Identification of Novel Compound Heterozygous Mutations in the \textit{ACADS} Gene of an Asymptomatic Korean Newborn with Short Chain Acyl-CoA Dehydrogenase Deficiency by Tandem Mass Spectrometry

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Short-chain acyl-CoA dehydrogenase deficiency (SCADD; OMIM \# 201470) is an autosomal recessive inborn error of mitochondrial fatty acid \(\beta\)-oxidation, presenting with a variety of clinical signs and symptoms. Developmental delay, hypertonia or hypotonia, ketotic hypoglycemia, and epilepsy are most frequently reported. In general, patients diagnosed through newborn screening have shown normal growth and development in contrast to those diagnosed as a result of clinically initiated evaluations. Here, the case of an asymptomatic Korean newborn with SCADD identified by tandem mass spectrometry is reported. The patient showed an elevated concentration of butyrylcarnitine detected on newborn screening. Urinary excretion of ethylmalonic acid was elevated by urine organic acid analysis. To confirm the diagnosis of SCADD, a direct sequencing analysis of 10 coding exons and the exon-intron boundaries of the \textit{ACADS} gene were performed. Genetic analysis of \textit{ACADS} showed the following novel compound heterozygous missense mutations: c.277C>A (p.Leu93Ile) on exon3 and c.682G>A (p.Glu288Lys) on exon6. These results will provide further evidence of mutational heterogeneity for SCADD.

Key Words: Short-chain acyl-CoA dehydrogenase deficiency, \textit{ACADS} gene, Novel mutations, Tandem mass spectrometry

Introduction

Short-chain acyl-CoA dehydrogenase deficiency (SCADD; OMIM\# 201470) is a rare fatty acid oxidation (FAO) disorder with variable clinical presentations.\textsuperscript{1,2} SCADD catalyzes the dehydrogenation of butyryl-CoA (C\textsubscript{4}-CoA) during the first step of the short-chain fatty acid \(\beta\)-oxidation spiral.\textsuperscript{1,2} Impaired SCAD activity results in accumulation of its substrate (C\textsubscript{4}-CoA) and the subsequent production of alternative metabolites, including the following: the corresponding carnitine-ester, i.e., butyryl carnitine (C\textsubscript{4}-C), the corresponding glycine-ester (butyryl glycine), butyrate, and ethylmalonic acid (EMA). C\textsubscript{4}-C, measured in blood, and EMA, measured in urine, are generally used as biochemical markers for SCADD.\textsuperscript{3} The clinical presentation is characterized by hypotonia, developmental delay, seizures, microcephaly, lethargy, scoliosis, and finally a combination of hypoglycemia, vomiting, poor feeding, and...
failure to thrive.\textsuperscript{39} Newborns with SCADD are now being identified due to widespread implementation of expanded newborn screening by tandem mass spectrometry.\textsuperscript{4} In general, patients diagnosed through newborn screening have shown normal growth and development in contrast to those diagnosed as a result of clinically initiated evaluations.\textsuperscript{4}

We recently examined an asymptomatic Korean newborn with SCADD with novel mutations of the \textit{ACADS} gene. Here, we report on this patient, with a review of the literature.

**Case Report**

The female patient was born at 39 weeks of gestation, with a birth weight of 3,700 g. She was the first child of healthy non-consanguineous Korean parents. The pregnancy with the patient was not complicated by hypertension or HELLP (Hemolysis, Elevated Liver Enzyme, and Low Platelet Count) syndrome. Her neonatal period was unremarkable. Birth height, weight, and head circumference were 58 cm (75-90th percentile), 3,700 g (75th percentile), and 39 cm (90-95th percentile), respectively. She was referred to our clinic on day 30 of life for abnormal newborn screening test result (butyrylcarnitine 1.79 umol/L; normal range <1.5 umol/L). She showed no symptoms or signs. Laboratory-investigations (L-carnitine, lactate/pyruvate, ammonia, glucose, blood pH, ketone, and transaminases) were normal, with the exception of slightly elevated CK (244 U/L, normal range: 5-217). Urinary organic acid analysis on admission showed increased amounts of EMA (77.92 mmol/mol Cr; normal range <14.6 mmol/mol Cr) and methylsuccinic acid (MSA; 21.58 mmol/mol Cr; normal range <8.8 mmol/mol Cr). Echocardiogram examination showed normal. On the basis of the findings of the urinary organic acid and tandem mass analysis, SCADD was strongly suspected. To confirm the diagnosis of SCADD, we performed a direct sequencing analysis of 10 coding exons and the exon-intron boundaries of the \textit{ACADS} gene. Informed consent was obtained by the parents. Genetic analysis of \textit{ACADS} revealed that she carries the compound heterozygous missense mutations c.277C>A (p.Leu93Ile) on exon3 and c.682G>A (p.Glu228Lys) on exon6 (Fig. 1). Both mutations are novel. These compound heterozygous mutations were derived from the mother (p.Glu228Lys) and father (p.Leu93Ile). In addition, seven known polymorphisms were detected, specifically c.321T>C, c360C>T, IVS5(-99)T>C, IVS6(-43)C>T, IVS7(+76), IVS8(+52) C>T, and c.990C>T. Based on the diagnosis of SCADD, parents of the patient were advise to feed the patient a low fat diet and the patient was to have frequent feedings and glucose levels monitored, especially during acute illness. A therapeutic attempt with high-dose oral riboflavin (100 mg/day) was attempted from her age of 10 months, resulting in a decrease in EMA (67.66 mmol/mol Cr) and MSA (11.98 mmol/mol Cr) excretion (Fig. 2). During the follow up, she presented with recurrent attack of acute gastroenteritis without severe metabolic crisis and serum CK and butyrylcarnitine levels were normalized. She presented normal development on Denver developmental screening test (DDST) and normal growth pattern, height 93 cm.

![Partial genomic DNA sequence of the ACADS gene of the patient.](image-url)
Urine EMA and MSA levels were shown at baseline and after 12 months of riboflavin treatment. EMA, ethylmalonoc acid; MSA, methylsuccinic acid.

Fig. 1. Urine EMA and MSA levels were shown at baseline and after 12 months of riboflavin treatment. EMA, ethylmalonoc acid; MSA, methylsuccinic acid.

Discussion

SCADD is an autosomal recessive inborn error of mitochondrial FAO. Mitochondrial FAO results in the sequential cleavage of two carbon units from fatty acids and represents an important source of energy for the body during times of fasting and metabolic stress. SCADD is a flavoprotein consisting of four subunits, each of which contains one molecule of its cofactor flavin adenine dinucleotide (FAD) as a prosthetic group. SCAD, a mitochondrial enzyme of the FAO system, mediates the metabolic transition from acyl-CoA with four- or six-carbon chains to 2-enoyl-CoA in the first step of the β-oxidation spiral. The SCAD enzyme is most active with butyryl-CoA as substrate, thus leading to elevated levels of butyrylcarnitine, butyrylglycine, and EMA, in body fluids and cells, when SCAD enzyme activity is reduced. The frequency of SCADD is unknown, but results from newborn screening (NBS) suggest frequencies varying between 1:33,000 and 1:340,000.

Years of NBS have resulted in a different understanding of SCADD. Whereas this condition was regarded a potentially life-threatening disorder prior to expanded NBS, patients identified by NBS seem to remain asymptomatic despite confirmation of severe enzyme deficiency. The clinical features in SCADD are extremely broad and difficult to correlate to the enzymatic defect; furthermore, they are often different from those seen in other FAO defects. The previously reported patients typically presented with neuromuscular symptoms, especially developmental delay, which is uncommon in the other β-oxidation defects. Neurological symptoms may be caused by EMA, which has been found to be toxic to neuronal cells, and free butyrate, which may cause encephalopathy. Butyric acid may also contribute to the disease course since it is well known to modulate gene expression in elevated levels due to its action as a histone deacetylase. In circumstances involving increased demand on mitochondrial FAO, such as prolonged fasting, concentrations of these potentially toxic metabolites may increase, resulting in reversible neurotoxicity. van Maldegem et al. reported one in three patients with SCADD with pathogenic mutation presented normal neurological outcome. In this study, the patient with SCADD did not share several attributes with previously reported patients including severe muscular hypotonia, hypoglycemia, and developmental delay. However, she had been hospitalized more than 10 times for gastroenteritis over 22 months. Biochemical markers of SCADD include increased urinary EMA and butyrylglycine, and increased plasma butyrylcarnitine. While elevated EMA in urine is characteristic of SCADD, it is not diagnostic, nor does the level of EMA correlate well with the degree of enzymatic defect in the patients. During times of metabolic stress, MSA (the hydrolyzed product of isomerization of ethylmalonyl-CoA by ethylmalonyl-CoA isomerase) may also be excreted in the urine. Some patients have been reported to have low serum or muscle carnitine levels, but this is not a consistent finding. The patient also showed an increased EMA and MSA levels during times of metabolic stress, but normal serum carnitine levels on sequential testing in this study.

Several inactivating variations in the gene encoding SCAD (ACADS; OMIM# 606885) have been identified in patients with SCADD. The ACADS gene is located on chromosome 12q22 and is approximately 13 kb of length with 10 exons and 1,236 nucleotides of coding sequence. Primary SCADD can result from multiple mutations or two common coding polymorphisms that have been described in the ACADS gene. Up to 70 different inactivating ACADS mutations have been reported so far. Most of these variations are of the missense type. In addition, two common missense variations, c.511C > T (p.Arg171Trp) and c.625G > A (p.Gly209Ser), are present in the normal population with allele frequencies of 3–8% and 22–43%, respectively, with variation in frequency among different ethnic groups. Thus, as much as 14% of the normal population is homozygous for one or compound heterozygous for both of these common ACADS gene variations. It has been suggested that these functional polymorphisms, when homozygous or heterozygous with a known pathogenic mutation, increase susceptibility to symptoms in certain environmental situations, such as fever. It has been demonstrated that homozygosity for one of the polymorphisms is associated with an increased incidence of elevated EMA excretion. Genetic analysis of patients with elevated levels of EMA in the urine revealed that approximately 69% were homozygous or compound heterozygous.
for these two variants.\(^1\) Genotype-phenotype or EMA-phenotype excretion correlations, however, have been inconsistent.\(^3,6,10\) In addition, the phenotypic expression of a given mutation is thought to depend on additional genetic or environmental determinants.\(^3\) Recently, there has been just one report of SCADD by biochemical and genetic findings in Korea and sequence analysis of the ACADS gene revealed novel homozygous missence mutations, c. 1031A>G (p.Glu344Gly) with increased concentration of butylcarnitine and urinary excretion of EMA.\(^16\)

The p.Leu39Ile and p.Glu288Lys, novel variants identified in this study, are expected to cause functional abnormalities by SIFT (Sorts Intolerant From Tolerant amino acid substitutions; <0.05 is predicted to be deleterious) with value of 0.00 and 0.04, respectively. Additional functional analysis in vitro might help validate these predictions.

Little firm data exist on the appropriate therapy for SCADD—indeed, there is no consensus on the need to treat this disease.\(^3\) Chronic management of SCADD should be similar to that of other FAO disorders, focusing on decreasing catabolic drive as well as providing alternative sources of energy.\(^6\) During acute crises, intravenous fluids with high dextrose concentrations (usually at least 10% to give 8–10 mg/kg/min of glucose intake) with or without intralipids can be used to reverse the catabolic state.\(^4\) Preventive measures, if necessary, likely include only avoidance of fasting.\(^6\) Since FAD is an essential cofactor for SCAD function, riboflavin supplementation has been suggested as a possible therapy for SCADD due to its potential ability to act as a chemical chaperone and stabilize mutant enzymes.\(^4\) In addition, riboflavin deficiency is a relatively common condition and could therefore be a common environmental factor reducing SCAD activity in susceptible SCADD individuals, resulting in clinical disease.\(^3\)

In some patients, it was found that riboflavin treatment seemed to be beneficial.\(^1\) A high dose of riboflavin (10 mg/kg/d, with a maximum of 150 mg/d) was thought to be sufficient to obtain the maximal attainable FAD levels in patients with SCADD.\(^5\) Such a treatment could be especially effective in those patients who have a relatively low FAD status at baseline.\(^3\) Even though the blood FAD level of the patient was not tested in this study, high-dose riboflavin treatment for 12 months was used due to aggravated biochemical findings; this resulted in a decrease in EMA and MSA excretion without side effects. However, long-term follow-up of this patient is needed to validate the efficacy of riboflavin supplementation. Also, the patient’s natural history and genetic background need to be further investigated in order to provide appropriate genetic counseling and management. These results will provide further evidence of mutational heterogeneity for SCADD.

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References


