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Original Article

Detection of allele frequencies of common c. 511C>T and c.625G>A variants in the *ACADS* gene in the Turkish population

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ABSTRACT

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare inborn error of mitochondrial fatty acid oxidation and protein misfolding disorder. Our aim was to detect the number of Turkish patients diagnosed with SCADD in the literature and to determine the allele frequencies of two common variants (c.511C>T and c.625G>A) in the Turkish population. Five Turkish patients with SCADD were reported in the literature from four unrelated families. We also investigated allele frequencies of common variants of c.511C>T and c.625G>A, which confer susceptibility to SCADD, which were found to be 1.7% and 20.2%, respectively. Both of these susceptibility variants were found to be high in the Turkish population as they are worldwide.

Key words: short-chain acyl-CoA dehydrogenase deficiency, ACADS, SCAD, SCADD, SCAD deficiency, ethylmalonic aciduria.

Short-chain acyl-CoA dehydrogenase (SCAD, OMIM 606885) deficiency (SCADD, OMIM 201470) is a rare autosomal recessive inherited disorder of mitochondrial fatty acid oxidation encoded by *ACADS* gene, located on chromosome 12q24.[1-12] It has variable clinical phenotypes ranging from asymptomatic individuals to severe neurological presentation.[1-12] The brain is the the most commonly affected organ, but muscles, liver, heart involvement are also reported.[1-12] Birth prevelance of SCADD from newborn screening was reported to be approximately 1 in 35.000-50.000 live births.[5-6] SCADD was first described in 1987 and thereafter the responsible gene was identified in 1990.[13,14] To date, nearly 70 pathogenic mutations and two common variants, c.511C>T and c.625G>A have been reported in the *ACADS* gene.[1-14] Genotype-phenotype correlation is weak and poorly understood.[1-14] Our aim was to detect the number of Turkish patients diagnosed with SCADD in the literature and to determine the allele frequencies of two common variants (c.511C>T and c.625G>A) in the Turkish population.

Material and Methods

Turkish patients diagnosed with SCADD through metabolic screening and mutation analyses were searched in the literature. Literature search was made via PubMed with the following key words: 'short-chain acyl-CoA deficiency', 'SCAD deficiency', 'SCAD', 'SCADD', '*ACADS*', 'ethylmalonic aciduria'. We used TUBITAK, IGBAM in-house exome database to find out the allelic distributions of c.511C>T and c.625G>A variants in the *ACADS* gene in the Turkish population. These two variants were questioned anonymously in a total of 1182 individuals. This exome database cohort includes a mixed group of unrelated individuals and some randomly selected family members. It is composed of patients with

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undiagnosed diseases, their healthy parents and healthy and/or affected siblings. In addition to these data, we analysed these allele frequencies in a control group of 89 healthy individuals to fulfill the Hardy-Weinberg equation.

Results

In the literature, we found five Turkish patients from four families. All five patients were diagnosed with metabolic screening followed by Sanger sequencing of *ACADS* gene (Table I). Age at diagnosis was 2 months-14 years. Male to female ratio was 2:3. Two of three families were consanguineous and consanguinity was not reported in one of the families. All patients except one had increased butyryl carnitine levels and all had increased ethylmalonic acid in urine. Two siblings had the homozygous c.1138C>T pathogenic variant and the homozygous c.625G>A susceptibility variant. One patient had a different homozygous c.1147C>T pathogenic mutation. Two patients had homozygous c.625G>A susceptibility variant, but no other causative mutations. All of the patients except one were symptomatic with mostly neurological problems. Three of them had neurological sequela.

In the in-house exome data of 1182 individuals, the heterozygote ratio of c.625G>A allele (pGly209Ser) was found to be 44% (hetRatio= 0.4391) and the homozygote ratio was calculated as 13% (homRatio= 0.1320). The heterozygote ratio of c.511C>T (p.Arg171Trp) variant was 3% (hetRatio= 0.0305) and the homozygote ratio was 0%. In 89 unrelated healthy Turkish individuals (control group), the allele frequency of c.625G>A (pGly209Ser) susceptibility variant was 20.2% and the c.511C>T (p.Arg171Trp) susceptibility variant was 1.7%.

Discussion

The clinical spectrum of SCADD is extremely heterogeneous. It can vary from being asymptomatic to having feeding problems, ketotic hypoglycaemia, metabolic acidosis, lethargy, weakness, hypotonia, microcephaly, developmental delay, speech delay, behavioural disturbances, epilepsy, myopathy, neuropathy, dysmorphic features and rarely optic atrophy, hepatic dysfunction, Reye syndrome, cardiomyopathy, arrhythmia.[1-18] Acute fatty liver of pregnancy, pre-eclampsia, maternal HELLP syndrome and premature birth have also been reported. SCADD is generally diagnosed when investigating neurological disorders and/or hypoglycaemia, by selective screening. Therefore, these patients were diagnosed later by selective screening and showed severe clinical symptoms including microcephaly, developmental delay, epilepsy and dysmorphic features. However, in recent years newborn screening by tandem mass spectrometry has led to identification of mostly asymptomatic newborns and the prevalence appeared higher than it was estimated.[3,6]

In spite of many patients already known in the literature, only five Turkish patients from four unrelated families were reported.[15-18] Bok et al.[15] reported a homozygous pathogenic c.1138C>T mutation with a homozygous c.625G>A susceptibility variant in two Turkish siblings, one of whom had transient cholestasis, maternal HELLP syndrome, premature delivery but normal mental development, slight hypotonia, active behaviour at three years of age while the other sibling was asymptomatic. Kiykim et al.[16] reported a homozygous c.625G>A susceptibility variant in a Turkish patient with speech delay, epilepsy and behavioural disturbances, without any other causative mutations. Okuyaz et al.[17] reported a homozygous c.625G>A susceptibility variant in a Turkish patient with infantile hypotonia, also without other causative mutations. We also recently reported a homozygous c.1147C>T pathogenic mutation in a Turkish patient with microcephaly, developmental delay, epilepsy and dysmorphic features.[18] All five patients were diagnosed with metabolic screening followed by direct sequencing of *ACADS* gene (Table I). Neurological problems were found in the majority of the patients and are accepted as the most important clinical findings.

Table I. Turkish patients diagnosed with SCADD in literature.

Patient No	References	Age at diagnosis (month)	Sex (Male/ Female)	Consanguinity	Tandem mass analyses (C4: N<1.1µmol/L)	Urine organic acid analyses (ethylmalonic acids: mmol/ molcreatinine; N<18)	Brain MRI	Mutation (*ACADS* gene)	Protein effect	Prognosis
I[a]	Bok et al	2	M	+	2.4/3.91/4.7	124-380	NA	c.1138C>T; c.1138C>T and c.625G>A;c.625G>A	p.R380W;p. R380W and p.G209S;p. G209S	Prematurity, cholestasis, hepatomegaly, maternal HELLP syndrome. Normal mental development, slight hypotonia, active behaviour at three years of age
II[a]	Bok et al	2	F	+	2/6.25	25-58	NA	c.1138C>T; c.1138C>T and c.625G>A;c.625G>A	p.R380W;p. R380W and p.G209S;p. G209S	Asymptomatic
III	Kiykim et al	168	M	NA	2.14	Increased ethylmalonic acid and methylsuccinic acid.	NA	c.625G>A;c.625G>A	p.G209S;p. G209S	Delayed speech, epilepsy and behavioral disturbances
IV	Okuyaz et al	8	F	-	N	Significant increase	N	c.625G>A;c.625G>A	p.G209S;p. G209S	Hypotonia, developmental delay
V	Kilic et al	48	F	+	2.32	720	Microcephaly and arachnoid cyst	c.1147C>T;c.1147C>T	p.R383C; p.R383C	Microcephaly, severe developmental delay, epilepsy, dysmorphic features

N: normal, NA: not available M: male, F: female, a: siblings

There is a remarkably high prevalence of homozygosity for *ACADS* variants in the general population, with frequencies of approximately 0.3% for the c.511C>T (p.R171W) and 5.5% for the c.625G>A (p.G209S) variant.[19,20] Additionally, 7% of the population was found to be homozygous for one of these variants or compound heterozygous for each. In Europe, two common variants c.511C>T;p.Arg171Trp and c.625G>A;p.Gly209Ser were reported as polymorphisms.[1,21] Each variant accounts for 3-8% and 22-43% of normal population, respectively. In a population study conducted in the United States of 694 samples, the allele frequency of the c.625G>A variant was found to be 22% and that of the c.511C>T variant was 3%.[19] Sequence analyses of the *ACADS* gene in 100 alleles from Danish controls revealed allele frequencies of 8% for c.511C>T and 21% for c.625G>A.[22] When the ExAc browser (The Exome Aggregation Consortium) was searched, the c.625G>A allele frequency was 25.9 % and the c.511C>T allele frequency was 3.1%. To date, there is no data for the allele frequencies of these two common variants in the Turkish population. In our study, the allele frequency of c.625G>A (pGly209Ser) variant was 20.2% and the c.511C>T (p.Arg171Trp) variant was 1.7% in our Turkish population based on the healthy control group composed of 89 unrelated individuals.

These two variants have not been directly associated with SCADD although they were reported to confer disease susceptibility when co-occurring with environmental or genetic factors.[1,3,21] Homozygosity for these variants solely or together with homozygous or heterozygous known pathogenic mutations, increase susceptibility to symptoms in certain environmental situations.[1,21] Although individuals with these two common genetic sensitivity variants in the general population are mostly asymptomatic, they can also present with severe neurological abnormalities. Environmental (e.g. fever, infection, metabolic stress, starvation, hypoglycaemia) and genetic (modifier genes) factors may be responsible for the clinical and genetic variability of this disease.

Our observation from our patient and the reported clinical cases suggest that patients with higher ethylmalonic acid (EMA) levels usually had more of a severe clinical condition. We agree that elevated EMA excretion is related to mitochondrial dysfunction and oxidative stress.[23-25] Homozygosity for one of these polymorphisms is associated with an increased incidence of elevated EMA excretion.[10]

In conclusion, SCADD is a rare fatty acid oxidation disorder. The allele frequencies of c.625G>A (pGly209Ser) and c.511C>T (p.Arg171Trp) susceptibility variants in the Turkish population are found to be very similar to that in the literature. In the future, population-specific mutations and genotype-phenotype correlations will be clearer when the number of reported patients increase.

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