be measured in intact fibroblasts in monolayer culture. Human serum is used as a source of transcobalamin to facilitate cellular uptake (Fowler and Jakobs 1998).

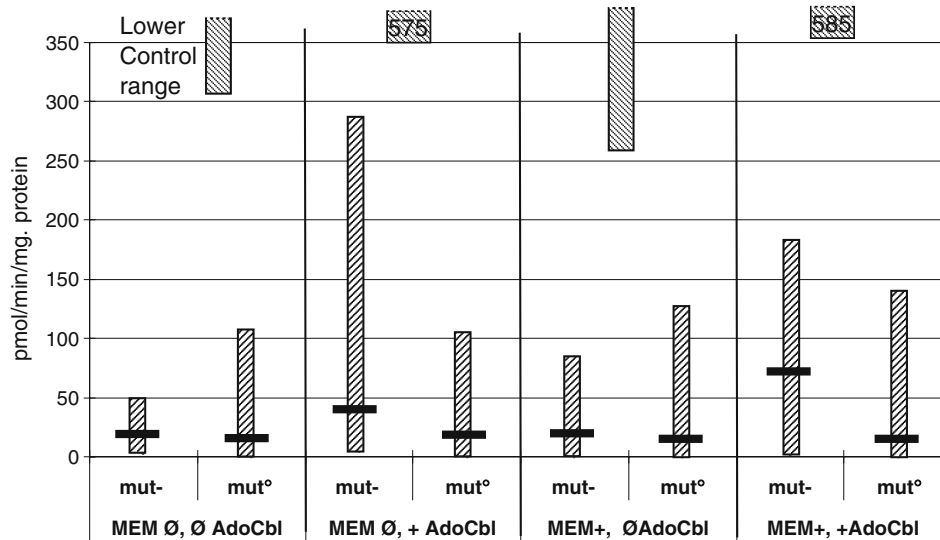
Complementation analysis is performed by measuring the incorporation of [ $^{14}\text{C}$ ]propionate into protein in patient cells mixed with cells of known complementation groups and treated with 40% polyethyleneglycol (PEG) which results in the formation of multinuclear cells (Fig. 6) (Zavadakova et al 2002).

In the investigation of combined MMAuria and homocystinuria the function of methionine synthase



**Fig. 4** Propionate incorporation assay in 66  $\text{mut}^0$  and 17  $\text{mut}^-$  cell lines. Intact fibroblasts in monolayer culture are incubated with [ $^{14}\text{C}$ ]propionate (0.2 mmol/L) in medium containing 15% serum for 16 h at 37°C. Then cells are harvested with trypsin followed by precipitation of protein with 5% trichloroacetic acid. Protein is solubilized in 1 mol/L NaOH. The protein concentration is determined by Lowry and radioactivity is measured by

scintillation counting. Activity is expressed as pmol propionate fixed per mg protein per 16 h. Shaded bars represent the ranges with median values depicted by solid bars. MEM 0 = fibroblasts grown in minimal essential medium without added hydroxocobalamin. MEM+ = fibroblasts grown in medium with 10 mg/L added hydroxocobalamin



**Fig. 5** Methylmalonyl-CoA mutase assay. Fibroblast extract is incubated with [<sup>14</sup>C]MMCoA (4 mmol/L) at pH 7.4, 37°C for 60 min, without (holomutase) and +50 μmol/L AdoCbl (total mutase). Free [<sup>14</sup>C]succinic acid is released by alkaline hydrolysis, unlabelled succinic acid is added as carrier and is separated by anion exchange HPLC. Fractions are collected and counted for radioac-

tivity. Activity is expressed as pmol succinate formed per minute per mg extract protein. Shaded bars represent the ranges, with median values depicted by solid bars. MEMØ=fibroblasts grown in minimal essential medium without added hydroxocobalamin. MEM+=fibroblasts grown in medium with 10 mg/L added hydroxocobalamin

can be determined by measurement of conversion of [<sup>14</sup>C]formate to methionine in intact fibroblasts in monolayer culture (Fowler et al 1997).

The results of these assays, the most important being the determination of propionate incorporation and MMCoA mutase activity in fibroblasts grown in medium with and without supplemented hydroxocobalamin, allow the reliable characterization of patients

with isolated MMAuria and identify the correct gene for mutation analysis (Table 4).

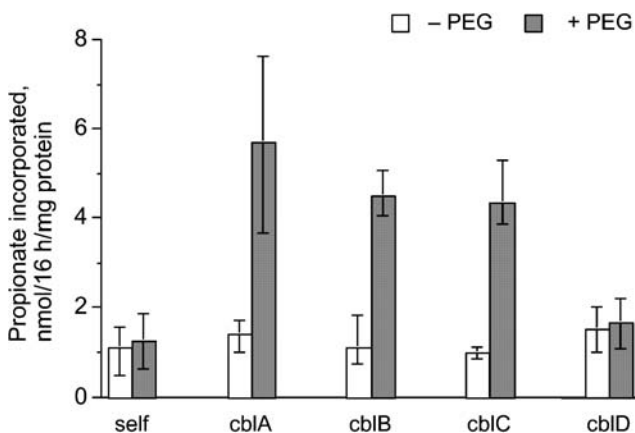
**Mutation analysis**

Many different complementation classes exist and these cannot be distinguished by clinical presentation and/or response to high-dose parenteral vitamin B<sub>12</sub> administration. Enzymatic and/or complementation analysis is necessary before proceeding to mutation analysis of single genes (Fig. 2). Table 2 summarizes present knowledge on genes and mutations relevant to isolated MMAuria.

Approaching 200 mutations of the *MUT* gene have been described in MMCoA mutase deficient patients (Lempp et al 2007; Worgan et al 2006). Most are private, although a few occur in between 10% and 20% of mutant alleles. The p.R694W mutation is associated with the mut<sup>-</sup> form (Lempp et al 2007). Two others have been identified in particular ethnic groups with a relatively high frequency (Worgan et al 2006).

In the *cblA* defect the p.R145X mutation was found to be present in 43% of mutant alleles and was associated with a common haplotype (Lerner-Ellis et al 2004).

The p.R186W mutation of the *MMAB* gene accounted for 33% of mutant alleles in *cblB* patients who were of European origin and presented with a severe clinical phenotype (Lerner-Ellis et al 2006a).



**Fig. 6** Complementation analysis of *cblD*-variant 2 cell line. Fibroblasts of a particular patient were mixed with fibroblasts belonging to known complementation groups, fused with polyethyleneglycol 1500 (PEG) treatment (shaded columns), and 3 days later propionate incorporation was measured. Parallel cultures of mixed unfused cells were used as background controls (unshaded columns). Self-fusions were used as a negative control



**Table 4** Methylmalonic acidurias: findings in fibroblast assays (N = normal)

	Medium OHcbl	Propionate incorporation	MMA-CoA mutase		CN-[ <sup>57</sup> Co]Cbl uptake		
			–AdoCbl	+AdoCbl	Total	AdoCbl	MeCbl
mut <sup>0</sup>	–	↓	±↓	↓	N	N	N
	+	↓	↓	↓	N	N	N
mut <sup>–</sup>	–	↓	±↓	↓	N	±N	N
	+	upto N	↓	±N	N	↓	N
cblA	–	↓	±↓	N	N	↓	N
	+	N	±↓	N	N	↓	N
cblB	–	↓	±↓	N	N	↓	N
	+	↓ or N	↓	N	N	↓	N
cblD-var2	–	↓	±↓	N	N	↓	N
	+	N	↓	N	N	↓	N

Mutations of the recently identified gene responsible for the cblD defect (*MMADHC*) have been described in four subjects with combined MMAuria and homocystinuria and in four subjects with the isolated MMAuria form of this defect (Coelho et al 2008).

At least 44 mutations have been described in the *MMACHC* gene responsible for the cblC defect (Lerner-Ellis et al 2006b, Nogueira et al 2008). The c.271dupA mutation occurred in about 40% of alleles and correlated with early-onset disease. In addition, two nonsense mutations, R111X and R132X, were found in 10–20% of mutant alleles. Interestingly, one of these (R132X) was associated with late-onset disease.

### Miscellaneous disorders with mildly elevated MMA

#### MCoA epimerase deficiency

Mutations of the gene (*MCEE*) coding for this enzyme deficiency have been reported in six patients. The first patient with MMA levels of 142–300 mmol/mol creatinine and increased propionylcarnitine (Bikker et al 2006) was also affected by sepiapterin reductase deficiency with a clinical phenotype reminiscent of that disorder. Dobson and colleagues (2006) reported a child previously thought to have MMA due to the cblA defect who presented at 13.5 months with severe metabolic acidosis requiring intensive care, having previously failed to thrive. MMA and methylcitrate were elevated in urine and plasma MMA was 11.1 μmol/L (normal <0.27). Urine MMA while on a normal protein intake with oral cobalamin was 180–1456 mmol/mol creatinine. Growth and mental development are normal. An older sister showed MMA levels of 95–166 mmol/mol creatinine on a self restricted low-protein intake but has remained asymptomatic.

It is likely that this enzyme deficiency leads to only a partial block in MMCoA catabolism owing to the ability of D-MMCoA to interconvert with L-MMCoA through deacetylation and acetylation of free MMA (Fig. 1). This is reflected in the relatively modest elevation of urine MMA as well as the only partially reduced levels of propionate incorporation into macromolecules in cultured fibroblasts. Grading and colleagues (2007) reported pathogenic mutations in 5 of 229 patients with unexplained MMAuria but there was no consistent clinical phenotype. Three of these five patients had two mutant alleles but propionate incorporation was decreased in only two. The other two subjects were only heterozygous for a single *MCEE* mutation and normal propionate incorporation was found in their cultured fibroblasts.

Thus conclusions on the clinical consequences of MMCoA epimerase deficiency cannot be made.

#### Atypical MMA with neurological symptoms due to *SUCL* mutations

The succinate-CoA ligase (*SUCL*) enzyme complex catalyses the conversion of succinyl-CoA to succinate in the tricarboxylic acid cycle (Fig. 1). Two forms of the complex exist, each comprising a common alpha unit and one of two different beta subunits which impose specificity for either GDP or ADP in the reaction. Mutations of the ADP-forming beta subunit *SUCLA2* are associated with depletion of mitochondrial DNA (Elpeleg et al. 2005) and deficiency of complexes I, III and IV of the respiratory chain. Further reported patients (Carrozzo et al. 2007; Ostergaard et al. 2007a) characterized by Leigh-like encephalomyopathy, dystonia and deafness have been shown to excrete mildly elevated levels of MMA. The mild MMAuria ranged from 16 to 212 mmol/mol cre-

atinine and is accompanied by a range of increased metabolites such as methylcitrate, 3-hydroxypropionate, propionylcarnitine, MMA, methylmalonylcarnitine, succinylcarnitine, lactate, citrate and succinate. Thus MMA is likely to constitute a marker for the disease rather than to be responsible for the phenotype.

Fatal lactic acidosis of neonatal onset with mtDNA depletion was recently described in two patients with mutations in the alpha subunit, *SUCLG1* (Ostergaard et al. 2007b) with mildly increased urinary MMA (68 mmol/mol creatinine) in one of them.

Further cases, some of which have been reported previously (Yano et al. 2003) presented with a milder phenotype including developmental delay, generalized muscular hypotonia and bilateral papillary oedema but without deafness (A. Burlina, C. Dionisi-Vici and R. Wevers, personal communication 2007). One of them showed increased urinary MMA (285 mmol/mol creatinine) and related organic acids and elevated propionylcarnitine and methylmalonylcarnitine (Yano et al 2003; patient 1).

In conclusion, several possible causes of elevated MMA are known (Table 3) and each must be carefully considered in relation to the clinical presentation and nutritional state of the patient. The diagnostic approach and methods described here allow a definitive classification of this heterogeneous group of disorders. It must be emphasized that such investigations need to be performed in laboratories with comprehensive experience. Reliable classification of these patients is essential for ongoing and future prospective studies on treatment and outcome.

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