

SESION 22 OCTUBRE 15:30-17:00

Causas de acidemia metilmalónica: diagnóstico diferencial

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Methylmalonic acidurias (MMAs) encompass a heterogeneous group of autosomal recessively inherited disorders characterized by the accumulation of methylmalonic acid in body fluids. Methylmalonic acid derives from its precursor propionyl-CoA, produced by the breakdown of branched-chain amino acids valine and isoleucine and of other propiogenic substrates such as methionine, threonine, odd-chain fatty acids and cholesterol. MMAs are caused by a defect of the mitochondrial enzyme methylmalonyl-CoA mutase or by one of the known defects in the uptake, transport, or synthesis of cobalamin (vitamin B12), the cofactor of methylmalonyl-CoA mutase.

Cobalamin must be obtained from the diet and is only present animal products. Absorption of cobalamin requires binding to specific proteins in saliva, proteolytic release and binding to the intestine and uptake into the enterocyte via a specific receptor. After release from its complex with intrinsic factor cobalamin binds to transcobalamin and enters the circulation as the TC-Cbl complex. This complex is taken up by cells by endocytosis and thereby incorporated into lysosomes. Following proteolytic action cobalamin is released into the cytosol and inside the cell it is converted into two active co-enzymes in the cytosol and in the mitochondrion. After transport into the mitochondria cobalamin is converted into adenosylcobalamin, the essential co-enzyme for methylmalonyl-CoA mutase, whose deficiency results in methylmalonic aciduria, Methylcobalamin is the essential co-factor for N5-methyltetrahydrofolate: homocysteine methyltransferase whose deficiency results in an increase of homocysteine with low levels of methionine

On the bases of biochemical, enzymatic and genetic complementation analyses, MMAs can be categorised into different forms (Fowler 2008). Patients may have a defect of the apoenzyme methylmalonyl-CoA mutase or defects in the synthesis of the cofactor vitamin B12 (cobalamin). To date, eight distinct genetic defects have been defined by somatic cell complementation analysis: mut, cblA, cblB, cblC, cblD, cblE, cblF, and cblG. Depending on their localization within the pathway, these complementation

defects can cause either isolated methylmalonic aciduria, combined methylmalonic aciduria and homocystinuria, or isolated homocystinuria (not discussed in this work).

Isolated MMAs, causes the so called “classical” form of MMA, usually characterized by a neurological distress with acute or rapidly progressive signs caused by the accumulation of toxic compounds proximal to the metabolic block (Deodato 2006). Patients may have a defect of the apoenzyme methylmalonyl-CoA mutase [mut (mut^o/mut^t)], or in its cofactor adenosylcobalamin (cblA, cblB, and less frequently cblD-methylmalonic aciduria). The diagnosis is based on the presence of massively increased urinary excretion of methylmalonic acid, methylcitric acid and derivatives of propionyl-CoA, and on the presence of increased propionyl-carnitine in blood. Onset of the disease is mostly in the neonatal period with recurrent vomiting, dehydration, respiratory distress, hypotonia, progressive lethargy, seizures and coma, leading to death if not promptly treated. The neurological picture is frequently characterized by a dystonic syndrome resulting from acute basal ganglia lesions, localized bilaterally in the globus pallidus, that usually occur during episodes of metabolic decompensation with severe ketoacidosis. Long-term survivors are at risk for development of chronic renal failure .

Increased levels of methylmalonic acid can also be observed in MMA with homocystinuria, caused by other defects of cobalamin metabolism (cblC, cblD and cblF defects) which impair the conversion of vitamin B12 into its two metabolically active forms, methylcobalamin and adenosylcobalamin. The most frequent form is cblC defect which usually presents with a different clinical picture from isolated MMA. The clinical features of the early-onset form, with appearance of symptoms within the first year of life, include a multisystemic disease with severe neurological signs associated with characteristic hematological, renal, and gastrointestinal manifestations. The long-term neurological outcome is usually poor. Patients with the later onset form present a milder clinical phenotype with moderate neurological regression and behavioral disturbances. More rarely, transcobalamin II deficiency, an inherited disorder of cobalamin transport that leads to intracellular cobalamin depletion with secondary impairment of methylmalonyl-CoA mutase and methionine synthetase activities, cause combined MMA with homocystinuria.

In the last few years, there has been a growing interest in this field which has greatly expanded the level of knowledge, providing the solution for many of the

unsolved problems of MMAs. These include the discovery of several genetic defects as well as the identification of new “atypical” forms.

In 2006 the gene *MMACHC* responsible for methylmalonic aciduria and homocystinuria cblC type has been identified (Lerner-Ellis 2006). The cblC locus was mapped to chromosome region 1p34.1 by linkage analysis. The authors showed the existence in C-terminal domain of *MMACHC* gene products of motifs similar to those seen in bacterial genes with cobalamin related functions such as TonB protein. However, function of the product of this gene remained unknown until 2008, when it was demonstrated in vitro that human *MMACHC* protein is a CblC decyanase, which catalyse the decyanation of CblC to cob(II)alamin by using reducing equivalents furnished by cytosolic diflavin oxidoreductases, supporting the idea that *MMACHC* acts as an intracellular cobalamin trafficking chaperone that carries out targeted delivery of cobalamin to and from other cobalamin related proteins (Kim 2008).

In 2008, Coelho et al. identified the gene *MMADHC* responsible for cblD disease. The cblD gene was localized to human chromosome 2q23.2 and very interestingly, it has been shown that mutations in different regions of *MMADHC* were associated with different biochemical phenotypes. Based on the presence in *MMADHC* protein of a putative cobalamin binding motif and of a putative mitochondrial targeting sequence, the authors hypothesized that the mutations localized toward the N-terminal part found in patients with isolated cblD methylmalonic aciduria (which includes also the former cblH defect), result in the formation of a shorter functional cblD protein lacking the putative mitochondrial leader sequence and causing deficient synthesis of adenosylcobalamine alone. Vice versa, the mutations localized toward the C-terminal part of the protein found in patients with isolated cblD homocystinuria, caused a deficient synthesis of methylcobalamin. The mutations found in patients with combined MMA and homocystinuria, predicted to lead to premature stop codons, would result in a defective protein lacking both functional domains therefore causing impaired synthesis of both adenosyl- and methyl-cobalamin.

In 2009, by linkage analysis of several families with cblF, Rutsch et al. (2009) identified a locus on chromosome 6q12-q13 and, and identified mutations in the *LMBRD1* gene, coding for a lysosomal membrane exporter for cobalamin. The genetic defect of cblF impairs the release of cobalamin from the lysosome into the cytoplasm, causing deficient methylmalonyl-CoA mutase and methionine synthase activities because of the inability to synthesize both adenosyl- and methyl-cobalamin. The

authors found that a 1-bp deletion was present in 18 of the 24 disease chromosomes, consistent with a common founder of European ancestry. All mutations were truncating, but the phenotype was variable, ranging from developmental delay to asymptomatic long-term survival excluding genotype/phenotype correlations.

Besides these well-defined conditions that primarily affect the metabolism of methylmalonic acid, recent discoveries have focused the attention on other defects responsible for “atypical” forms of MMAs, caused by deficient activity of succinyl-CoA synthetase (SCS), the enzyme complex that catalyzes the conversion of succinyl-CoA to succinate in Krebs’s cycle. SCS is composed by an alpha-subunit (coded by *SUCLG1*) and by two beta-subunits (coded by *SUCLA2* and *SUCLG2*). Deficiency of the SCS activity has been associated with mutations in two out of the three subunits composing the enzyme. Several patients with different genetic background have been reported to be mutated in *SUCLA2* (Elpeleg 2005, Carrozzo 2007, Ostergard 2007a) and only two families are characterized by mutations in *SUCLG1* (Ostergard 2007b, Ostergard 2009). Defects in either genes can be found at the metabolite level and are defined by mildly elevated methylmalonic acid and C4-dicarboxylic-carnitine (succinyl-carnitine) concentrations in body fluids, in association with variable lactic acidosis, multiple defect of respiratory chain enzymes, and mtDNA depletion. The clinical pattern in *SUCLA2* patients is highly homogeneous and the diagnosis should be considered in patients with early onset encephalomyopathy, dystonia, deafness, and Leigh-like MRI abnormalities mainly affecting the putamen and the caudate nuclei. Patients with *SUCLG1* mutations are clinically heterogeneous, showing either a severe form with neonatal multiorgan failure and early death or a phenotype similar to that of *SUCLA2* mutation.

Recently, a new genetic defect caused by mutation in the methylmalonyl-CoA epimerase gene has been reported in subjects with mild MMA (Bikker 2006, Dobson 2006). The deficiency of methylmalonyl-CoA epimerase explained the in vitro biochemical findings of decreased propionate incorporation in cultured fibroblasts and fully normal activity of methylmalonyl-CoA mutase. As some of the reported patient did not seem to be more severely affected it has been suggested that isolated methylmalonyl-CoA epimerase deficiency may not have a large clinical impact, or could even be considered a non-disease.

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Convulsiones neonatales con respuesta a piridoxina y piridoxal-5-fosfato. Neonatal epileptic encephalopathy due to inherited disorders affecting intracellular availability of pyridoxal phosphate (vitamin B₆).

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Pyridoxal phosphate (PLP, vitamin B₆) is an essential cofactor of more than 100 enzyme-catalysed reactions in the body, mostly involving amino acids. Condensation of the highly reactive aldehyde group of PLP with the alpha-amino group of various amino acids is required for transamination reactions in several metabolic pathways including the biosynthesis of several neurotransmitters. Dietary vitamin B₆ enters the blood stream as pyridoxine, pyridoxamine or pyridoxal which are phosphorylated by hepatic pyridoxal kinase; pyridoxine phosphate and pyridoxamine phosphate are then converted to PLP by pyridox(am)ine-5'-phosphate oxidase (PNPO). PLP re-enters the circulation bound to albumin, is released by the tissue non-specific isoenzyme of alkaline phosphatase, and may cross the blood-brain barrier and enter target cells as pyridoxine. Within the cell it needs to be re-phosphorylated by pyridoxal kinase to active PLP.

Intracellular deficiency of PLP may be caused by a variety of mechanisms, many of which are amenable to treatment. **Primary deficiency of PNPO** frequently manifests before birth with foetal distress, intrauterine seizures and prematurity. Neonates may show apparent signs of asphyxia and develop intractable seizures. Metabolic work-up typically shows evidence of a deficiency of multiple PLP-dependent enzymes such as elevated threonine and glycine in blood and CSF, a decrease of serotonin in plasma, or a decrease of homovanillic and 5-hydroxyindoleacetic acids in CSF. However, some children may have normal results of metabolic investigations. An important clue for diagnosis is a therapeutic trial with oral PLP (30 mg/kg/d in three doses for at least one day); PNPO deficiency is confirmed by mutation analysis in the *PNPO* gene. PNPO deficiency does not respond to oral treatment with pyridoxine as the conversion of pyridoxine phosphate to PLP is deficient.

The rather more common phenotype of **pyridoxine-dependent seizures** was recognised as a secondary depletion of PLP due to accumulation of L-delta 1-piperidine 6-carboxylate (P6C) in primary alpha-aminoadipic semialdehyde/P6C dehydrogenase deficiency in pipercolic acid metabolism. Affected children show a somewhat milder phenotype with mostly normal prenatal course (although intrauterine seizures may be noted) and few prenatal complications but intractable neonatal seizures. Metabolic investigations are usually normal except for specific elevations of pipercolic acid in CSF and blood as well as alpha-aminoadipic semialdehyde in urine. Oral administration of 50–100 mg (rarely 500 mg) pyridoxine usually causes rapid cessation of seizures. However, as in PNPO deficiency, the therapeutic effect may be combined with transient but potentially life-threatening additional effects (particularly in children previously treated with antiepileptic drugs) such as severe unresponsiveness, hypothermia, hypotension and respiratory arrest. Therapeutic trials thus should be undertaken only under close surveillance within the hospital. P6C dehydrogenase deficiency may be confirmed by identification of mutations in the *ALDH7A1* (antiquitin) gene.

Secondary depletion of PLP is also observed in **hyperprolinaemia type II** (L-delta 1-pyrroline-5-carboxylate [P5C] dehydrogenase deficiency) where it is due to accumulation of P5C. Epileptic seizures due to intracellular PLP deficiency are also observed in **hypophosphatasia**, the genetic deficiency of tissue non-specific alkaline phosphatase.