Clinical, Biochemical, and Genetic Heterogeneity in Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

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SHORT-CHAIN ACYL-COENZYME A (CoA) dehydrogenase (SCAD; MIM 606885) deficiency (SCADD, MIM 201470) is an autosomal recessive inborn error of mitochondrial fatty acid β-oxidation, presenting with a variety of clinical signs and symptoms. Developmental delay, hyper- and hypotonia, ketotic hypoglycemia, and epilepsy are most frequently reported.1-9 SCAD is the first enzyme of the short-chain fatty acid β-oxidation spiral, which catalyzes the dehydrogenation of butyryl-CoA (C4-CoA).

When SCAD activity is impaired, its substrate (C4-CoA) will accumulate. The substrate C4-CoA can be converted into different metabolites including (1) the corresponding carnitine (C)-ester, ie, butyrylcarnitine (C4-C), (2) the corresponding glycine-ester (butyrylglycine), (3) butyrate, and (4) ethylmalonic acid (EMA). Butyrylcarnitine can be measured in blood, whereas butyrylglycine and EMA can be found in urine.

For editorial comment see p 993.

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be measured in urine. The latter 3 metabolites may all be elevated in SCADD, although to different extents.

The diagnosis of SCADD may be confirmed by enzyme activity measurements in muscle, fibroblasts and/or lymphocytes, and by DNA studies. Assuming Hardy-Weinberg equilibrium, the allele frequency based on the prevalence of homozygotes is 0.3% and 5.6% for the c.511C>T and 23.5% for the c.625G>A variant, respectively. These SCAD gene variants have been found in the general population with a prevalence of homozgyosity and heterozygosity of approximately 0.3% and 5.6% for the c.511C>T and 5.5% and 31.3% for the c.625G>A variant, respectively. Assuming Hardy-Weinberg equilibrium, the allele frequency based on the prevalence of homozgyosity is 5.5% for the c.511C>T and 23.5% for the c.625G>A variant, respectively. Both gene variants are considered to play a modifying role in the pathogenesis of clinical SCADD, by conferring susceptibility for clinical disease.

The purpose of our study was to calculate the prevalence of SCADD in the Netherlands and to document and summarize the genetic, biochemical, and clinical characteristics of the largest group of SCADD patients and their SCADD relatives published so far. Within this group, we determined the relation of genotype to biochemical as well as clinical phenotype. In addition, we used the results of our study to discuss newborn screening for SCADD.

**METHODS**

**Patients**

For this study, SCADD was defined by the presence of (1) increased C4-C level in plasma and/or increased EMA level in urine under nonstressed conditions on at least 2 occasions, in combination with (2) a mutation and/or the c.511C>T or c.625G>A susceptibility variants in all ACADS allele. Only SCADD patients in whom sequence analysis of all exons and flanking intronic sequences had been performed were included. Patients were included only if clinical information, including development scores and school performance as assessed by the treating physician, could be obtained. Unless a complete recovery was achieved or the patients had died, only patients who had been seen within the last year were included.

All 8 metabolic centers in the Netherlands participated in this study. Patients were identified using the SCAD DNA database from the main participating center (Academic Medical Center, Amsterdam), which is the only Dutch center...
where DNA analysis for SCADD is performed, and by contacting all Dutch metabolic centers. A cross-check on missing patients was performed by consulting the Dutch Diagnosis Registration Metabolic Disorders database, a national registry of all patients diagnosed in the Dutch metabolic centers. This search did not reveal any additional SCADD patients, indicating that the complete cohort of Dutch patients meeting the inclusion criteria were available for the study.

Written informed consent to use anonymous patient information for this study was obtained from the parents and/or legal representatives of all patients participating in this study. The study was reviewed and approved by the Medical Ethics Committee of the Academic Medical Center.

Prevalence Calculation
The number of patients diagnosed from January 2003 until January 2006 was used to calculate the birth prevalence in the Netherlands. The birth rate used for this calculation was 200,000 per year (Dutch Central Bureau for Statistics).

DNA Analyses
Mutation analysis of the ACADS gene was performed by sequence analysis of all exons and flanking intronic sequences amplified by polymerase chain reaction from genomic DNA isolated from either fibroblasts or lymphocytes from the patients. Details on primer composition and polymerase chain reaction conditions are available on request. In case of newly identified mutations, sequence analysis of 100 control alleles was performed to rule out the possibility of a polymorphism. Based on their genotype, we divided the patients into 3 groups: mutation/mutation, mutation/variant, and variant/variant.

Biochemistry
The level of EMA in urine was analyzed by gas chromatography/mass spectrometry of its methoxime/trimethylsilyl derivative as part of the organic acid analyses. It was considered to be increased in cases where the concentrations were 15 µmol/mmol or more of creatinine for children younger than 2 years and 8 µmol/mmol or more of creatinine for children aged 2 years or older.

The level of C4-C in blood was determined as its butyl ester using electrospray tandem mass spectrometry as part of the acylcarnitine analyses. The C4-C concentration was quantitated by signal comparison with 1H2-C2-carnitine as an internal standard. Reference ranges consisted of the 95th percentile obtained. In this way, an upper reference range of 0.58 µmol/L was defined.

Clinical Signs and Symptoms, Patient Characteristics, and Applied Treatment
Information about the patients and the applied treatment was obtained by interviewing the physician of each patient and/or by reviewing the medical charts. Age at first presentation, country of ancestry, symptoms, developmental scores, school performance, clinical course, and applied treatment were summarized. The country of ancestry was used to study potential founder effects. Developmental delay was defined as severe if the IQ was measured or estimated to be lower than 50 or if there was more than 50% delay in developmental milestones. Epilepsy was defined as severe in the case of persistent seizures that were refractory to drugs. Behavioral disorders were classified as severe when they resulted in the impossibility of attending a regular school or having social contacts. Hypoglycemia was defined as measured blood glucose concentrations of 45 mg/dL (2.5 mmol/L) or less or 46 to 63 mg/dL (2.6-3.5 mmol/L) in combination with hypoglycemic symptoms, and was classified as severe if glucose concentrations were 27 mg/dL (1.5 mmol/L) or less.

Family Studies
Parents of patients seen in one of the participating centers (Academic Medical Center, Amsterdam) were asked to participate in SCAD DNA studies and, if relevant, also to include their other children. The DNA analyses were performed for 37 relatives (20 parents and 17 sibs) of 10 patients. Biochemical analyses were performed for relatives with an ACADS genotype identical to the proband. Relatives were labeled as having SCADD when they carried the same ACADS genotype as the proband and had increased levels of C4-C and/or EMA. If a parent of a proband was determined as having SCADD, clinical information was obtained from this parent and, if available, from his/her parents.

Genotype to Phenotype Relation
Results of biochemical studies were used to explore the relation between genotype and biochemical phenotype. Results of clinical studies were used to explore the relation between genotype and clinical phenotype.

Statistical Analysis
The Mann-Whitney test was used to compare genotypes, expressed as an ordinal scale, in patients with and without specific clinical symptoms, ie, developmental delay, epilepsy, and behavioral disturbances. The Kruskal-Wallis test was used to compare the biochemical results and the percentage of patients with severe, complicated, and uncomplicated symptoms in the 3 different genotype groups. The level of significance was set at P<.05. Analyses were done using Graphpad Prism 3.0 and SPSS 12.0.1 (SSPS Inc, Chicago, Ill) software.

RESULTS
Patients
Thirty-one patients who were diagnosed with SCADD in the Netherlands between January 1987 and January 2006 were included in this study.

Calculated Prevalence
The majority of patients (n=25) developed symptoms in the first 3 years of life. Twelve (40%) of the patients included in this study were diagnosed in the Netherlands in the past 3 years. This implies that at least 4 new SCADD patients are born each year in the Netherlands, resulting in a birth-prevalence of at least 1:50,000 (95% confidence interval [CI], 1:29,000-1:87,000).
Genotype
All patients and their genotypes are presented in Table 1. In total, 12 different mutations and 2 variants were identified in the Dutch patient group, including 7 mutations that have not been reported previously. Of the newly identified mutations, 5 were missense, 1 was nonsense, and 1 affected splicing. Sequence analysis of 100 control alleles did not reveal any of these mutations. The most common mutation was the c.1058C>T mutation, found in 8 patients of Dutch ancestry (Table 1 and Table 2).

Biochemistry
The EMA values under nonstressed conditions ranged from near normal values to greater than 400 μmol/mmol of creatinine. The C4-C values ranged from normal values to greater than 4 μmol/L (Table 1).

Clinical Signs and Symptoms, Patient Characteristics, and Applied Treatment
Patients presented with a variety of clinical signs and symptoms. Most frequently encountered signs and symptoms were developmental delay (n = 16), which was nonsevere in the majority of patients (n = 15), epilepsy (n = 11, nonsevere in all), behavioral disorders (n = 8, nonsevere in 5), and hypoglyce-
mia (n=6, nonsevere in 5) (Table 2 and FIGURE 1).

Overall, 5 patients (patients 17 and 19 in the mutation/variant group, and patients 23, 25, and 30 in the variant/variant group) (Table 2) had 1 or more severe symptoms. Additional diagnoses, which were considered to be a more likely cause of the signs and symptoms, were made in 2 of them (patients 17 and 23, Table 2). Overall, 7 patients (patients 1 and 2 in the mutation/mutation group; patients 14 and 16 in the mutation/variant group; patients 27, 29, and 31 in the variant/variant group) had 3 or more different symptoms recorded. An additional diagnosis that was considered to be a more likely cause of the clinical signs and symptoms was made in 2 patients (patients 1 and 16, Table 2). There were 19 patients who had

### Table 2. Clinical Signs and Symptoms and Genotype in 31 Dutch SCADD Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Onset Symptoms, Sex/Age</th>
<th>Country of Ancestry</th>
<th>Developmental Delay</th>
<th>Epilepsy</th>
<th>Hypoglycemia</th>
<th>ACADS Gene Mutations on Both Alleles (Mutation/Mutation Group)</th>
<th>ACADS Gene Mutation on One Allele and Gene Variant on the Other Allele (Mutation/Variant Group)</th>
<th>Behavioral Disorders</th>
<th>Other Symptoms</th>
<th>Duration of Follow-up, y</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>F/0 d</td>
<td>Turkish</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Microcephaly, multiple dysmorphic features, cutis laxa, hypertonia</td>
<td>Food refusal, hypertonia</td>
<td></td>
<td></td>
<td>6</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>2</td>
<td>M/1 wk</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Transient infantile hepatic dysfunction</td>
<td></td>
<td></td>
<td>7</td>
<td>Normal</td>
</tr>
<tr>
<td>3†</td>
<td>M/2 mo</td>
<td>Turkish</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>11</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>4</td>
<td>F/14 y</td>
<td>Maroc</td>
<td></td>
<td>–</td>
<td>–</td>
<td>Fatigue</td>
<td>Fatigue</td>
<td></td>
<td></td>
<td>4</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>M/1 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>11</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>6</td>
<td>F/7 y</td>
<td>India</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>18</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>F/1 mo</td>
<td>Turkish</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>3</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>8</td>
<td>M/9 mo</td>
<td>Turkish</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Mild pulmonary stenosis</td>
<td></td>
<td></td>
<td>8</td>
<td>Stable</td>
</tr>
<tr>
<td>9</td>
<td>M/8 mo</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>Microcephaly, clubfeet</td>
<td>–</td>
<td></td>
<td></td>
<td>7</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>F/3 mo</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>Transient hypertonia</td>
<td>–</td>
<td></td>
<td></td>
<td>4</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>M/1 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>5</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>12</td>
<td>F/4 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>4</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>13</td>
<td>M/1 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Transient hypertonia</td>
<td></td>
<td></td>
<td>8</td>
<td>Normal</td>
</tr>
<tr>
<td>14</td>
<td>F/1 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Facial dysmorphism, exercise intolerance</td>
<td></td>
<td></td>
<td>3</td>
<td>Normal</td>
</tr>
<tr>
<td>15</td>
<td>F/1 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Failure to thrive, spasticity</td>
<td>Failure to thrive</td>
<td></td>
<td>11</td>
<td>Stable</td>
</tr>
<tr>
<td>16‡</td>
<td>F/6 mo</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Dysmorphism, hypotonia</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>17§</td>
<td>M/0 mo</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>–</td>
<td>Dysmorphism, hypotonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18††</td>
<td>M/3 mo</td>
<td>Dutch</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>Failure to thrive</td>
<td></td>
<td></td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>19</td>
<td>M/11 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td>–</td>
<td>Vomiting, fatigue</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>M/8 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Vomiting</td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>21</td>
<td>M/1 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>M/2 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>23‡</td>
<td>F/1 y</td>
<td>Maroc</td>
<td></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td>Failure to thrive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>F/2 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>F/3 y</td>
<td>Turkish</td>
<td></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td>Failure to thrive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>M/3 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Fatigue</td>
<td></td>
<td></td>
<td>9</td>
<td>Stable</td>
</tr>
<tr>
<td>27</td>
<td>M/2 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Hypertonia</td>
<td></td>
<td></td>
<td>9</td>
<td>Ameliorated</td>
</tr>
<tr>
<td>28</td>
<td>M/2 y</td>
<td>Dutch</td>
<td></td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>29</td>
<td>M/2 y</td>
<td>Maroc</td>
<td></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Dysmorphic features, scoliosis</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>30</td>
<td>M/2 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>M/2 y</td>
<td>Dutch</td>
<td></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: ACADS, acyl-coenzyme A dehydrogenase gene; SCADD, short-chain acyl-coenzyme A dehydrogenase deficiency.
*Congenital disorder of glycosylation type 1x was diagnosed during follow-up.
†Angelman syndrome was diagnosed during follow-up.
‡Mitochondrial disorder of unknown origin was diagnosed during follow-up.
§Severe.
¶Recurrent urinary tract infections due to a subpelvic ureter stenosis was diagnosed during follow-up.
‖Adrenal insufficiency was diagnosed during follow-up.
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uncomplicated symptoms. Further investigations in one of these patients (patient 20) revealed recurrent urinary tract infections that were caused by a subpelvic ureter stenosis, and his symptoms disappeared after surgical intervention. An additional diagnosis, which was more likely the cause of the signs and symptoms, was therefore made in a total of 5 out of 31 patients.

Follow-up after the first presentation ranged from 1 to 18 years. No patients died during follow-up. In 2 patients (both from the variant/variant group) progressive clinical deterioration was recorded, 12 patients had no change in symptoms during follow-up, 8 patients had amelioration of symptoms, and 9 had complete recovery (Table 2).

Eighteen patients (patients 1, 2, 4, 5, 7, 8, 11-15, 17, 19-26, and 27) were treated, in almost all cases temporarily, with riboflavin (vitamin B2). In 6 patients (patients 16, 19, 20, and 25-27), carnitine had been given. In all cases, parents and/or representatives were given instructions to avoid fasting. In 8 patients (patients 8 and 10, 11, 21, 23, and 25-27), a cornstarch feed (long-acting carbohydrates) was given before bedtime. No consistent clinical improvement was noted in relation to riboflavin, carnitine, cornstarch, or avoidance of fasting.

Family Studies
Of the 37 relatives (20 parents and 17 siblings) tested, 9 relatives (a parent of Figure 1. Clinical Signs and Symptoms in 31 Dutch Patients With Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

![Figure 1](https://jama.jamanetwork.com/)

Clinical Signs and Symptoms

<table>
<thead>
<tr>
<th>No. With Symptom at Presentation</th>
<th>Developmental Delay</th>
<th>Epilepsy</th>
<th>Behavioral Disorder</th>
<th>Hypoglycemia</th>
<th>Hypotonia</th>
<th>Dysmorphic Features</th>
<th>Fatigue</th>
<th>Failure to Thrive</th>
<th>Recurrent Vomiting</th>
<th>Hypertonia</th>
<th>Food Refusal</th>
<th>Microcephaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only symptoms reported in more than 1 of the total number of 31 patients are included.

**Table 3.** Genotype, EMA, C4-C, and Clinical Signs and Symptoms in 6 SCADD Patients and 9 Relatives With an ACADS Genotype Identical to the Proband

<table>
<thead>
<tr>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Age at Sample Collection</th>
<th>EMA, µmol/mmol creatinine‡</th>
<th>C4-C, µmol/L‡</th>
<th>Clinical Signs and Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.988C&gt;T, c.625G&gt;A</td>
<td>c.1147C&gt;T</td>
<td>7 mo</td>
<td>261-414</td>
<td>3.22</td>
<td>Epilepsy, food refusal, hypertonia</td>
</tr>
</tbody>
</table>

Sibling 2

Patient 3| c.1138C>T, c.1138C>T | 2 mo | 124-380 | 2.4-4.7 | Transient infantile hepatic dysfunction |

Sibling 4| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Parent 4| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Patient 5| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Sibling 5| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Patient 14| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Sibling 14-1| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Sibling 14-2| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Patient 21| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Sibling 21| c.14067 | 15 y | 25-58 | 2.0-6.25 | No |

Abbreviations: ACADS, acyl-coenzyme A dehydrogenase gene; C4-C, butyrylcarnitine; EMA, ethylmalonic acid; NA, not analyzed; SCADD, short-chain acyl-coenzyme A dehydrogenase deficiency.

*Gene variants in regular type and mutations in bold type.
†Normal range of EMA: 0-2 y: 0-15 µmol/mmol creatinine; 2 y: 0-8 µmol/mmol creatinine.
‡Maximum normal concentration of C4-C: <0.58 µmol/L.
§Newly identified mutations.
| Reported previously by Bok et al. |"
patients 4, 5, and 21, and a sibling of patients 2, 3, 5, and 21, and 2 siblings of patient 14) were found to have a ACADS genotype identical to the proband. Except for the father of patient 21 (variant/variant group) all relatives were found to have increased levels of C4-C and/or EMA (Table 3). Eight of the 9 relatives had no clinical symptoms. The only one with symptoms was a sibling of patient 3 (mutation/mutation group), who had transient food refusal in her first year of life.

**Genotype to Phenotype Relation**

The EMA and C4-C values were highest in the 3 patients in the mutation/mutation group, less increased in the mutation/variant group, and the lowest values were found in the patients with gene variants only. A statistically significant association between EMA as well as C4-C concentrations and the genotype (P=.002 in both) was detected (Figure 2).

The genotype of the SCADD patients and SCADD relatives was not related to the nature or the severity of symptoms (Table 3 and Figure 3).

**COMMENT**

Our study presents the largest group of SCADD patients reported so far. As extended diagnostic studies are more likely to be performed and clinical information is more likely to be available in the case of severe symptoms, our study population might represent the more severely affected SCADD patients.

Based on the number of patients diagnosed in the last 3 years, a birth prevalence for the Netherlands of at least 1:50 000 was calculated. The true prevalence may well be higher, in view of the strict inclusion criteria used in our study. Furthermore, metabolic studies are generally performed in only a minority of patients with symptoms found in SCADD, such as mild developmental delay and epilepsy. Indeed, results of newborn screening studies performed in the United States indicate a higher prevalence. Birth-prevalences as high as 1:33 000 were estimated for classic SCADD, defined as SCADD with mutations on both alleles and very high C4-C levels as detected by using high cut-off levels (4-10 SD above the mean) for C4-C.32 In the group of 31 patients reported herein, only 3 (9.7%) were found to have mutations on both alleles of the ACADS gene and are thus comparable with SCADD in its classic form. If one assumes that the prevalence of classic SCADD (mutation/mutation group) and SCADD with a mutation/variant or variant/variant genotype (nonclassic SCADD) is similar in the United States and the Netherlands, this would imply that the birth-prevalence of SCADD in the Netherlands, including both forms, might be approximately 10 times as high, which would result in a birth-prevalence of SCADD of approximately 1:3300. This prevalence is even higher than the prevalence of phenylketonuria, which is assumed to be one of the most common inborn errors of metabolism with a birth-prevalence of 1:10 400 to 1: 4500 if all variant forms of phenylketonuria are included.33

Our study revealed 7 newly identified ACADS gene mutations, resulting in a total number of 22 different

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ACADS mutations, apart from the 2 ACADS gene variants, published so far (Table 4). The biochemical features of patients with the newly identified missense mutations, combined with the fact that these mutations have not been observed in 100 control alleles, strongly suggests that these are inactivating mutations. The c.1058C>T mutation was found in 8 of the 20 patients with mutations, all of Dutch ancestry, suggesting a founder effect. This mutation has been reported previously, also in a patient of Dutch ancestry.

Clinical signs and symptoms at presentation were highly variable, with developmental delay (nonsevere in almost all patients), epilepsy, behavioral disturbances, and hypoglycemia being the most frequently reported (Table 2 and Figure 1). Most patients presented with more than one of these symptoms. Except for behavioral disorders these symptoms also were frequently noted in patients reported previously. Most of the severely affected patients belonged to the variant/variant group (Table 2), which is in line with other studies. The overrepresentation of variant alleles in severely affected SCADD patients may be the result of a selection bias, since it is more likely that a full metabolic screening is performed in patients with severe clinical symptoms. Because approximately 6% of the general population is homozygous or compound heterozygous for the c.625G>A and/or c.511C>T ACADS gene variants, the apparent association between clinical symptoms and the presence of variant alleles might be coincidental. Therefore, the detection of homozygosity or compound heterozygosity for these gene variants in patients with severe clinical symptoms should not preclude a full diagnostic work-up for other potential causes of the symptoms. Further diagnostic studies in SCADD patients with either a mutation/mutation or mutation/variant genotype also may be indicated. The importance of further studies is illustrated by patients 1, 16, 17, and 18 in whom additional diagnoses were made that are more likely to be causing the clinical symptoms. These findings, as well as the results of the family studies reported herein, demonstrate that even missense mutations can occur without any clinical significance.

The clinical course was rather similar in most patients. In general, symptoms developed early in life, which also was reported in previous studies. Complete recovery of symptoms was reported in 9 of the 31 patients reported herein and in 8 of the 10 patients in whom outcome was reported previously, suggesting that in a substantial number of patients, SCADD is associated with transient clinical symptoms. In our study, no consistent improvement was reported in response to riboflavin, carnitine, cornstarch, or avoidance of fasting. However, more studies are necessary to assess the effect of treatment, in particular of riboflavin therapy.

Although a significant association was found between genotype and biochemical phenotype, our study did not reveal an association between genotype and clinical features in SCADD. This finding suggests that modifying factors may be involved in the pathogenesis of clinical SCADD, as previously suggested for the c.625G>A and c.511C>T susceptibility alleles. Our observation that SCADD is often associated with transient clinical symptoms might be related to a temporary nature of these factors. Neurological symptoms may be caused by EMA, which was found to be toxic to neu-

Table 4. Newly Identified and Previously Reported Mutations and Variants in the ACADS Gene

<table>
<thead>
<tr>
<th>Reference</th>
<th>DNA Mutation</th>
<th>Coding Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>IVS1-6C&gt;A</td>
<td>Putative splicing error</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.268G&gt;A</td>
<td>p.G90S†</td>
</tr>
<tr>
<td>Gregersen et al,1998</td>
<td>c.274G&gt;T</td>
<td>p.G92C†</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.310-312delGAG</td>
<td>p.E104del†</td>
</tr>
<tr>
<td>Naito et al,1990</td>
<td>c.319C&gt;T</td>
<td>p.R107C</td>
</tr>
<tr>
<td>Koeberi et al,2003</td>
<td>c.332C&gt;T</td>
<td>p.S111F</td>
</tr>
<tr>
<td>Koeberi et al,2003</td>
<td>c.409C&gt;T</td>
<td>p.Q137X</td>
</tr>
<tr>
<td>Seidel et al,2003</td>
<td>c.417G&gt;C</td>
<td>p.T139C†</td>
</tr>
<tr>
<td>Current study</td>
<td>c.505A&gt;C</td>
<td>p.T169P</td>
</tr>
<tr>
<td>Gregersen et al,1998</td>
<td>c.529T&gt;C</td>
<td>p.W177R††</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.575C&gt;T</td>
<td>p.A192V††</td>
</tr>
<tr>
<td>Current study</td>
<td>c.796C&gt;T</td>
<td>p.Q266X</td>
</tr>
<tr>
<td>Current study</td>
<td>c.815G&gt;A</td>
<td>p.R272H</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.973C&gt;T</td>
<td>p.R325W††</td>
</tr>
<tr>
<td>Current study</td>
<td>c.988C&gt;T</td>
<td>p.R330C</td>
</tr>
<tr>
<td>Current study</td>
<td>c.989G&gt;A</td>
<td>p.R330H</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.1058C&gt;T</td>
<td>p.S353L††</td>
</tr>
<tr>
<td>Corydon et al,2001</td>
<td>c.1138C&gt;T</td>
<td>p.R380W††</td>
</tr>
<tr>
<td>Current study</td>
<td>c.1170C&gt;G</td>
<td>p.I390M††§</td>
</tr>
</tbody>
</table>

**Variants**

<table>
<thead>
<tr>
<th>Reference</th>
<th>DNA Mutation</th>
<th>Coding Effect</th>
</tr>
</thead>
</table>

Abbreviation: ACADS, acyl-coenzyme A dehydrogenase gene.
*Nomenclature according to the human genome variation society.
†Mutant short-chain acyl-CoA dehydrogenase protein was found to result in undetectable activity after expression in Escherichia coli or COS-7 cells.
‡DNA analysis performed by Corydon and Gregersen in Aarhus, Denmark.
§Expression studies performed by Wockley in Rochester, NY.
nal cells, and free butyrate, which may cause encephalopathy.\textsuperscript{2,3,39} During circumstances with increased demand on mitochondrial fatty acid oxidation, such as prolonged fasting, concentrations of these potential toxic metabolites may increase, resulting in reversible neurotoxicity.

The relatively benign clinical course observed in many of the SCADD patients implies that SCADD does not meet the first Frankenburg screening criterion stating that the disease or condition screened for should be serious or potentially serious.\textsuperscript{2,9} In addition, 7 of the 8 relatives identified in this study with an ACADS genotype identical to the proband and increased C4-C and/or EMA levels were free of symptoms. This observation also implies that it is not possible to differentiate SCADD patients from nondiseased SCADD individuals. Therefore, SCADD does not meet the third Frankenburg criterion for screening. This is in line with the observation that all 7 individuals detected by newborn screening in Australia with probable SCADD remained free of symptoms without any treatment during subsequent follow-up of 2 to 7 years (written communication, B. Wilcken, The Children’s Hospital at Westmead, Westmead, Australia). Furthermore, 17 putative SCADD patients as well as 2 of the 3 patients with confirmed SCADD based on homozygous mutations, detected by newborn screening programs in the United States, did not develop any clinical symptoms during the first years of life.\textsuperscript{2,36} Although the results of our study suggest that SCADD is relatively common, thus meeting the second Frankenburg criterion, we believe that SCADD should not be included in neonatal screening programs at this time. Indeed, screening for SCADD may have negative consequences placing families at risk for increased stress and parent-child dysfunction.\textsuperscript{37}

However, infants already identified by SCADD newborn screening should be included in long-term follow-up studies to obtain more information to decide about the relevance of screening for SCADD.

Unfortunately, both the Wilson and Jungner and the Frankenburg criteria have limitations in the context of newborn screening using tandem mass spectrometry.\textsuperscript{28} However, no criteria have been published since and they are still applied in the discussion on newborn screening.\textsuperscript{28,39} The results of a new approach for recommending conditions for newborn screening by using an expert panel recently were published by the American College of Medical Genetics.\textsuperscript{28} In this report, SCADD was not included in the core panel of diseases for which screening is considered mandatory. SCADD was included in the group of secondary targets because it is in the differential diagnosis of a condition in the core panel and it is of clinical significance. However, the results of our study and newborn screening studies demonstrate that SCADD lacks clear clinical significance in many patients, implying that SCADD should not be included in the group of secondary targets and it does not qualify for newborn screening at this time.

This study has several limitations in addition to the underestimate of prevalence given the use of a clinically identified rather than screened population. As data were collected retrospectively, no sequential neuropsychological and motor development tests were performed. In addition, clinical information was scored by different physicians. To further elucidate the clinical spectrum of SCADD, more prospective and long-term studies, including formal neuropsychological and motor development testing, are necessary.

In summary, the results of newborn screening studies as well as our data suggest that SCADD is far more common than assumed previously. In the Dutch patient cohort, clinical symptoms are nonspecific, generally uncomplicated, often transient, and not related to the ACADS genotype. These observations, in combination with the observation that almost all relatives diagnosed with SCADD, as well as almost all individuals found by neonatal screening, remain asymptomatic, suggest that an association between symptoms and SCADD is often spurious. In some individuals carrying ACADS gene variants and/or mutations, environmental or other genetic factors may result in true SCADD-related clinical pathology. In many other individuals, however, SCADD may only be a lifelong biochemical phenomenon. Because SCADD does not meet major newborn screening criteria, it is not suited for inclusion in newborn screening programs at this time.

Author Contributions: Dr van Maldegem had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data: van Maldegem, Niezen-Koning, Hogeveen.

Analysis and interpretation of data: van Maldegem, Wanders, Wijburg, Waterham.

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Critical revision of the manuscript for important intellectual content: Duran, Ijlst, Waterham.

Statistical analysis: van Maldegem.

Obtained funding: van Maldegem, Wijburg.

Administrative, technical, or material support: van Maldegem, Duran, Niezen-Koning, Hogeveen, Ijlst, Waterham.

Study supervision: Wanders, Wijburg.

Financial Disclosures: None reported.

Funding/Support: This work was supported by a research grant to Drs van Maldegem and Wijburg from the “Metabole Fonds” of the Academic Medical Center, Amsterdam.

Role of the Sponsor: The research sponsor had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; or preparation, review, or approval of the manuscript.

Acknowledgment: We thank H. D. Bakker, MD, PhD, P. G. Barth, MD, PhD, R. F. M. de Coo, MD, PhD, P. M. van Hasselt, MD, PhD, J. B. C. de Klerk, MD, PhD, T. J. de Koning, MD, PhD, L. Langius, MD, PhD, S. M. Maas, MD, PhD, J. P. Rake, MD, PhD, M. E. Rubio-Gozalbo, MD, PhD, G. P. A. Smit, MD, PhD, M. E. J. Wergdam-den Boer, MD, PhD, and J. M. B. Wennis, MD, PhD, for providing data on their patients. N. G. G. M. Abeling for assistance on biochemical analyses, and I. H. van der Lee, MD, PhD, of the Centre for Pediatric Clinical Epidemiology for assistance on statistical analyses.

REFERENCES


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