A Novel Mutation in the OXCT1 Gene Causing Succinyl-CoA:3-Ketoacid CoA Transferase (SCOT) Deficiency Starting with Neurologic Manifestations

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Davoud AMIRKASHANI[1],
Mostafa ASADOLLAHI[2, 3],
Rozita HOSSEINI[4],
Saeed TALEBI[5],
Zahra GOLCHEHRE[2, 3],
Mohammad KERAMATIPOUR[2, 3]

1. Division of Endocrinology and Metabolism, Ali Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
2. Watson Genetic Laboratory, Tehran, Iran
3. Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Pediatric Nephrology, Ali Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
5. Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

Corresponding Author
Keramatipour M. MD. PhD
Department of Medical Genetics,

Abstract

Succinyl-CoA:3-oxoacid CoA-transferase (SCOT) deficiency is an inborn error of ketone body utilization characterized by intermittent ketoacidosis crises. This study reports the first Iranian patient with SCOT deficiency who presented with seizure and hypotonia at birth. Accordingly, she was consequently re-hospitalized due to hypotonia and respiratory distress. Laboratory tests revealed hyperammonemia, ketonuria, and metabolic acidosis. Besides, the plasma glucose level was normal without any other abnormality. Despite treatment with high-dose bicarbonate, severe acidosis persisted. Poor response to treatment raised a significant diagnostic challenge among specialists until genetic investigation identified a homozygous nonsense mutation (c.79G>T; p.Gly27*) in the OXCT1 gene (NM_000436), causing SCOT deficiency. Genetic studies help clinicians achieve a definite diagnosis of such metabolic disorders. In this case, the accurate and early diagnosis of SCOT deficiency opened new therapeutic possibilities, including frequent carbohydrate-rich meals and low fat and protein diet. Moreover, our findings expand the mutational and clinical spectrum of SCOT deficiency.

Keywords: Succinyl-CoA:3-oxoacid CoA transferase deficiency; Iran; Next-generation sequencing (NGS), OXCT1 gene
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Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email:keramatipour@sina.tums.ac.ir

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Introduction

Ketone bodies, including β-hydroxybutyrate (bHB), acetoacetate (AcAc), and acetone, are small lipid-derived molecules that provide a circulating energy source for tissues during fasting or prolonged exercise (1). Ketone bodies are produced in hepatic tissue by breaking down fatty acids and ketogenic amino acids and transported into extrahepatic tissues for terminal oxidation. Ketolysis does not progress in the liver. During the process of ketolysis, bHB is primarily oxidized to AcAc. Then, with succinyl-CoA donating its CoA to AcAc, AcAc is converted into acetoacetyl-CoA by SCOT. This enzyme is expressed in all mammalian cells that harbor mitochondria except those of hepatocytes. Finally, acetyl-CoA acetyltransferase1 (ACAT1) breaks acetoacetyl-CoA into two acetyl-CoAs, which are involved in the tricarboxylic acid (TCA) cycle as substrates to supply ATP (1-5). A sketch of ketone body metabolism is shown in Figure 1(5).

Figure 1. A sketch of ketone body metabolism (Puchalska, P., & Crawford, P. A. (2017)).

SCOT deficiency is characterized by recurrent episodes of severe ketoacidosis and is usually asymptomatic between the episodes, even though permanent ketosis or ketonuria is a pathognomonic feature of this disorder. Tachypnea is often accompanied by hypotonia and lethargy, and 30 % of patients become symptomatic within a few days of birth. Most patients present symptoms later in the first year. A few patients have had cardiomegaly on admission. Blood glucose, lactate, and ammonia concentrations are generally normal, though there have been reports of hypoglycemia in neonates and hyperglycemia in older children (6-8).

Urinary organic acid analysis and acylcarnitine analysis show non-specific profiles in this disorder. Hence, in vitro diagnosis methods, such as enzyme assay and mutation analysis, are essential for a definite diagnosis.

This study presents an Iranian infant with a novel homozygous nonsense mutation in OXCT1 causing Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency with neurologic manifestations at first.

Case presentation

The patient is a nine-month-old Iranian girl born to consanguineous parents (Figure 2) with normal growth parameters in February 2020. She was referred to our hospital with recurrent, refractory metabolic acidosis. She was not discharged from the hospital at birth due to seizures and hypotonia. The patient went home after ruling out sepsis and conducting common interventions such as anticonvulsants and hydration. She was re-hospitalized at three months of age due to hypotonia remaining for several days and respiratory distress. Laboratory test results showed metabolic acidosis (PH=7.01, PCO2= 12.1 mmHg, HCO3=3.3 mmol/L, BE= -19.6 mmol/L) with an anion gap of 15, hyperammonemia (180 µmol/lit), and ketonuria (4+). The plasma glucose level was normal without any other abnormality. How accurate was the diagnosis of renal tubular acidosis (RTA)? However, after metabolic evaluations,

organic academia (OA) treatment started with biotin, hydroxy cobalamin, L-carnitine, bicarbonate, and Na-benzoate. Despite high-dose bicarbonate treatment, severe acidosis persisted. Therefore, after consultation with a nephrologist, peritoneal dialysis was performed. After some days, metabolic data showed a significant increase in ketones, lactic acid, and adipic acid (Table 1). These results led to some challenges between nephrologists and metabolic specialists regarding diagnosis (OA or RTA). Finally, after several dialyzes and a high-dose bicarbonate infusion, she was discharged with a cocktail of organic acidemia, poly citrate solution, and low protein formula.

The patient was admitted for the third time with tonic-clonic seizure, respiratory distress, and severe metabolic acidosis (anion gap=18). She was not able to sit at the age of 11 months. Metabolic status was re-evaluated immediately and only showed an increase in ketone level in urinary organic acids analysis without any other helpful clues. Acidosis was severe and refractory. Therefore, dialysis was performed as per the previous admission. The catastrophic status of the infant in every hospitalization convinced us to carry out a genetic study.

Genetic investigation

At the first step of genetic investigations, the patient's blood sample was collected in an EDTA tube. According to the manufacturer's instructions, genomic DNA was extracted from whole blood using a Blood SV-mini kit (GeneAll Biotechnology Co., LTD, South Korea). According to manufacturer instructions, library preparation was performed using the Twist Human Core Exome kit (Twist Bioscience, USA). Sequencing of libraries was done by high-throughput paired-end sequencing using the NovaSeq sequencing platform (Illumina Inc., CA, USA).

Sequencing data were analyzed using the Genome Analysis Toolkit (GATK-v3.4.0), and detected variants were annotated. Proper filtering and the pathogenicity interpretation of a short list of variants were performed based on the ACMG (American College of Medical Genetics and Genomics) guidelines for variant interpretation (9). The above investigation resulted in the detection of a novel homozygous nonsense variant (c.79G>T; p.Gly27*) in exon 2 of the OXCT1 gene (NM_000436). Mutations in this gene cause Succinyl CoA:3-oxoacid CoA transferase deficiency with autosomal recessive inheritance. Although the detected variant in this patient has not been previously reported for its pathogenicity, null variants (including nonsense variants) in the OXCT1 gene are a known disease mechanism.

The homozygosity of the detected variant in the patient and heterozygosity in her parents were validated by Sanger sequencing (Figure 3). This finding was consistent with the autosomal recessive pattern of inheritance.

In addition, multiple lines of in silico computational analysis (Mutation Taster, CADD, and DANN) support the deleterious effect of this variant on the gene or gene product(s). The variant is absent in population databases (gnomAD, ExAC, 1000G, and our local database), and it was classified as a pathogenic variant based on ACMG guidelines. The genetic diagnosis was confirmed based on this classification and the consistency of the patient phenotype with the associated disease.

Table 1. Urinary organic acids profile of patient.

Urinary Organic acids (urine by GC-MS and LC-MS/MS)		
Urinary metabolite	Patient (mmol/molcrt)	Normal range(mmol/molcrt)
Lactic acid	303	<60
Acetoacetic acid	949	<5
3-hydroxy butyric acid	1125	<5
Adipic acid	39.4	<6

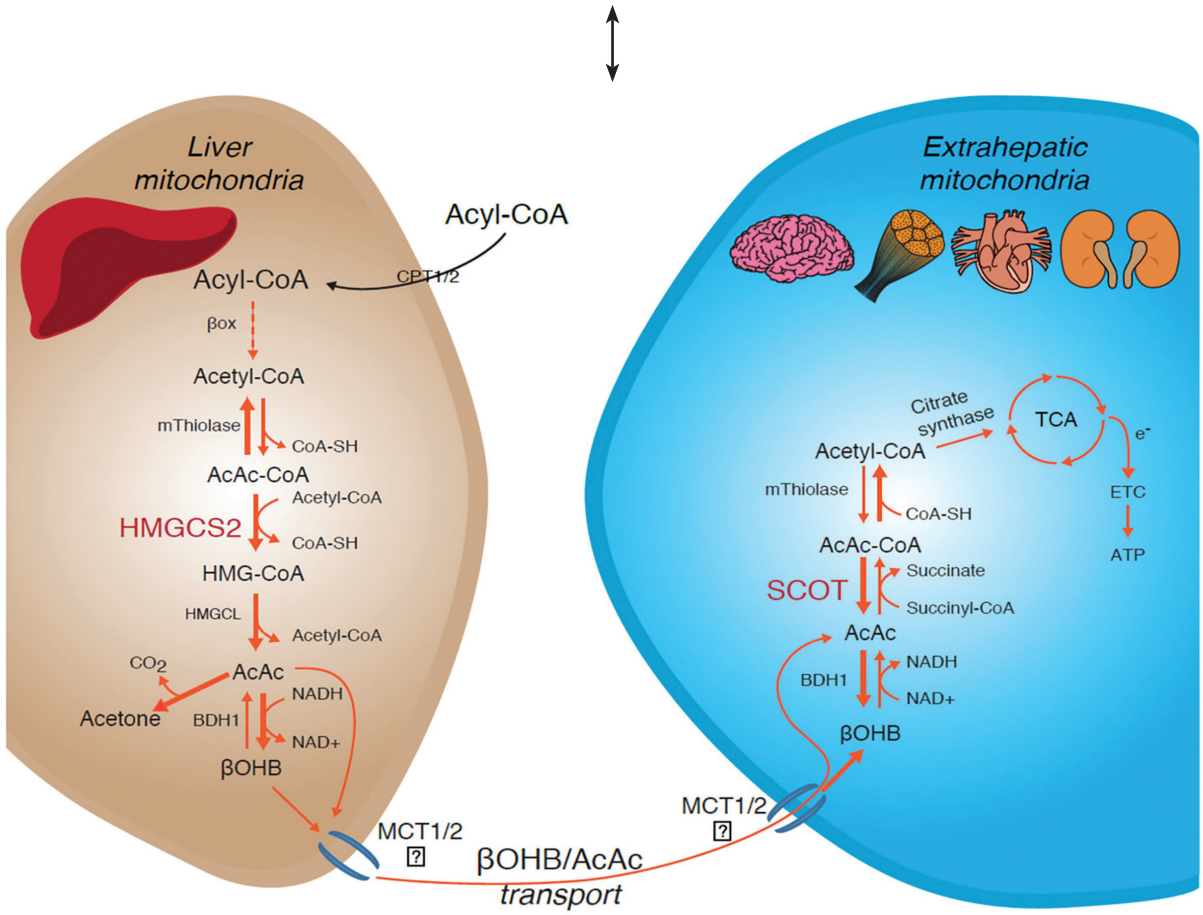

Figure 1. *A sketch of ketone body metabolism.*

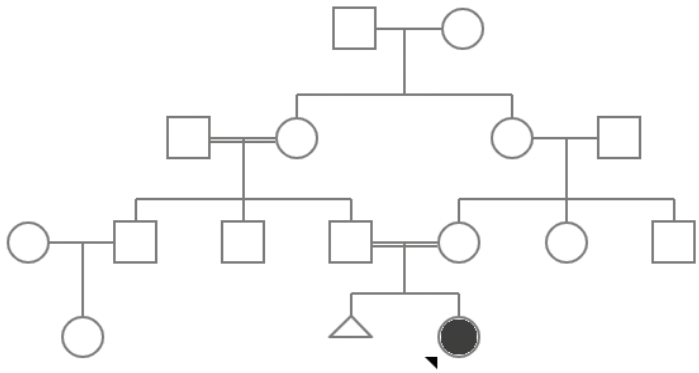

Figure 2. *Pedigree of investigated patient. Proband has been shown in filled square.*

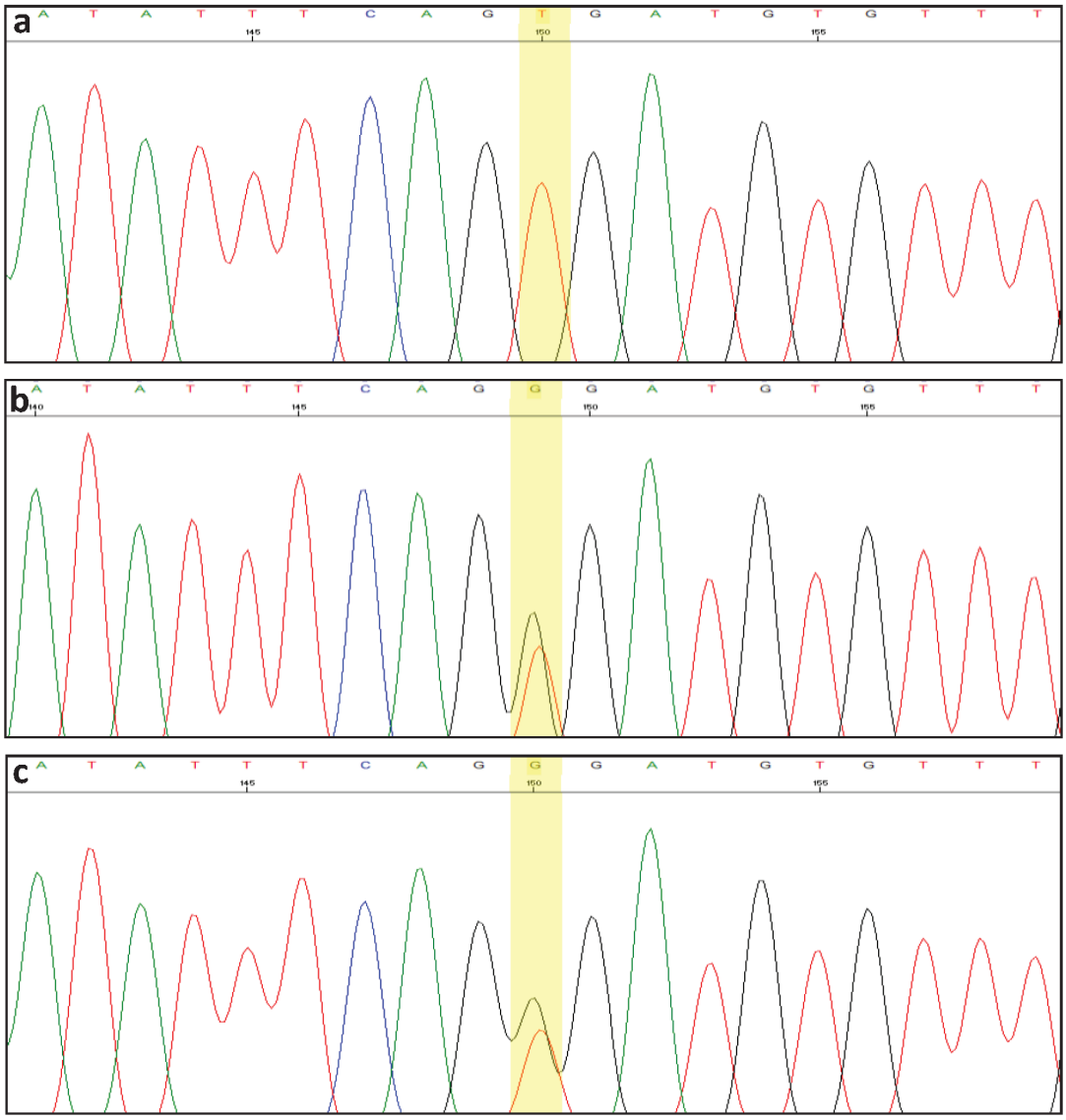

Figure 3. *Homozygosity is shown in the patient for variant c.79G>T in OXCT1 gene by Sanger sequencing* (**a**). *Heterozygosity is shown in his mother* (**b**) *and his father* (**c**).

Discussion

After diagnosis, treatment with frequent carbohydrate-rich meals and low fat and protein diet, including cornstarch during mild infections and intercurrent illnesses, is advisable to prevent metabolic decompensation. Therefore, treatment is easy; if these cases are not missed, they can be saved from fatal episodes (10, 11).

Although common clinical presentations of SCOT deficiency are explained in different literature, such as hypo/hyperglycemia, permanent or sudden ketonuria, and metabolic acidosis, a physician should not rule out SCOT deficiency just with the absence of ketonuria or abnormal plasma glucose. Because typically not associated with significant abnormalities in blood glucose, but refractory and severe metabolic acidosis is characteristic of this disorder either permanently or suddenly, and permanent ketosis or ketonuria will be a pathognomonic feature of this disorder (7, 8, 12, 13).

In contrast with most organic acidemias, no diagnostic metabolites are observed in blood and urine samples from SCOT-deficient patients. Although ketone body levels are elevated (13), equivocal symptoms with different diagnoses, such as organic academia, diabetic ketoacidosis, glycogen storage disorders, renal tubular acidosis, and adrenal and pituitary insufficiency may lead to misdiagnosis and prolong proper treatment. Therefore, a genetic study will be necessary for narrowing the differential diagnosis, in addition to the history, clinical examinations, and para-clinical data (14, 15).

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Author's Contribution

The authors confirm contribution to the paper as follows:

study conception and design: Davoud Amirkashani,mohammad keramatipour<rozita hoseini,said talebi,zahra golchehre and mostafa asadollahi

data collection: Davoud Amirkashani

analysis and interpretation of results: Davoud Amirkashani,mohammad keramatipour,

draft manuscript preparation: Davoud Amirkashani,zahra golchehre,mohammad keramatipour. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors have no conflict of interest to disclose.

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