Severe Neonatal Metabolic Decompensation in Methylmalonic Acidemia Caused by CblD Defect

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Abstract CblD disorder is an autosomal recessive, rare, heterogeneous disease with variable clinical presentations, depending on the nature and location of the *MMADHC* gene mutations. Mutations in *MMADHC* lead to three distinct phenotypes: cblD-MMA, cblD-HC, and cblD-MMA/HC. To date, 18 cblD patients have been reported. Six of them were affected by cblD-MMA, but only three had a known clinical history. One of these patients presented with a metabolic decompensation at 11 months; the second one, born prematurely, was diagnosed with cblD after being treated for intracranial hemorrhage, respiratory distress syndrome, necrotizing enterocolitis, and convulsions at birth; the third one was diagnosed at 5 years of age.

Here we present a case of a cblD-MMA patient who had an acute neonatal onset with severe hyperammonemia requiring hemodiafiltration. To the best of our knowledge, this is the first cblD-MMA patient who presented acutely in

the newborn period. He has developed well upon treatment with B₁₂, carnitine, and hypoproteic diet. At present time, at the age of 7, he shows normal growth and cognitive development. Thus, it is likely that the aggressive treatment of this child with hemodiafiltration might have prevented him from long-term neurological sequelae. Overall, this case shows that even severe, neonatal-onset patients may display a vitamin B₁₂-responsive MMA. Furthermore, it suggests that an early treatment with vitamins might be beneficial for patients presenting with neonatal-onset hyperammonemia regardless of the suspected disease and before receiving the biochemical diagnosis.

Abbreviations

Ado-Cbl	Adenosylcobalamin
Cbl	Cobalamin
HC	Homocystinuria
Met-Cbl	Methylcobalamin
MLS	Mitochondrial leader sequence
MMA	Methylmalonic aciduria
MMADHC	Methylmalonic aciduria cblD type with homocystinuria gene

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Introduction

CblD disorder (OMIM 277410) is one of the seven known defects of the intracellular cobalamin (Cbl) metabolism which serves for the production of two coenzymes through two different pathways: one leading to the synthesis of adenosylcobalamin (Ado-Cbl), coenzyme of the mitochondrial methylmalonyl-CoA mutase (EC 5.4.99.2), and the other to the synthesis of methylcobalamin (Met-Cbl), a coenzyme of the cytosolic methionine synthase

(EC2.1.1.13). Only the initial steps are common to both pathways (Watkins and Rosenblatt 2011). Patients with defects in the common pathway (cblF, cblC, and cblD-MMA/HC) have methylmalonic aciduria (MMA) and homocystinuria (HC) and most frequently present with neurological and hematological problems. Patients with an aberrant Ado-Cbl pathway (cblA, cblB, and cblD-MMA) are usually characterized by methylmalonic aciduria (MMA), hyperammonemia, ketosis, and metabolic acidosis. Those ones characterized by a defective Met-Cbl pathway (cblD-HC, cblE, cblG) have only isolated homocystinuria and mainly show neurological symptoms and megaloblastic anemia (Watkins and Rosenblatt 2011).

CblD is a unique, apparently very rare, genetic condition due to mutations of a protein with two distinct functional domains, which interact with either cytosolic or mitochondrial targets (Stucki et al. 2012). Mutations of this gene, named *MMADHC* and identified in 2008 by Coelho et al. (2008), may lead to three different phenotypes on the basis of the nature and location of the mutation: those affecting the N-terminal domain of the protein result in cblD-MMA, while those affecting the C-terminal domain of the protein cause cblD-HC. Furthermore, mutations decreasing the level of the protein result in cblD-MMA/HC (Coelho et al. 2008; Miousse et al. 2009). Recently, Stucki et al. (2012) have confirmed that a single protein does exist and interacts with both cytosolic and mitochondrial substrates. Moreover, the same authors have shown that there is a delicate balance between Met-Cbl and Ado-Cbl synthesis depending on the relative strength of the mitochondrial leader sequence (MLS). In this regard, the first 61 amino acids of the protein are required to target the cblD protein to the mitochondria (MLS), whereas the synthesis of Ado-Cbl requires an intact sequence downstream from Met62, and synthesis of Met-Cbl needs only an intact sequence downstream from Met116.

Here, we report, for the first time, the case of a cblD-MMA patient who had a severe acute onset in the neonatal period and was then followed up for 7 years with a favorable evolution.

Case Report

The patient is a male, born at term (39 + 5 gestational age) after a normal pregnancy and delivery. He is the second child of healthy, first-degree cousin parents from Indian Punjab. Their first child, a girl, is healthy. Amniotic fluid at birth was meconium stained. Birth weight was 3,250 g and Apgar score 7–10.

At 4 days of life, the clinical examination revealed hypotonia, hyporeactivity, polypnea, and weight loss >10% (2,800 g). Blood tests showed metabolic acidosis (pH 7.32,

BE-14) and a positive C-reactive protein. In the suspicion of sepsis, intravenous treatment with ampicillin, gentamicin, ceftazidime, and bicarbonates was started. Nevertheless, his clinical conditions worsened and at 6 days he had severe respiratory distress and progressive neurological involvement with hypo-/hypertonia, myoclonic jerks, and coma. Blood tests showed severe hyperammonemia (1190 $\mu\text{mol/L}$), hyperlactacidemia (6 mmol/L), pH 7.35, BE-8, and ketonuria (Ketostix ++++). An intoxication type metabolic disease was suspected and immediately an intravenous treatment with glucolipidic calories (100 Kcal/kg) was started in combination with a loading IV dose of arginine 250 mg/kg and sodium benzoate 250 mg/kg in 2 h, followed by a maintenance of 250 mg/kg/24 h of both, associated to IV administered carnitine 100 mg/kg/day and hydroxocobalamin 1 mg/day. Hemodiafiltration was started 6 h later when plasma ammonia increased to 1348 $\mu\text{mol/L}$. After 24 h, ammonia was normalized (53 $\mu\text{mol/L}$). Specific biochemical tests were performed before hemodiafiltration during the 6 h of IV treatment and their results were received after 24 h. The plasma aminoacidogram showed depletion of threonine (54 $\mu\text{mol/L}$; ref.range 141–213), isoleucine (8 $\mu\text{mol/L}$; ref.range 31–47), phenylalanine (28 $\mu\text{mol/L}$; ref.range 45–65) and alanine (135 $\mu\text{mol/L}$; ref.range 239–345); glycine (205 $\mu\text{mol/L}$; ref.range 178–248), and glutamine (283 $\mu\text{mol/L}$; ref.range 243–809) were in the low-normal range; ornithine (523 $\mu\text{mol/L}$; ref.range 39–61), arginine (409 $\mu\text{mol/L}$; ref.range 53–71), and lysine (293 $\mu\text{mol/L}$; ref.range 107–163) were found increased. Total homocysteine was 6 $\mu\text{mol/L}$ (ref.range 3–15). Urinary orotic acid was absent. Urinary organic acid analysis showed elevated urinary lactate (1157 mmol/mol creatinine) and MMA (5875 mmol/mol creatinine; ref.range <2). Treatment with carnitine and hydroxocobalamin was continued while arginine and sodium benzoate were discontinued. A hypoproteic diet through nasogastric drip feeding (4 g protein/day) was started and then continued by mouth. In the subsequent days, his neurological features improved with normalization of muscle tone, development of normal neonatal reflexes, and good suction. When the patient was discharged at 3 months of life, he was in good general conditions with a weight of 4.860 kg and a natural protein intake of 5.1 g/day (577 Kcal).

In vitro studies showed that propionate incorporation in fibroblasts was low (0.13 nmol/10 h/mg protein; ref.range 1.94 ± 1.21) and normalized after OHCbl incubation (2.30 nmol/10 h/mg protein; ref.range 2.29 ± 1.51). MMA CoA mutase activity was normal (0.89 nmol/min/mg prot; ref.range 0.89 ± 0.43) and complementation analysis excluded cblA and cblB defects. Molecular analysis of the *MMADHC* gene showed a homozygous mutation (c.57-64del8). Analysis of parents' DNA confirmed that the mutations were in separate alleles. This mutation has been

previously identified in homozygosity in two other patients and functionally characterized (Stucki et al. 2012).

Protein intake was maintained restricted with progressive slow increase on the basis of MMA plasma and urinary concentrations. MMA levels never decreased to normal values although incorporation of propionate after addition of hydroxocobalamin was normal in vitro. Hydroxocobalamin was administered daily. During the first 5 months of his life, it was given as a single IM injection once every 7 days and orally once a day in the following 6 days. After the age of 5 months, it was given orally every day. In the subsequent years, we did not go back to IM administration because the patient showed plasma B₁₂ levels at periodic evaluations always between 1,800 and 4,000 pg/ml, well over the normal range (191–663).

At his last examination at the age of 7 years, weight was 29 kg (90th–97th) and height was 123 cm (50th–75th). He never showed metabolic decompensations and was never anorectic. During the first 6 years, MMA plasma values were always below 140 μ mol/L (ref. range 0.04–4.00) with the exception of a value of 213 μ mol/L at the age of 30 months, associated to a high urinary level of 3,000 mmol/mol creatinine (n.v. <2). In the last year, plasma and urinary values have increased mildly (Fig. 1).

At present, he is being treated with carnitine 100 mg/kg/day and hydroxocobalamin 1 mg/day orally. His natural protein intake is formally 12 g/day (0.4 g/kg/day), but we have reasons to suspect that the family does not observe a strict dietary compliance. Brain MRI performed just after diagnosis at 2 months of age showed bilateral hyperintensity of the caudate nuclei (Fig. 2a, b). At 7 years of age, neurological examination is normal, he walks, runs, and jumps; the administration of the WISC III Scale of Intelligence at 7 years of age showed normal performance subscales (performance IQ 90) and reduced verbal scores (verbal IQ 63) because of the language barrier due to the Indian origin; a nonverbal intelligence test (Leiter-R) was then administered and it showed a normal and homogeneous profile with total IQ 96. Brain MRI was repeated at 7 years and was found to be normal (Fig. 2c, d).

Discussion

To the best of our knowledge only 18 cbID patients have been reported in the literature so far (6 cbID-MMA/HC, of whom two were brothers, 6 cbID-HC and 6 cbID-MMA) (Goodman et al. 1970; Cooper et al. 1990; Suormala et al. 2004; Coelho et al. 2008; Miousse et al. 2009; Stucki et al. 2012). The clinical history of the seven most recently reported patients is not known in detail (Stucki et al. 2012). The age of diagnosis of the other 11 patients (5 cbID-MMA/HC, 3 CbID-HC, 3 cbID-MMA) ranged from 22 days, for a cbID-MMA/HC patient presenting with poor feeding and

seizures, to 14 years, for another cbID-MMA/HC patient presenting with psychotic crisis. One of the three cbID-MMA patients had an acute onset with decompensation at 11 months (Cooper et al. 1990). The second one presented with self-limited metabolic acidosis in the first days of life and survived without diagnosis until 5 years of age, when he was evaluated because of vomiting and muscle weakness, and eventually diagnosed and treated (Miousse et al. 2009). The third patient (Suormala et al. 2004) had a complicated history characterized by low gestational age (32 weeks), intracranial hemorrhage, respiratory distress syndrome, necrotizing enterocolitis, and convulsions at birth; neither the age of MMA diagnosis, nor the starting age of the treatment with carnitine, hydroxocobalamin, and protein restriction are known. The treatment led to normalization of EEG traces and reduced urinary MMA. Interestingly, this patient shares with our case the same geographic origin and the same genotype. They both have a clinically isolated MMA presentation and show an apparent response to B₁₂. Another patient with the same genotype has been reported by Stucki et al. (2012), but the clinical picture is not known. These authors studied both their patient's cell lines and those of Suormala's patient and found that these cells could not normalize the adenosylcobalamin synthesis not even upon transfection with either cbID wild type or a more efficient construct (cbID_MLS_ALDH2), in which the MLS targets the mitochondria more efficiently than the wild type. This effect was negatively correlated with the levels of *MMADHC* mRNA found in the cells. Thus they speculated that in this experimental condition the shorter protein may interfere with the formation of functional complexes needed for AdoCbl synthesis or that, alternatively, it might interact with full-length protein or with binding partners located in the cytosol or at the mitochondrial membrane.

The clinical presentation of our patient was suggestive of a sepsis. The presence of meconium-stained amniotic fluid and a positive C-reactive protein further confirmed the suspicion. The very high hyperammonemia might be indicative of a urea cycle disorder, while the age of onset (4–6 days) together with the associated metabolic acidosis and ketosis were instead more suggestive of organic aciduria. Hemodiafiltration was started when plasma ammonia had not decreased after 6 h of conventional treatment and the risk of further deterioration with permanent neurological damage or death became possible. Due to the acute-onset conditions, we never formally tested this patient's responsiveness to B₁₂. However, his clinical evolution, showing no acute decompensations in 7 years, and the detection of MMA levels in plasma and urine comparable to those found in vitamin B₁₂ deficiency rather than in B₁₂-unresponsive MMA (Fowler et al. 2008), led us to believe that this patient was at least partly responsive to vitamin B₁₂, in vivo. Although propionate

Fig. 1 Urinary (*solid line*) (n.v. < 2 mmol/mol creatinine) and plasma (n.v. 0.04–4.00 μmol/L) (*broken line*) MMA during the 7 year follow-up

Fig. 2 Bilateral hyperintensity of the caudate nuclei in brain MRI at 2 months of life (**a, b**) and at 7 years (**c, d**). The hyperintensity of the heads (**a**) and the tails of the caudate nuclei (**b**) is absent at the age of 7 years (**c, d**)

incorporation was completely normalized in vitro after the addition of OHCbl, it is possible that his in vivo responsiveness was only partial thereby promoting residual MMA production. In this regard, also the patient reported by Suormala et al. (2004) was treated with protein restriction and still had a residual excretion of MMA. Likewise, also this patient's family compliance had not been optimal.

Of the three cbID-MMA patients that have been reported in the literature (Cooper et al. 1990; Suormala et al. 2004; Miousse et al. 2009), none of them presented with an acute neonatal decompensation with severe hyperammonemia and coma comparable to what we have found in our patient. They presented with a late-onset condition, probably more consistent with a vitamin-responsive disorder. In this regard, it is a common knowledge that B₁₂-responsive MMA patients have a milder later onset and very rarely present in the neonatal age (Manoli and Venditti 2010; Burlina et al. 1999). This notion might lead neonatologists to underestimate the importance of a prompt vitamin treatment of the newborn with an acute metabolic presentation, before having made a specific diagnosis. However, a number of patients with B₁₂-responsive MMA, who presented severely in the neonatal age, have also been reported (Hori et al. 2005). Thus, taken all together, this case argues in favor of starting an early vitamin treatment in patients presenting with a neonatal-onset hyperammonemia independently of the suspected disease and before having received a specific biochemical diagnosis. Neonatal screening for methylmalonic acidemia – including mutase deficiency and Cbl defects associated with increased MMA plasma levels – is performed in most European and US expanded neonatal screening programs (Loeber et al. 2012; Sun et al. 2012). The importance of such programs is once more highlighted by our findings showing a favorable and long-term outcome of a CblID-MMA patient with acute neonatal onset. It is likely that our patient could have avoided the hyperammonemic coma and hemodiafiltration, if he had undergone early screening. The evolution of this patient was quite favorable without any noticeable handicap at the age of 7 years. We strongly believe that both early B₁₂ administration and aggressive treatment with hemodiafiltration prevented him from long-term neurologic sequelae.

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Synopsis

CblID defect with unusual acute neonatal onset requiring hemodiafiltration.

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