GAVIN PUBLISHERS

OPEN ACCESS

Annals of Case Reports

Baillat M, et al. Ann Case Report 14: 290.

DOI: 10.29011/2574-7754/100290

Case Report

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

Marine Baillat[1], Aline Cano[1*], Cécile Acquaviva[2], Florent Darriet[3], Marguerite Gastaldi[3,4], Brigitte Chabrol[1]

[1]Reference Center for Inborn Errors of Metabolism, La Timone Hospital, University Hospital, Marseille, France

[2]Reference Center for Inborn Errors of Metabolism, Groupement Hospitalier Est - Hospices Civils de Lyon, University Hospital, Lyon, France

[3]Biochimie métabolique, La Timone Hospital, University Hospital, APHM, Marseille, France

[4]Center for cardiovascular research and nutrition, INRA, INSERM, Aix-Marseille University, Marseille France.

[*]**Corresponding author:** Dr Aline Cano, Reference Center for Inborn Errors of Metabolism, La Timone Hospital, APHM, 264 Rue Saint Pierre, 13005 Marseille, France. Tel: +33-491386967; Fax: +33-491386809; Email: aline.cano@ap-hm.fr

Citation: Baillat M, Cano A, Acquaviva C, Darriet F, Gastaldi M, et al. (2020) Misleading Organic Acid Excretion in MAT Deficiency: Third Case of C.951C>T Compound Heterozygosity. Ann Case Report 14: 290. DOI: 10.29011/2574-7754/100290

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 Tiglylglycine (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 ketoacidotic 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, pCO_2 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 Succinyl-Coa-3-Oxoacid 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.

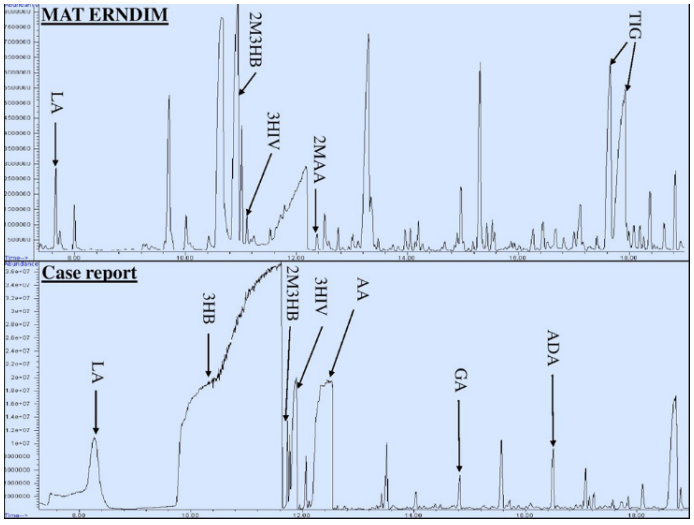

Figure 1: Urinary organic acid profiles comparison between a MAT control sample from Sheffield ERNDIM (European research network for evaluation and improvement of screening-diagnosis-treatment of inherited disorders of metabolism) qualitative organic acid scheme (n° 197, 2012) and our present case report 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 acetoacetate (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 q23.11), 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.

Case reports	ACAT1 Mutations	Mutations effects	MAT urinary organic acid profile
Present case	c.951C>T / c.814C>T	Exon 10 skipping/ Nonsens	2M3HB
[11]	c.951C>T / c.556G>T	Exon 10 skipping/ Missense	2M3HB
[16]	c.951C>T / c.949G>A	Exon 10 skipping/ Missense	TIG

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

normally splices transcripts were detected, the mutant protein did not retain any activity and the patient had an increased urinary excretion of 2M3HB, 2MAA and TIG. This emphasizes the hypothesis that residual MAT activity can explain unusual urinary organic acid profile. The three patients with c.951C>T mutation shown in table 1 are compound heterozygous. As a result, they all have a misleading organic acid expression. This atypical profile is probably due to c.951C>T mutation that leads to a residual MAT activity.

However, we didn't measure MAT activity in our patient. Indeed, diagnosis is nowadays performed on genetic panels instead of MAT activity analysis in skin fibroblasts. It should be noted that MAT deficiency patients with mild mutations develop ketoacidotic attacks as severely and with the same frequency as those seen in patients with null mutations, but their blood acylcarnitine and urinary organic acid profiles may show unspecific levels even during acute attacks [5,18,19]. Genotype and clinical phenotype do not seem correlated: the same expression of the disease is observed with null or residual MAT activity, and siblings with the same mutations exhibit different clinical presentations [20-22]. However, a correlation between biochemical phenotype and genotype may exist.

Conclusion

We report here the third case of a MAT deficiency compound heterozygous patient carrying the c.951C>T mutant allele and no specific organic acid profile. This case highlights the need for screening for MAT deficiency in a context of clinical suspicion of ketolysis disorder, even in the absence of typical biochemical abnormalities in plasma and urine. This misleading organic acid excretion might be the result of a MAT residual activity.

Acknowledgement

No conflict of interest for each author.

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

References

1. Daum RS, Lamm PH, Mamer OA, Scriver CR (1971) A « new » disorder of isoleucine catabolism. Lancet Lond Engl 2: 1289-90.

2. Abdelkreem E, Harijan R, Yamaguchi S, Wierenga RK, Fukao T (2019) Mutation update on ACAT1 variants associated with mitochondrial acetoacetyl-CoA thiolase (T2) deficiency. Hum Mut 40: 1641-1663.

3. Nguyen KN, Abdelkreem E, Colombo R, Hasegawa Y, Can NT, et al. (2017) Characterization and outcome of 41 patients with beta-ketothiolase deficiency: 10 years' experience of a medical center in northern Vietnam. J Inherit Metab Dis 40: 395-401.

4. Grünert SC, Schmitt RN, Schlatter SM, Gemperle-Britschgi C, Balcı MC, et al. (2017) Clinical presentation and outcome in a series of 32 patients with 2-methylacetoacetyl-coenzyme A thiolase (MAT) deficiency. Mol Genet Metab 122: 67-75.

5. Abdelkreem E, Otsuka H, Sasai H, Aoyama Y, Hori T, et al. (2016) Beta-ketothiolase deficiency: resolving challenges in diagnosis. J Inborn Errors Metab Screen 4: 1-9.

6. Wojcik MH, Wierenga KJ, Rodan LH, Sahai I, Ferdinandusse S, et al. (2018) Beta-ketothiolase deficiency presenting with metabolic stroke after a normal newborn screen in two individuals. JIMD Rep 39: 45-54.

7. Sasai H, Aoyama Y, Otsuka H, Abdelkreem E, Nakama M, et al. (2017) Single-nucleotide substitution T to A in the polypyrimidine stretch at the splice acceptor site of intron 9 causes exon 10 skipping in the ACAT1 gene. Mol Genet Genomic Med 5: 177-184.

8. Otsuka H, Sasai H, Nakama M, Aoyama Y, Abdelkreem E, et al. (2016) Exon 10 skipping in ACAT1 caused by a novel c.949G>A mutation located at an exonic splice enhancer site. Mol Med Rep 14: 4906-4910.

9. Fukao T, Mitchell G, Sass JO, Hori T, Orii K, et al. (2014) Ketone body metabolism and its defects. J Inherit Metab Dis 37: 541-551.

10. Fukao T, Yamaguchi S, Wakazono A, Orii T, Hoganson G, et al. (1994) Identification of a novel exonic mutation at -13 from 5' splice site causing exon skipping in a girl with mitochondrial acetoacetyl-coenzyme A thiolase deficiency. J Clin Invest 93: 1035-1041.

11. Fukao T, Horikawa R, Naiki Y, Tanaka T, Takayanagi M, et al. (2010) A novel mutation (c.951C>T) in an exonic splicing enhancer results in exon 10 skipping in the human mitochondrial acetoacetyl-CoA thiolase gene. Mol Genet Metab 100: 339-344.

12. van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiter PNJ, Turkenburg M, et al (2014) Monocarboxylate transporter 1 deficiency and ketone utilization. N Engl J Med 371: 1900-1907.

13. Bergersen LH and Eid T (2014) Monocarboxylate transport matters. N Engl J Med 371: 1931-1932.

14. Kano M, Fukao T, Yamaguchi S, Orii T, Osumi T, et al. (1991) Structure and expression of the human mitochondrial acetoacetyl-CoA thiolase-encoding gene. Gene 109: 285-290.

15. Lin S and Fu XD (2007) SR proteins and related factors in alternative splicing. Adv Exp Med Biol 623: 107-122.

16. Kılıç-Yıldırım G, Durmuş-Aydoğdu S, Ceylaner S, Sass JO (2017) Beta-ketothiolase deficiency: an unusual cause of recurrent ketoacidosis. Turk J Pediatr 59: 471-474.

17. Fukao T, Zhang G-X, Sakura N, Kubo T, Yamaga H, et al. (2003) The mitochondrial acetoacetyl-CoA thiolase (T2) deficiency in Japanese patients: urinary organic acid and blood acylcarnitine profiles under stable conditions have subtle abnormalities in T2-deficiency patients with some residual T2 activity. JIMD 26: 423-431.

18. Fukao T, Maruyama S, Ohura T, Hasegawa Y, Toyoshima M, et al. (2012) Three japanese patients with beta-ketothiolase deficiency who share a mutation, c.431A>C (H144P) in ACAT1 : subtle abnormality in urinary organic acid analysis and blood acylcarnitine analysis using tandem mass spectrometry. JIMD Rep 3: 107-115.

19. Fukao T, Scriver CR, Kondo N, t2 Collaborative Working Group. (2001) The clinical phenotype and outcome of mitochondrial acetoacetyl-CoA thiolase deficiency (β-ketothiolase or T2 deficiency) in 26 enzymatically proved and mutation-defined patients. Mol Genet Metab 72: 109-114.

20. Abdelkreem E, Akella RRD, Dave U, Sane S, Otsuka H, et al. (2017) Clinical and mutational characterizations of ten indian patients with beta-ketothiolase deficiency. JIMD Rep 35: 59-65.

21. Fukao T, Yamaguchi S, Orii T, Hashimoto T (1995) Molecular basis of beta-ketothiolase deficiency: mutations and polymorphisms in the human mitochondrial acetoacetyl-coenzyme A thiolase gene. Hum Mutat 5: 113-120.

22. Thümmler S, Dupont D, Acquaviva C, Fukao T, de Ricaud D (2010) Different clinical presentation in siblings with mitochondrial acetoacetyl-CoA thiolase deficiency and identification of two novel mutations. Tohoku J Exp Med 220: 27-31.