Abstract

Background: Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare inherited mitochondrial fatty acid oxidation disorder associated with variations in the ACADS (Acyl-CoA dehydrogenase, C2 to C3 short chain) gene. SCADD has highly variable biochemical, genetic and clinical characteristics. Phenotypes vary from fatal metabolic decompensation to asymptomatic individuals.

Subject and methods: A Romani boy presented at 3 days after birth with hypoglycaemia, hypotonia and respiratory pauses with brief generalized seizures. Afterwards the failure to thrive and developmental delay were present. Organic acids analysis with gas chromatography-mass spectrometry (GS/MS) in urine and acylcarnitines analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS) in dried blood spot were measured. Deoxyribonucleic acid (DNA) was isolated from blood and polymerase chain reactions (PCRs) were performed for all exons. Sequence analysis of all exons and flanking intron sequences of ACADS gene was performed.

Results: Organic acids analysis revealed increased concentration of ethylmalonic acid. Acylcarnitines analysis showed increase of butyrylcarnitine, C4-carnitine. C4-carnitine was 3.5 times above the reference range (<0.68 µmol/L). Confirmation analysis for organic acids and acylcarnitine profile was performed on the second independent sample and showed the same pattern of increased metabolites. Sequence analysis revealed 3-bp deletion at position 310-312 in homozygous state (c.310_312delGAG). Mutation was previously described as pathogenic in heterozygous state, while it is in homozygous state in our patient.

Conclusions: In our case clinical features of a patient, biochemical parameters and genetic data were consistent and showed definitely SCAD deficiency.

Key words: SCAD deficiency; short chain acyl-CoA dehydrogenase deficiency; screening; acylcarnitine; polymorphism, genetic

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Case report of short-chain acyl-CoA dehydrogenase deficiency (SCADD)

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Introduction

Short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) is an autosomal recessive inborn error of mitochondrial fatty acid disorder (FAO) (1–3). Birth prevalence of SCADD from newborn screening in California was reported to be 1 in 34,632 (4). Similar data was reported from the New England newborn screening program, approximately 1 in 33,000 (5). Birth prevalence of SCADD with a mutation/variant genotype in the Netherlands as high as > 1:1000 was calculated. This is in contrast with lower number of patients diagnosed clinically (6).

SCAD is encoded by the ACADS (Acyl-CoA dehydrogenase, C2 to C3 short chain) gene, and a number of mutations have been associated with deficient enzyme activity (7–10). The ACADS gene maps to the 12-chromosome (12q24) and spans 14 kb of genomic deoxyribonucleic acid (DNA), its 1.9 kb coding region consists of 10 exons (7). SCAD is the first enzyme of the mitochondrial short-chain β-oxidation spiral catalyzing the dehydrogenation of C4 and C6 fatty acids (7). Impaired SCAD activity results in accumulation of butyrylcarnitine (C4-car-
nitine), butyrylglycine, ethylmalonic acid and methylsuccinic acid in blood and urine (4). C4-carnitine, measured in blood, and ethylmalonic acid, measured in urine, are generally used as biochemical markers for SCADD, although accumulation of four-carbon carnitine esters, C4-carnitine, occurs in two other inherited conditions: isobutyryl-CoA dehydrogenase (IBD) deficiency (11) and ethylmalonic encephalopathy (12).

The first two cases of SCADD were reported in 1987 in two neonates who excreted increased concentration of urinary ethylmalonate (5). SCADD is a heterogeneous condition that has been associated with various clinical phenotypes ranging from fatal metabolic decompensation in infancy to asymptomatic individuals (13). Clinical symptoms include development delay, hypotonia, epilepsy, behavioural disorders and hypoglycaemia (3). Most SCADD patients have been diagnosed as a result of investigations for neurological symptoms and/or hypoglycaemia (1,10,14,15). However, many asymptomatic patients have been diagnosed through newborn screening programs.

In this report, we present a first case of SCADD diagnosed in Slovenia, with until now the second described deletion in ACADS gene. Genetic analysis is in accordance with biochemical findings and clinical picture of the described patient.

**Patient and methods**

**Case history**

The Romani child was born to no consanguineous parents after uneventful pregnancy by spontaneous delivery at 36 weeks of gestation with birth weight of 2550 g (25-50th percentile), birth length 46 cm (10-50th percentile), head circumference 33 cm (50th percentile) and Apgar scores 1 and 5 minutes after birth of 9/9, respectively. On the first day of life low blood glucose concentration (1.9 mmol/L) was measured. Parenteral infusion of 10% glucose solution was performed to maintain normal blood glucose concentration. Three days after birth hypotonia and respiratory pauses with brief generalized seizures occurred. At 4 months of age the failure to thrive and moderate developmental delay with muscular hypertrophy were noticed. Informed consent for genetic testing was obtained from parents and all analyses were performed as a part of diagnostic procedure according to the principles of the Helsinki Declaration.

**Materials**

Random urine sample without addition of preservative was collected for organic acids analysis, creatinine was measured and urine was stored at -20 °C prior the analysis. Analysis was done within 3 days. For the determination of acylcarnitines capillary blood was spotted directly on Whatman 903 filter paper (Whatman GmbH, Dassel, Germany). Blood samples were allowed to dry at room temperature for at least 4 hours. They were stored at 8 °C prior the analysis and analysis was done within 3 days. Urine samples and dried blood spots were collected at 6th day after birth (sampling I) and again at 13th day after birth (sampling II). Peripheral venous blood sample for DNA isolation (6 mL) was obtained by venipuncture into EDTA tubes (Becton Dickinson, Milan, Italy).

**Biochemical methodology**

Creatinine was determined by Jaffe reaction on Beckman Coulter AU 400 analyzer (Beckman Coulter, Brea, CA, USA). Qualitative determination of organic acids was performed on gas chromatography–mass spectrometry (GC/MS) system (Agilent Technologies, Wilmington, USA). Urine sample was oxidized with hydroxyl-amine (O-Ethylhydroxy-lamine hydrochloride, Sigma-Aldrich, Steinheim, Germany). An internal standard, 2-phenylbutyric acid (Sigma-Aldrich, Steinheim, Germany), was added in concentration of 100 mmol/mol creatinine. Urine was acidified with HCl (Riedel-de Haën, Sigma-Aldrich, Seele, Germany), saturated with NaCl (Sigma-Aldrich, Steinheim, Germany) and organic acids were extracted using ethyl-acetate (Sigma-Aldrich, Steinheim, Germany). Ethyl-acetate layer was separated and evaporated under the steam of nitrogen. The dried residue was dissolved in pyridine (Sigma-Aldrich, Steinheim, Germany), derivatised with BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide, Sigma-Aldrich, Steinheim, Ger-
many) and injected into GC/MS system. Chromatographic conditions were as followed: initial temperature 70 °C, initial time 2 minutes, rate 3.5 °C/min, final temperature 270 °C, final time 2 minutes. The obtained spectra were compared with the known library spectra (W8N08 library).

Determination of acylcarnitines from filter paper were performed on tandem mass spectrometer 3200Q Trap (ABSCIEX, Singapore) with Chromsystems MassChrom Aminoacid and Acylcarnitines from dried blood spot commercial reagents kit (Grüfelfing, Germany). The sample preparation was based on extraction followed by derivatisation to butyric esters. Positive electro spray ionization and multiple reactions monitoring (MRM) mode were used.

### Molecular diagnose

Molecular studies were performed on DNA extracted from peripheral blood leukocytes using FlexiGene isolation kit (Qiagene, Hilden, Germany) from patient and his parents. Polymerase chain reaction (PCR) primers (Eurofins MWG Operon, Ebersberg, Germany) were designed on our own according to the established laboratory protocol (Table 1), covering whole coding regions and intron/exon boundaries of \(\text{ACADS}\) gene. All PCR reactions were performed with GoTaq G2 Flexi DNA polymerase (Promega, Madison, USA) using 100 ng of double stranded DNA, 0.2 mM of each dNTPs (Life Technologies, Carlsbad, USA), 0.32 μM of primers, 2 mM of MgCl\(_2\) (Promega, Madison, USA), 1X green buffer (Promega, Madison, USA) and 0.75 U of polymerase (Promega, Madison, USA) in a final reaction volume of 25 μL. The thermo cycling procedure consisted of initial denaturing step at 95°C for 2 min followed by 35 cycles of 94°C for 30 s, annealing step at 58°C for 30 s, extension step at 72°C for 30 s and final extension at 72°C for 7 min. Sequence analysis of all exons and flanking intron sequences of \(\text{ACADS}\) gene was performed using 3500 genetic analyzer (Applied Biosystem, USA). All identified mutations/variants were validated in additional independent round of PCR and once again sequenced.

| Primer | Sequence |
|--------|----------|
| \(\text{ACADS e1F/R}\) | gcagtcgaqagcgtcgttcc |
| \(\text{ACADS e2F/R}\) | caaggacacagcagaacctgg |
| \(\text{ACADS e3F/R}\) | cctccccctgtgagatgtgg |
| \(\text{ACADS e4F/R}\) | tcagctacatgcccccact |
| \(\text{ACADS e5AF/R}\) | gcctacccagtagaaca |
| \(\text{ACADS e5BF/R}\) | gtaggccccctgagacaacag |
| \(\text{ACADS e6F/R}\) | gctctagacacccctctcct |
| \(\text{ACADS e7F/R}\) | agcttttgagacccctctactt |
| \(\text{ACADS e8F/R}\) | tgcctacccagtagaaca |
| \(\text{ACADS e9F/10R}\) | acagagcccctgacacacac |
| \(\text{ACADS e10F/11R}\) | gtaggccccctgagacaacag |

\(\text{ACADS}\) - acyl-CoA dehydrogenase, C-2 to C-3 short chain

### Results

Organic acids measurement in the first urine sample with GC/MS revealed increased concentration of ethylmalonic acid. Confirmation analysis for organic acids from the second urine sample showed also slightly increased methylsuccinic acid (qualitative analysis). Butyrilglycine was not present (Table 2). Acylcarnitines analysis in dried blood spot

| Specific metabolite | Sampling I 6th day of life | Sampling II 13th day of life |
|---------------------|---------------------------|-----------------------------|
| Ethylmalonic acid   | +                         | ++                          |
| Methylsuccinic acid | -                         | +                           |
| Butyrilglycine      | -                         | -                           |

++ highly increased ; + slightly increased; - not present

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showed increased of butyrylcarnitine, C4-carnitine. C4-carnitine was 3.5 times above the reference range (< 0.68 µmol/L) (Table 3). Acylcarnitine profile was confirmed from the second dried blood spot card. Metabolic findings were consistent with SCADD. In order to confirm the diagnosis, the DNA was isolated from peripheral blood. Sequence analysis of all exons and flanking intron sequences of ACADS gene revealed 3-bp in frame deletion at position 310-312 in homozygous state (NM_000017.2: c.310_312delGAG). The mutation results in the loss of glutamine residue at position 104 of the mature SCAD protein (p.Glu104del). The sequence variant is considered as a mutation according to Mutation-Taster prediction program (http://www.mutation-taster.org/). Mutation analysis of parents DNA showed the same mutation in heterozygous state. Parents are not consanguineous, but they are coming from the same community of Romani people.

Discussion

We describe a first patient of Romani origin with SCADD diagnosed in Slovenia presented with hypoglycaemia, hypotonia, seizures, failure to thrive and developmental delay. Clinical signs of a patient were consistent with the most frequent signs, reported in the literature (3,4,16). Due to observed clinical signs, diagnostic tests for selective screening of metabolic disorders were done. Increase of the biochemical hallmarks, C4-carnitine and ethylmalonic acid suggested SCADD. Diagnosis was confirmed with mutational analysis and homozygous deletion of 3 nucleotides at position 310-312 was detected. This mutation was to our knowledge for the first time described in homozygous state. Corydon et al. previously described the mutation as pathogenic in one patient with hypotonia and developmental delay but in heterozygous state, the second mutation was not found. Clinical signs presented in first week of life as hypotonia and developmental delay. Fibroblast SCAD activity was undetectable (7). According to the literature, molecular effect of specific SCAD variations on cellular function is unclear (1). Since our nucleotide variation is 3 bp deletion on both alleles we believe that it has higher impact on a clinical picture than other described mutations in the ACADS gene.

We are aware of limitations of this case report. There were scarce data on neurological evaluation of a patient and enzyme activity has not been measured. We also don’t have information about parents history related with certain signs and symptoms suggestive for SCADD. Additionally, we don’t have results for biochemical analyses for parents (organic acids and acylcarnitine analysis).

The disorder has been reported in infants, children and adults (7,17). Most symptomatic cases of SCADD have been diagnosed due to large diagnostic work-up on children with non-specific findings such as development delay, failure to thrive and myopathy (18). However, the clinical relevance of SCADD is questionable and still under discussion. The reasons are the variability and the nature of the symptoms and signs and existence of many patients without signs of the disease that are diagnosed through newborn screening programs (4,14,19). Those patients may remain asymptomatic even with the same genotype as symptomatic patients (13). Moreover, there were alternate explanations for clinical findings when present (4). The reported spectrum of clinical signs is difficult to correlate to enzymatic defect (20-22). Pederson et al. propose that SCADD should be considered as a disorder of protein folding that can lead to clinical disease in combination with other genetic and environmental factors (1). Also the need to treat affected individuals is unclear. It was shown that only patients with history of hypoglycaemia had development of hypoglycaemia during fasting test (2). Different proteomic studies search for biomarkers that will help to distinguish asymptomatic

| Acylcarnitine | Concentration (µmol/L) | Reference range (µmol/L) | Sampling |
|--------------|------------------------|--------------------------|----------|
| C4           | 2.40                   | 0.11-0.68                | 6th day of life |
| C4           | 1.71                   | 0.11-0.68                | 13th day of life |

Table 3. Acylcarnitine analysis in dried blood spots of a patient; samples were collected on the 6th and 13th day of life.

C4: C4-carnitine, butyrylcarnitine
SCAD deficiency from individuals at risk to develop symptoms (23,24) and genome-wide association studies identify genetic variants associated with relative levels of C4-carnitine (25).

The described cases of SCADD indicate that thorough workup off all patients with neurological signs like development delay, seizures and hypotonia is required. The true relationship between this enzyme deficiency and clinical outcomes will be determined only by long-term follow-up data on SCADD patients diagnosed by selective screening and those diagnosed by newborn screening.

In our case clinical picture of a patient, biochemical parameters and genetic data were concordant and showed definitely SCAD deficiency. We hope that this will contribute to the better understanding of this disease.

Potential conflict of interest

None declared.

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