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Original article

Malonyl-CoA decarboxylase deficiency: Long-term follow-up of a patient new clinical features and novel mutations

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Abstract

Background: Malonyl-CoA decarboxylase (MLYCD, EC 4.1.1.9) deficiency is a rare autosomal recessive disorder that is widely diagnosed by neonatal screening. *Methods:* We report long term follow up of a patient with MLYCD deficiency showing signs of neonatal hypoglycemia, mental retardation, developmental delay and rheumatoid arthritis. Brain MRI revealed patchy, symmetrical hyperintensity of the deep white matter with periventricular white matter and subcortical arcuate fibers being spared. *MLCYD* gene sequence analysis was done to identify possible mutations. Expression analyses at mRNA and protein levels were also performed. Further, immunocytochemical studies were implemented to check for its subcellular localization. *Results: MLYCD* gene sequencing identified a novel compound heterozygous mutation (c.22 T>A, p.M1K, c.454 C>A; pH152N) in our patient and a heterozygous mutation in the healthy mother c.22 T>A; pM1K. Reduced expression of RNA and protein levels was observed. Immunocytochemical analysis showed diffused staining across the cytoplasm with apparent signs of intracellular mislocalization to the nucleus. Results also indicated subcellular colocalization of MLCYD with mitochondria was scant compared to control. *Conclusion:* Our patient was identified with a novel compound heterozygous *MLYCD* mutation at the N-terminal helical domain. This study indicates that protein mislocalization is a characteristic feature of MLYCD deficiency in our patient.

Keywords: Malonyl CoA decarboxylase; Mitochondrial disorder; Mental retardation; Low fat diet therapy; Rheumatoid arthritis

1. Introduction

Malonyl-CoA, a product of acetyl-CoA carboxylase (ACC) is a metabolic intermediate in lipogenic tissues that include liver and adipose tissue, where it is involved in the de novo fatty acid synthesis and elongation [1]. In nonlipogenic tissues as cardiac and skeletal muscle, cytosolic malonyl-CoA acts as a key regulator of mitochondrial fatty acid β-oxidation by potentially inhibiting carnitine palmitoyl transferase 1 (CPT), thus maintaining the balance between lipid and glycogen metabolism [1]. Malonyl-CoA decarboxylase (MLYCD, E.C. 4.1.1.9), a 55 kDa enzyme catalyzes the conversion of malonyl-CoA to acetyl-CoA and carbon dioxide thus providing a route for disposal of malonyl-CoA from mitochondria and peroxisomes, whereas in the cytosol the malonyl-CoA pool is regulated by the balance of MLYCD and acetyl-CoA carboxylase activities [1]. Earlier studies demonstrated that Malonyl-CoA could substantially inhibit the other isoforms of CPT, *CPT1B* in the heart [2] and *CPT1C* in the brain [3].

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MLYCD is encoded by the gene *MLYCD* localized on chromosome 16q24 [4,5]. The highest tissue specific expression of human MLYCD mRNA was found in the heart and skeletal muscle and to a lesser extent in liver, kidney and pancreas [6]. Expression of rat MLYCD has been detected in at least the mitochondria and cytosol, and possibly in peroxisomes as well [7]. However, despite of numerous studies, the subcellular localization of MLYCD has always been a controversy with emergence of conflicting study reports [6–8].

Human MLYCD deficiency (OMIM 248360) also known as malonic aciduria is a rare autosomal recessively inherited inborn error of fatty acid metabolism that is characterized by cardiomyopathy, seizures, hypoglycemia, mental retardation, developmental delay and in some cases neonatal death [9–13]. Although central nervous system (CNS) manifestations are characteristic for MLYCD deficiency, little is still known about the role of MLYCD in the brain. In an adult rat brain, MLYCD expression was found in neurons and microglia of the frontal cortex and hippocampus. In the cerebellum MLYCD labelling was detected in several cell types but not in neurons [14]. Recent studies by Forese et al. show that the crystal structure of MLYCD constitutes two domains, an N-terminal helical domain that is involved in oligomerization and a C-terminal catalytic domain [15]. The elucidated structure gives us a better understanding of the various mutations in the *MLYCD* gene causing the deficiency [15].

Several cases have been reported in literature [7–12,16–24] regarding the metabolic crisis associated with MLYCD deficiency, albeit very little is known about the long term outcome of such cases through subsequent dietary therapy prescribed [22,24–25]. Here we describe the long term follow up of a patient affected by malonic aciduria upon neonatal onset. Molecular analysis of the *MLYCD* gene showed two novel heterozygous mutations. Concurrently we also present the evidence of mislocalization of MLYCD that we hypothesize could be due to the compound heterozygous mutation within the predicted mitochondrial targeting sequence.

2. Case presentation

Our 17 year old patient is the daughter of healthy non-consanguineous Finnish parents, was born at 39 weeks by C-section due to breech presentation. Family history shows one healthy boy and an early miscarriage. Birth weight was 3800 g (height-50 cm, head circumference-37.5 cm) with Apgar score 10. Neonatally, the baby had feeding difficulties, failure to thrive and somnolence on day 3. Patient had hypoglycemia 2.1 and 2.6 mmol/L, respectively and she had two series of focal seizures, which responded to fenobarbitone. Plasma ammonia was normal (36 μmmol/L) and alanine transferases were ALAT (<30 U/L) normal. Electroencephalogram showed centroparietal focal spikes, but normal background. Brain computer tomography revealed nonspecific findings: hypodensic white matter and brain magnetic resonance imaging was normal.

At the age of 6 months, she had a generalized developmental delay, hypotonia, mild motoric asymmetry, and potential loss of acquired skills. Urinary organic acids screening showed increased excretion of malonic acid (110 mmol/mol creatinine; reference <20 mmol/mol creatinine) and methyl malonicacid (5 mmol/mol creatinine; reference <5 mmol/mol creatinine). Repeated fasting amino acid analysis did not show any significant abnormalities and cerebrospinal fluid amino acids were normal. Plasma creatine kinase was normal and the patient had no vacuolated lymphocytes. Additional, acylcarnitine analysis by tandem mass spectrometry showed elevated levels of malonylcarnitine (1.1 μM; reference <0.1 μM). However, her mother's malonylcarnitine concentration was 0.04 μM. The malonyl-CoA decarboxylase enzyme activity in fibroblasts of the patient was normal and did not show any significant difference from the controls.

When she was 18 months old, development was delayed, accompanied by hypotonia, mild dystonia, and a delayed mental development. At 2 years (yrs), her cerebral spinal fluid (CSF) malonate concentrations were considerably higher compared to serum malonate which indicates cerebral production of malonate (Table 1). At the age of 3 and 4 years, the child had developed muscle hypotonia and mild ataxia and able to produce few words but had difficult in understanding abstract matters suggesting a mild mental retardation. The audiometry was normal and had clonic reflexes. She was given dietary therapy from low fat to high-fat diets, as the high fat diet increased malonic aciduria so a moderate low fat diet was continued (Fig. 1A).

At 12 years of age her brain MRI imaging revealed normal spinal fluid spaces and symmetrical hyperintensity of the deep and periventricular white matter. Corpus callosum was thin but there was not a uniform constriction of U-fibres. Subcortical white matter spaces appeared to be constricted. The short echo spectrum showed increased myoinositol, which suggests glial cell damage (Fig. 1B). At 16 years of age, the patient was diagnosed with rheumatoid arthritis, which was HLA-B27 positive, RF negative, and had an increased anti-nuclear antibody 320, but no signs of iritis.

Table 1
Methylmalonate and malonate levels in serum and Cerebral spinal fluid (CSF) of patient.

	Serum (μmol/L)	CSF (μmol/L)
Methylmalonate	2.8 (reference <0.34)	13 (reference 0.15–0.55)
Malonate	40 (reference 14.2)	180 (reference 4.5)