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# Short-chain acyl-CoA dehydrogenase gene mutation (319C>T) presents with clinical heterogeneity and is candidate founder mutation in Ashkenazi Jewish population

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**Abstract**

We report seven children (4 male, 3 female), three homozygous for 319C>T mutation and four compound heterozygous for the 319C>T and 625G>A variant in the short-chain acyl-CoA dehydrogenase (SCAD) gene (*ACADS*). All seven are of Ashkenazi Jewish origin in which group we found a 319C>T heterozygote frequency of 1:15 suggesting this may be a founder mutation with a predicted homozygous birth rate of 1:900 or due to selective advantage. Phenotype is variable with onset from birth to early childhood. Features include hypotonia (6/7), developmental delay (5/6), myopathy (4/7) with multicore changes in two and lipid storage in one, facial weakness (3/7), lethargy (4/7), and congenital abnormalities (3/7). One female with multiminicore myopathy had progressive external ophthalmoplegia, ptosis, and cardiomyopathy with pneumonia and respiratory failure. Two compound heterozygous brothers presented with psychosis, optic atrophy, pyramidal signs, and multifocal white matter abnormalities on MRI brain suggesting additional genetic factors. Elevated butyrylcarnitine (4/6), ethylmalonic aciduria (6/6), methylsuccinic aciduria (3/4), decreased butyrate oxidation in lymphoblasts (2/4), and decreased SCAD activity in fibroblasts or muscle (3/3) were shown. Expression studies of 319C>T in mouse liver mitochondria confirmed this to be disease causing. 625G>A is a common variant conferring disease susceptibility. Five parents were compound heterozygous for 319C>T and 625G>A, suggesting either reduced penetrance or broad clinical spectrum. The wide clinical and biochemical phenotypic variability of this 319C>T mutation suggests that this is a complex multifactorial/polygenic condition that should be screened for in individuals with multicore myopathy, particularly among the Ashkenazi population.


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## Introduction

Short-chain acyl-CoA dehydrogenase (SCAD) (EC 1.3.99.2) is the first enzyme of mitochondrial short-chain β-oxidation which catalyzes the dehydrogenation of C4–C6 fatty acids [1]. SCAD deficiency is an inherited autosomal recessive disorder which was first reported in 1987 [2]. Since then approximately 25 patients have been identified worldwide based upon reduced or absent SCAD activity *in vitro* [2–18] and the presence of ethylmalonic aciduria (EMA) which is believed to have only limited specificity [19]. It is a heterogeneous disorder in which the clinical phenotype varies from fatal metabolic decompensation in infancy with failure to thrive, developmental delay, hypotonia and seizures [6] to more subtle later onset progressive myopathy to asymptomatic individuals [10]. Another phenotype characterized by progressive external ophthalmoplegia with ptosis, cardiomyopathy, contractures, scoliosis and multicore myopathy has also been described [11]. Two additional individuals presenting with adult-onset proximal lipid storage myopathy [20,21] and with SCAD deficiency in muscle in both patients, but not in fibroblasts in one patient in whom it was measured [3], are now more likely to have multiple acyl-CoA dehydrogenase deficiency [6]. All patients with documented SCAD deficiency excrete increased concentrations of ethylmalonic acid in their urine, originating from the accumulation of butyryl-CoA which is the substrate for SCAD [22].

Subunits of the SCAD enzyme are nuclear encoded and synthesized in the cytosol as precursor proteins that are then imported into the mitochondrial matrix. The newly imported SCAD proteins are proteolytically processed, folded and assembled into the biologically active homotetramer (168 kDa) which contains one molecule of FAD per subunit [23]. The SCAD gene (*ACADS*) was cloned in 1989 [23] and subsequently two pathogenic mutations were identified in one patient with SCAD deficiency [4].These included C>T transitions at positions 136 and 319 of the coding region of *ACADS* which resulted in the substitution of Arg-22 and Arg-83 of the mature SCAD with Trp and Cys, respectively. In subsequent studies of two unrelated patients with SCAD deficiency, Gregersen et al. [9] identified three further disease-causing mutations confirmed by a marked reduction in SCAD activity after expression in COS-7 cells. These included the pathogenic mutation 1147C>T (Arg359Cys) in one patient with the disease susceptibility polymorphism 625G>A on the same allele and the 511C>T disease susceptibility polymorphism in the second allele. The second SCAD-deficient patient was a compound heterozygote for two disease-causing mutations, namely 274G>T (Gly68Cys) and 529T>C (Trp153Arg).

Presently, 22 disease-causing mutations have been reported in *ACADS*, all of which are of the missense type with the exception of one (a 3-bp deletion) [4,9,12,15,24]. In addition to these rare disease-causing mutations in *ACADS* which lead to a complete deficiency of SCAD activity, two common SCAD variations have been identified that are highly prevalent in the Western European population and encode proteins with decreased catalytic activity and/or thermostability, namely, 625G>A (Gly185Ser) and 511C>T (Arg147Trp). The G185S and R147W variant proteins, respectively, showed 86% and 69% activity of the mean wild-type SCAD value, respectively, when expressed in *Escherichia coli* at 37 °C [12]. Expression of the variant R147W SCAD protein, encoded by the 511T-625G allele, in COS-7 cells showed 45% activity at 37 °C in comparison with the wild-type protein, comparable levels of activity at 26 °C, and 13% activity when incubated at 41 °C. In contrast, expression of the variant G185S SCAD protein, encoded by the 511C-625A allele, in COS-7 cells demonstrated higher than normal activity at 26 and 37 °C and 58% activity at 41 °C [9]. These variations are overrepresented in homozygous or in compound heterozygous form in patients with elevated ethylmalonic acid concentrations (>18 mmol/mol creatinine) in the urine (69% versus 14% in the general Western European populations) [9,12,25]. The majority of patients reported with apparent SCAD deficiency carry only the common variant genotypes (625A/625A, 511T/511T, and 625A/511T) or a genotype defined by common variations in one allele and rare mutations in the other [9,12]. In another study in the Netherlands, 1036 screening cards of 5- to 8-day old newborns were analyzed to determine the frequency of the 625G>A variant and found 5.5% homozygosity and 31.3% heterozygosity for the 625G>A variant [26]. In the US, analysis of 694 newborn blood spots revealed that the allele frequency of the 625G>A variant was significantly higher (22%) than that of the 511C>T variant (3%) [27]. These gene variants were detected in either homozygous or compound heterozygous form in 7% of the study population. Additionally, the frequentcy of the 625G>A allele in the Hispanic population (30%) was significantly higher than that of the African–American (9%) and Asian (13%) subpopulations. Because the common *ACADS* variations are detected in up to 14% of the general population, they cannot be sufficient to cause SCAD deficiency. Biogenesis experiments at two temperatures have revealed that some of the SCAD variant proteins (R22W, G68C, W153R, and R359C) cause severe misfolding, whereas others (R147W, G185S, and Q341H) demonstrated less severe temperature-sensitive folding defects [28]. Based on the magnitude of the *in vitro* defects, these SCAD proteins have been characterized as folding-defective variants and mild folding variants, respectively. Pulse-chase experiments have demonstrated that the variant SCAD proteins either triggered proteolytic degradation by mitochondrial proteases or, particularly at elevated temperatures, aggregation of non-native conformers, which may play a role in the pathogenesis of SCAD deficiency. Thus it has been hypothesized that the 625G>A and

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511C>T variations, may in combination with other genetic and/or environmental factors, especially fever, trigger disease in some individuals [9].

We had previously described a unique variant phenotype characterized by progressive external ophthalmoplegia with ptosis, cardiomyopathy, contractures, scoliosis, and multicore myopathy in an individual who was homozygous for the 319C>T SCAD mutation. The purpose of this study was to characterize the clinical phenotypic spectrum arising from homozygosity or heterozygosity for this disease-causing mutation, with or without the 625 G>A disease susceptibility variation.

## Materials and methods

### *Patients*

The clinical features of 7 patients (two related) with EMA aciduria and/or deficient SCAD activity in cultured skin fibroblasts or biopsied muscle are summarized in Table 1 and their corresponding biochemical and molecular data are outlined in Table 2. The clinical report of patient E460-1 has been previously published [11]. All studies were performed with informed patient consent and the approval of the respective institutional review boards.

### *Controls*

DNA was isolated from anonymized lymphoblastoid cells of 105 individuals of Ashkenazi Jewish origin which were obtained from a cell bank repository in Jerusalem. The Human Subjects Review Board in Jerusalem approved the use of this control material [29].

### *Analytical methods*

Serum acylcarnitines were measured by fast-atom bombardment tandem mass spectrometry [30]. Urinary organic acids were identified as their trimethylsilyl derivatives by gas–liquid chromatography, and were confirmed by gas chromatography-mass spectroscopy (GCMS) [31]. Urinary acylglycines were measured by stable isotope dilution GCMS [32]. Skin fibroblast cultures from the patients and controls subjects, at less than 12 passages, were grown to confluence in α-minimum essential medium supplemented with 10% fetal calf serum. Fibroblast protein concentration was determined using the Lowry method [33]. Substrate oxidation rates of [1-$^{14}$C]butyrate in cultured skin fibroblasts were measured as previously described [34]. Acyl-CoA dehydrogenase activity was measured using the ETF-reduction assay [35] with modifications [36]. The patients' fibroblasts were assayed in conjunction with a known SCAD mutant cell line that contained two *ACADS* alleles with known mutations [4] and a normal fibroblast control cell line. Assays were performed with butyryl-CoA and octanoyl-CoA as substrates, before and after inactivation with specific antiserum to medium-chain acyl-CoA dehydrogenase (MCAD) activity. A polyclonal rabbit antiporcine MCAD antibody was used.

### *SCAD gene sequence analysis*

Genomic DNA was isolated from blood samples and cultured skin fibroblasts by standard methods [37]. Fragments covering the *ACADS* 5′-flanking promoter-region and each of the 10 *ACADS* exons including flanking intron sequences were produced by PCR and sequenced in both directions [12].

### *Expression of SCAD variant enzymes in COS-7 cells*

SCAD cDNA containing normal wild-type (wt) sequence was cloned into the pcDNA3.1(+) expression vector (Invitrogen). The gene variation 319C>T was introduced by PCR based *in vitro* mutagenesis and the

Table 1
Clinical features in individuals with SCAD deficiency and 319C>T mutation

| Patient # | E365 | E401a[a] | E401b[a] | E460-1[b] | E486 | E499-1 | E541 |
|---|---|---|---|---|---|---|---|
| Sex | M | M | M | F | M | F | F |
| Ashkenazi Jewish | + | + | + | + | + | + | + |
| Age at onset | 4 m | 6 y | 4 y | Birth | Birth | Birth | Birth |
| Presenting features | DD | psych | psych | H, neck, and facial weakness | H DD | H poor feeding, acidosis | H dysmorDD |
| Optic atrophy | – | + | + | – | – | – | – |
| Opthalmoplegia | – | – | – | +, ptosis | – | – | – |
| Developmental delay | + | + | + | – | + | + motor | |
| Speech delay | + | – | – | – | + | – | |
| Hypotonia | + | + | – | + | + | + | + |
| Myopathy | – | + | – | + | + | + | – |
| Facial weakness | + | – | – | + | – | + | – |
| Cerebellar ataxia | NR | + | NR | - | – | – | NR |
| Pyramidal signs | NR | + | + | – | – | NR | NR |
| Psychosis | NR | + | + | – | – | NR | Autism |
| Lethargy | – | + | + | – | + | – | + |
| Feeding difficulties | – | – | – | – | + | + | – |
| Congenital abnormalities | Short stature | NR | NR | Sutural cataracts | NR | – | + dysmor |
| Cardiomyopathy | – | – | – | + | – | – | – |
| Age @ July 2002 | 5 y 2 m | 27 y | 27 y | 19 y | 4 y | 3 y 9 m | 6 y |
| MRI findings | NR | multifoc. WM abn. | multifoc WM abn. | | Normal | ND, CT normal | Normal |
| Other medical | | | | Recurrent pneumonia | | Normal EEG | FIRDA on EEG with episodic lethargy |

abn., abnormalities; DD, developmental delay; dysmor, dysmorphic features; H, hypotonia; multifoc, multifocal; psych, psychosis; FIRDA, frontal intermittent rhythmic delta activity; NR, not recorded; resp dist, respiratory distress; WM, white matter.

[a] Identical twin brothers.

[b] Previously published in Ref. [11].

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Table 2
Biochemical and molecular features in individuals with SCAD deficiency and 319C>T mutation

| Patient # | E365 | E401a | E401b | E460-1 | E486 | E499-1 | E541 |
|---|---|---|---|---|---|---|---|
| Elevated urine OA | | MS | MS | MS | | | Glutaric, DCA |
| Elevated urine acylglycines | | | | Hexanoyl- and butyrylglycine | | ND | |
| Urine EMA[a] | | 80 | Elevated | >1000 | 65–222 | 120–150 | 42–70 |
| Serum acyl-carnitines | | Elevated butyrylCn | Elevated butyrylCn | Elevated butyrylCn in fibroblasts | Elevated butyrylCn | Normal | Normal |
| [$^{14}$C]butyrate oxidation in lymphoblasts | | 40–50% | 40–50% | | | Normal | Normal |
| %Fibroblast SCAD activity | Decreased | | | 1% | | ND | |
| %Muscle SCAD activity | | 3–6% | | ND | | ND | |
| Muscle biopsy | | Lipid storage | | Multiminicore myopathy | | Multiminicore myopathy | |
| Allele 1 | 319C>T 625G | 319C>T 625G | 319C>T 625G | 319C>T 625G | 319C>T 625G | 319C>T 625G | 319C>T 625G |
| Allele 2 | 319C>T 625G | 625G>A | 625G>A | 319C>T 625G | 319 C>T 625 G | 625G>A | 625G>A |
| *Mother* | | | | | | | |
| Allele 1 | 319C>T | 625G>A | 625G>A | 319C>T625G | | 625G | 319C>T 625G |
| Allele 2 | 625G>A | 625G>A | 625G>A | 625G | | 625G>A | 625G>A |
| *Father* | | | | | | | |
| Allele 1 | 319C>T 625G | 319C>T 625G | 319C>T 625G | 319C>T 625G | | 319C>T 625G | 625G>A |
| Allele 2 | 625G | 625G>A | 625G>A | 625G | | 625G>A | 625G>A |

Cn, carnitine; DCA, dicarboxylic aciduria; EMA, ethylmalonic aciduria; MS, methylsuccinic acid; ND, not done; OA, organic acids; SCAD, short-chain acyl-CoA dehydrogenase.
[a] EMA expressed in mmol/mol creatinine.

presence of the variation was confirmed by DNA sequencing. *In vitro* transcription/translation of wt- and 319C>T *ACADS* was performed using $^{35}$S-Methionine. The synthesized radioactive labeled wt- and Arg83Cys variant SCAD proteins were incubated with isolated mitochondria from the −/− SCAD mouse [38] and the biogenesis, including formation of tetrameric SCAD enzyme protein, was followed for 260 min by native and denaturing gel electrophoresis exactly as described previously [28].

### *Detection of the 319C>T gene mutation in control DNA from Ashkenazi Jews*

A 360-bp genomic fragment which encompassed exon 3 and its flanking regions was amplified using a forward primer 5′-TCACATGGCCTG AGTTTCTG and a reverse primer: 5′-AGAGAGATCAGG GCTGGG AG and sequenced as described above

## Results

We report 7 children (4 male, 3 female), three of whom are homozygous for the 319C>T mutation and four of whom are compound heterozygotes for the 319C>T mutation and disease susceptibility variation 625G>A. The clinical phenotype is highly variable with age of presentation from birth to early childhood. The common clinical features include hypotonia (6/7), developmental delay (5/6), myopathy (4/7), facial weakness (3/7), lethargy (4/7), and various congenital abnormalities (3/7) (Table 1). Three of the four children with myopathy underwent muscle biopsies, one demonstrating lipid storage and two with ultrastructural features of multiminicore myopathy. One previously reported homozygous female (E460-1) with multiminicore myopathy had decreased mitochondria in the region of the multicores and demonstrated progressive external opthalmoplegia, ptosis, facial weakness, and weakness of the muscles of mastication with severe limb girdle and axial myopathy and muscle wasting, scoliosis, progressive joint contractures and wheelchair dependence by 5 years of age [11]. She also had evidence of a cardiomyopathy with generalized mild left ventricular dysfunction on echocardiogram and evidence of biatrial hypertrophy on EKG during an episode of pneumonia with respiratory failure at 9 years of age. The spectrum of congenital abnormalities include short stature in one male homozygote (E365), congenital sutural cataracts in one female homozygote (E460-1) and dysmorphic facial features in a female compound heterozygote (E451). Two compound heterozygote identical twin brothers (E401a, E401b) were highly distinctive presenting at 4–6 years of age with psychosis, optic atrophy, pyramidal signs, and multifocal white matter abnormalities on MRI examination of the brain. One brother (E401a) also had cerebellar ataxia. This clinical presentation may suggest additional genetic variables in this highly consanguinous population group, given its striking variance with previously reported cases of SCAD deficiency. Another girl (E451) was diagnosed with autistic spectrum disorder and has had repeated episodes of lethargy with vomiting lasting more than one week at a time which were precipitated by surgery with anesthesia, gastroenteritis, and a burn. During these episodes her EEG demonstrated frontal intermittent rhythmic delta activity with generalized slowing,

Biochemical analysis revealed ethylmalonic aciduria (6/6), methylsuccinic aciduria (3/4), elevated butyrylcarnitine concentrations in the serum of three of five children

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and in the cultured skin fibroblasts of one, decreased butyrate oxidation in lymphoblasts (2/4) and decreased SCAD activity in fibroblasts or muscle where measured (3/3) (Table 2). In the 319C>T homozygous female with multiminicore myopathy (E460-1), urinary organic acids intermittently revealed very large amounts of EMA and methylsuccinic acid with elevated butyrylglycine, 2-methylbutyrylglycine and tiglylglycine, underlining the intermittent nature of the excretion of EMA which appears to be highly dependent upon the degree of intercurrent metabolic stress. Of note, the highest concentrations of EMA appeared to occur in the 319C>T homozygotes. Patient E460-1 demonstrated complete absence of SCAD activity in cultured skin fibroblasts and no SCAD protein was detectable by Western blot analysis [11]. One compound heterozygous male (E401a) also had markedly reduced SCAD activity in muscle in the range of 3–6% residual activity with evidence of lipid storage suggesting that the combination of the disease-causing mutation 319C>T with the susceptibility variation 625G>A leads to a similar destabilizing effect on the SCAD protein.

Of particular note, all seven children were of Ashkenazi Jewish origin. In our subsequent population screening survey of 105 individuals of Ashkenazi Jewish descent, seven individuals were found to be heterozygous for the 319C>T mutation suggesting a high carrier frequency of 1:15 and a homozygote frequency of 1:900.

The mitochondria biogenesis experiments revealed that the Arg83Cys SCAD protein was not able to form tetrameric SCAD enzyme as compared to the wt-SCAD, confirming the 319C>T *ACADS* mutation to be disease-causing. The mitochondria biogenesis experiment was performed at 26 and 37 °C in order to determine the possible temperature sensitivity of the Arg83Cys SCAD mutant protein. At both temperatures the formation of tetrameric Arg83Cys SCAD was negligible compared to wild-type SCAD protein, as judged by native gel electrophoresis [28]. However, the tendency to form aggregates, as judged by SDS gel electrophoresis of the precipitate after lysis of mitochondria, was enhanced significantly at 37 °C compared to 26 °C, documenting that the 319C>T mutation leads to a severe folding defective mutant SCAD protein.

## Discussion

SCAD deficiency presents with striking clinical and biochemical heterogeneity which may in part be attributable to the presence of disease-causing mutations leading to markedly reduced or absent SCAD activity or, alternatively, to the presence of susceptibility variations in the SCAD protein which may predispose to reduced SCAD activity under specific conditions of stress [9]. The common disease susceptibility variation, 625G>A, as seen in our current series of patients, is unlikely to lead to clinically relevant SCAD deficiency in and of itself. However, it is possible that other genetic factors such as the presence of the disease-causing 319C>T mutation on the other allele, as well as cellular and environmental factors are involved in reducing the level of catalytic activity of this variant enzyme below a critical threshold, thereby resulting in clinical symptomatology. Depending upon the nature of these factors, a deficiency may either be consistently expressed or expressed in response to intermittent stressors such as elevated temperature or metabolic acidosis as seen with infection or may be due to variable efficiency of the cellular protein handling system [39,40]. The 319C>T mutation, in homozygous form, resulted in a 1% residual SCAD activity in the cultured skin fibroblasts of patient E460-1 suggesting that this is a severe disease-causing mutation. In compound heterozygous form, the 319T/625A mutations resulted in a 3–6% residual SCAD activity in the muscle of patient E401a. Even though SCAD activities in fibroblasts may give inconsistent results [9,12], which may not be directly comparable to activities in muscle, our data may suggest that the compound heterozygosity genotype produces a similarly severe biochemical phenotype in terms of reduced SCAD activity, though further comparisons of SCAD activities in muscle and their corresponding activity in fibroblasts should be done.

Though there is clinical heterogeneity in the presentation of the 319C>T *ACADS* mutation, the clear targets of this disorder are the neuromuscular system, as manifested by the variegate myopathy, as well as early development, as manifested by the frequent developmental delay and associated congenital abnormalities. The ultrastructural finding of multiminicore disease of muscle in two of three children biopsied is intriguing given the congenital nature of this myopathy. Multiminicore disease is a relatively rare congenital myopathy characterized by marked hypotonia with evidence of antenatal onset in 30% of cases [41]. Weakness is most pronounced axially and proximally, often more severely affecting the shoulder girdle and mild facial involvement is frequent [41–47]. Varying degrees of scoliosis have been noted in all patients older than 10 years in a series of 19 cases and respiratory failure developed in half of these children after 10 years correlating strongly with the degree of scoliosis [41]. Cardiac involvement was felt to occur mainly secondary to respiratory involvement as seen in our patient E460-1. Progressive external ophthalmoplegia and ptosis may also be seen in more severely affected patients as in our case. The course may be one of steady improvement to non-progressive or progressive. With the exception of one family in which two generations were affected, inheritance appears to be autosomal recessive or sporadic in this pathological disorder [41]. The pathologic finding is multiple, small foci of decreased or absent oxidative enzyme reaction in otherwise normal or atrophic fibres of both types, with type I fibre predominance [44,46]. The lesions are marked by myofilament disruption accompanied by Z-disc streaming, in which mitochondria are absent. Ultrastructural and histological examination of the muscle in the remaining four children of our series would be highly informative given the remarkable prevalence of multiminicore changes in two of three children biopsied to date and the relative rarity of mul-

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timinicore disease in the general population. Among the previously reported children with SCAD deficiency, there have been other cases characterized by moderately severe progressive myopathy [3,5] with ptosis presenting as early as 8 months of age in one child [3]. Similar to our Case E460-1, a 16-year-old girl has been reported who presented with failure to thrive, marked muscle hypotonia, severe progressive scoliosis with ventilatory restriction, marked muscle wasting, facial weakness and ptosis but good intellectual performance [8]. Muscle biopsies with histologic and ultrastructural examination in all of these cases would be informative in determining whether the finding of mulitminicore myopathy is common to all SCAD deficient patients or whether it is more characteristic of the 319C>T mutation or only present in a subgroup of affected patients with other genetic variables. Furthermore, as it has been suggested that the mitochondrial changes precede the myofilament disruption [42], it is interesting to speculate on the role of defective SCAD activity in predisposing to these congenital structural myopathic changes in utero.

On further review of our series of children, early onset hypotonia (6/7) is the most common feature, often presenting at birth or in the neonatal period (4/6) also suggesting an antenatal onset. Early onset developmental delay (5/6) including both motor and/or speech delay may suggest involvement of both the central and the peripheral nervous systems. The finding of dysmorphic facial features in one compound heterozygote male may be attributable to the immature muscle development associated with the congenital myopathic facies. The additional congenital abnormalities of short stature and sutural cataracts would again imply an antenatal onset. Previously described central nervous system abnormalities in children with proven SCAD deficiency have included cognitive delay, hyperactivity, microcephaly, seizures, hypertonia and hyperreflexia [2,3,6,7,12]. This may, in certain cases, be related to intermittent episodes of metabolic decompensation precipitated by fasting, infection and fever, and characterized by metabolic acidosis, hypoglycemia and/or hyperammonemia [2,7,14] or to the chronic excessive accumulation of abnormal metabolites such as EMA leading to secondary deleterious effects on intermediary energy metabolism. Another intriguing feature has been the presence of autistic spectrum disorder in one of the girls (E541). The highly distinctive presentation of psychosis, optic atrophy, pyramidal signs and multifocal white matter abnormalities on MRI examination of the brain in the two compound heterozygous identical twin brothers would more likely suggest the presence of additional genetic factors. The broad phenotypic heterogeneity is further underlined by the fact that five of the parents are compound heterozygous for the 319C>T and the 625G>A *ACADS* variants, suggesting that this combination may be compatible with milder phenotypes and child bearing. The more severe childhood presentations may relate to significant early environmental stressors such as fever with infectious illnesses and other genetic modifiers.

Insight into the pathophysiology of this disorder has been provided by the murine model for autosomal recessive SCAD deficiency which has been identified and characterized in BALB/cByJ mice [38,48]. These mice have undetectable SCAD activity, severe organic aciduria with elevated ethylmalonic and methylsuccinic acids and *N*-butyrylglycine, and develop a fatty liver upon fasting or dietary fat challenge. These mice also develop hypoglycemia after an 18-hour fast and have elevated urinary and muscle butyrylcarnitine concentrations. Fasted mutant mice developed microvesicular steatosis and swollen irregular mitochondria in hepatocytes and equivocal increased staining of mitochondria in skeletal muscle both before and after fasting, when compared to control mice [49]. This model has been useful in understanding the impact of SCAD deficiency on cerebral energy metabolism and on development. Mutant BALB/cBYJ mice, compared with control BALB/cJ mice were found to have low levels of acetyl-CoA and high levels of lactate [50]. Fasting aggravated this condition by further decreasing acetyl-CoA and increasing lactate levels in the mutant mice. Free carnitine concentrations were significantly decreased in liver with fasting. Long-chain acylcarnitines were found to be significantly lower in the brain of mutant mice. L-Carnitine treatment increased cerebral CoA-SH concentrations and both hepatic and cerebral acetyl-CoA levels in the mutant mice. In further studies, Rao and Qureshi [51] attempted to decompensate the acyl-CoA metabolism in SCAD-deficient mice by chronic treatment with a riboflavin-deficient diet to potentiate the SCAD deficiency. These riboflavin-deficient mutant mice were shown to have reduced hepatic and cerebral free and esterified carnitines, indicating a potentiation of the secondary carnitine deficiency. Hepatic ammonia levels but not cerebral ammonia or glutamine levels were elevated, indicating a tendency toward secondary hyperammonemia. The brain choline acetyltransferase activity was significantly reduced in the striatum, implying a reduced availability of cerebral acetyl-CoA or a decreased cerebral transport of choline. Most of these changes were partially or completely restored by concomitant treatment with acetyl-L-carnitine. Such a reduction in central cholinergic neurotransmission on a chronic or recurrent basis may contribute to the underlying developmental delay, seizures and hypotonia seen in SCAD-deficient children.

In developmental studies of SCAD-deficient mice, depressed mRNA expression and enzyme activity for the urea cycle enzymes carbamyl phosphate synthetase I (CPS) and argininosuccinate synthetase (AS) at 6 days of age during neonatal development have been found [52]. These two enzymes are considered to be the rate-limiting steps for *in vivo* urea synthesis and ammonia disposal. This may help to explain a particular vulnerability of young children with SCAD deficiency to hyperammonemia during metabolic decompensations [2]. Other factors contributing to central nervous system injury include the possibility of hypoglycemia during acute decompensations, as documented in a child homozygous for the 625G>A variation [14].

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Gluconeogenesis might be compromised because of a lack of reducing equivalents from the oxidation of fatty acids in the fasting or stressed state, which has also been demonstrated in the SCAD-deficient mouse model [50]. Alternatively, gluconeogenesis may be reduced as a result of decreased pyruvate carboxylase activity in liver and kidney, the major regulatory enzyme and the flux-generating step in the pathway of gluconeogenesis. This would be due to the reduced production of acetyl-CoA from fatty acid oxidation, as acetyl-CoA is an allosteric activator of pyruvate carboxylase [53]. Reduction of pyruvate carboxylase activity may decrease anaplerosis in brain and muscle as it plays a role in these tissues in the maintenance of 4-carbon intermediates in the citric acid cycle. Furthermore, the accumulation of butyryl-CoA, which may be hydrolyzed, may result in the intracellular accumulation of the cytotoxic butyric acid. It is expected that butyryl-CoA and its derivative EMA which are produced in high concentrations in the liver of SCAD deficient patients, can penetrate into brain because short-chain fatty acids are able to cross the blood–brain barrier [54]. Finally, it has been shown in young rats that EMA significantly inhibits the activity of mitochondrial CK at 1.0 mM and higher concentrations in the brain, but has no effect on cytosolic CK [55,56]. In contrast, both mitochondrial and cytosolic CK from skeletal muscle and cardiac muscle are not affected. It has been suggested that EMA reduces mitochondrial CK activity by oxidation of fundamental thiol groups of the enzyme necessary for its function. Mitochondrial CK is crucial for buffering ATP levels and transport between the sites of ATP generation and consumption by ATPases, which are essential for brain energy metabolism [57]. Of interest, it has also been shown that the antioxidants glutathione and ascorbic acid prevent the inhibitory effect of EMA on mitochondrial CK when co-incubated with EMA. More recently, it has been demonstrated that ethylmalonic acid, *in vitro*, significantly inhibited the electron transport chain and creatine kinase activities in human skeletal muscle [58].

Corydon et al. [59,60] mapped the SCAD gene to the distal part of chromosome 12 (12q22qter) by fluorescence *in situ* hybridization and concluded that it is a single-copy gene. The predominant Ashkenazi Jewish origin of this group of children with the 319C>T *ACADS* mutation and the high prevalence of this mutation among the Ashkenazi Jewish population suggests that this may be an ancient founder mutation. Currently there are about 13–14 million Jews in the world [61]. About 80% are Ashkenazim who originated in current Israel, migrated to the Rhineland in the 9th century and beginning in the 14th century moved into present-day Poland, Lithuania, Belarus, the Ukraine, and Russia [62]. Based on modern genetic mapping and cloning methodologies, a number of diseases of high frequency have been described in this population group. As an example, over 90% of cases of early-onset autosomal dominant idiopathic torsion dystonia (ITD) in Ashkenazim are estimated to have arisen from a single mutation that arose approximately 350 years ago in ITD carriers originating mostly from Lithuania and Belarus and resulting in a current heterozygote frequency of 1/1000–1/3000 among Ashkenazim [63]. While the size of the Ashkenazi population at the time of the calculated origin of this mutation is not known, historical sources suggest a relatively small population. Based upon Polish socioeconomic data from the middle of the 18th century which suggested that the more wealthy segment of the Jewish population usually had between 4 and 9 children who reached adulthood, it has been concluded that the effective population size of the founders of the current Ashkenazim may have been as low as a few thousand individuals from the wealthier fraction of the original population [62]. Thus it has been suggested that a single mutation that occurred once in an ancestral population of about 3000 people would be coamplified with population expansion to reach the claimed Ashkenazi ITD frequency of 1/1000–1/3000 [63]. Another explanation would be some as yet unknown selective advantage of the dystonia gene. Among the many autosomal recessive disorders of the Ashkenazim, strong linkage disequilibrium has also been detected in Bloom syndrome [64] and familial dysautonomia [65] which have carrier frequencies of 1/110 and 1/30 among Ashkenazim, respectively. In our current cohort of anonymous individuals, we have an extraordinarily high carrier frequency of the 319C>T *ACADS* mutation of 1/15 among Ashkenazim. Whether this high gene frequency is due to an ancient founder effect alone or whether a selective advantage has been involved will require further historical and linkage disequilibrium or haplotype analysis and more extensive population screening. With a predicted homozygote frequency of 1:900, we would expect to see nearly 2700 patients in Israel alone among the Ashkenazim. This may underline reduced penetrance of the clinical phenotype or relate to the broad clinical spectrum.

In conclusion, the wide clinical and biochemical phenotypic variability in presentation of the 319C>T *ACADS* mutation suggests that this is a complex multifactorial polygenic condition that should be screened for in individuals with multiminicore myopathy, particularly among the Ashkenazi population, given the extremely high heterozygote frequency in this group. The findings of hypotonia, early onset developmental delay, and multiminicore myopathy with EMA warrant a full biochemical and molecular investigation for underlying SCAD deficiency. Early diagnosis is critical particularly for the aggressive treatment of acute catabolic episodes to prevent irreversible cerebral injury with secondary cognitive impairment and seizures and for the avoidance of fasting, the prompt management of fever and the early treatment of infections in order to reduce the risk factors for metabolic decompensation. On a chronic basis, intervention with a high carbohydrate, low fat, frequent feeding diet and supplementation with cofactors such as riboflavin [66] and antioxidants such as vitamin C and E may also be of benefit as well as the prevention of riboflavin and carnitine deficiency.

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