Published in final edited form as:
Mol Genet Metab. 2005 ; 86(1-2): 34–43. doi:10.1016/j.ymgme.2005.07.020.

Genetic and Genomic Systems to Study Methylmalonic Acidemia

R.J. Chandler and **C. P. Venditti***
Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, M.D. 20892

Abstract

Methylmalonic acidemia (MMAemia) is the biochemical hallmark of a group of genetic metabolic disorders that share a common defect in the ability to convert methylmalonyl-CoA into succinyl-CoA. This disorder is due to either a mutant methylmalonyl-CoA mutase apoenzyme or impaired synthesis of adenosylcobalamin, the cofactor for this enzyme. In this article, we will provide an overview of the pathways disrupted in these disorders, discuss the known metabolic blocks with a particular focus on molecular genetics, and review the use of selected model organisms to study features of methylmalonic acidemia.

Keywords

methylmalonic acidemia; cobalamin deficiency; methylmalonyl-CoA mutase; MMAA; MMAB; *C. elegans*; mouse models; molecular genetics; genomics

Introduction

The clinical foundation for understanding the phenotype of methylmalonic acidemia derives from a series of reports published in the late 1960s [1,2,3]. Oberholtzer et al. described two unrelated patients with methylmalonic acidemia [1]. The first affected individual was initially suspected to have a renal tubular dysfunction syndrome. This patient had frequent attacks of dehydration and acidosis, and perished during a decompensation at age 2 years. On postmortem examination, a fungal pneumonia was noted and a peculiar histopathological appearance of the kidneys was documented. Specifically, the kidneys were shrunken; the tubules were diminutive and had increased interstitial tissue with a lymphocytic infiltration. The second child, a 6 year-old female, displayed a similar phenotype with respect to acid-base instability. Classical analytic chemical methods, such as paper chromatography, melting point analysis, and reactivity with diazotized p-nitroaniline, were used to demonstrate that she produced large amounts of methylmalonic acid in her urine, blood and cerebral spinal fluid (CSF). Of note, she had a CSF: plasma methylmalonic acid (MMA) gradient, with concentrations of MMA equal to 1.55 mM in her plasma and 1.575 mM in her CSF. The authors also recognized that the metabolic acidosis was only partly explained by the plasma MMA levels. A propensity toward ketosis was demonstrated, with an exquisite sensitivity to oral propionic acid. The child had problems with growth and motor skills in the early years, but when assessed at age 5 years, had a normal IQ.

*Corresponding author. Fax: +1 301 402 2170. Email address: venditti@mail.nih.gov (C. Venditti).

The same year Stokke et al. studied the third child born to a family that had two infants perish in the newborn period with overwhelming acidosis and coma [2]. Using a newer method of GC/MS, they demonstrated that the patient produced MMA in enormous amounts. Whole body metabolism was studied in the index case with C^{14}-valine and H^3-MMA. The patient did not respond to parenteral cobalamin but did demonstrate a clinical improvement when treated with a simple hyperalimentation consisting of elemental amino acids and glucose given IV, and fats and carbohydrates administered by nasogastric feeding, prior to perishing from an intercurrent infection. In the next year, a patient with a similar phenotype of intermittent ketoacidosis and severe methylmalonic aciduria was proven to respond to vitamin B12, firmly establishing a role for the vitamin in human intermediary metabolism [3,4]. The early studies on patients with methylmalonic acidemia generated theories to explain the metabolic perturbations seen in these individuals, demonstrated fundamental precursor relationships, described a renal lesion seen in the patients, demonstrated that MMA is likely produced *de novo* or concentrated in the nervous system, showed that the disorder could be treated with precursor restriction and possibly hyperalimentation, proved that the block was located at the methylmalonyl-CoA mutase (MCM, EC 5.4.99.2) step and was co-factor responsive in some patients. These papers provided the foundation of current therapies for methylmalonic acidemia.

Scientific studies of methylmalonic acidemia have provided a paradigm for the importance of human genetics and the investigation of rare disorders as a means to elucidate fundamental aspects of metabolism. Over the past three decades, great progress has been made in understanding and treating this group of disorders. However, the challenges faced by physicians caring for the early patients, such as the propensity toward metabolic decompensation, growth and feeding problems, renal disease and premature death, still exist [5,6,7,8]. The use of model systems to study methylmalonic acidemia may help guide the development and testing of newer therapies for this devastating disorder. In this review, we will update the reader on the molecular genetics of isolated methylmalonic acidemia and highlight the use of model systems to study MMAemia and cobalamin metabolic disorders.

Overview of the Pathway

An overview of the major pathway for propionyl-CoA metabolism into the Krebs cycle is depicted in Figure 1 and the location of the intracellular steps of cobalamin metabolism are displayed in Figure 2. Minor metabolic pathways for propionyl-CoA metabolism exist [9,10], are activated in patients with blocks in the pathway and are likely to be more significant in methylmalonic acidemia than in propionic acidemia [10]. The biotin-dependent propionyl-CoA carboxylase reaction may be the least likely reaction in the sequence to be reversed because of the consumption of ATP by the reaction and the direction of the equilibrium *in vivo* [11]. Methylmalonic acidemia is always associated with secondary perturbations of propionyl-CoA metabolism. These perturbations include elevated propionylcarnitine, propionylglycine, 2-methylcitrate, and increased concentrations of propionate in the blood. Methylmalonic acidemia also shares many features with propionic acidemia, both clinically and metabolically. The source of bicarbonate in the propionyl-CoA fixation reaction is not clearly defined and may represent a way to modulate the regional production of methylmalonic acid in certain body compartments. The propionyl-CoA carboxylase reaction and propionic acidemia will not be discussed in this review.

D-Methylmalonyl-CoA Racemase (MCR) or D-Methylmalonyl-CoA Epimerase

D-methylmalonyl-CoA (D-MMCoA) is formed as a product of the propionyl-CoA carboxylase reaction. D-MMCoA requires racemization prior to becoming a substrate for the methylmalonyl-CoA mutase reaction and a deficiency of D-methylmalonyl-CoA racemase (MCR, EC 5.1.99.1) has long been postulated as a potential etiology of hereditary

methylmalonic acidemia. The one patient suspected of having a defect at this step was later shown to have a lesion at the methylmalonyl-CoA mutase locus [12]. The enzyme has been purified from a number of sources and shown to have enzymatic activity on D-MMCoA. The human MCR homologue had eluded direct characterization until several years ago. Bobik and Rasche pioneered a novel approach to identify this gene [13] that others have also successfully used to find other cobalamin metabolic genes [14,15]. This genomic method relies on the operon principle in that bacteria tend to organize genes involved in a metabolic pathway or specialized enzymatic reaction in the same transcriptional unit. After analyzing the genomic sequences of several species of bacteria, conserved genes near the methylmalonyl-CoA mutase enzyme were noted [13]. The authors hypothesized that one of the genes was a MCR and proved this by demonstrating the MCR gene product catalyzed the conversion of D-MMCoA to L-MMCoA. They also then identified the human homologue of MCR and demonstrated enzymatic activity in an *in vitro* assay. The use of model organisms and genomic principles has been demonstrated to be a powerful method of finding conserved genes involved in methylmalonyl-CoA metabolism.

The exact role MCR may have in human metabolism remains to be defined. One theory has suggested that the enzymatic function of the racemase reaction can be bypassed by a “free” MMA shunt and offers an explanation as to why there have been no patients with methylmalonic acidemia that harbor genetic lesions at this step identified to date [16]. The formal resolution of the role that this enzyme plays in human metabolism may require the identification of patients who harbor mutations in the MCR gene. A linked assay to assess the function of this enzyme has been described [17]. If such patients can be found and are asymptomatic, the mutant alleles they harbor will need to be directly assayed for activity. These individuals will need to be carefully studied and stressed *in vivo* by substrate loading before dismissing the role that this enzyme may have in methylmalonyl-CoA metabolism.

An enzyme deficiency at this step may also be studied in model organisms as an alternative and supporting means of examining the importance of the reaction. The gene has been identified in *C. elegans* and studied in a deletion mutant, which is viable and relatively resistant to oxidative stress [18]. A murine homologue is predicted to exist and construction of a mouse model may also be useful. Alternatively, deficiency of this enzyme may be lethal and not observed in living patients or its function may be redundant. The striking conservation of this enzyme across the phyla suggests it may have an important function, which has yet to be formally studied in man.

D- Methylmalonyl-CoA Hydrolase

The generation of free MMA requires hydrolysis of the methylmalonyl-CoA thioester into free coenzyme A and methylmalonic acid. This reaction has received some study in previous years. Kovachy, Copley and Allen purified the enzyme, D-methylmalonyl coenzyme A hydrolase, which hydrolyzes D-methylmalonyl coenzyme A to free methylmalonic acid and coenzyme A [19]. The kinetic constants for this enzyme were defined experimentally. Several years later, Shimomura et al. purified and characterized 3-hydroxyisobutyryl-coenzyme A hydrolase, an enzyme that acts in the terminal portion of valine metabolism [20]. These authors noted that 3-hydroxyisobutyryl-coenzyme A hydrolase had activity toward D-MMCoA and commented on the similarities in the molecular weight and pH optima for the two enzymes. The gene encoding a specific D-methylmalonyl coenzyme A hydrolase has not been identified in humans. However, if overlapping activities for the 3-hydroxyisobutyryl-coenzyme A hydrolase and D-methylmalonyl coenzyme A hydrolase exist, the former may represent a functional modifier in methylmalonic acidemia.

Bacterial operon-based gene finding and studies on this enzyme in other model organisms may help elucidate the exact role that this protein has in D-methylmalonyl coenzyme A metabolism. Of note, a succinate to propionate conversion pathway has been described in *E. coli* [21].The pathway is encoded in an operon organized as follows: a methylmalonyl-CoA mutase, an MMAA-like protein, a methylmalonyl-CoA decarboxylase and finally a propionyl-CoA:succinyl-CoA transferase protein. This may indicate that bacteria use an alternative metabolic pathway to catabolize the thioester once it is formed. Studies in higher organisms are necessary to determine if such a hydrolase pathway is present in humans.

MMAA

Bobik and Rasche described two genes that clustered with methylmalonyl-CoA mutase in bacterial operons [13]. One was proven to be an MCR; the other was considered to function as an accessory protein needed for the methylmalonyl-CoA mutase enzyme. Dobson et al. used bioinformatics and orthology relationships to independently identify the same latter gene as a candidate for *cblA* deficiency [14]. To prove causation, they characterized this gene, called MMAA, and sequenced the DNA from patients with *cblA* lesions at this locus. Mutations in a number of patients were identified, including premature terminations and non-conservative missense changes. Dobson et al. speculated that the gene product could function as part of a mitochondrial pore, allowing cobalamin to enter into the mitochondria. A mitochondrial leader sequence was suggested by informatic analysis but the gene product has not been formally proven to localize to the mitochondrial inner space or to the mitochondrial membranes. At this point, a role for MMAA has not been defined and there are no direct functional or biochemical assays for this protein. However, a bacterial homologue of MMAA, *meaB*, has been shown to associate with the methylmalonyl-CoA mutase enzyme *in vitro* [22]. These authors have suggested that the function of *meaB*, and by inference MMAA, is to protect the MCM enzyme from inactivation during catalytic cycles. Such a role would certainly be feasible for the human gene product. Determining the function of this gene might be accomplished by a number of biochemical and genetic approaches, such as direct purification, targeted over-expression or ablation in mice. Multiple mutations in various regions of the gene have been identified, which will help guide future structure and function studies.

MMAB

In *cblB* class methylmalonic acidemia the defective gene is MMAB [15]. It encodes an ATP:cob(I)alamin adenosyltransferase of the *pduO* family [23]. It should be noted that multiple enzymatic solutions to this reaction occur in nature [24] and that humans and mice only appear to possess a *pduO*-like family. Two approaches were used to identify the gene defective in *cblB* deficiency. Dobson et al. used informatics to identify the human homologue of the *pduO* gene and then directly sequenced the DNA from patient cell lines of the *cblB* class [15]. Leal et al. performed direct complementation of a bacterial *pduO* mutant with a bovine cDNA expression library, isolated the bovine homologue and then the human gene homologue which they called ATP: cob(I)alamin adenosyltransferase (ATR) [23]. Recently, methionine synthase reductase (MSR), an enzyme thought to have a primary role in the reductive reactivation of methionine synthase, has been demonstrated to localize to the mitochondria and associate with the MMAB gene product *in vitro* [25]. A model for MSR involvement in the ATP:cob(I)alamin adenosyltransferase reaction has been proposed. A bacterial homologue of MMAB has been crystallized and homology considerations have afforded insight into the nature of mutations observed at this locus [24].

Methylmalonyl-CoA Mutase (MCM)

The MCM step has received the most attention of any of the steps in this pathway. The enzyme has been studied extensively in a variety of species [26]. The *P. shermani* MCM has been

crystallized and the structure solved at 2 Angstroms, allowing enzymatic and mutational mechanisms to be inferred in the human condition [27]. The human MCM gene was identified by Ledley et al., who screened an expression library with mutase antibodies to isolate the first human cDNA [28]. Over the last 10 years a number of studies have described the spectrum of mutations observed at the mutase locus in human patients [29,30]. Several assays exist to measure the function of this enzyme, including an automated HPLC assay [31].

The mutase enzyme exists as a homodimer, possibly with unequivalent active sites [32]. The enzyme has several functional domains from N- to C- terminus including: a mitochondrial importation signal, a dimerization domain (inferred from studies with mutant alleles), a coenzyme-A ester binding pocket, a spacer or linker region, and a cobalamin binding motif. Multiple mutational mechanisms operate at the MCM locus including premature stop codons [30,33,34], missense mutations [35,36,37], splice site mutations [38], mRNA stability mutations [33], deletions [30] and isodisomy [39]. Kinetic properties of mutant enzymes historically had been studied using cell lines and tissues from affected patients. Few groups have undertaken direct biochemical characterization of mutant alleles. Crane and Ledley examined selected mutant cDNAs in a yeast over-expression system [36] while others have used bacterial hosts to produce the enzyme [40,41]. The advantage of the yeast system is that the enzyme can be expressed with very high specific activities under standard growth conditions and can be processed and targeted to the mitochondria as it would in a vertebrate [42]. Kinetic analysis can be performed with either approach, although purified enzyme is always the preferred starting material for Km and Vmax determination. Detailed biochemical characterization of mutant alleles at this locus will be important because most patients are allelic compounds. Interallelic interactions between mutant subunits can occur and create an intermediate or mut- deficiency state that might otherwise be unexpected from *de novo* prediction algorithms [40,43]. This phenomenon makes correlations between genotype and phenotype possible for only a subset of missense alleles or when patients are homozygous.

Other Lesions

At least two other genetic entities in the above pathway can be associated with isolated methylmalonic acidemia, *cblD* (variant 2) deficiency [44] and *cblH* deficiency [45]. The biochemical and genetic etiology of these disorders has not yet been determined.

Genetic Systems To Study Methylmalonic Acidemia

Studies on bacterial systems have defined B12 transport, synthetic pathways and enzymatic functions of dependent enzyme systems and have been extensively reviewed in the past [46]. Below, we will focus on uses and potential applications of widely used eukaryotic model organisms.

Yeast

S. cervisiae may be useful to model certain aspects of methylmalonic acidemia and disordered propionate metabolism. Yeast do not possess a methylmalonyl-CoA mutase gene and use a cobalamin-independent methionine synthase. Furthermore, yeast use the methylcitrate cycle to metabolize propionyl-CoA [47]. In this pathway, 2-methylcitrate is formed from propionyl-CoA and oxaloacetate. A dehydration of 2-methylcitrate generates methylisocitrate that is subsequently acted upon by a methylisocitrate lyase to yield succinate and pyruvate. The enzymatic details of these steps have not yet been fully elicudated. The cycle may be viewed as enabling an organism to oxidize propionate to pyruvate. Yeast harboring mutations in the *pdh* gene, a putative methylcitrate dehydratase, are sensitive to exogenous propionate [48]. The exact basis for this sensitivity is not known but may be relevant to the understanding of the targets of propionyl-CoA toxicity in mammalian systems. For example, in the

aforementioned mutant, increased methylcitrate production may be causing the growth defect observed in the *pdh* mutant.

The method of chemogenomic profiling may be employed to study the sensitivity of yeast to propionate and methylmalonate [49,50]. This method should allow the identification of yeast genes that confer heightened sensitivity or resistance to an exogenously added chemical or metabolite such as MMA. Yeast strains harboring gene(s) that confer resistance or sensitivity to MMA should be easily identified by whole genome parallel analysis. These strains might then be studied to determine whether they have mammalian homologues that may be targets of MMA toxicity.

Because yeast do not harbor B12 synthetic or metabolic capabilities, they may be used to reconstitute the mutase pathway in a controlled fashion in the mitochondria. Yeast can produce a functional MCM apoenzyme when the gene is expressed from an inducible promoter [42]. Other than MCM, genes in the pathway have not been assayed for function using yeast overexpression systems to date. Specific mutations may be easier to study in yeast and supramolecular complexes between the methylmalonyl-CoA mutase and other genes, such as MMAA might also be produced, detected and studied. Alternatively, yeast genes suspected of participating in the methylcitrate cycle might be expressed in mutant cell lines derived from patients or knock-out mice in an attempt to “engineer” a new propionate pathway in a different organism. Metabolic pathway re-engineering has been used with success in other disorders [51], such as PKU, and may represent a novel therapeutic approach for patients with propionate spectrum metabolic disorders.

C. elegans

C. elegans is an attractive model to use because of easy maintenance, a short life span, extensive biological characterization, and the ability to easily knock genes down by RNA interference (RNAi). In addition, there are numerous research tools available for *C. elegans* including a centralized, comprehensive database (www.wormbase.org) that has not only sequence information, but a wide spectrum of genomic and genetic data, such as RNAi experiments, microarray data and global expression clustering, mutants and mutant phenotypes for each gene and large scale two-hybrid data sets [52]. Whole genome RNAi experiments have been performed on wild-type and sensitized backgrounds, a centralized mutant stock center exists, and an effort to create deletion mutants of every gene is underway.

We have performed an analysis of the *C. elegans* genome to identify putative homologues of genes involved in propionate and cobalamin metabolism. Highly conserved homologues of all the genes known to be involved in the conversion of propionate to succinate - PCCA, PCCB, MCR, MCM, MMAA and MMAB (Table 1) - are predicted to exist, as well as homologues of methionine synthase reductase and methionine synthase. Each potential *C. elegans* homologue has a protein-to-protein BLAST score of less than e-35 and a reverse BLAST analysis indicates the existence of only one homologue of each gene in the human database [53]. The existence of such highly conserved homologues in *C. elegans* is strong evidence that this primitive animal possesses the same pathway for the conversion of propionate to succinate as is found in humans. Furthermore, the existence and functional demonstration of MCR enzymatic activity in *C. elegans* supports this hypothesis [18].

Functional verification of the pathway was assessed after substrate loading wild-type animals with 10 mM propionic acid. The nematodes were removed by centrifugation and the supernatant was analyzed by GC/MS. After propionate loading, the worms showed a 15-fold increase in methylmalonate production from baseline [54]. This increase in MMA production was not observed in negative controls consisting of the bacterial feeding strain incubated with 10 mM propionate; demonstrating the increase in MMA was not due to the bacterial feeding

strains. RNAi directed against PCCA, PCCB, MCR, MCM, MMAA and MMAB produce no visibly detectable phenotype. Typically, RNAi experiments in *C. elegans* are measured by the appearance of visually observable phenotypes such as egg laying defects or lack of motor coordination. Given the severe phenotype that these mutations cause in humans, the lack of a visual mutant phenotype is surprising. The absence of a phenotype may be explained by the nematodes ability to expel the methylmalonate into its surrounding environment, either by diffusion or secretion, or by low-penetrance of the RNAi-dependent phenotype. Substrate loading after RNAi against known and suspected genes involved in propionate metabolism, followed by metabolite analysis, may allow for rapid characterization of gene(s) suspected to play a role in cobalamin utilization. *C. elegans* appears to possess a MCM and a cobalamin-dependent methionine synthase as well as all other known cobalamin metabolic enzymes, such as MSR, MMAA and MMAB. Therefore homologues of the genes mutated in *cbl* deficiency syndromes, such as *cblC*, *cblD* (variant 1 and variant 2), *cblH* and *cblF*, may be present in *C. elegans* and may cause a chemical phenotype when knocked down or out in wild type animals. *C. elegans* may be a valuable tool in determining the underlying genetic defect in these disorders. Finally, suspected enhancers and suppressors of the pathway may be studied in a similar manner. Community-wide efforts are currently underway to create knock-outs of all the genes in the *C. elegans* genome, which should facilitate the use of this versatile animal in the study of MMA as well as other human inborn errors of metabolism where homologues are known or suspected to exist.

Murine

Two mouse models of *mut*-class methylmalonic acidemia have been created [54,55]. The phenotype of the affected animals mirrors the most extreme end of the human syndrome, with 100% fatality in the affected animals by 24–36 hours of life. The animals perish with massive elevations of MMA in the urine, plasma and tissues. The mice prove that the pathway of propionyl-CoA metabolism operates in a manner similar to humans. The existence of a functionally equivalent pathway had been previously suspected based on homology between the mouse and human genes and the observation that the mouse gene could correct the propionate incorporation defect seen in human cells by transfection [56].

The mouse models have afforded a description of some aspects of disordered metabolism seen in methylmalonic acidemia. For example, the animals do not develop severe ketonuria as assessed by dipstick analysis [55]. Immediately after birth, the animals have significant methylmalonic aciduria suggesting that MMA accumulates prenatally. When measured on embryonic day 16, the *mut*- mice have plasma MMA concentrations that exceed 100 uM [54]. These metabolite levels argue against the theory that the placenta provides complete protection (detoxification) from MMA *in utero*. The MMA tissue content has been followed during the life of the *mut*- miceand suggests that a substantial amount of MMA may derive from skeletal muscle as well as the liver, kidney and brain [54]. The production of MMA by the muscle may provide one possible explanation of why liver transplantation and combined liver-kidney transplantations does not completely alleviate aberrant metabolite production in this disorder.

The murine models created to date have disease-related phenotypes but are too severe to study the long-term effects of methylmalonic acidemia. Over-expression of a well characterized mutant or synthetic MCM allele via a transgenic construct, either as a BAC or transgene driven by a heterologous promoter, may rescue the lethal phenotype. Alternatively, transgenic rescue with a wild-type gene under the control of an inducible or a tissue-specific promoter may prove useful in creating a conditional-on model to study the effects of MMA on certain organs. For instance, it may be possible to simulate a liver transplantation by transgenic rescue using a liver specific promoter regulating MCM production or assessing the metabolic capacity of the

skeletal muscle to restore homeostasis by driving gene expression only in that location. Conditional–off alleles may also be useful to examine organ specific contributions to metabolism. Finally, the knocking-in of selected human mutation(s) into the MCM locus, such as those that participate in interallelic complementation or mutations that have predominately cobalamin Km effects, may allow for a versatile model of a partial deficiency state to be developed. The current models may be useful for proof of principle gene and stem cell therapy experiments and possibly, *in utero* correction.

Human

Studies on methylmalonic acidemia began with clinical investigations and there still is a great deal to learn about the disorders by continued study of the existing patient populations. Before new and novel therapies are considered, careful clinical characterization of the patients with methylmalonic acidemia, particularly the adult and transplanted individuals, should be pursued. Varied regimens to treat the condition have been employed, including renal, hepato - and combined hepato-renal transplantation [57]. These therapies may be viewed as precursors to cell based therapies and patients who have undergone transplantation should be carefully studied prior to and after the procedures. Others have advocated that a registry of such patients be created so longer-term outcomes and complications can be described [58]. Liver and combined liver/kidney transplantation can completely eliminate the inherent tendency toward ketoacidotic crises but the patients are not cured of the biochemical phenotype and can still exhibit progression of the disease after transplant. For example, one patient who received a liver transplant subsequently developed renal failure requiring transplantation [59] and another had a metabolic stroke 5 years after transplantation [60]. Transplant patients may enable the study of organ specific contributions to whole body MMA metabolism. A formal consideration might also be given to the potential of damaging a transplanted kidney in the setting of liver-kidney and kidney transplants due to the magnitude of the MMA elevations. Long-term outcome studies on the patients will be required to determine the significance of these metabolic perturbations. The renal disease of methylmalonic acidemia and the propensity of the patients to develop metabolic strokes also require further investigation and may benefit from animal models that replicate the *mut*- state.

As mentioned above, precise correlations between genotype and enzymatic activity have yet to be undertaken for most mutations seen in isolated methylmalonic acidemia. This activity will be an important undertaking in an era of tandem mass spectrometry based newborn screening that promises to identify patients with MMA by virtue of increased propionylcarnitine in the newborn blood spot. Continued studies on patients will be of utmost importance in making such correlations and organized efforts to form registries will be needed to assist these endeavors. Human genetic studies may also aid in the localization and identification of genes involved in the known cobalamin disorders, particularly *cblC*, *cblD* (and variants), *cblF* and *cblH*, as well as other syndromes that can feature elevated MMA levels, such as the combined malonic-methylmalonic acidemia syndrome [61], and transient methylmalonic aciduria [62].

Other Eukaryotic Model Systems

As a greater diversity of genomes becomes sequenced, so will the opportunities to study cobalamin pathways and enzymes in other species. Database analysis indicates that Zebrafish, Fugu, Canis, Bos Taurus, Swine, Great Apes, Gallus, and Rattus appear to harbor a functional methylmalonyl-CoA mutase. Any of these organisms may provide a natural model of MMA or in the future, be used to develop one.

References

1. Oberholzer VG, Levin B, Burgess EA, Young WF. Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. Arch Dis Child 1967;42:492–504. [PubMed: 6061291]
2. Stokke O, Eldjarn L, Norum KR, Steen-Johnsen J, Halovorsen S. Methylmalonic acidemia: A newborn error of metabolism which may cause fatal acidosis in the neonatal period. Scand J Clin Lab Invest 1967;20:313–328.
3. Rosenberg LE, Lilljeqvist AC, Hsia YE. Methylmalonic aciduria. An inborn error leading to metabolic acidosis, long-chain ketonuria and intermittent hyperglycinemia. N Engl J Med 1968;278:1319–1322. [PubMed: 5648598]
4. Rosenberg LE, Lilljeqvist A, Hsia YE. Methylmalonic aciduria: metabolic block localization and vitamin B 12 dependency. Science 1968;162:805–807. [PubMed: 5686220]
5. Matsui SM, Mahoney MJ, Rosenberg LE. The natural history of the inherited methylmalonic acidemias. N Engl J Med 1983;308:857–61. [PubMed: 6132336]
6. van der Meer SB, Poggi F, Spada M, Bonnefont JP, Ogier H, Hubert P, Depondt E, Rapoport D, Rabier D, Charpentier C, et al. Clinical outcome of long-term management of patients with vitamin B12-unresponsive methylmalonic acidemia. J Pediatr 1994;125:903–908. [PubMed: 7996362]
7. Baumgarter ER, Viardot C. Long-term follow-up of 77 patients with isolated methylmalonic acidaemia. J Inherit Metab Dis 1995;18:138–142. [PubMed: 7564229]
8. Nicolaides P, Leonard J, Surtees R. Neurological outcome of methylmalonic acidaemia. Arch Dis Child 1998;78:508–512. [PubMed: 9713004]
9. Leonard JV. Stable isotope studies in propionic and methylmalonic acidaemia. Eur J Pediatr 1997;156 (Suppl 1):S67–S69. [PubMed: 9266219]
10. Barshop BA, Yoshida I, Ajami A, Sweetman L, Wolff JA, Sweetman FR, Prodanos C, Smith M, Nyhan WL. Metabolism of 1–13C-propionate in vivo in patients with disorders of propionate metabolism. Pediatr Res 1991;30:15–22. [PubMed: 1909779]
11. Reszko AE, Kasumov T, Pierce BA, David F, Hoppel CL, Stanley WC, Des Rosiers C, Brunengraber H. Assessing the reversibility of the anaplerotic reactions of the propionyl-CoA pathway in heart and liver. J Biol Chem 2003;278:34959–34965. [PubMed: 12824185]
12. Kang ES, Snodgrass PJ, Gerald PS. Methylmalonyl coenzyme A racemase defect: another cause of methylmalonic aciduria. Pediatr Res 1972;6:875–879. [PubMed: 4643536]
13. Bobik TA, Rasche ME. Identification of the human methylmalonyl-CoA racemase gene based on the analysis of prokaryotic gene arrangements. Implications for decoding the human genome. J Biol Chem 2001;276:37194–37198. [PubMed: 11481338]
14. Dobson CM, Wai T, Leclerc D, Wilson A, Wu X, Dore C, Hudson T, Rosenblatt DS, Gravel RA. 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 U S A 2002;99:15554–15559. [PubMed: 12438653]
15. Dobson CM, Wai T, Leclerc D, Kadir H, Narang M, Lerner-Ellis JP, Hudson TJ, Rosenblatt DS, Gravel RA. Identification of the gene responsible for the cblB complementation group of vitamin B12-dependent methylmalonic aciduria. Hum Mol Genet 2002;11:3361–3369. [PubMed: 12471062]
16. Montgomery JA, Mamer OA, Scriver CR. Metabolism of methylmalonic acid in rats. Is methylmalonyl-coenzyme a racemase deficiency symptomatic in man? J Clin Invest 1983;72:1937–1947. [PubMed: 6643681]
17. Bobik TA, Rasche ME. HPLC assay for methylmalonyl-CoA epimerase. Anal Bioanal Chem 2003;375:344–349. [PubMed: 12589497]
18. Kuhnl J, Bobik T, Procter JB, Burmeister C, Hoppner J, Wilde I, Luersen K, Torda AE, Walter RD, Liebau E. Functional analysis of the methylmalonyl-CoA epimerase from Caenorhabditis elegans. Febs J 2005;272:1465–1477. [PubMed: 15752362]
19. Kovachy RJ, Copley SD, Allen RH. Recognition, isolation, and characterization of rat liver D-methylmalonyl coenzyme A hydrolase. J Biol Chem 1983;258:11415–11421. [PubMed: 6885824]

20. Shimomura Y, Murakami T, Fujitsuka N, Nakai N, Sato Y, Sugiyama S, Shimomura N, Irwin J, Hawes JW, Harris RA. Purification and partial characterization of 3-hydroxyisobutyryl-coenzyme A hydrolase of rat liver. J Biol Chem 1994;269:14248–14253. [PubMed: 8188708]
21. Haller T, Buckel T, Retey J, Gerlt JA. Discovering new enzymes and metabolic pathways: conversion of succinate to propionate by Escherichia coli. Biochemistry 2000;39:4622–4629. [PubMed: 10769117]
22. Korotkova N, Lidstrom ME. MeaB is a component of the methylmalonyl-CoA mutase complex required for protection of the enzyme from inactivation. J Biol Chem 2004;279:13652–13658. [PubMed: 14734568]
23. Leal NA, Park SD, Kima PE, Bobik TA. Identification of the human and bovine ATP:Cob(I)alamin adenosyltransferase cDNAs based on complementation of a bacterial mutant. J Biol Chem 2003;278:9227–9234. [PubMed: 12514191]
24. Saridakis V, Yakunin A, Xu X, Anandakumar P, Pennycooke M, Gu J, Cheung F, Lew JM, Sanishvili R, Joachimiak A, Arrowsmith CH, Christendat D, Edwards AM. The structural basis for methylmalonic aciduria. The crystal structure of archaeal ATP:cobalamin adenosyltransferase. J Biol Chem 2004;279:23646–23653. [PubMed: 15044458]
25. Leal NA, Olteanu H, Banerjee R, Bobik TA. Human ATP:Cob(I)alamin adenosyltransferase and its interaction with methionine synthase reductase. J Biol Chem 2004;279:47536–47542. [PubMed: 15347655]
26. Banerjee R, Ragsdale SW. The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. Annu Rev Biochem 2003;72:209–247. [PubMed: 14527323]
27. Mancia F, Keep NH, Nakagawa A, Leadlay PF, McSweeney S, Rasmussen B, Bosecke P, Diat O, Evans PR. How coenzyme B12 radicals are generated: the crystal structure of methylmalonyl-coenzyme A mutase at 2 A resolution. Structure 1996;4:339–350. [PubMed: 8805541]
28. Ledley FD, Lumetta M, Nguyen PN, Kolhouse JF, Allen RH. Molecular cloning of L-methylmalonyl-CoA mutase: gene transfer and analysis of mut cell lines. Proc Natl Acad Sci U S A 1988;85:3518–3521. [PubMed: 2453061]
29. Ledley FD, Rosenblatt DS. Mutations in mut methylmalonic acidemia: clinical and enzymatic correlations. Hum Mutat 1997;9:1–6. [PubMed: 8990001]
30. Acquaviva C, Benoist JF, Pereira S, Callebaut I, Koskas T, Porquet D, Elion J. 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. [PubMed: 15643616]
31. Riedel B, Ueland PM, Svardal AM. Fully automated assay for cobalamin-dependent methylmalonyl CoA mutase. Clin Chem 1995;41:1164–1170. [PubMed: 7628092]
32. Willard HF, Rosenberg LE. Interactions of methylmalonyl CoA mutase from normal human fibroblasts with adenosylcobalamin and methylmalonyl CoA: evidence for non-equivalent active sites. Arch Biochem Biophys 1980;200:130–139. [PubMed: 6102452]
33. Ogasawara M, Matsubara Y, Mikami H, Narisawa K. Identification of two novel mutations in the methylmalonyl-CoA mutase gene with decreased levels of mutant mRNA in methylmalonic acidemia. Hum Mol Genet 1994;3:867–872. [PubMed: 7951229]
34. Ledley FD, Jansen R, Nham SU, Fenton WA, Rosenberg LE. Mutation eliminating mitochondrial leader sequence of methylmalonyl-CoA mutase causes muto methylmalonic acidemia. Proc Natl Acad Sci U S A 1990;87:3147–3150. [PubMed: 1970180]
35. Crane AM, Jansen R, Andrews ER, Ledley FD. Cloning and expression of a mutant methylmalonyl coenzyme A mutase with altered cobalamin affinity that causes mut- methylmalonic aciduria. J Clin Invest 1992;89:385–391. [PubMed: 1346616]
36. Crane AM, Ledley FD. Clustering of mutations in methylmalonyl CoA mutase associated with mut- methylmalonic acidemia. Am J Hum Genet 1994;55:42–50. [PubMed: 7912889]
37. Acquaviva C, Benoist JF, Callebaut I, Guffon N, Ogier de Baulny H, Touati G, Aydin A, Porquet D, Elion J. N219Y, a new frequent mutation among mut(degree) forms of methylmalonic acidemia in Caucasian patients. Eur J Hum Genet 2001;9:577–582. [PubMed: 11528502]

38. Berger I, Shaag A, Anikster Y, Baumgartner ER, Bar-Meir M, Joseph A, Elpeleg ON. Mutation analysis of the MCM gene in Israeli patients with mut(0) disease. Mol Genet Metab 2001;73:107–110. [PubMed: 11350191]
39. Abramowicz MJ, Andrien M, Dupont E, Dorchy H, Parma J, Duprez L, Ledley FD, Courtens W, Vamos E. Isodisomy of chromosome 6 in a newborn with methylmalonic acidemia and agenesis of pancreatic beta cells causing diabetes mellitus. J Clin Invest 1994;94:418–421. [PubMed: 7913714]
40. Janata J, Kogekar N, Fenton WA. Expression and kinetic characterization of methylmalonyl-CoA mutase from patients with the mut- phenotype: evidence for naturally occurring interallelic complementation. Hum Mol Genet 1997;6:1457–1464. [PubMed: 9285782]
41. Champattanachai V, Ketudat Cairns JR, Shotelersuk V, Keeratichamroen S, Sawangareetrakul P, Srisomsap C, Kaewpaluek V, Svasti J. Novel mutations in a Thai patient with methylmalonic acidemia. Mol Genet Metab 2003;79:300–302. [PubMed: 12948746]
42. Andrews E, Jansen R, Crane AM, Cholin S, McDonnell D, Ledley FD. Expression of recombinant human methylmalonyl-CoA mutase: in primary mut fibroblasts and Saccharomyces cerevisiae. Biochem Med Metab Biol 1993;50:135–144. [PubMed: 7903149]
43. Qureshi AA, Crane AM, Matiaszuk NV, Rezvani I, Ledley FD, Rosenblatt DS. Cloning and expression of mutations demonstrating intragenic complementation in mut0 methylmalonic aciduria. J Clin Invest 1994;93:1812–1819. [PubMed: 7909321]
44. Suormala T, Baumgartner MR, Coelho D, Zavadakova P, Kozich V, Koch HG, Berghauser M, Wraith JE, Burlina A, Sewell A, Herwig J, Fowler B. The cblD defect causes either isolated or combined deficiency of methylcobalamin and adenosylcobalamin synthesis. J Biol Chem 2004;279:42742–42749. [PubMed: 15292234]
45. Watkins D, Matiaszuk N, Rosenblatt DS. Complementation studies in the cblA class of inborn error of cobalamin metabolism: evidence for interallelic complementation and for a new complementation class (cblH). J Med Genet 2000;37:510–513. [PubMed: 10882753]
46. Roth JR, Lawrence JG, Bobik TA. Cobalamin (coenzyme B12): synthesis and biological significance. Annu Rev Microbiol 1996;50:137–181. [PubMed: 8905078]
47. Pronk JT, van der Linden-Beuman A, Verduyn C, Scheffers WA, van Dijken JP. Propionate metabolism in Saccharomyces cerevisiae: implications for the metabolon hypothesis. Microbiology 1994;140(Pt 4):717–722. [PubMed: 7912143]
48. Epstein CB, Waddle JA, Hale Wt, Dave V, Thornton J, Macatee TL, Garner HR, Butow RA. Genome-wide responses to mitochondrial dysfunction. Mol Biol Cell 2001;12:297–308. [PubMed: 11179416]
49. Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, Astromoff A, Davis RW. Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat Genet 1999;21:278–283. [PubMed: 10080179]
50. Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, Jaramillo DF, Chu AM, Jordan MI, Arkin AP, Davis RW. Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc Natl Acad Sci U S A 2004;101:793–798. [PubMed: 14718668]
51. Sarkissian CN, Shao Z, Blain F, Peevers R, Su H, Heft R, Chang TM, Scriver CR. A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. Proc Natl Acad Sci U S A 1999;96:2339–2344. [PubMed: 10051643]
52. Harris TW, Chen N, Cunningham F, Tello-Ruiz M, Antoshechkin I, Bastiani C, Bieri T, Blasiar D, Bradnam K, Chan J, Chen CK, Chen WJ, Davis P, Kenny E, Kishore R, Lawson D, Lee R, Muller HM, Nakamura C, Ozersky P, Petcherski A, Rogers A, Sabo A, Schwarz EM, Van Auken K, Wang Q, Durbin R, Spieth J, Sternberg PW, Stein LD. WormBase: a multi-species resource for nematode biology and genomics. Nucleic Acids Res 2004;32:D411–417. [PubMed: 14681445]
53. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–3402. [PubMed: 9254694]
54. Venditti, C.; Chandler, R.; Tsai, M.; Werhrli, N.; Deering, R.; Kaestner, K.; Giaever, G.; Kazazian, H. Genetic and Genomic Systems to Study Methylmalonic Acidemia (MMA). The American Society of Human Genetics, 54th Annual Meeting; Toronto, Canada. 2004. p. 337

55. Peters H, Nefedov M, Sarsero J, Pitt J, Fowler KJ, Gazeas S, Kahler SG, Ioannou PA. A knock-out mouse model for methylmalonic aciduria resulting in neonatal lethality. J Biol Chem 2003;278:52909–52913. [PubMed: 14555645]

56. Wilkemeyer MF, Crane AM, Ledley FD. Primary structure and activity of mouse methylmalonyl-CoA mutase. Biochem J 1990;271:449–455. [PubMed: 1978672]

57. van 't Hoff WG, Dixon M, Taylor J, Mistry P, Rolles K, Rees L, Leonard JV. Combined liver-kidney transplantation in methylmalonic acidemia. J Pediatr 1998;132:1043–1044. [PubMed: 9627602]

58. Leonard JV, Walter JH, McKiernan PJ. The management of organic acidaemias: the role of transplantation. J Inherit Metab Dis 2001;24:309–311. [PubMed: 11405351]

59. Nyhan WL, Gargus JJ, Boyle K, Selby R, Koch R. Progressive neurologic disability in methylmalonic acidemia despite transplantation of the liver. Eur J Pediatr 2002;161:377–379. [PubMed: 12111189]

60. Chakrapani A, Sivakumar P, McKiernan PJ, Leonard JV. Metabolic stroke in methylmalonic acidemia five years after liver transplantation. J Pediatr 2002;140:261–263. [PubMed: 11865284]

61. Gregg AR, Warman AW, Thorburn DR, O'Brien WE. Combined malonic and methylmalonic aciduria with normal malonyl-coenzyme A decarboxylase activity: a case supporting multiple aetiologies. J Inherit Metab Dis 1998;21:382–390. [PubMed: 9700595]

62. Sniderman LC, Lambert M, Giguere R, Auray-Blais C, Lemieux B, Laframboise R, Rosenblatt DS, Treacy EP. Outcome of individuals with low-moderate methylmalonic aciduria detected through a neonatal screening program. J Pediatr 1999;134:675–680. [PubMed: 10356133]

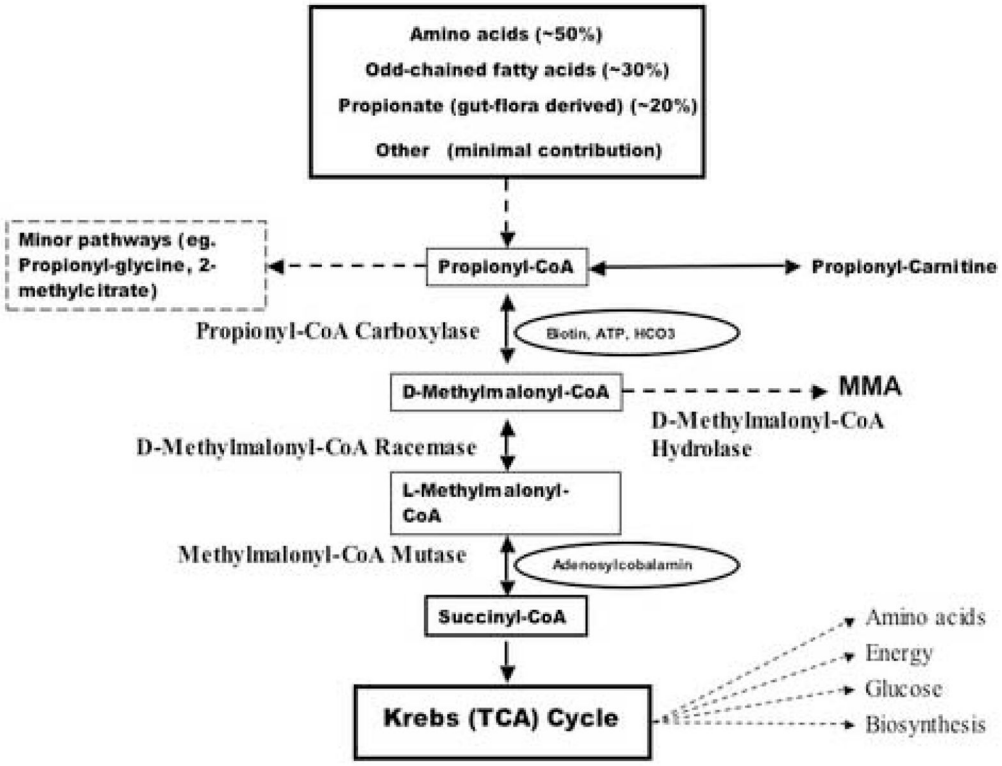

Figure 1.

Major pathway of the conversion of propionyl-CoA into succinyl-CoA. The precursors are indicated with their approximated contribution to whole body propionate metabolism. The biotin-dependent enzyme, propionyl-CoA carboxylase, converts propionyl-CoA into D-methylmalonyl-CoA that is then racemized into L-methylmalonyl-CoA and isomerized into succinyl-CoA, a Krebs cycle intermediate. The location of the D-methylmalonyl-CoA hydrolase is indicated as are minor pathways of propionyl-CoA metabolism, such as the glycine conjugation pathway and 2-methylcitrate formation. The L-methylmalonyl-CoA mutase reaction requires adenosylcobalamin, an activated form of vitamin B12. The formation of adenosylcobalamin requires intracellular metabolism as outlined in the next figure.

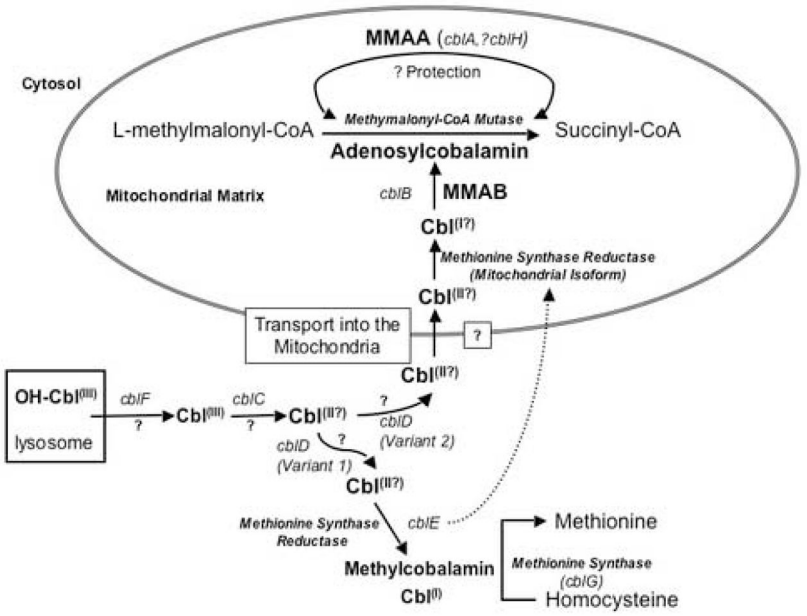

Figure 2.

Pathway of cellular processing of cobalamin (OH-Cbl). The class and genes associated with isolated methylmalonic acidemia are *cblA* (MMAA), *cblB* (MMAB), *cblD variant 2 (unknown gene), cblH* (*unknown gene*) and MCM. Question marks indicate unknown genes or poorly defined cellular processes. The position of the MMAA gene product reflects the probable role that this protein has in the protection of MCM [22]. A mitochondrial isoform of the methionine synthase reductase gene may protect or interact with the product of the MMAB gene [25] and is shown with a dashed line.

Table 1

Predicted Propionate and Cobalamin Homologues in *C. elegans*. Blast-P values are of the predicted C. Elegans homologues vs human proteins. The *C. elegans* MCRacemase/Epimerase has been expressed in *E. coli* and the enzymatic activity has been verified [18]

Human Gene	C. elegans Gene	C. elegans Protein	Blastp e-value	% Similarity
Propionyl-CoA carboxylase alpha subunit (PCCA)	F27D9.5	WP:CE04451	5.2e-215	94.6
Propionyl-CoA carboxylase beta subunit (PCCB)	F52E4.1	WP:CE07269	1.4e-212	95.3
Methylmalonyl-CoA Epimerase (MCR)	D2030.5	WP:CE09082	2.3e-45	79.6
Methylmalonyl-CoA Mutase (MCM)	ZK1058.1	WP:CE30404	7.29e-280	97.8
Methylmalonic aciduria Type A (MMAA)	T02G5.13	WP:CE31822	1.2e-89	82.5
Methylmalonic aciduria Type A (MMAB)	C26E6.11	WP:CE01159	5.7e-35	53.7
Methionine Synthase	R03D7.1	WP:CE0109	0	99.8
Methionine Synthase Reductase (MSR)	R13A5.9	WP:CE2711	9.2e-76	90.2