Mutations of \textit{ACADS} Gene Associated with Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

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Abstract. Short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) is an autosomal recessive disorder of mitochondrial fatty acid oxidation associated with mutations in the \textit{ACADS} gene (Acyl-CoA Dehydrogenase, Short-chain, OMIM #606885). SCADD is a heterogeneous condition that has been associated with various clinical phenotypes ranging from fetal metabolic decompensation in infancy to asymptomatic individuals. Here, the first Korean neonate diagnosed with SCADD by biochemical and genetic findings is reported. The patient has remained asymptomatic by avoiding hypoglycemia. An increased concentration of butylcarnitine was detected on newborn screening. Subsequent urine organic acid analysis showed increased urinary excretion of ethylmalonic acid. To confirm the presence of the genetic abnormality, all the coding exons of the \textit{ACADS} gene and flanking introns were amplified by the polymerase chain reaction (PCR). Sequence analysis of the \textit{ACADS} gene revealed novel homozygous missence mutations, c. 1031A>G (p.E344G) in exon 9. In summary, the first Korean patient with confirmed SCADD by genetic analysis is reported with novel mutation.

Introduction
Short-chain acyl-CoA dehydrogenase (SCAD) is member of the acyl-CoA dehydrogenase (ACAD) family of mitochondrial enzymes involved in the B-oxidation system. It catalyzes the first step in mitochondrial B-oxidation of fatty acids four to six carbons in length [1,2]. Short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) is a rare autosomal recessive disorder of mitochondrial fatty acid oxidation, first reported in 1987 [3]. The clinical symptoms of primary SCADD include failure to thrive, metabolic acidosis, ketotic hypoglycemia, developmental delay, seizures, and neuromuscular symptoms such as myopathy and hypotonia in infants, children, and adults [4,5]. Because of the enzyme defect, increased level of C4-acylcarnitine in the plasma and increased level of ethylmalonic acid (EMA) in the urine are observed and suggest the diagnosis of SCADD. However, these findings are also found in individuals who carry one of two common polymorphisms identified in the SCAD coding region [6,7]. Therefore, genetic analysis is helpful in the diagnosis. The SCAD gene is located on chromosome 12q22 and is approximately 13kb long with 10 exons and 1236 nucleotides in the coding sequence [8]. Several inactivating mutations in the gene encoding SCAD (\textit{ASCADS}; OMIM #606885) have been identified in patients with SCAD deficiency. Overall, approximately 60 mutations in \textit{ACADS} are known [9], with two common variants. The A 511C >T polymorphism located in exon 5 leads to substitution of tryptophan for arginine at position 147 of the mature enzyme (R147W; position 171 in the precursor); the A 625G > A variant in exon 6 substitutes serine for glycine at position 185 of the mature protein (G185S; position 209 in the precursor protein). Both \textit{ACADS} sequence variants are relatively common in the healthy population [7].

Recently, with newborn screening (NBS) by tandem mass spectrometry (MS/MS), several metabolic disorders are detected early in life, which allows immediate...
initiation of treatment and monitoring, particularly during times of illness [10]. Therefore, individuals with SCADD identified through newborn screening have a chance to remain asymptomatic [11].

In this article, the first biochemically and genetically confirmed Korean patient with SCADD is reported. The patient was found to have a novel homozygous missense mutation of the ACADS gene.

**Material and Methods**

**Clinical and biochemical analysis.** A female neonate was admitted to the hospital for evaluation of an abnormal tandem mass spectrometry analysis performed three days after birth at a local hospital. When the patient was 10 days of age, the following studies were performed: liquid chromatography tandem mass spectrometry (LC-MS/MS),
plasma amino acids, urine organic acids, and quantitative acylcarnitine profile.

**Mutation analysis.** Genomic DNA was extracted from the peripheral blood leukocytes of the patient, using the Wizard Genomic DNA Purification Kit according to the manufacturer’s protocol (Promega, Madison, WI, USA). We failed to obtain the DNAs from the parents. The coding exons and exon-intron boundaries of the *ACADS* gene were amplified by the polymerase chain reaction (PCR) on a thermal cycler (Applied Biosystems, Foster City, CA, USA) using primer pairs designed by the authors (sequences available upon request). Direct sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction kit on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). The sequences were analyzed using the Sequencher program (Gene Codes Corp., Ann Arbor, MI, USA) with comparison to reference sequences. All numbering of nucleotide positions was done according to the *ACADS* cDNA sequence (GenBank accession NM 000017.2). Sequence variation was described according to the recommendations by the Human Genome Variation Society (www.hgvs.org/mutnomen). Novel mutations were confirmed by sequencing 50 control chromosomes.

**Results**

**Clinical and biochemical findings.** The patient was born at 39 weeks of gestation by vaginal delivery and weighed 2.52 kg. Physical examination revealed no abnormal findings. A newborn screening test showed increased concentrations of plasma C4-acylcarnitine (C4): 2.85 µmol/L (cut-off, 1.2). Serum tests of liver function performed at 10 days of age gave the following results: aspartate aminotransferase 44 [reference range (RR) - 40] U/L; alanine aminotransferase 31 (RR, 0-40) U/L; alkaline phosphatase 223 (RR, 42-98) U/L; ammonia 75.5 (RR, 11.2-48.2) mmol/L, and lactate 6.07 (RR, 0.5–2.2) mmol/L. Assay of urine organic acids revealed a significant increase in the urinary excretion of ethylmalonic acid (98.0 mmol/mol creatinine (RR, 1.7-14.6) and mild elevations of 3-hydroxyisobutyric acid, fumaric acid, 3-hydroxyisobaleric acid, aconitic acid, and hippuric acid. Quantitative acylcarnitine profiling in plasma showed prominent accumulation of C4, 3.59 µmol/L (RR, 0-1.2) (Fig. 1). The patient was suspected as having SCADD based on the biochemical results at 10 days after birth, without any symptoms. The problems associated with SCADD were explained to the parents and they were instructed to keep the patient from fasting for long durations.

**Mutation findings.** A homozygous c.1031A>G mutation of the *ACADS* gene was identified. The c.1031A>G transition resulted in amino acid substitution of Glu to Gly at codon 344 (p.E344G) in exon 9. Fifty healthy normal subjects were screened by direct sequencing and none carried the c.1031A>G mutation of the *ACADS* gene (Fig. 2). In silico analysis of the sequence variations was performed. The p. E344G variation showed a ‘possibly damaging change’ in the PolyPhen prediction of the functional effects of human nsSNPs (http://genetics.bwh.harvard.edu/pph/) and ‘not tolerated change’ in the SIFT analysis (http://sift.jcvi.org/). The novel mutation was a major amino acid substitution.

**Discussion**

A novel *ACADS* mutation was identified in a neonate affected by...
SCAD deficiency. SCADD illustrates several of the clinical and public policy issues currently facing the detection of many inborn errors of metabolism that are identified by expanded newborn screening with tandem mass spectrometry. Clinical presentation of SCADD includes failure to thrive, metabolic acidosis, developmental and cognitive delay, seizures, and neuromuscular symptoms [4,5]. These symptoms have been attributed to intermittent episodes of metabolic decomposition precipitated by fasting, infection, and fever, and are characterized by metabolic acidosis, hypoglycemia and/or hyperammonemia [3,12,13]. Fatty acid oxidation defects, especially MCAD (medium-chain acyl-Co A dehydrogenase) deficiency, provide a classic example of how patients can derive great benefit from early detection [14,15].

Early diagnosis may be critical, particularly since the avoidance of fasting, the prompt management of fever, and the early treatment of infections are likely to reduce the risk for metabolic decompensation and aggressive treatment of acute catabolic episodes is advocated when necessary [16]. However, patients with the same genotype and laboratory findings of symptomatic SCADD have been found to be asymptomatic, especially those identified by newborn screening. Elevation of butylcarnitine, or more correctly, “C4 carnitine” in blood spot samples and elevated excretion of ethylmalonic acid in urine can predict patients with SCADD [7]. However, these findings can be nonspecific, and are observed in a number of other inborn errors of metabolism. In the present case, the concentration of C4 in MS/MS was greatly increased and SCADD was diagnosed from the results of analysis of the urine organic acids (elevated ethylmalonic acid).

There have been no reports of Korean patients with SCADD, thus this is the first case in Korea of SCADD that was confirmed by genetic analysis and biochemical abnormalities. The mutation identified in this study is a novel homozygous mutation, c.1031A>G (p.E344G) of exon 9. This c.1031A>G transition results in amino acid substitution of Glu to Gly. Glutamic acid, with a hydrophathy index of -3.5, is one of the most hydrophilic amino acids [17]. Glycine is more hydrophobic than glutamic acid, and as a result the p.E344G mutation of ACADS may have influenced short-chain acyl-CoA dehydrogenase; this is because hydrophilic amino acids tend to be located closer to protein surfaces. Although the enzymatic activities of each component of SCAD were not determined in the patient, we assume that the ACADS mutations influenced protein stability and function.

The common SCAD gene (ACADS) mutations in European populations are c.511C>T (Arg147Trp) and c.625G>A (Gly185Ser) [18,19]. In Japan, two asymptomatic patients with SCADD were diagnosed by newborn screening and they had the G108D mutation, a novel one. The p.E344G mutation of ACADS has been attributed to intermittent catabolic episodes is advocated and aggressive treatment of acute catabolic episodes is advocated when necessary [16]. However, these findings can be nonspecific, and are observed in a number of other inborn errors of metabolism. In the present case, the concentration of C4 in MS/MS was greatly increased and SCADD was diagnosed from the results of analysis of the urine organic acids (elevated ethylmalonic acid).

Therefore, molecular analysis can aid tandem mass spectrometry in the differential diagnosis and confirmation of SCADD.

In summary, a Korean female patient who, as a result of MS/MS newborn screening, was suspected as having SCADD had the diagnosis confirmed by biochemical and genetic analysis. A novel ACADS gene mutation was detected. Further study may be needed to understand the functional and structural changes of the proteins involved in this disorder and their association with clinical findings.

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