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Mini-Review: Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

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Abstract

Short chain acyl-CoA dehydrogenase deficiency (SCADD) is a disorder of mitochondrial fatty acid oxidation that leads to the accumulation of butyrylcarnitine and ethylmalonic acid in blood and urine. Originally described with a relatively severe phenotype, most patients are now diagnosed through newborn screening by tandem mass spectrometry and remain asymptomatic. Molecular analysis of affected individuals has identified a preponderance of private inactivating point mutations and one common one present in high frequency in individuals of Ashkenazi Jewish ancestry. In addition, two polymorphic variants have been identified that have little effect on enzyme kinetics but impair folding and stability. Individuals homozygous for one of these variants or compound heterozygous for one of each often show an increased level of ethylmalonic acid excretion that appears not to be clinically significant. The combination of asymptomatic affected newborns and the frequent variants can cause much confusion in evaluating and treating individuals with SCADD. The long term consequences and the need for chronic therapy remain current topics of contention and investigation.

Introduction

Short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) is an autosomal recessive inborn error of mitochondrial fatty acid oxidation. Mitochondrial fatty acid oxidation results in sequential cleavage of two carbon units from fatty acids and represents an important source of energy for the body during times of fasting and metabolic stress. It also serves continually as an energy source for the heart. Energy production after release of free fatty acids from adipose tissue is a complicated process that occurs in four basic stages: the carnitine cycle; β -oxidation; the electron-transfer pathway; and in liver, the synthesis of ketone bodies.

The acyl-CoA dehydrogenases (ACADs) are a family of enzymes that catalyze the α,β -dehydrogenation of acyl-CoA esters, transferring electrons to electron transferring flavoprotein

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(ETF). In humans, very long, medium, and short chain acyl-CoA dehydrogenases (VLCAD, MCAD, and SCAD) catalyze the first step in the β -oxidation cycle with substrate optima of 16, 8, and 4 carbon chains, respectively. SCAD is a homotetramer with each monomer containing a non-covalently bound flavin adenine dinucleotide (FAD) molecule as a prosthetic group [1,2,3]. It is encoded in the nuclear genome as a precursor protein, but similar to all of the ACADs, it functions in mitochondria [4,5,6]. The ACADs are postulated to share a common ordered Bi Bi type kinetic mechanism. Reduction of the enzyme via dehydrogenation of the substrate occurs when a glutamate residue acting as a catalytic base abstracts the α -carbon proR hydrogen of substrate as a proton. Electrons from this reaction are passed to electron transfer flavoprotein and then directly into the electron transport chain via electron transfer flavoprotein-ubiquinone oxidoreductase (also designated electron transport flavoprotein dehydrogenase) [7,8,9]. SCAD is most active *in vitro* with hexanoyl- and butyryl-CoA as substrates, but its physiologic role is specific to butyryl-CoA as none of the other ACADs are active with this substrate *in vivo*. In the absence of SCAD, the byproducts of butyryl-CoA accumulation, including butyrylcarnitine, butyrylglycine, ethylmalonic (EMA) acid, and methylsuccinic acid, accumulate in blood, urine, and cells [10]. EMA, the hallmark of SCAD deficiency, is likely formed by the carboxylation of excess butyryl-CoA by propionyl-CoA carboxylase.

Primary SCADD, the topic of this review, is attributed to alterations in the *SCAD* gene. Secondary SCADD deficiency occurs in defects of the electron transfer flavoprotein, electron transfer flavoprotein-ubiquinone oxidoreductase, and flavin adenine dinucleotide deficiency. It may also manifest in disorders of oxidative phosphorylation as evidenced by accumulation of ethylmalonic acid in the blood and urine of some of these patients. Primary SCADD can result from multiple mutations or two common coding polymorphisms that have been described in the *SCAD* gene. Genotype-phenotype correlations, however, have been inconsistent [11, 12].

Clinical Presentation

The first published case of what was initially considered to be SCADD was a 53-year-old woman with progressive myopathy whose symptoms began in her forties [13]. She was later felt to have a multiple acyl-CoA dehydrogenation defect [14]. True SCADD was first reported in two neonates who were found to have increased urinary ethylmalonate excretion; the diagnosis was confirmed enzymatically in skin fibroblasts [15]. One of these infants died of overwhelming neonatal acidosis as would be typical for an organic acidemia. Further case reports have helped to define the clinical, biochemical, and pathological phenotype as being heterogenous [14,16]. Using fairly strict biochemical and molecular criteria, a birth prevalence of at least 1:50,000 has been estimated in the Netherlands [17].

A range of phenotypes has now been ascribed to primary SCADD including failure to thrive, metabolic acidosis, ketotic hypoglycemia, developmental delay, seizures, and neuromuscular symptoms such as myopathy and hypotonia. The disorder has been reported in infants, children, and adults [14,18]. In the largest series of patients published to date, 69% of 114 patients had developmental delay. Three large sub-groups of patients were identified: 1) failure to thrive with feeding difficulties and hypotonia (23 patients, 20%); 2) developmental delay and seizures (25 patients, 25%); and 3) developmental delay and hypotonia without seizures (34 patients, 30%). Four individuals were asymptomatic, identified either through family studies or newborn screening [19]. In another report, 16/31 patients exhibited developmental delay, 12 had epilepsy, and 10 showed a behavioral disorder. Only 8 patients had a history of hypoglycemia. Seventeen of the patients were reported to ultimately improve (8/17) or completely normalize (9/17) over time. No consistent correlation was noted between phenotype or EMA excretion, and no consistent improvement with therapy could be documented. In addition, asymptomatic

relatives of patients with identical genotypes were identified [17]. Another study of 10 affected individuals of Ashkenazi Jewish ancestry revealed 8 cases with developmental delay and 4 with significant myopathy (including biopsy proven mini-multicore disease). Again, asymptomatic family members with the same genotype as the patient were found [20]. Acute fatty liver of pregnancy with preeclampsia and HELLP syndrome in the mother of an affected fetus has also been described [21,22].

Newborns with SCAD deficiency are now being identified due to widespread implementation of expanded newborn screening by tandem mass spectrometry. In general, patients diagnosed through newborn screening have shown normal growth and development in contrast to those diagnosed as a result of clinically initiated evaluations [23,24,25]. Moreover, the former usually have complete deficiencies while the latter mostly have gene variants of unknown significance (see next section).

Molecular Genetics and Functional Implications of SCAD mutations

Understanding the molecular basis of SCADD is key to deciphering its variable clinical picture. The *SCAD* gene is located on chromosome 12q22 and is approximately 13 kb long with 10 exons and 1,236 nucleotides of coding sequence [26,27]. SCAD, like all of the ACADs, is a flavoprotein that is synthesized in the cytosol as a precursor and transported to the mitochondria for further processing by proteolytic cleaving of an N-terminal mitochondrial targeting sequence into a mature form [27,28]. The crystal structure of recombinant rat SCAD has revealed it to be a homotetramer arranged as a dimer of dimers that is highly conserved with the other ACAD structures. A glutamic acid residue located at amino acid position 368 of the mature SCAD protein (homologous to position 376 in MCAD) acts as the catalytic base to initiate the catalytic reaction [29]. Mutation of this residue in the SCAD enzyme to a Gln or Ala inactivates the enzyme. Each ACAD enzyme also has amino acid residues specific to its particular function. In *SCAD*, Gln-254 and Thr-364 appear to shorten the substrate binding pocket and contribute to its substrate specificity [30].

At least 35 inactivating mutations and two polymorphic variants have been reported in the *SCAD* gene [17,19]. A 511C>T polymorphism located in exon 5 leads to an amino acid substitution of tryptophan for arginine at position 147 of the mature enzyme (R147W; position 171 in the precursor protein). A 625G>A variant in exon 6 substitutes a serine for a glycine at position 185 of the mature protein (G185S; position 209 in the precursor protein). Both polymorphic variants are relatively common in the general population. In a study of 694 newborns in the United States, approximately 6% were 625G>A homozygous, 0.3% were 511C>T homozygous, and 1% were compound heterozygous. The allele frequencies for the 625G>A and 511C>T polymorphisms are 3% and 22%, respectively. In the U.S., 7% of the population is estimated to be homozygous for one of the polymorphisms or compound heterozygous for one of each [12]. Based on this frequency alone, it is clear that homozygosity for one of the polymorphisms cannot by itself be sufficient for the development of clinical symptoms.

It has been demonstrated that homozygosity for one of the polymorphisms is associated with an increased incidence of elevated EMA excretion. In one European study, 14% of controls were homozygous for one of the polymorphisms as compared to 69% of 133 subjects with an increased urinary EMA excretion [11]. Since most individuals who are homozygous for the polymorphisms are asymptomatic, it has been suggested that the presence of the polymorphisms may represent a susceptibility state that requires one or more other genetic or environmental factors to be present for development of disease. If this is true, their contribution to any clinical symptoms must be small or it would be readily discernable due to the frequency of the alleles in the general population.

Nearly all reported *SCAD* mutations are missense, an unusual spectrum of mutations compared to genetic diseases including other fatty acid oxidation defects. There are a number of multiply identified mutations and one common mutation in the Ashkenazi Jewish population (319 C>T, carrier frequency 1:8) [20]. For those alleles that have been directly tested, the mutations have all led to complete inactivation of mutant enzyme, impaired biogenesis of the enzyme, or formation of aggregates of misfolded protein within mitochondria [31]. The level of dysfunction is often adversely affected by other external factors such as temperature and pH [32]. In these studies, the level of protein dysfunction was found to correlate well with biochemical phenotype (i.e., level of EMA excretion) but not clinical phenotype. Functional studies of the polymorphic variant *SCAD* enzymes have been somewhat mixed. Expression of the R147W and G185S variants in a prokaryotic system led to stable proteins that could be purified and had similar, but not identical catalytic properties to the wild type enzyme. However, both variant proteins show impaired folding and increased aggregation in mitochondrial import studies, especially at elevated temperatures. In a recent study, analysis of the *SCAD* gene in 114 patients revealed 29 variations (26 missense, one start codon, and two stop codon variations). *In vitro* import studies of variant *SCAD* proteins in isolated mitochondria from *SCAD* deficient mice demonstrated an increased tendency of the abnormal proteins to misfold and aggregate compared to the wild type. In other disorders, this has been shown to lead to gain-of-function (cellular toxicity) phenotypes. However, no correlation was found between the clinical phenotype and the degree of *SCAD* dysfunction [19].

A mouse deficient of *SCAD* activity was originally identified by screening animals from a non-specific mutagenesis program. It fairly faithfully reproduces the biochemical phenotype of the human disease with plasma accumulation of butyrylcarnitine and urinary EMA excretion [33]. While the animals appeared asymptomatic, their brain EEG pattern was abnormal [34]. In addition, fasting-induced fatty liver that reversed with resumption of caloric intake was observed. The combination of a mutant *SCAD* allele in heterozygous fashion with heterozygous mutations in other *ACAD* genes leads to cold intolerance in the compound heterozygotes that is not present in any of the single heterozygotes, emphasizing the possibility for mutations in *SCAD* to produce symptoms in combination with other genetic and environmental factors [35,36].

Pathophysiology of SCADD

Most individuals have not presented with the classic picture of hypoketotic hypoglycemia, recurrent rhabdomyolysis, or cardiomyopathy that characterize many other fatty acid oxidation disorders. A 14 month old with *SCADD* was reported to have hypoglycemia, but ketonuria was also present, suggesting that ketone body formation is not significantly impacted in *SCADD* [37]. One explanation for the lack of classical biochemical findings is that since *SCAD* is only needed at the end of the β -oxidation cycle, there may be sufficient stimulation of gluconeogenesis and ketogenic capability from activity of the fatty acid oxidation pathway preceding it. Overlap of substrate specificity of *SCAD* and *MCAD* may also partially compensate for the deficiency of the former enzyme.

It is interesting to note that most symptomatic patients with *SCADD* have presented with predominantly neurologic manifestations, especially developmental delay, which is uncommon in the other β -oxidation defects. This has led to speculation of a direct neurotoxic effect related to *SCAD* deficiency. EMA has been postulated to play a role in the pathogenesis of *SCADD* since it has been shown to significantly inhibit creatine kinase activity in the cerebral cortex of Wistar rats without impacting levels in the skeletal or heart muscle [10]. EMA has also been shown to inhibit electron transport chain activity *in vitro* [38]. Butyric acid, which accumulates in *SCADD*, may also contribute to the disease course since it is well known to modulate gene expression in elevated levels due to its action as a histone deacetylase [39].

Its highly volatile physical property as free acid may also add to its neurotoxic qualities. Differentiating between cause and effect of symptoms (especially relatively common and non-specific ones such as developmental delay) vs. coincidental association in SCADD remains one of the major challenges in this deficiency.

Finally, the finding that most mutations identified in SCADD patients are point mutations that nearly all lead to some measure of intra-mitochondrial aggregation of the abnormal protein suggests the possibility that the protein aggregation itself may play a role in the development of symptoms in SCADD. Such a mechanism of pathogenesis is now well recognized in a number of disorders with neurologic phenotypes such as Alzheimer disease [40,41].

Diagnosis of SCADD

Biochemical markers of SCADD include increased urinary EMA and butyrylglycine, increased plasma butyrylcarnitine, and decreased SCAD activity in skin fibroblasts or muscle, though some enzymatic assays are not sufficiently sensitive for reliable measurements [11]. During times of metabolic stress, methylsuccinate (the hydrolyzed product of isomerization of ethylmalonyl-CoA by methylmalonyl-CoA isomerase) may also be excreted in the urine [14, 15]. Some patients have been reported to have low serum or muscle carnitine levels, but this is not a consistent finding [16]. An initial biochemical evaluation for SCADD should include a urine organic acid profile, plasma acylcarnitine profile, and plasma carnitine levels. While elevated EMA in urine is characteristic of SCADD, it is not diagnostic. Other disorders with urinary EMA include glutaric acidemia type II, EMA encephalopathy and some defects of mitochondrial oxidative phosphorylation. It is also important to note that characteristic biochemical findings of SCADD may be normal in affected individuals when they are well and may only manifest during times of physiologic stress such as fasting and illness. Thus, a negative evaluation does not eliminate the possibility of the disorder [22,42].

DNA mutational analysis, starting with identification of the common polymorphic variants and proceeding to full gene sequencing if normal, is the most appropriate next diagnostic test. Full gene sequencing even in the presence of the polymorphisms is warranted if the fibroblast acylcarnitine profile suggests full deficiency. While genotype/phenotype correlations have not been consistent in SCADD, mutation analysis does seem to correlate well with biochemical findings. Patients homozygous for inactivating mutations generally show greater abnormalities in their biochemical profiles and significantly decreased SCAD activity, whereas individuals with only the polymorphic variants have less altered biochemical and enzymatic findings. Individuals with an inactivating mutation on one allele and a polymorphic variant on the other tend to have intermediate biochemical and enzymatic abnormalities [22]. Measurement of SCAD activity levels in lymphocytes, fibroblasts, or muscle may be helpful when clinical symptoms and/or biochemical findings appear to be inconsistent with DNA sequencing results. Characterization of the acylcarnitine profile in tissue culture media from cultured fibroblasts has been shown to reliably reflect cellular SCAD activity and is more readily available on a clinical service basis.

Newborn screening for SCADD requires specific comment. Elevation of butyrylcarnitine, or more correctly, “C4 carnitine”, can be predicted to be elevated in newborn blood spots from patients with SCADD. Normal cutoff values can be appropriately adjusted to result in identification of most individuals who have either an inactivating mutation on both *SCAD* alleles or an inactivating mutation on one with a polymorphic variation on the other. Newborns homozygous for the 625G>A polymorphism have a higher butyrylcarnitine concentration on blood spots than controls [12]. A concentration threshold to separate these individuals from the other two abnormal genotype groups has not always been clear, but more recent experience seems to indicate that this may be possible [43].

Data from approximately 362,000 newborns in New South Wales and Australian Capital Territory, Australia from 1998–2002 indicates that the overall prevalence of inborn errors did not increase significantly following the introduction of MS/MS screening (compared to data from 1982–1998), but that rates of certain diagnoses did increase. However, there was a rise in the rates of MCADD and SCADD [23]. All of the SCADD babies identified in this study have remained healthy. It must be remembered that isobutyryl-CoA dehydrogenase deficiency also leads to elevated C4 acylcarnitine in newborn screening by MS/MS of blood spots and thus must be differentiated from SCADD. The American College of Medical Genetics has published an online algorithm delineating the appropriate response to an elevated C4 acylcarnitine on newborn screening [44].

Management

Little firm data exists on the appropriate therapy for SCADD and there is not a consensus on the need to treat it. Chronic management of SCADD, if instituted, should be similar to other fatty acid oxidation disorders, focusing on decreasing catabolic drive as well as providing alternative sources of energy. During acute crises, intravenous fluids with high dextrose concentrations (usually at least 10% to give 8–10 mg/kg/min of glucose intake) with or without intralipids can be used to reverse the catabolic state. This is especially important if nausea or vomiting are present, which often prevent the patient from tolerating oral fluids. Hypoglycemia is uncommon but can be treated in the same fashion. Clinical course does not seem to be altered significantly by chronic therapies and in general, patient symptoms have improved with age. Preventive measures, if necessary, likely include only avoidance of fasting. The need for a low fat diet has not been substantiated.

It has been argued that increased metabolite elimination through formation of butyrylcarnitine (with carnitine supplementation) may be preferable to excretion of EMA, but there is little data in support of this hypothesis. Moreover, while SCAD-deficient mice have low blood and tissue carnitine levels, this finding has not been consistent in humans [45]. Since FAD is an essential cofactor for SCAD function, riboflavin supplementation has been suggested as a possible therapy for SCADD due to its potential ability to act as a chemical chaperone and stabilize mutant enzyme. One report described clinical improvement in a 5-year old female with a refractory seizure disorder and metabolic findings consistent with SCADD but the relationship of the change to therapy was not clear [46]. Another neonate with two inactivating SCAD mutations, hypertonicity, staring spells, and jitteriness was treated initially with frequent low fat feedings, riboflavin (200 mg/day), and carnitine (50 mg/kg/day) and subsequently did well [47]. This patient, now a teenager, and a subsequently affected sibling diagnosed in the neonatal period both have had no additional signs or symptoms. (Personal communication, Michael Bennett, PhD). In one 2-year old patient with SCADD who eventually died at three years of age from muscular atrophy and recurrent bronchopneumonia, carnitine and riboflavin did not have any apparent effect on the biochemical or clinical profile [48]. Thus, the need and efficacy of carnitine and riboflavin supplementation in SCADD remains unproven [17].

Discussion

SCADD demonstrates several of the clinical and public policy issues currently facing many inborn errors of metabolism identified by expanded newborn screening with tandem mass spectrometry, including deficiency of short/branched chain- and isobutyryl-CoA dehydrogenases as well as 3-methylcrotonyl-CoA carboxylase. Issues surrounding these disorders echo similar problems faced in earlier decades of newborn screening with diseases such as histidinemia and the Duarte variant galactosemia. Specifically, how does one best differentiate between a benign biochemical phenotype, a clinical disorder with incomplete penetrance, and a clinically relevant part of a multi-factorial disorder?

Fatty acid oxidation defects provide a classic example of how patients can derive great benefit from early detection. Prior to newborn screening, approximately 25% of known MCADD patients died and another 30–40% of cases exhibited variable developmental delay [49]. Although some patients were completely asymptomatic, the morbidity and mortality is clearly significant. In contrast, patients identified before the onset of symptoms and managed with appropriate preventive urgent care services have little morbidity and rare mortality with significant cost benefits to the health care system [50]. There is less certainty about the advantages of early detection of SCADD. Most symptomatic cases have come to light due to large diagnostic work-ups on children with non-specific findings such as developmental delay, failure to thrive, and myopathy. Some of these patients are found to have molecular testing, biochemical findings, and decreased enzyme levels which are consistent with SCADD, at which point they are labeled with the diagnosis. However, patients with the same genotypes and laboratory findings have been found to be asymptomatic, especially through newborn screening. Clearly, the issue of cause and effect requires more scrutiny. In the meantime, both symptomatic patients and those diagnosed on newborn screening should receive appropriate counseling about potential risks and current guidelines for management.

The example of isovaleric acidemia due to isovaleryl-CoA dehydrogenase (IVDH) deficiency offers some additional insight into the problem. The long history of this disorder and its clinical, biochemical, and molecular study leave no doubt that this deficiency can lead to catastrophic consequences in affected individuals. Yet, individuals with only mild elevations of isovaleryl-CoA-related metabolites in plasma, urine, and fibroblasts are now routinely identified by MS/MS newborn screening and nearly half of the mutant *IVD* gene alleles sequenced from these infants have a common recurring missense mutation, 932C>T; A282V [51]. Nearly all of these individuals have remained asymptomatic with mild or no dietary protein restriction with or without carnitine supplementation. If this group of asymptomatic patients had been recognized prior to the cases of penetrant disease, it may well have confounded the true association of IVDH deficiency with metabolic acidosis. Thus, a real association between the non-specific neuromuscular presentations reported in most patients with SCADD may exist, even if it is infrequent.

The curious finding that nearly all patients identified with SCADD have point mutations that lead to protein misfolding may provide some insight into a possible pathologic effect of SCADD. The loss of SCAD activity clearly can lead to the accumulation of abnormal organic acids and the true risk relative to this loss of function may be that of acute metabolic acidosis with metabolic stress. The risk may be lower than for isovaleric acidemia, but there is little other difference between the two disorders in this regard. In contrast, aggregation of abnormally folded SCAD protein in patient cells is distinct and may lead to otherwise unexpected cellular toxicity. Examples of misfolding defects that are known to lead to neurotoxicity include superoxide dismutase, β -amyloid protein, and α -synuclein. Moreover, SCAD misfolding is aggravated by environmental factors that may vary from person to person and interact with as of yet uncharacterized factors to cause disease in some individuals.

The lack of an easily discernible and consistent phenotype in SCADD has clouded its continued inclusion in the panel of disorders identified through newborn screening by MS/MS. Nevertheless, an argument can currently be made for its retention. The risk for acute metabolic decompensation with acidosis appears to be small but not null, and knowledge of the enzyme deficiency will expedite recognition and treatment of such an episode. Moreover, continued uncertainty over long term consequences of SCADD can only be addressed by larger, collaborative studies of individuals prior to symptoms. While this is not a classic indication for implementation of newborn screening, recognition of the disorder comes at no additional expense to an existing test. Appropriate education and reassurance of families (and primary care physicians) should allow collection of vital clinical data with minimal psychosocial

impact. In this regard, SCADD could prove to be a valuable model to study other secondary target diseases identified coincidentally with a technology aimed at another disorder. Continuing efforts to follow outcomes in such disorders and elucidate possible underlying mechanisms of disease will ultimately prove to be invaluable in guiding both newborn screening protocols as well as clinical management for physicians and families.

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