

CASE REPORT

Biochemical profiling in two siblings with mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency

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Abstract

We report the biochemical profiling in two siblings with mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency. Organic aciduria typical of this rare inborn error metabolism was found when the elder sibling presented with an episode of severe ketoacidosis at 20 months of age, which consisted of excessive excretion of ketones, tiglylglycine, 2-methyl-3-hydroxybutyrate, and 2-methylacetoacetate. Blood acylcarnitines profile showed elevation of C₅OH-carnitine, which represents 2-methyl-3-hydroxybutyrylcarnitine. A similar biochemical profile was identified in the younger sibling during screening although he had only mild clinical symptoms. Both patients reported a favourable outcome on follow-up.

Keywords: mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency, urine organic acids, acylcarnitines, ketoacidosis

INTRODUCTION

Mitochondrial 2-methylacetoacetyl-CoA Thiolase (EC 2.3.1.9.) is an important enzyme responsible for cleavage of 2-methylacetoacetyl-CoA in isoleucine catabolism, acetoacetyl-CoA formation in hepatic ketogenesis and acetoacetyl-CoA cleavage in ketolysis in extrahepatic tissues.¹ Over 40 cases of 2-methylacetoacetyl-CoA thiolase deficiency have been reported worldwide since it was first described by Keating *et al* in 1972.^{2,3} The clinical manifestation is characterized by intermittent ketoacidosis, triggered by a precipitating factor such as an intercurrent illness, in an otherwise healthy child.¹ We reported here biochemical profiling of two siblings with this rare inborn error.

CASE REPORTS

Patient 1

The patient is a 2-year-old girl who was well until the age of 22 months. Following a 3 days of fever and cough, she had a severe episode of metabolic acidosis. She developed severe tachypnoea and dehydration due to frequent

vomiting and poor oral intake which progress into peripheral shock. She was lethargic but otherwise there was no localizing neurological signs. She required mechanical ventilation support due to deteriorating consciousness upon admission to hospital. Her liver was enlarged to 8cm below the right costal margin. The arterial blood pH was 7.166; pCO₂ 3.5kPa; pO₂ 17.0kPa; bicarbonate 1.3mmol/L; BE -27mmol/L; and anion gap 33.5mmol/L. She had significant ketonuria (3+). Other investigations on admission were: blood ammonia 24.8μmol/L (normal range < 80μmol/L); blood lactate 0.63mmol/L (normal range < 2.50mmol/L); Na⁺ 137mmol/L; K⁺ 2.8mmol/L; Cl⁻ 105mmol/L; Ca²⁺ 2.10mmol/L; mg²⁺ 1.22mmol/L; PO₄⁻ 0.69mmol/L; Hb 11.4g/dL; total white count 17.2 x10⁹/L; platelet count 340 x 10⁹/L. The liver function test and plasma amino acids quantitative analysis were normal. There was no lumbar puncture performed. She did not develop any hypoglycemia.

She was treated for presumed meningo-encephalitis. She received iv ceftriaxone and iv acyclovir for 7 days, large amounts of volume expanders and iv sodium bicarbonate. Her

condition improved and she was extubated on the 3rd day. Mild compensated metabolic acidosis persisted when feeding was restarted on the 5th day and she was prescribed oral Shohl's solution for a short duration. When she was reviewed 2 weeks later, she was well and the ketonuria, metabolic acidosis and hepatomegaly had all resolved.

In view of parental consanguinity, screening for inborn errors metabolism was requested. The profiling of urine organic acids was done by gas chromatography mass spectrometry (GC-MS) method on fresh urine sample collected during the acute phase. The profiling of blood acylcarnitines was done by tandem mass spectrometry (MS/MS) method on dried blood spots collected on a Guthrie paper. The results are shown in figures 1 and 2. The biochemical findings were consistent with mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency.

She was treated with modest dietary protein restriction and oral carnitine supplementation. She was well and developmental milestones were normal during her last clinic visit at 25 months. Her weight was 13.1kg (50th centile), height 84cm (25th centile) and head circumference 48.5cm (50th centile). MRI brain was done and revealed normal findings. Her urine organic acids excretion pattern remained abnormal even when

she was well.

Patient 2

This 6-month-old male sibling was studied after the diagnosis was established in his sister (Patient 1). He was born at term with a birth weight of 3.8kg. He had roving nystagmus detected when he was 3-months old. Otherwise he had normal neurological examination and developmental milestones. He was having mild upper respiratory infection when the following results of investigation were obtained: arterial blood pH 7.365; bicarbonate 10.8mmol/L; BE -14.6mmol/L; anion gap 23.3mmol/L; ammonia 93.6µmol/L; lactate 2.26mmol/L and urine ketone was negative. Other routine biochemistry and haematology tests were normal. Metabolic acidosis was easily corrected with iv sodium bicarbonate. His blood acylcarnitines and urine organic acids analyses demonstrated similar profiles as in his sister. This is shown in figure 1(c) and figure 2(c).

He was followed up at 9 months. He was healthy except for the nystagmus, which was still present. His weight was 10.2kg (75th centile), height 75cm (75th centile) and head circumference 45.5cm (50th centile). His MRI brain also revealed normal findings.

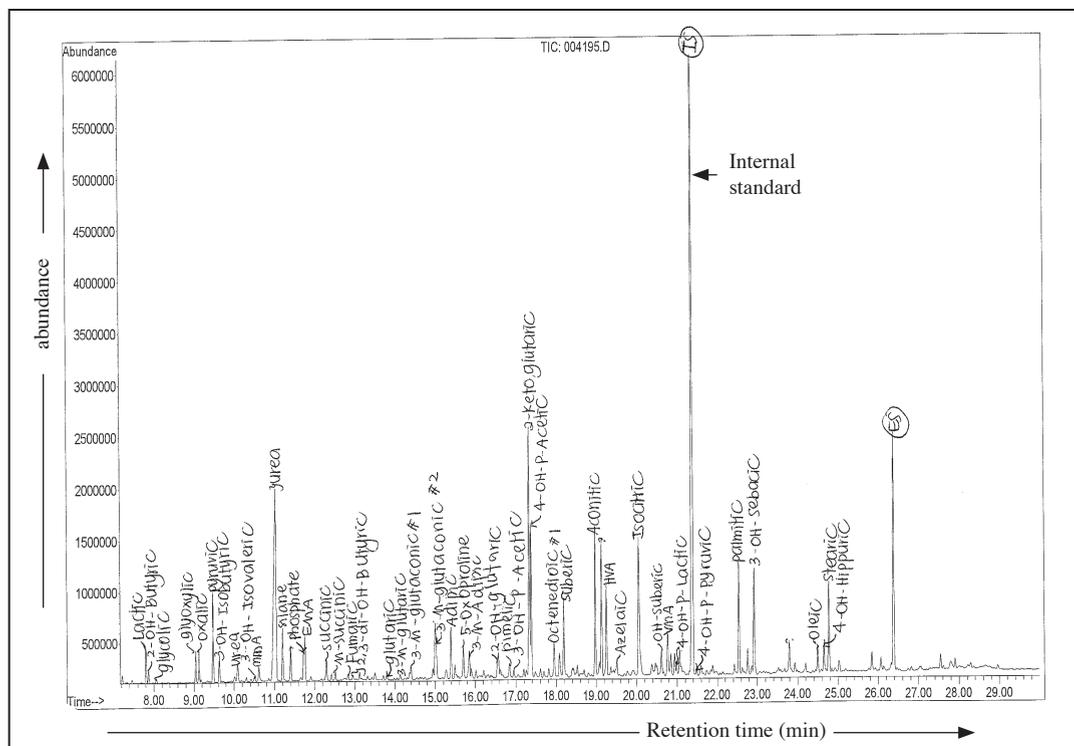


Figure 1(a). Urine organic acids chromatogram of a normal control

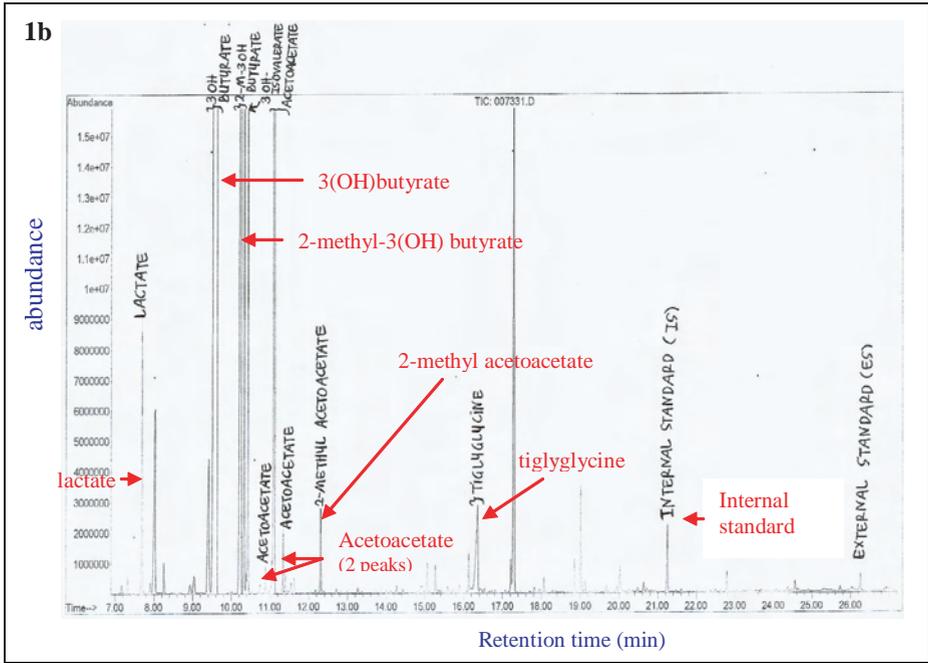


Figure 1(b). Urine organic acids chromatogram for Patient 1

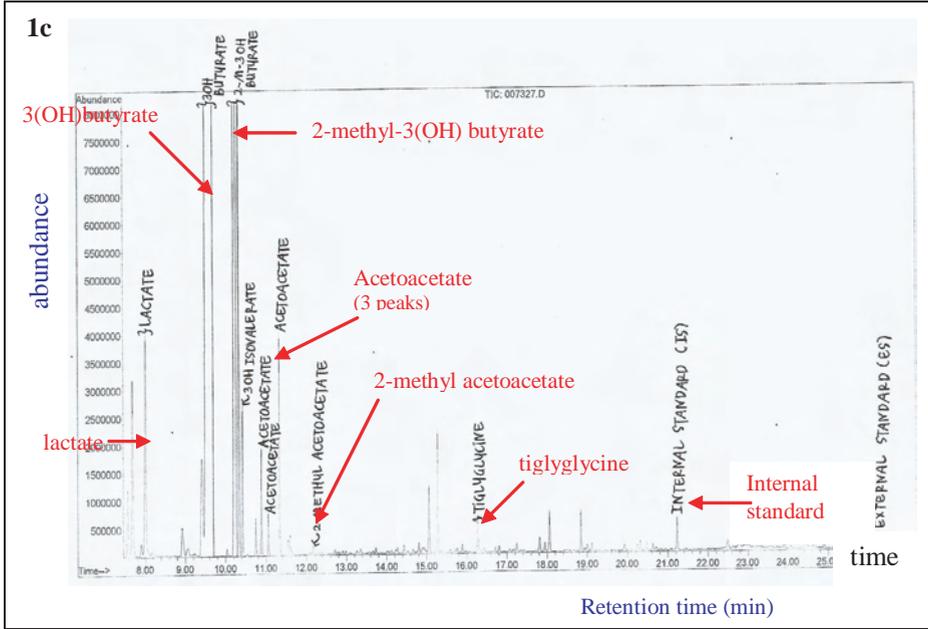


Figure 1(c). Urine organic acids chromatogram for Patient 2

FIG. 1 (a) Urine organic acids chromatogram of a normal control. The analytes are separated based on their retention times. The identity of each analyte is determined by mass spectral analysis. The height of the peak corresponds to the abundance of each analyte. n-Pentadecanoic acid (PDA, 1mg/mg of creatinine) is added as an internal standard. Abnormal analytes are significant if their peaks are higher than the internal standard. In a normal control, small peaks of various metabolites such as lactate, glycolic, succinate, etc are usually present. (b) Urine organic acids chromatogram for Patient 1. The urine which was collected during an acute illness shows presence of following metabolites: lactate, 3-hydroxybutyrate, acetoacetate, 2-methyl acetoacetate, 2-methyl-3-hydroxybutyrate and tiglyglycine, all in an abnormally high abundance in comparison to the internal standard. (c) Figure 1(c). Urine organic acids chromatogram for Patient 2. Patient 2's urine shows similar pattern as Patient 1 although he only had mild clinical symptoms.

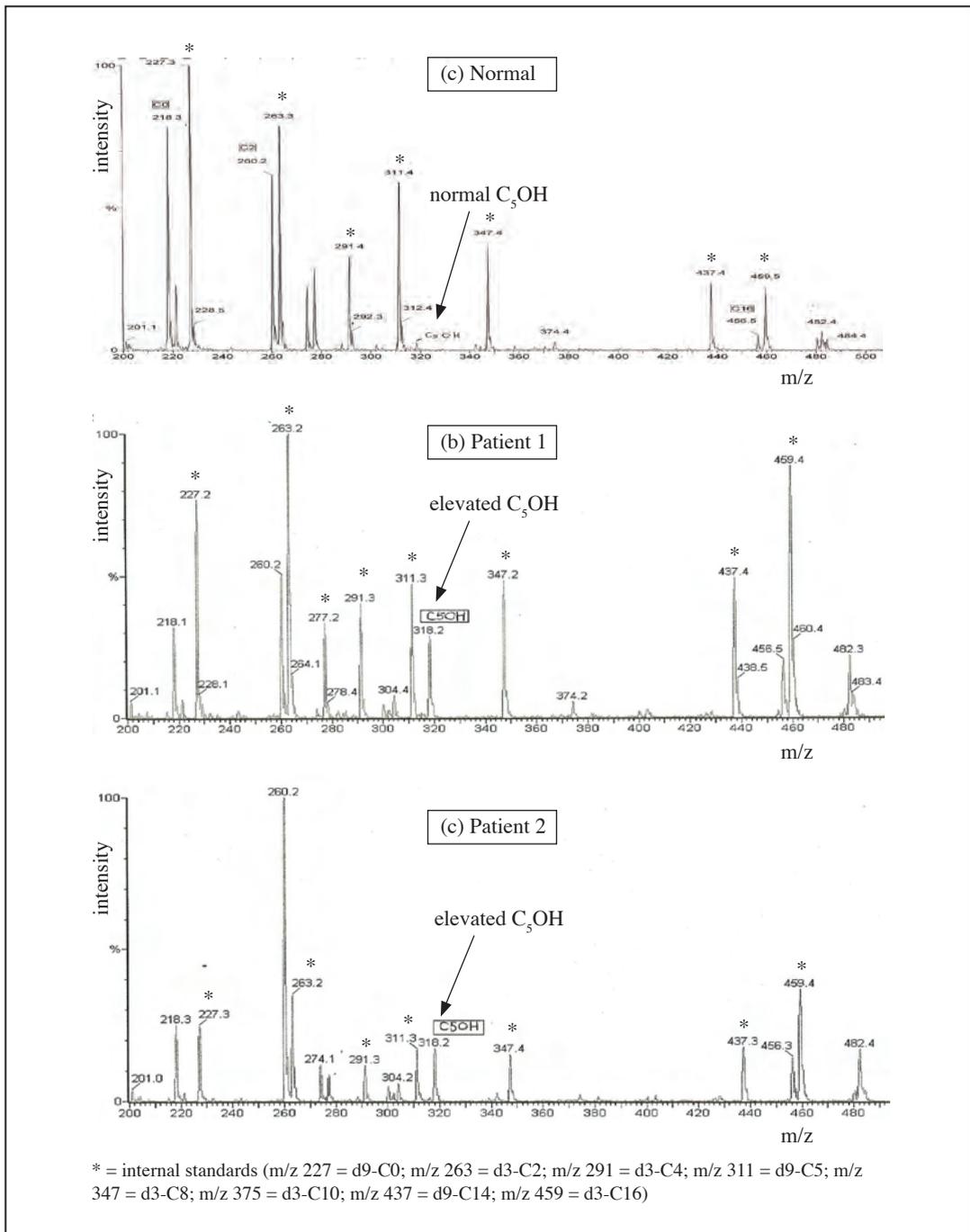


FIG. 2 (a). Blood acylcarnitines profile of a normal control. The analytes are separated according to their mass (m/z). A number of stable and isotopically labelled acylcarnitines are added in known amounts as internal standards. The ratio between labelled standards and analytes provides the concentration of the analyte in the sample. (b) Blood acylcarnitines profile of Patient 1. The profile shows increased C₅OH (representing 2-methyl-3-hydroxybutyrylcarnitine). (c) Blood acylcarnitines profile of Patient 2 that shows similar finding as in Patient 1.

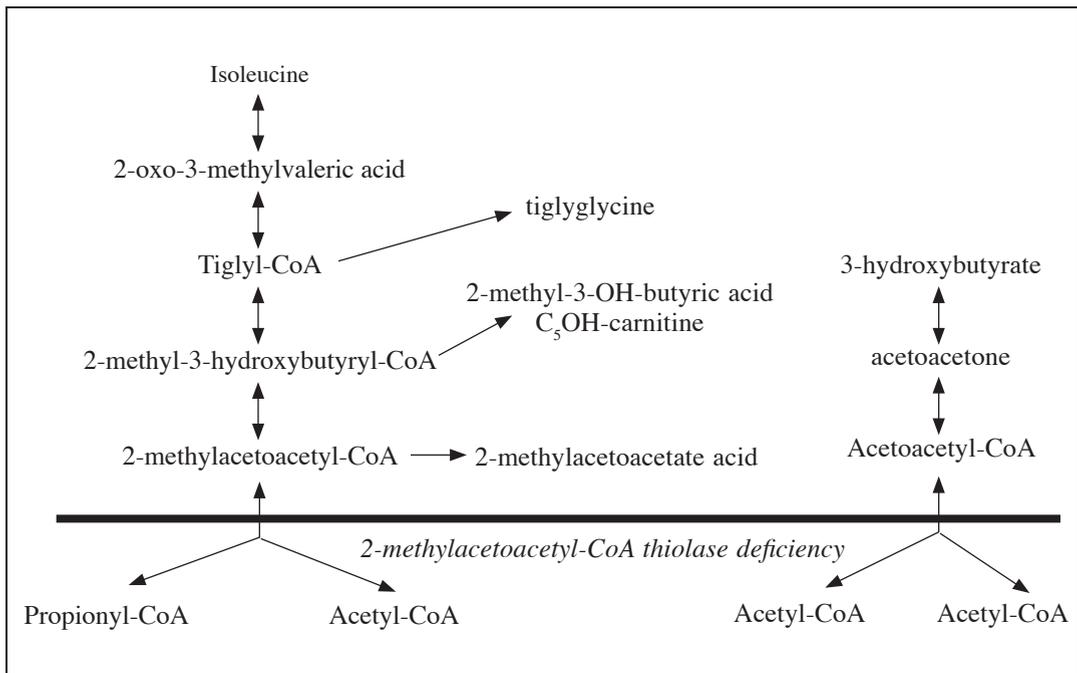


FIG. 3. Defective thiolysis of 2-methylacetoacetyl CoA in isoleucine catabolism resulting in accumulation of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate and tiglylglycine. Impaired thiolysis of acetoacetyl-CoA to acetyl-CoA in extrahepatic tissues results in accumulation of acetoacetate and 3-hydroxybutyrate. Deficiency of 2-methylacetoacetyl-CoA thiolase has a greater impact on extrahepatic ketolysis than hepatic ketogenesis.

DISCUSSION

We highlight here the importance of including blood acylcarnitines and urinary organic acid analysis in the investigation of severely ketoacidotic children, especially if there are other risk factors such as consanguinity and family history to indicate the possibility of an underlying inborn error. In our patients, the presence of large quantities of metabolites from isoleucine degradation pathway in their body fluids indicates an underlying inherited metabolic disease rather than non-specific ketonuria, which is not uncommon in an acutely ill child. There are several known organic acidemias due to defective isoleucine and other branched-chain amino acid catabolism such as methylmalonic acidemia, propionic acidemia, isovaleric acidemia and mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency. All of them present similarly with acute ketoacidosis episodes in childhood, frequently precipitated by acute catabolic stress. Profiling of blood acylcarnitines and urine organic acids will differentiate these different disorders.

Mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency is due to mutation in

the *ACAT1* gene. This enzyme is a short-chain-specific thiolase. It has an important role in isoleucine degradation and ketone body metabolism.^{1,3} A cytosolic form of 2-methylacetoacetyl-CoA thiolase is also in existence. However, it is encoded by a different gene (*ACAT2*, EC 2.3.1.9.), and has a different function, which is involved in cholesterol synthesis.¹

The blood acylcarnitines profile may provide supportive evidence for mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency.^{1,4,5} Acylcarnitines profiling can be done by using dry blood spots on a Guthrie card, which is easy to collect and can be mailed rapidly to the diagnostic centre. An abnormal acylcarnitines profile should be followed up with a careful analysis of urine organic acids that is able to confirm the diagnosis of mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency. Confirmation of the diagnosis by enzyme assay is desirable especially in cases that do not show typical metabolic abnormalities. Mitochondrial 2-methylacetoacetyl-CoA thiolase is K^+ -dependent for its activation, whereas the cytosolic thiolase is not. A specific direct assay of the K^+ -activated mitochondrial thiolase

activity using acetoacetyl-CoA as substrate can be performed in cultured fibroblasts.⁶

According to Fukao *et al*, the median age at onset for the first ketoacidotic episode in mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency is 15 months. As many as 50% of them had experienced recurrent episodes of ketoacidosis.³ The main advantage of diagnosing this disorder early is that adverse clinical consequences such as death during acute ketoacidotic episode and long term cognitive impairment are avoidable with appropriate intervention. The most important aspect of medical care of patients with this disorder is timely institution of supportive therapy during acute ketoacidosis. Long term management consists of avoidance of fasting, provision of an “emergency dietary regimen” during acute febrile illnesses to reduce catabolism, oral carnitine supplementation and modest protein restriction. A favourable outcome is expected in most patients if they are diagnosed early.

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