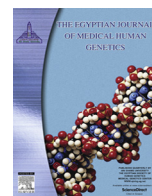


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The Egyptian Journal of Medical Human Genetics

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Case Report

Two Libyan siblings with beta-ketothiolase deficiency: A case report and review of literature



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ARTICLE INFO

Article history:

Available online 4 January 2017

Keywords:

T2 deficiency

ACAT1

Mutation

Transient expression analysis

Ketone bodies

Metabolic encephalopathy

ABSTRACT

Beta-ketothiolase (mitochondrial acetoacetyl-CoA thiolase, T2) deficiency is an autosomal recessive disorder characterized by impaired metabolism of ketones and isoleucine. In this study, we report on the first two siblings with T2 deficiency from Libya. Both siblings presented with ketoacidosis, but the severity and outcomes were quite distinctive. T2 deficiency in patient 1, the younger sister, manifested as recurrent severe episodes of ketoacidosis during the first year of life. She unfortunately experienced neurodevelopmental complications, and died at 14 months old, after her 5th episode. In contrast, patient 2, the elder brother, experienced only one ketoacidotic episode at the age of 4 years. He recovered uneventfully and has continued to achieve age-appropriate development to date. Upon analysis, the siblings' blood acylcarnitine profiles had shown increased levels of C5:1 and C5-OH carnitine. *ACAT1* mutational analysis revealed patient 2 is homozygotic for a novel mutation–c.674C > A (p.Ala225Glu); this mutation was then confirmed by familial analysis. Transient expression analysis of c.674C > A mutant T2 cDNA revealed neither potassium ion-activated acetoacetyl-CoA thiolase activity, which represents T2 activity, nor mutant T2 protein. Therefore, this mutation is truly pathogenic. Interestingly, the incidence of T2 deficiency may be high among the Arab population. This disease should be considered in the differential diagnosis for unexplained ketoacidosis in children. Patients with T2 deficiency could have a favorable outcome if diagnosed and treated early.

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1. Introduction

Beta-ketothiolase deficiency (Online Mendelian Inheritance in Man [OMIM] 203750, 607809), a defect in mitochondrial acetoacetyl-CoA thiolase (T2) (EC 2.3.1.9, gene symbol *ACAT1*), is an autosomal recessive disease that affects isoleucine catabolism

and ketone body utilization [1,2]. Since Daum et al. first characterized T2 deficiency in 1971, more than 100 patients have been diagnosed with the disease worldwide [3]. T2 deficiency ordinarily manifests as intermittent ketoacidotic episodes between the ages of 6 and 18 months; patients are usually asymptomatic between episodes. A distinctive laboratory finding is an increased urinary excretion of isoleucine-catabolic intermediates 2-methyl-3-hydroxybutyrate (2M3HB), 2-methylacetoacetate (2MAA), and tiglylglycine (TIG). In addition, blood acylcarnitine analysis usually shows elevated C5:1 and C5-OH carnitine levels [1,2]. Nevertheless, some T2-deficient patients demonstrated atypical clinical and laboratory features. The severity and frequency of episodes vary among patients; however, the outcome is commonly favorable, unless a ketoacidotic episode causes life-threatening or irreversible sequelae [1,3].

The human *ACAT1* gene is located at chromosome 11q22.3–23.1. It spans nearly 27 kb and contains 12 exons and 11 introns.

Abbreviations: 2MAA, 2-methylacetoacetate; 2M3HB, 2-methyl-3-hydroxybutyrate; MS/MS, tandem mass spectrometry; SCOT, succinyl-CoA:3-oxoacid CoA transferase; T2, mitochondrial acetoacetyl-CoA thiolase; TIG, tiglylglycine; TKB, total ketone body.

Peer review under responsibility of Ain Shams University.

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<http://dx.doi.org/10.1016/j.ejmhg.2016.11.001>

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T2 cDNA is approximately 1.5 kb in length and encodes a precursor protein consisting of 427 amino acids [4]. *ACAT1* mutations are highly diverse; more than 70 mutations have been identified to date. Except for p.Arg208* among the Vietnamese, no other common *ACAT1* mutation patterns have yet been recognized [3,5].

There are few studies on T2 deficiency in the Arab population. Several cases have been detected by tandem mass spectrometry (MS/MS), but the diagnoses were seldom confirmed by an enzyme assay or genetic analysis [6–18]. In this study, we report on the first two Libyan siblings, sister and brother, with T2 deficiency. We describe their clinical and laboratory features and characterize a novel *ACAT1* mutation. We also discuss the current situation of T2 deficiency among Arab population and review main characteristics of this disorder, which may point to diagnostic clues.

2. Patients and methods

Patient 1: The female patient was the third of four children born to first cousins parents of Arab ancestry in Libya. She was delivered at full term following an uneventful gestation, weighing 3 kg. There was no prior remarkable family history, as her elder brother, patient 2, was asymptomatic at that time. The patient thrived until 20 days of age when she was admitted to a hospital with vomiting, diarrhea, dehydration, and acidotic breathing. Symptomatic management, including intravenous fluids, was given; she recovered uneventfully. A hydrolyzed milk formula was prescribed for presumed cow's milk protein intolerance. No additional investigation was performed for this episode.

At 7 months of age, the patient was admitted to an intensive care unit with dehydration, acidotic breathing, and lethargy. This was preceded by fever, vomiting, and diarrhea for 2 days. Physical examination revealed hepatomegaly, marked hypotonia, hyporeflexia, and abnormal pupillary response to light, showing no reaction on the right and a sluggish reaction on the left. Arterial blood gases revealed a severe metabolic acidosis: pH 6.8, HCO_3^- 3 mmol/L, base excess –24, and pCO_2 15 mmHg. Ketonuria was detected. A blood count demonstrated leukocytosis ($31 \times 10^3/\mu\text{l}$), mild anemia (9.6 gm/dl), and normal platelets. Other investigations showed a blood glucose of 2.5 mmol/L, lactate 2 mmol/L, and ammonia 145 $\mu\text{mol/L}$; her electrolytes and liver and kidney function tests revealed no significant abnormalities. Management for an assumed central nervous system infection, such as meningitis or encephalitis, was implemented. After 12 days, her general condition was stabilized. She was then referred to a metabolic unit, where an organic aciduria was suspected. A subsequent acylcarnitine analysis of dried blood spots showed increased C5:1 (0.34 $\mu\text{mol/L}$; cut-off point 0.12) and C5-OH (2.68 $\mu\text{mol/L}$; cut-off point 0.47) levels. Precautionary measures, including avoidance of prolonged fasting, protein restriction (1.5 g/kg/day), carnitine supplementation (100 mg/kg/day), and early recognition of illness symptoms, were prescribed.

Follow-up demonstrated a neurodevelopmental delay, irritability, progressive dystonia, choreic movements, and nystagmus. At 12 months of age, a brain magnetic resonance imaging was conducted. This showed cerebral atrophy and bilateral hyperintense areas involving caudate and putamen nuclei on T2-weighted images. An electroencephalogram showed hypsarrhythmia. Two mild ketoacidotic episodes, precipitated by infections, recurred at 9 and 12 months of age. At 14 months of age, the patient experienced her final episode, which was more severe than her 2nd episode. She succumbed to her disease at that time.

Patient 2: The male patient, the elder brother of patient 1, was the firstborn child of his parents. There were no remarkable antenatal events. He was born at full term with a birth weight of 4 kg. He was well until 4 years and 2 months of age when he was admit-

ted to an intensive care unit with vomiting, acidotic breathing, lethargy, and generalized convulsions. This was preceded by fever, sore throat, and poor oral intake for 5 days. Arterial blood gases showed pH 7.11, HCO_3^- 3.4 mmol/L, base excess –20, and pCO_2 4.3 mmHg. Urinary ketone body testing was positive. Other investigations revealed severe hypoglycemia (0.95 mmol/L), ammonia 144 $\mu\text{mol/L}$, and lactate 1.67 mmol/L. Patient 2 was provisionally diagnosed with T2 deficiency because his sister (patient 1) had already been diagnosed with this disease one month before. Therefore, a timely blood acylcarnitine analysis was performed. It demonstrated increased C5:1 (0.41 $\mu\text{mol/L}$) and C5-OH (4.13 $\mu\text{mol/L}$) levels. He was treated successfully with appropriate intravenous glucose, fluids, bicarbonate, and electrolytes. The previously-described precautionary measures were reinforced. At his current age of 7.5 years, he has not suffered of any further episodes. He has attained age-appropriate growth and development. Recently, dried blood spots were obtained from the living family members for an *ACAT1* mutational analysis.

This study was approved by the Ethical Committee of the Graduate School of Medicine, Gifu University, Japan. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consents were obtained from all participants or their parents for inclusion in the study.

2.1. Mutation analysis

Small pieces (1.2×1.2 mm) were used for direct PCR amplification of DNA from dried blood specimens. The 12 *ACAT1* exons, with flanking intron regions, were amplified by primer pairs as described before [19]. PCR conditions for all segments, using a Biotaq™ HS DNA Polymerase (Bioline Reagents Ltd, London, UK), were 95 °C for 10 min; 40 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min; followed by 72 °C for 7 min. The 12 amplified fragments were sequenced using a BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) per the manufacturer's directions. The genomic *ACAT1* sequence (GenBank accession NG_009888.1) was used as a reference.

2.2. Transient expression analysis

A pCAGGS eukaryote expression vector was used for transient expression analysis of T2 cDNA, as previously described [19–21]. In brief, we used a Kod-Plus-Mutagenesis Kit® (Tyobo Co., Osaka, Japan) to construct full-length mutant cDNA p.Ala225Glu. Wild-type and mutant constructs were transfected via Lipofectamine® 2000 (Invitrogen, San Diego, CA, USA) into 5×10^5 SV40-transformed T2-deficient fibroblasts. After incubation at 37 °C for 72 h, cells were harvested and stored at –80 °C until needed. After that, cells were freeze-thawed and sonicated in 50 mM sodium phosphate (pH 8.0) and 0.1% Triton X-100. This was followed by centrifugation at 10,000g for 10 min. Supernatants from cell extracts were used for enzyme assay and immunoblot analysis.

We spectrophotometrically monitored the decrease of acetoacetyl-CoA absorbance at 303 nm, which is caused by acetoacetyl-CoA thiolysis to acetyl-CoA. Acetoacetyl-CoA thiolase activity, was measured in the absence and presence of potassium ions. Potassium ions specifically stimulate T2; hence, the difference represents T2 activity [19,21]. We calculated the average and standard errors of three independent experiments. An immunoblot analysis was performed as previously described [19,22]. We used a mixture of an anti-T2 and anti-succinyl-CoA:3-oxoacid CoA transferase (SCOT) antibodies as the first antibody. Serial dilution samples extracted from the wild-type (10, 5, 2.5, and 1.25 μg) were

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