High prevalence of short-chain acyl-CoA dehydrogenase deficiency in the Netherlands, but no association with epilepsy of unknown origin in childhood

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Abstract

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is an autosomal recessive inborn error of metabolism, most frequently associated with developmental delay and/or epilepsy. Most SCADD patients carry common SCAD-encoding gene (ACADS) variants or ACADS variants in combination with a mutation. The ACADS variants are considered to confer susceptibility to development of clinical disease. The most common ACADS mutation in the Netherlands is the c.1058C>T. C4-Carnitine, one of the biochemical SCADD characteristics is increased in almost all patients. Epilepsy is a frequent finding in childhood and often remains unexplained. However, studies for inborn errors of metabolism such as SCADD are often not performed. Because the prevalence of SCADD is much higher than the number of patients detected, patients with epilepsy related to SCADD may remain undiagnosed.

To test this hypothesis and to further estimate the extent of the Dutch SCADD population, we performed a case-referent study in 131 paediatric patients with epilepsy and 909 anonymous newborns. To investigate the presence of the 2 common ACADS variants and the c.1058C>T mutation and to measure C4-Carnitine, DNA PCR-RFLP analysis and electrospray tandem mass spectrometry were performed on blood spot samples.

Overall, the 2 ACADS variants and c.1058C>T mutation were detected in either homozygous or compound heterozygous forms in 9.2% of the epilepsy and 7.5% of the reference group. In none of the epilepsy patients C4-Carnitine was increased. This implies that the birth prevalence of SCADD with a mutation/variant genotype in the Netherlands is > 1:1000, but does not support an association between SCADD and epilepsy. Therefore, SCADD studies cannot be recommended in epilepsy of unknown origin in childhood.
Introduction

Short-chain acyl-CoA dehydrogenase (SCAD) is the first enzyme of the short-chain β-oxidation spiral catalyzing the dehydrogenation of short-chain fatty acids. SCAD deficiency (SCADD) is a relatively newly recognized inborn error of fatty acid oxidation and has been associated with a remarkable variety of clinical symptoms. SCADD is probably one of the more common inborn errors of metabolism.1

Butyryl-CoA (C4-CoA) is the main substrate for the SCAD enzyme and accumulates in mitochondria of patients with decreased SCAD activity, which subsequently leads to increased levels of butyrylcarnitine (C4-C) and ethylmalonic acid (EMA). EMA can best be measured in urine, whereas C4-C can best be measured in plasma and blood spots dried on filter paper. These 2 metabolites constitute the major biochemical features of SCADD.

Diagnosis of SCADD is usually confirmed by DNA analyses.1 The majority of SCADD patients are homozygotes or compound heterozygotes for two common SCAD-encoding gene (ACADS) variants, or for ACADS variants in combination with an inactivating mutation.1-4 Homozygosity for ACADS variants has been found in the general population with a remarkably high prevalence of approximately 0.3% for the c.511C>T and 5.5% for the c.625G>A variant,5,6 and is considered to confer susceptibility to development of clinical disease.7,9 More than 70 different ACADS mutations have been reported so far.1-3,7,8,10-15 In the majority of Dutch patients one specific mutation, the c.1058C>T, is present next to the c.625G>A variant on the other allele.1 The c.1058C>T mutation is located on the c.625G and c.511C ACADS allele. Overall an ACADS genotype consisting of this specific mutation and/or ACADS variants was present in 61% of the Dutch SCADD patients.1

Most of the reported SCADD patients were initially investigated because of neurological symptoms and/or hypoglycemia.1,4-7 In our previous study on 31 Dutch SCADD patients, developmental delay was reported in 52% and epilepsy in 35% of the patients.1 Behavioral disorders and hypoglycemia were reported in 26% and 19% of this patient group respectively. No correlation between genotype and phenotype could be established and in some patients other diagnoses, explaining the symptoms initially leading to metabolic studies, were identified.

Epilepsy is a frequent finding in childhood. Studies from Europe and North America report rates from 3.6 to 6.5/1000 children.16-21 Diagnostic studies fail to establish the cause of the epilepsy in up to 30% of the patients.22 However, in patients with idiopathic childhood epilepsy, studies for inborn errors of metabolism such as SCADD as a potential cause are often not performed. This, plus the fact that epilepsy is a frequent symptom in SCADD and the observation that the prevalence of SCADD is much higher than the number of patients detected by metabolic studies,1 suggests that patients with epilepsy related to SCADD may well remain undiagnosed.

In order to test this hypothesis we studied a group of pediatric patients with epilepsy for the presence of SCADD. This way we aimed to achieve better knowledge on the
need for SCADD studies in epilepsy patients. In addition we aimed to further estimate the extent of the group of SCADD individuals in the Netherlands by establishing the frequency of the c.511C>T variant and the c.1058C>T mutation in the Dutch population.

**Patients and Methods**

**Patients and material**

We performed a case-referent study in 131 pediatric patients with epilepsy and 909 anonymous newborns. Blood spots of the reference group were obtained from the RIVM (National Institute for Public Health and the Environment, the Dutch Institute involved in newborn screening). The sample of newborn screening cards used was drawn proportionally to the number of live births in each of the 14 screening districts, guaranteeing a demographic representation of the Dutch population. As Dutch newborn screening cards are stored for 5 years for reasons of possible need for further testing, the obtained screening cards were consequently >5 years old. The newborn screening cards had been stored at 4°C and were analyzed anonymously.

The study was performed in a pediatric patient group, with patients aged 16 years or younger at the time of participation. As all patients in the Dutch SCADD patients group were < 8 years when epileptic symptoms started, we restricted the epilepsy group to those with a debut of epilepsy < 8 years. Exclusion criteria were a known or suspected cause of the epilepsy and previously performed metabolic studies including SCADD investigations.

Patients with epilepsy were recruited via pediatricians and neurologists within the AMC Amsterdam, and 3 Dutch epilepsy centres. Patient and symptom characteristics were obtained by taking questionnaires answered by the parents or legal representatives.

Written informed consent was obtained from the patients and/or parents/legal representatives of all patients. The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam and the RIVM.

**DNA polymerase chain reaction restriction- fragment length polymorphism analysis**

DNA was extracted from bloodspot samples using Chelex 100 (BioRad) essentially as described before, but with the following modifications. Bloodspot samples (6 mm diameter) were washed overnight at 4°C with 1 mL of sterile water. The next day, the supernatant was discarded, 200 μL of Chelex (50 g/L, pH 10.6) was added and the sample was incubated at 56°C for 30 min. Subsequently, the samples were mixed for 10 s, centrifuged (3 min, 10 000 x g), incubated during 8 min at 96°C, mixed again for 10 s, and centrifuged (3 min, 10 000 x g). 14.5μL of supernatant was used in a 25 μL PCR reaction.
The extracted DNA was subjected to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis to determine the presence of the c.625G>A, and c.511C>T variant, and c.1058C>T mutation in the *ACADS* gene. Concerning the c.625G>A variant, a RFLP assay as described before was used. In order to identify the c.511C>T variant and the c.1058C>T mutation, new PCR-RFLP assays were developed.

The c.511C>T variant creates a *Mva*I restriction site. Exon 5 contains an additional *Mva*I site 42 bp downstream used as an internal control for restriction. The upstream endogenous *Mva*I site was destroyed by a C>A change (bold) in the forward primer in order to increase the size of the restriction product. For the PCR-RFLP analysis, exon 5 of the *ACADS* gene is amplified from 14.5 µL extracted DNA in a 25 µL PCR reaction as described before, except for a 1.25 mmol/L MgCl₂ concentration. The following primers were used: forward 5'- tgtaaaacgacggccagt CGT GCG CTG AGC ACT GGG TCT-3', reverse 5'- caggaaacagctatgacc TCG AAG CCT CCC AGG CAT TGG TGA-3'. Like the reverse primer for the PCR-RFLP analysis of the c.625G>A variant, the forward and reverse primer for determination of the c.511C>T variant have an M13-rev and -21M13 extension (small characters), which can be used for sequence analysis.

The c.1058C>T mutation creates an *Eco*130I (*Sty*I) restriction site. Exon 9 contains an additional *Eco*130I (*Sty*I) site 18 bp upstream used as an internal control for restriction. For this PCR-RFLP analysis, exon 9 of the *ACADS* gene is amplified from 14.5 µL extracted DNA in a 25 µL PCR reaction as described before with 1.0 mmol/L MgCl₂ using 5'- tgtaaaacgacggccagt GGG AAG GCT CTG ACT GTA CC -3' as a the forward and 5'- caggaaacagctatgacc CAG GAT CTG GAT GGC CTG AG-3' as the reversed primer. These primers also have an M13-rev and -21M13 extension (small characters).

For DNA amplification the PTC 100 thermocycler was programmed as follows: Denaturation for 2 minutes at 96°C, followed by 5 cycles of 30 s at 96°C, 30 s at 55°C, and 30 s at 72°C and subsequently 30 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, with a final step of 15 minutes at 72°C.

**RFLP c.625G>A.** To 25 µL PCR product 2.9 µL Buffer M and 5U *Dde*I (Roche) were added followed by incubation overnight at 37°C. The non-digested PCR fragment of the c.625G>A variant comprises 226 bp. The digested PCR product shows 2 restriction fragments of 208 and 18 bp in a non-carrier of the c.625G>A variant and 3 fragments of 180, 28, and 18 bp when the c.625G>A variant is present on both alleles. Consequently, in case of heterozygosity for the c.625G>A variant fragments of 208, 180, 28, and 18 bp will be observed.

**RFLP c.511C>T.** To 25µL PCR product 2.9 µL Buffer R and 10 U *Mva*I (Fermentas) were added followed by incubation overnight at 37°C. The non-digested PCR fragment of the c.511C>T variant comprises 174 bp. The digested PCR product shows 2 restriction fragments of 208 and 18 bp in a non-carrier of the c.625G>A variant and 3 fragments of 180, 28, and 18 bp when the c.625G>A variant is present on both alleles. Consequently, in case of heterozygosity for the c.625G>A variant fragments of 208, 180, 28, and 18 bp will be observed.

**RFLP 1058C>T.** To 25 µL PCR product 2.9 µL Buffer O and 10 U *Eco*130I (*Sty*I) (Fermentas) were added followed by incubation at 37°C. The non-digested PCR fragment of the
Figure 1. PCR-RFLP analysis of the c.511C>T variant

**Upper part,** Schematic representation of the relevant part of the ACADS gene. The primers are indicated by horizontal arrows; positions of the MvaI restriction sites are indicated by vertical arrows. **Lower part,** Ethidium bromide-stained agarose gel showing PCR-RFLP analysis using DNA extracted from dried blood spots of reference subjects (1-16) and a heterozygous (c.511C>T/C) control subject (C). Two reference subjects (8, 13) and the control subject show heterozygosity for the c.511C>T variant. PCR products were either directly loaded (−) or digested with MvaI before electrophoresis. M=100 bp marker.
Figure 2. PCR-RFLP analysis of the c.1058C>T mutation

Upper part, Schematic representation of the relevant part of ACADS. The primers are indicated by horizontal arrows; positions of the Eco130I restriction sites are indicated by vertical arrows. Lower part, Ethidium bromide-stained agarose gel showing PCR-RFLP analysis using DNA extracted from dried blood spots of reference subjects (1-16) and a heterozygous (c.1058C>T/C) control subject (C). One reference subject (4) and the control subject show heterozygosity for the c.1058C>T mutation. PCR products were either directly loaded (⁻) or digested with Eco130I before electrophoresis. M=100 bp marker.
c.1058C>T mutation comprises 238 bp. The digested PCR product shows 2 fragments of 181 and 57 bp in a non-carrier and 4 fragments of 181, 163, 57, and 18 bp when the c.1058C>T mutation is present on one of the alleles form (Figure 2).

The restriction fragments were analyzed on a 2% (w/v) agarose gel by ethidium bromide staining. Before loading 1/10 volume loading dye (ficoll 400 150g/L, orange G 1.5g/L, and 0.14g/L Xylene Xyanol FF) was added to the samples. As a reference a 100 bp marker (Invitrogen) was used in order to estimate the size of the fragments.

**Acylcarnitine analyses**

In order to identify individuals with SCADD due to an ACADS genotype not included in the ACADS analysis, C4-C analysis was performed in all blood spots. From all blood spots samples with a diameter of 6 mm, equivalent to 10 µL of blood were punched for determination of C4-carnitine and C0-carnitine levels using electrospray tandem mass spectrometry. The acylcarnitines were extracted with methanol and analyzed as their butyl esters using (2H3)-, (2H3)-C3-, (2H3)-C8-, (2H3)-C16- carnitine as internal standards. The reference range used for the C4-carnitine values was 0.14-0.87 µmol/L.

**Statistical analyses**

SPSS (SPSS 12.0.1), Graphpad Prism 3.0, and CIA (version 1, 1989) software programs were used for analyzing the data from the DNA and acylcarnitine analyses.

The necessary sample size for this study was calculated from an estimated percentage for ACADS genotypes homozygous or compound heterozygous for the ACADS variants and/or the c.1058C>T mutation of approximately 6% in de reference group, based on

**Table 1. ACADS genotypes in study populations**

<table>
<thead>
<tr>
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<th>Current study</th>
<th>Nagan study</th>
<th>Previous study</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Total</td>
<td>909</td>
<td>100</td>
<td>694</td>
</tr>
<tr>
<td>511C/C</td>
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<td>511T/C</td>
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<td>2</td>
</tr>
<tr>
<td>625G/G</td>
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<td>59.7</td>
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</tr>
<tr>
<td>625A/G</td>
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<td>35</td>
<td>221</td>
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<tr>
<td>625A/A</td>
<td>48</td>
<td>5.3</td>
<td>39</td>
</tr>
<tr>
<td>1058C/C</td>
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<td>99.3</td>
<td>221</td>
</tr>
<tr>
<td>1058T/C</td>
<td>6</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1058T/C+625A/G</td>
<td>1</td>
<td>0.1</td>
<td>7</td>
</tr>
<tr>
<td>625A/G+511T/C</td>
<td>18</td>
<td>2</td>
<td>7</td>
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</table>
previous studies.\textsuperscript{1,5,6} A percentage 3 times as high, i.e. 18\%, in the patient group was supposed to indicate an association between these specific ACADS genotypes and epilepsy. Using a two-tailed $\alpha=0.05$ and power=80\% we estimated that a sample size of at least 65 patients and 260 reference subjects were required.

To estimate the association between the ACADS genotypes and epilepsy, the odds ratio was calculated. As methods for obtaining plasma and the time period between blood puncture and C4-C analysis were different between the epilepsy and reference group, the C4-C results were not compared between these 2 groups. In 311 blood spots from the reference group acylcarnitine analyses were performed in the first year after they were obtained (>5 years after heel puncture) and in 598 blood spots in the fifth year after they were obtained (>9 years after heel puncture). To compare C4-C values between 5-year-old blood spots and 9-year-old blood spots within each of the 14 screening districts in the reference group, the Mann-Whitney test was used.

\section*{Results}

\textbf{Incidence of c.511C>T and c.625G>A ACADS gene variants and the c.1058C>T mutation in the Dutch population reference group}

The distribution of genotypes determined for the c.511C>T variant was clearly in favour of the wild type alleles. In 81 of the 909 individuals (8.9\%) one copy of the c.511C>T

\begin{table}
\centering
\begin{tabular}{lcc}
\hline
 & Observed frequency (%) & Expected frequency (%) \\
\hline
Total & 100 & 100 \\
511C/C & 91.0 & 91.1 \\
511T/C & 8.9 & 8.7 \\
511T/T & 0.1 & 0.2 \\
625G/G & 59.7 & 59.7 \\
625A/G & 35 & 35 \\
625A/A & 5.3 & 5.3 \\
1058C/C & 99.3 & 99.4 \\
1058T/C & 0.7 & 0.6 \\
\hline
\textbf{Combinations} & & \\
1058T/C+625A/G & 0.1 & 0.07 \\
625A/G+511T/C & 2 & 1.04 \\
\hline
\end{tabular}
\caption{Expected genotype frequencies calculated from the observed allele frequencies assuming Hardy-Weinberg equilibrium}
\end{table}
variant was identified and 1 individual (0.1%) was homozygous for this variant (Table 1). Homozygosity and heterozygosity for the c.625G>A variant was far more prevalent, with percentages of 5.3 and 35 % respectively. In 18 of the 909 individuals (2%) compound heterozygosity for the 2 different variants was established. In 6 of the 909 individuals (0.7%) 1 copy of the c.1058C>T mutation was identified. One of these 6 also carried 1 copy of the c.625G>A gene variant.

Overall, the c.511C>T and c.625G>A ACADS variants and the c.1058C>T ACADS mutation were detected in homozygous and compound heterozygous forms in 68/909 (7.5%, 95% CI 5.9-9.4%) subjects from the Dutch population.

Expected genotype frequencies in the Dutch population assuming Hardy-Weinberg equilibrium

Based on the observed allele frequencies of 4.6, 22.8, and 0.3% for the c.511C>T and c.625G>A ACADS variant and c.1058C>T mutation respectively, expected genotype frequencies were calculated assuming Hardy-Weinberg equilibrium (Table 2).

Demographic data of the epilepsy study group

Data of 131 epilepsy patients (53 girls and 78 boys) were studied. Age at study inclusion ranged between 0 and 16 years (median 9 years). Age of first epileptic insult ranged from 0 to 6 years (median 3 years). In all patients, the epilepsy was defined as idiopathic.

<table>
<thead>
<tr>
<th>Table 3. ACADS genotypes in the epilepsy and reference group</th>
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<tbody>
<tr>
<td><strong>Epilepsy group</strong></td>
</tr>
<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>511C/C</td>
</tr>
<tr>
<td>511T/C</td>
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<tr>
<td>511T/T</td>
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<tr>
<td>625G/G</td>
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<tr>
<td>625A/G</td>
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<tr>
<td>625A/A</td>
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<tr>
<td>1058C/C</td>
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<tr>
<td>1058T/C</td>
</tr>
<tr>
<td><strong>Combinations</strong></td>
</tr>
<tr>
<td>1058T/C+625A/G</td>
</tr>
<tr>
<td>625A/G+511T/C</td>
</tr>
<tr>
<td>511T/T, 625A/A, 1058T/C+625A/G, 625A/G+511T/C</td>
</tr>
</tbody>
</table>

* Not significantly different (OR 1.3, 95% CI 0.7-2.4)
Development was normal in 92/131 (70%) patients and 39/131 (30%) had delayed cognitive and/or motor function.

Incidence of c.511C>T and c.625G>A ACADS gene variants and the c.1058C>T mutation in the epilepsy study group and comparison with the reference group

In 12 of the 131 patients (9.8%) one copy of the c.511C>T variant was identified, but none of the epilepsy patients was homozygous for this variant (Table 3). The c.625G>A variant was identified in heterozygous form in 48 (36.1%) and in homozygous form in 10 (7.5%) epilepsy patients. One of the patients (0.8%) carried 1 copy of the c.1058C>T mutation. Overall, the c.511C>T and c.625G>A ACADS variants and the c.1058C>T ACADS mutation were detected in homozygous and compound heterozygous forms in 12/131 (9.2%, 95% CI 4.8-15.5%) epilepsy patients. This was comparable to the reference group (7.5%, Table 3). Within the epilepsy group, these combined ACADS genotypes were established in
4/39 (10%, 95% CI 2.9-24.2%) patients with developmental delay and in 8/92 (8.7%, 95% CI 3.8-16.4%) patients without developmental delay.

C4 carnitine levels in the Dutch population and epilepsy study group

C4-C values in the 9-year-old blood spots were significantly (p<0.0001) lower in 12 out of 14 screening districts and significantly higher (p<0.0001) in 2 out of 14 screenings districts, compared to the values in 5-year-old blood spots from the same districts. Therefore, the results of the analyses of the 9-year-old blood spots were taken to be unreliable and are not included in this paper.

In the collection of 311 5-year-old blood spots, 11 were found to have C4-C values above the upper limit of 0.87 μmol/L (Figure 3). The highest C4-C value was established in the only reference subject with one 1 copy of the c.625G>A variant in combination with 1 copy of the c.1058C>T mutation. None of the other 10 subjects with increased C4-C values carried more than 1 copy of an ACADS variant or the c.1058C>T mutation.

None of the epilepsy patients was found to have a blood spot C4-C concentration above the upper limit of 0.87 μmol/L (Figure 4). Therefore an elevation in C4-C concentration in blood spots was not identified more often in the patient compared to the reference group.

Figure 4. C4-Carnitine in blood spots from 131 epilepsy patients. The dotted line indicates the upper limit of normal values (0.87 μmol/L).
Discussion

The current study is the first one investigating the prevalence of the c.511C>T ACADS variant and the c.1058C>T ACADS mutation in the Netherlands. In addition, it is the first study investigating a potential association between SCADD and one of its most frequently associated symptoms: epilepsy.

A number of 131 patients and 909 reference subjects were included, resulting in >98% power to detect a difference of 12% between the 2 groups.

Homozygosity and heterozygosity for the c.511C>T ACADS variant in the Dutch population (0.1 and 8.9% respectively) were found to be comparable to numbers in the US population (Table 1).6 The prevalence of the most common ACADS variant, the c.625G>A, in the Netherlands, has been investigated previously.5 The current study provided similar results (5.3 and 35% respectively, Table 1). As these results also are comparable to numbers in the US population, it appears that both ACADS variants, which are supposed to confer susceptibility for the development of SCADD, show a proportional representation throughout the Western world. This implies that every 1:14 Western newborns should be considered to be susceptible to the development of SCADD. In the Netherlands with an annual birth rate of 180 000,26 this would correspond to approximately 12 500 newborns with supposed susceptibility for SCADD each year.

In addition we investigated the prevalence of the c.1058C>T mutation, which is supposed to represent a Dutch founder mutation.1 The results of the current study do provide evidence for a founder mutation as 0.7% percent of the reference group was identified with 1 c.1058C>T copy, all patients carrying the c.1058C>T mutation were of Dutch ancestry, and as this mutation has not been reported in patients without Dutch ancestors.1 Based on the established frequencies for this Dutch mutation and the ACADS variants, a birth-prevalence as high as 1:1000 for an ACADS genotype, which is compound heterozygous for 1 of the ACADS variants and the c.1058C>T mutation, can be calculated. This ACADS genotype has been associated with biochemical features (increased C4-C and/or EMA) of SCADD in all previously reported cases1 and was associated with the highest C4-C concentration in the current study. For the Netherlands this would imply that in addition to approximately 12 500 newborns with supposed susceptibility for SCADD, 180 newborns with SCADD would be born every year. The results of our study demonstrate that the combined prevalence of all SCADD related genotypes in the Netherlands amounts to at least 1:1000, which is significantly higher than our previous estimate of 1:3300.1 This is in strong contrast with the less than 40 patients with SCADD that have been diagnosed in the Netherlands within the last decades.

Failure to diagnose SCADD may be explained if SCADD often presents with clinical signs and symptoms generally not leading to metabolic studies in urine or plasma for increased EMA and/or C4-C concentrations. As epilepsy appears to be a common clinical symptom in SCADD, and as uncomplicated epilepsy in childhood is usually not followed by diagnostic tests for metabolic diseases, a high prevalence of SCADD in patients with
unexplained epilepsy might in part explain the high number of unrecognized SCADD patients.

We therefore investigated the presence of the most common ACADS genotypes (representing 61% percent of the ACADS genotypes in the Dutch SCADD patient group) and the presence of an increased C4-C (as present in almost all Dutch SCADD patients with at least one ACADS mutation) in pediatric patients with epilepsy of unknown origin and reference subjects. However, our study failed to demonstrate an association between homozygosity or compound heterozygosity for the c.511C>T and c.625G>A ACADS gene variant and/or the Dutch c.1058C>T ACADS mutation and/or increased C4-C in blood spots on the one hand, and epilepsy of unknown origin in childhood on the other hand.

A first limitation of our study, intrinsic to our study design, is that we compared ACADS genotypes rather than SCADD defined by the presence of increased biochemical characteristics (increased C4-C and/or EMA) in combination with an ACADS genotype. A second limitation is that we cannot rule out an association of epilepsy with the studied ACADS genotypes with an OR smaller than 2.4. However, given the results of our study, the probability of an OR larger than 2.4 is 2.5% only, and therefore not very likely.

In conclusion, the results of our study show that the prevalence of SCADD in the Netherlands is at least 1 in every 1000 newborns, but do not provide evidence for an association between SCADD and epilepsy. Therefore, SCADD does not seem to be an important risk factor for the development of epilepsy and metabolic investigations aimed to diagnose SCADD in epilepsy of unknown origin in childhood cannot be recommended.

Acknowledgments

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