Misleading Organic Acid Excretion in MAT Deficiency: Third Case of C.951C>T Compound Heterozygosity

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Received Date: 18 December, 2019; Accepted Date: 31 January, 2020; Published Date: 05 February, 2020

Abstract

Mitochondrial Acetoacetyl-Coa Thiolase (MAT) deficiency is an inherited disease of isoleucine and ketone body metabolism caused by mutations in ACAT1 gene. Patients suffer from ketoacidotic crisis, from the early childhood. This crisis starts with vomiting, followed by dehydration and tachypnea, usually triggered by infections. This disease is biochemically characterized by urinary excretion of 2-Methylacetoacetic Acid (2MAA), 2-Methyl 3-Hydroxybutyric Acid (2M3HB) and Tiglyglycine (TIG). We report here the case of a fourteen-month-old boy who was having severe ketoacidotic crisis, triggered by a gastroenteritis infection. His urinary organic acid analysis only revealed a massive ketosis, with no increased levels of MAT characteristic metabolites. Because of these factors, a SCOT deficiency was first suspected.

Molecular analysis finally revealed a MAT deficiency. The patient is compound heterozygous for two previously-reported mutations in ACAT1 gene: a missense mutation (c.951C>T (p.Asp317Asp)) and a nonsense mutation (c.814C>T (p.Gln272*). This was the third case of MAT deficiency compound heterozygous patient with a c.951C>T mutant allele. The interesting thing is that the two cases which had already been reported also presented a misleading urinary excretion of 2MAA, 2M3HB and TIG. This lack of typical organic acid excretion may be the result of a MAT residual activity, due to the c.951C>T mutation. That implies that MAT deficiency must be sought even in cases without urinary excretion of characteristic abnormal metabolites when SCOT deficiency had already been ruled out.

Keywords: Beta-Ketothiolase; C.951c>T Mutation; Ketoacidosis; Ketolysis Disorders; Mitochondrial Acetoacetyl-Coa Thiolase Deficiency; Organic Acidemia

Introduction

The first case of Mitochondrial Acetoacetyl-Coa Thiolase (MAT) deficiency was described in 1971 [1]. MAT deficiency is a rare autosomal recessive inherited disorder of isoleucine catabolism and ketone body metabolism due to biallelic mutations in ACAT1(NM_000019) gene. Until now, at least 159 patients have been diagnosed [2]. MAT deficiency is characterized, in early childhood (usually between 6 and 18 months), by severe ketoacidotic attacks triggered by an inter-current illness (gastroenteritis or respiratory infection) or excessive protein intake [3]. Between episodes, patients are asymptomatic. During acute metabolic crises, patients frequently have tachypnea, altered mental status and dehydration secondary to acidosis; seizures and hypotension are uncommon [4]. Ketoacidotic episodes are recurrent in 43% of patients [3]. Hypo or hyper glycemia and increased blood ammonia level may occur, but these parameters are usually normal [5]. Outcomes vary from normal development to severe cognitive impairment or even death after an acute episode of ketoacidosis [4].

Indeed, MAT deficiency can be revealed by metabolic stroke and neurological injury, as described by various authors with metabolic stroke mostly affecting basal ganglia [4,6,7]. MAT converts 2-methylacetoacetyl-CoA into propionyl-CoA and Acetyl-CoA. MAT deficiency leads to accumulation of 2MAA,
2M3HB and TIG which are excreted in urine [6,8]. Plasma increase of tiglylcarnitine (C5:1) and 2-methyl-3-hydroxybutyrylcarnitine (C5:OH) is also specific of MAT deficiency. The step catalyzed by MAT in ketolysis can also be catalyzed to some extent by another mitochondrial enzyme: medium-chain 3-ketoacyl-CoA thiolase [9]. Thus, even in the absence of any MAT function, some ketolysis is still possible and sufficient in basal conditions. Therefore, situations leading to excessive ketogenesis play an important role in ketoacidosic decompensations [3]. Here, we report a case of a patient with MAT deficiency revealed by a metabolic crisis with major ketoacidosis but without urinary excretion of characteristic abnormal metabolites.

Case Report

The patient is a fourteen-month-old boy born on term from non-consanguineous parents. He had normal growth and development. He was admitted to emergency department because of an episode of diarrhea and vomiting (gastroenteritis) which had begun four days earlier. First examination revealed a tired child, dehydrated, tachypneic with respiratory distress. Blood pressure was normal (95/47 mmHg) but his cardiac frequency was slightly elevated (150 beats per minute). Urine dipstick test indicated marked ketosis. The blood analysis revealed a severe metabolic acidosis (pH 7,03, pCO₂ 15 mmHg, bicarbonates 4,2 mmol/L, base excess -24,8 mmol/L), with normal ammonia level (79 µmol/L) and no hypoglycemia (blood glucose 1,38 g/L). He was treated with symptomatic measures (parenteral hydration). L-carnitine and vitaminotherapy (B12, biotin) were started because of suspicion of organic acidemia. Ketoacidosis progressively normalized within 24 hours. He clinically progressively recovered and had normal clinical examination 3 days after admission.

Metabolic investigations were performed during acute decompensation. Urine organic acid analysis and acyl carnitine profile in plasma only revealed a massive ketosis. Other metabolic explorations were normal. According to the lack of specific abnormal metabolites associated with marked ketosis, a Succiny1-Coa-3-Oxoaic Coa Transferase (SCOT) deficiency or Monocarboxylate Transporter 1 (MCT1) defect were suspected. Molecular investigation was performed to confirm diagnosis by testing, thanks to next generation sequencing, a panel of genes involved in ketolysis defects: ACAT1 (NM_000019), OXCT1 (NM_000436) and SLC16A1 (NM_003051). Surprisingly two heterozygous mutations in ACAT1 gene were identified: a nonsense mutation c.814C>T (p.Gln272*) and a silent mutation c.951C>T (p.Asp317Asp), both previously described as pathogenic [10,11]. On stable conditions, another urinary organic acid chromatography indicated only trace amount of 2M3HB. The patient is currently treated by L-carnitine and has a non-protein restriction diet and uncooked corn-starch intake before night fasting. He follows a specific personalized protocol in case of acute illness.

Discussion

MAT and SCOT are the main ketolytic enzymes. Permanent ketosis is pathognomonic for SCOT deficiency [5,9]. Compared to MAT deficiency, no characteristic urinary organic acids excretion has been described in SCOT deficiency, except for large amounts of 3-hydroxybutyrate and acetoacetate. Fifty percent of SCOT deficiency patients develop their first ketoacidotic crisis in the neonatal period. However, patients with mild SCOT mutations may have non-ketotic periods. Furthermore, in MAT deficiency, blood acylcarnitine analysis generally reveals increased C5-OH and C5:1 level. In our patient’s case, there was neither urinary excretion of 2M3HB, 2MAA nor TIG, and acylcarnitine level was within normal ranges. (Figure 1) shows urinary organic acid profile comparison among typical MAT deficiency and our present case report during the acute episode.

Figure 1: Urinary organic acid profiles comparison between a MAT control sample from Sheffield ERNDIM (European research network for evaluation and improvement of screening-diagnosis-treatment of inherited disorders of metabolism) qualitative organic acid scheme (n’ 197, 2012) and our present case report during the acute episode. Chromatogram profiles were zoomed on part (7,5 min-18 min) containing major differences. LA: lactate, 3HB: 3-OH-butyrate, 2M3HB: 2-methyl-3-OH-butyrate, 3HIV: 3-OH-isovalerate, 2MAA: 2-methyl-acetoacetate, AA: Acetoacetate, GA: Glutaric acid, ADA: Adipic acid, TIG: Tiglylglycine.

Therefore, a SCOT deficiency was firstly clinically suspected. Surprisingly, molecular testing revealed a nonsense mutation c.814C>T (p.Gln272*) and a silent mutation c.951C>T (p.Asp317Asp), resulting in the final diagnosis of MAT deficiency. This case highlights the need for searching for MAT deficiency in a context of clinical suspicion of ketolysis disorder, even in the absence of typical biochemical abnormalities either by enzymatic
assays or by molecular testing [5]. Moreover, as SCOT deficiency, other different diseases, such as 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase (MHBD) deficiency and MCT1 deficiency, can mimic the clinical or biochemical signs of MAT deficiency, but with some differences [5]. Patients with MHBD deficiency present urinary excretion of 2M3HB and TIG but no 2MAA [5,9]. MHBD deficiency is usually clinically different from MAT deficiency: it is a X-linked severe neurodegenerative disorder with a wide clinical heterogeneity including ketoacidosis decompensations.

Less severe cases have been described with mild neurological involvement and ketoacidosis crisis. Diagnosis can be performed by molecular analysis or enzymatic assay [5]. MCT1 deficiency was described as a cause of disorder of ketone utilization for the first time in 2014 [12]. MCT1 transports monocarboxylate metabolites, such as lactate, pyruvate, and ketone bodies, across the cellular membrane [5,12,13]. Patients with MCT1 deficiency have massive excretions of 3-hydroxybutyrate and acetocetate (ketone bodies), but show no increase in 2M3HB, 2MAA or TIG excretion. Clinical symptoms during ketoacidosis crisis are similar to those of MAT deficiency. ACAT1 gene is located on chromosome 11 (11q22.3 to 11q23.11), spans 27kb and is composed of 12 exons and 11 introns [14]. Nowadays, 105 mutations responsible for MAT deficiency have been identified [2].

The c.951C>T mutation was first described in a compound heterozygous patient whose urinary organic analysis only revealed an increase of 2M3HB, without TIG or 2MAA excretion and a deficient MAT activity in cultured fibroblasts [11]. The c.951C>T substitution is located within an exonic splicing enhancer sequence, which is the recognition site for SF2/ASF protein. SF2/ASF is a serine-and arginine-rich protein, member of the SR family, involved in efficient mRNA splicing and subsequent metabolism of mRNA [8,15]. The C in position 951 is the first nucleotide of exon 10 and participates to the recognition of exon 10 with the splice acceptor site of intron 9. The c.951C>T mutation causes exon 10 skipping, which result in nonsense-mediated mRNA decay. More recently, another compound heterozygous patient with c.951C>T mutant allele was described: urinary organic acid chromatography revealed only trace amount of TIG [16]. (Table 1) exhibits the urinary organic pattern of the three patients.

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Table 1: Comparison of urinary excretion between 3 cases reports showing compound heterozygosity for c.951 C>T mutation.

Mutation c.814C>T was also described in a compound heterozygous patient and is associated with exon 8 skipping [10]. The C to T translation causing Gln272 to termination codon was identified within exon 8, 13 bp from the 5’ splice of intron 8. In fibroblasts from this patient, mRNA with p.Gln272X mutation was prematurely terminated. It was unstable, degraded quickly and undetectable and MAT activity was absent. Thus, authors determined that mutant MAT derived from the mRNA with exon 8 sequence deleted had no thiolase activity. Atypical urinary organic acid excretion and/or acylcarnitine profile have been reported with other MAT genotypes. Studies described that some patients with MAT deficiency and MAT residual activity only show subtle biochemical abnormalities under stable conditions, but higher alterations during ketoacidotic crisis [17]. A patient with a mild mutation (c.1A>G) and MAT residual activity (11%) showed no increased urinary excretion of 2MAA, 2M3HB or TIG [3].

Another mutation of a splice acceptor site: c.941-9T>A, caused an aberrant splicing and exon 10 skipping [7]. Two patients homozygous for the c.941-9T>A mutation have been described: the first only had a urinary excretion of 2M3HB, and the second, with a normal acyl-carnitine profile, displayed only ketosis in initial urine organic acid collected during the first acute attack. Furthermore, in these patients, 2M3HB was excreted along during several episodes, with ketosis but without TIG. Enzymatic analysis revealed a residual MAT activity in these patients. The c.431A>C mutation which retains a residual MAT activity of 25% is associated with trace amounts of TIG and elevated 2M3HB in urine on acute conditions but only trace amounts of both acids on stable conditions without any increase in C5-OH or C5:1 carnitine’s [18]. Furthermore, it has been shown that the c.951C>T mutant allele could produce normally MAT spliced transcripts [2,8].

These may have residual activity and contribute to atypical biochemical profiles of MAT deficiency patients. On the other hand, we can compare the effects of the c.949G>A mutation which is located in the same codon as c.951C>T mutation [8]. It decreased the effect of an ESE and caused exon 10 skipping. While...
References


Acknowledgement

No conflict of interest for each author.

The content of the article has not been influenced by sponsors.
