Short-chain acyl-CoA dehydrogenase deficiency
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Summary
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Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a relatively newly recognized autosomal recessive inborn error of metabolism, for which many issues still need to be elucidated. The SCAD enzyme participates in the final β-oxidation cycle of mitochondrial fatty acid oxidation (FAO), a pathway central to the provision of energy for the mammalian organism. SCADD is most frequently reported to be associated with neurological symptoms and/or hypoglycemia, although the spectrum of clinical symptoms is remarkably broad. Most SCADD patients carry common SCAD-encoding gene (ACADS) variants or ACADS variants in combination with an ACADS mutation. The ACADS variants are considered to confer susceptibility to development of clinical disease and their high prevalence suggests that SCADD might be far more prevalent than initially assumed. SCADD is biochemically characterized by increased levels of C4-carnitine (C4-C) in plasma and ethylmalonic acid (EMA) in urine. The pathophysiology of SCADD is still speculative and the efficacy of a potential treatment has never been systematically studied. Nevertheless screening for SCADD had been included in newborn screening programs in most United States and Australian states.

This thesis focuses on the clinical, biochemical, genetic, epidemiological, pathophysiological, and therapeutic aspects of SCADD in order to increase knowledge of this intriguing disorder and to provide better care for the patients.

In Chapter 2, the results of a study on the prevalence of the c.625G>A ACADS variant in the Netherlands as well as the correlation of C4-C levels with the c.625G>A variant are reported. The screening cards of 1036 newborns were analyzed, and homozygosity and heterozygosity for the c.625G>A variant was established for 5.5% and 31.3% of these individuals, respectively. No significant differences were found for C4-C concentrations or for ratios of C4-C to free carnitine in blood spots among newborns who were homozygous, heterozygous, or non-carriers for this ACADS variant. Therefore, a high frequency of the c.625G>A ACADS variant in the Dutch population, but no correlation with significantly increased C4-C levels in blood spots taken between the 5th and 8th days of life was demonstrated. This latter observation might be the result of the relatively late timing of newborn screening in the Netherlands, which implies that some FAO disorders may be missed at that stage. If the c.625G>A variant is associated with clinical SCADD, the high frequency of the variant suggests a possible involvement of SCADD in the pathogenesis of common disorders, probably in relation to other genetic and/or environmental factors. However, homozygosity for the c.625G>A ACADS variant might be only a biochemical phenomenon, representing a non-disease.

Chapter 3 provides extensive data on clinical, biochemical, and genetic features of SCADD based on a group of 31 Dutch SCADD patients and their 8 SCADD relatives. In addition, these data are used to discuss newborn screening for SCADD.
A birth-prevalence of at least 1:50,000 was calculated, which suggested that SCADD is more common than previously assumed. Most patients presented before the age of 3 years, with non-specific, generally uncomplicated and often transient symptoms. Developmental delay, epilepsy, behavioral disorders, and hypoglycaemia were the most frequently reported. The ACADS genotype, subdivided into mutation/mutation (mut/mut), mutation/variant (mut/var) and variant/variant (var/var) genotype groups showed a statistically significant association with EMA and C4-C levels but not with clinical characteristics. Seven out of 8 SCADD relatives were free of symptoms.

These data and data of newborn screening studies show that SCADD lacks clinical significance in many patients as well as individuals diagnosed by newborn screening and show that it is not possible to differentiate between diseased and non-diseased individuals. SCADD therefore does not meet major newborn screening criteria and is not suited for inclusion in newborn screening programs at the present time.

**Chapter 4** provides insight into the pathophysiological consequences of SCADD through a retrospective study of 15 fasting and 6 fat-loading tests in 15 SCADD patients. Three patients developed hypoglycemia during fasting and all of these had originally presented with hypoglycemia. The EMA excretion increased in response to fasting and fat-loading, and plasma C4-C remained stable. Concentrations of all other studied metabolites in plasma and urine, including those related to ketogenesis, and all relevant hormones remained normal during all tests. Test results did not differ between the patients with a mut/mut, mut/var, and var/var genotype.

The metabolic profiles of the 3 patients who developed hypoglycemia resemble those of patients with idiopathic ketotic hypoglycemia. Because an unexplained episode of hypoglycemia will generally be followed by a metabolic work-up and because SCADD is relatively prevalent, SCADD may very well be diagnosed coincidentally and thus be causally unrelated to the hypoglycemia. If SCADD has other pathological consequences, the accumulation of potentially toxic metabolites such as EMA is most likely involved. However, the results indicate that there is no clear pathophysiological significance, irrespective of genotype. This further supports the claim that SCADD is not suited for inclusion in newborn screening programs.

**Chapter 5** presents results of a study on flavin adenine dinucleotide (FAD) status and the effects of riboflavin supplementation in a prospective open-label cohort study involving 16 SCADD patients, subdivided into mut/mut, mut/var, and var/var genotype groups. Blood FAD levels were normal in all patients before therapy but significantly lower in the mut/var and var/var groups as compared with the mut/mut group. Riboflavin treatment caused a decrease in EMA excretion in the mut/var group and a subjective clinical improvement in 4 patients from this group. However, this improvement persisted after treatment was stopped. These results indicate that high-dose riboflavin treatment may improve the biochemical features of SCADD, at least in patients with a mut/var genotype.
and low FAD levels. A clinically relevant effect of riboflavin could not be demonstrated and therefore general use of riboflavin cannot be recommended.

The biochemical response to exercise and the effects of high-dose riboflavin therapy on the observed response in 3 SCADD patients, all with mut/var genotypes and symptoms of exercise intolerance or fatigue, are presented in Chapter 6. C4-C concentrations in plasma increased in response to exercise, while EMA excretion remained stable. One patient, who responded clinically to riboflavin therapy, was retested during riboflavin therapy. During this test, the increases in plasma C4-C in response to exercise were not observed anymore. However, 2 years after stopping riboflavin therapy, C4-C was again found to increase in response to exercise in this patient, even though she was still without clinical symptoms. As C4-C increased in SCADD patients during exercise and EMA increased during fasting, preferred tissue-specific pathways might exist. In addition, high-dose riboflavin therapy may prevent C4-C increase during exercise but this does not appear to be related to any clinical effect in this particular patient.

Chapter 7 presents a review of the clinical aspects of SCADD. SCADD generally presents early in life and is most frequently associated with developmental delay, hypotonia, epilepsy, behavioral disorders, and hypoglycemia. However, these symptoms often ameliorate, they can disappear spontaneously during follow-up, and they were found to be unrelated to the ACADS genotype. In addition, in some cases symptoms initially attributed to SCADD could later be explained by other causes. Finally, SCADD relatives of SCADD patients as well as almost all SCADD individuals diagnosed by neonatal screening, remained asymptomatic during follow-up.

Based on these observations, one may question the clinical relevance of SCADD. We therefore searched the database of the laboratory for metabolic diseases in our centre for the most frequently reported symptoms of patients for whom metabolic studies were requested. These symptoms were comparable to those in the SCADD patients group, suggesting that the association might indeed be coincidental.

The potential lack of clinical consequences implies that the diagnosis SCADD should never preclude extension of the diagnostic workup for other potential causes of the observed symptoms and patients and parents should be clearly informed about the potential irrelevance of the disorder. Furthermore, to date, SCADD does not qualify for inclusion in newborn screening programs. More studies are needed to establish the relevance of SCADD and to solve the question of whether SCADD is involved in a multifactorial disease or if it represents a non-disease.

In view of the debate on the clinical significance of SCADD and to further estimate the extent of the Dutch SCADD population, a case-referent study among 131 pediatric patients with epilepsy and 909 anonymous newborns was performed (Chapter 8). The 2 ACADS variants and the most common Dutch ACADS mutation, c.1058C>T, were
detected in either homozygous or compound heterozygous forms in 9.2% of the epilepsy group and in 7.5% of the reference group. In none of the epilepsy patients C4-C was increased. These data imply that the birth-prevalence of SCADD with a mut/var genotype in the Netherlands is even higher than 1:1000 but provide no evidence for an association between SCADD and epilepsy. Therefore SCADD studies in epilepsy of unknown origin in childhood cannot be recommended.

Finally in chapter 9, it is concluded that SCADD should be considered a very common metabolic variant. The ACADS genotype correlates with the biochemical phenotype, i.e. the degree of EMA and C4-C increase, and the latter can be influenced by the FAD status and by external circumstances that stimulate FAO, particularly fasting. Considering SCADD as a metabolic variant implies that SCADD should not be included in newborn screening programs. A practical guideline in the case of elevated EMA and/or C4-C is presented. This guideline illustrates that for clinically identified patients with increased EMA and/or C4-C, clinically relevant disorders that are associated with these biochemical characteristics should be excluded. This may, as a consequence, result in the diagnosis of SCADD, after which patients and/or parents should be informed that SCADD is a metabolic variant and additional diagnostic studies for potential causes of the clinical signs and symptoms should be performed.