Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

A985G mutation incidence in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in Brazil

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Genet. Mol. Res. 8 (2): 487-493 (2009)
Received December 29, 2008
Accepted March 12, 2009
Published May 5, 2009

ABSTRACT. In view of the serious consequences of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and the absence of information about its incidence in the Brazilian population, we examined the frequency of the A985G mutation in the MCAD gene. A retrospective analysis was made of data on 1722 individuals (844 females) genotyped for the A985G mutation in the MCAD gene, using genomic DNA extracted from peripheral blood leukocytes and genotyping with PCR-RFLP; 0.41% of these individuals were heterozygous for the A985G mutation. The mutant homozygous genotype was not found. The 985G mu-

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tant and 985A normal alleles had allelic frequencies of 0.0020 and 0.9980, respectively. Given the A985G allele frequency, genotyping would be recommended in cases of family history of MCAD deficiency and sudden infant death syndrome, and when there is suspicion of medium-chain fatty acid metabolic alterations; genetic counseling should be offered in cases involving 985GG and A985G individuals and consanguineous marriages.

Key words: A985G mutation; MCAD deficiency; Molecular screening; Medium-chain acyl-CoA dehydrogenase deficiency; Brazil

INTRODUCTION

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a recessive autosomal disease, caused by a decrease in mitochondrial beta-oxidation of fatty acids into energy, being clinically and genetically well defined nowadays (Seddon et al., 1997; Thomason et al., 1998; Derks et al., 2005). MCAD deficiency represents one of the major causes of sudden death syndrome in childhood, therefore being potentially fatal (Opdal and Rognum, 2004). In cases of MCAD deficiency, a few days after birth until the first weeks, a previously healthy newborn begins to express a metabolic disease, suddenly progressing to hypoglycemia, acute encephalopathy and lethargy after periods of fasting and infections (Seddon et al., 1997; Raghuveer et al., 2006). Studies show that one-third of carriers of this enzyme deficiency manifest symptoms until the third day of life and that 29% of cases are fatal in the first acute disease episode (Seddon et al., 1997; Souza et al., 2002).

About 20 mutations have been described in the MCAD gene (Opdal and Rognum, 2004). However, the most prevalent genetic cause in diagnosed children is homozygosity for the A985G missense mutation in the MCAD gene (Seddon et al., 1997; Thomason et al., 1998; Opdal and Rognum, 2004; Marsden et al., 2006), located on chromosome 1p31 (OMIM, 2008). This mutation causes the substitution of a lysine for a glutamic acid at position 304 of the protein, and is related to about 90% of MCAD deficiency cases (OMIM, 2008). Meta-analysis studies show that 985GG homozygous have 1% probability of infant sudden death occurrence in the United States, while in Australia these values are as high as 3%. This reduces the likelihood to 0.1% among A985G heterozygous individuals who may, very rarely, present asymptomatic or mild MCAD deficiency (Marsden et al., 2006). Despite the founder factor existing in Western Europe, the papers published so far indicate an incidence of MCAD deficiency in the range of 1:6500 to 1:20,000 (Seddon et al., 1997; Wajner et al., 2001).

Neonatal screening is suggested in several countries due to the incidence of this disorder, the potentially fatal consequences and simplicity of treatment (Seddon et al., 1997). The tests targeted at the diagnosis of MCAD deficiency must be easy to perform, effective and of high predictive value, allowing specific preventive therapies to be initiated in time (Raghuveer et al., 2006). The tracking program choice will depend on the mutation frequency in the observed population and available financial and methodological resources (Seddon et al., 1997; Seymour et al., 1997; Wajner et al., 2001). In Europe and the United States, tandem mass spectrometry is the reference technique for the diagnosis,

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which screens mainly for octanoylcarnitine, but also the urinary metabolites octanoic acid, medium-chain cis-4-decenoic and dicarboxylic acids and hexanoylglycine (Thomason et al., 1998; Marsden et al., 2006; Raghuveer et al., 2006). In Brazil, the molecular test for the 985G mutation is not included in the guidelines of the Neonatal Screening National Program offered by the Unified Health System of the Ministry of Health (Ministério da Saúde, 2002). Consequently, this mutation is evaluated on a smaller scale and only by private laboratories, where blood samples collected on filter paper are analyzed using the polymerase chain reaction (PCR) technique (Souza et al., 2002), which requires less investment in resources when compared to mass spectrometry in tandem (Marsden et al., 2006).

The treatment of patients with MCAD deficiency includes regular intake of carbohydrates, low fat intake, therapy with L-carnitine, emergency diet during a crisis, and avoidance of long periods of fasting (Thomason et al., 1998; Souza et al., 2002). Thus, the morbidity and mortality can be prevented in a simple and low-cost way (Thomason et al., 1998; Souza et al., 2002; Haas et al., 2007). Once a metabolic crisis crops up, treatment should start immediately to minimize neurological sequelae (Raghuveer et al., 2006). For this reason, as in every neonatal screening test, it is suggested that the test be done between the 3rd and 30th days of life, preferably, between the 5th and 7th days of life (Ministério da Saúde, 2002; Marsden et al., 2006).

In view of the serious consequences of MCAD deficiency and the absence of records about its incidence in the Brazilian population, the aim of this study was to determine the A985G mutation frequency in the MCAD gene in the Brazilian population and from this result evaluate the need and importance of a neonatal screening program for MCAD deficiency in Brazil.

MATERIAL AND METHODS

Population sample

The study is based on a retrospective analysis of data recorded from 1722 individuals who were subjected, upon medical request, to the molecular study of A985G mutation in the MCAD gene, from 2003 to 2007, at the Hermes Pardini Institute, Department of Human Genetics in Belo Horizonte, Minas Gerais. In this study, only individuals whose medical examination requested MCAD molecular screening were included. We analyzed the gender and genotype for the A985G mutation in the MCAD gene. To select the population studied, the ethnicity criterion was disregarded, since the Brazilian population is one of the most diverse in the world, making it impossible to precisely define the ethnic origin of individuals based only on morphological parameters (Parra et al., 2003). The study was a survey of A985G mutation genotyping results, with epidemiological and statistical purposes, in accordance with the procedures of the Ethics in Research National Commission (CONEP).

Biological samples and A985G mutation genotyping

Capillary blood samples obtained from the heel of newborns or venipuncture samples in individuals over 28 days old were placed on FTA Card® filter paper and subjected to DNA extraction, according to manufacturer instructions (Whatman England Classic® FTA).

Genomic DNA was extracted from peripheral blood leukocytes, collected on filter paper. The A985G mutation was examined by PCR-RFLP (restriction fragment length polymorphisms), using primers F: 5' ATATCATTTATGCTGGCTGAAATGGCCA 3' and R: 5' ACCAGAATCAACCTCCCAAG 3' and subsequent enzymatic digestion with *Nco*I. In the absence of A985G mutation, the restriction site is not present, resulting in a fragment of 87 bp, while the mutant allele is cleaved into two fragments of 61 and 26 bp. The digestion product was seen on 7% acrylamide gels and developed with Syber Green®. For comparative purposes and quality control, positive and negative controls and a blank reaction were placed on each gel.

Statistical analysis

The clinical and molecular characteristics of the subjects studied were analyzed by the Biostatic 4.0 software, using the chi-square test. Differences were considered to be significant when $P < 0.05$.

RESULTS

Population - age and gender

In the population studied of 1722 individuals, both genders were equally represented, as it consisted of 844 (49%) female subjects and 878 (51%) male subjects. There was a wide variation in age in the population studied, where there were 1452 (84.3%) newborns, 253 (14.7%) children (from 29 days to 12 years of age) and 17 (1%) adults (13 to 70 years) who had an average age of 17.12 ± 10.50 days, 2.90 ± 1.45 months and 32.22 ± 13.90 years, respectively.

Prevalence of A985G mutation

A985G mutation was detected in heterozygosity in 7 (0.41%) subjects (Table 1), which results in a frequency of 1:246. We could not find the mutant homozygous genotype in the sample studied. The wild homozygous genotype (A985A) was present in 99.6% of the studied population. Among A985G heterozygous subjects, 5 were males (0.57%) and 2 were females (0.24%). The 985G mutant alleles and 985A normal alleles showed allelic frequencies of 0.0020 and 0.9980, respectively (Table 1). In the population studied, there was no significant difference in the genotypic ($P = 0.28$) and allele ($P = 0.28$) frequencies between males and females.

Table 1. Genotypic and allelic frequency of A985G mutation in medium-chain acyl-CoA dehydrogenase gene in the studied population.

		Population (N)	Male (N)	Female (N)
Genotypes	A985A	0.9959 (1715)	0.9943 (873)	0.9976 (842)
	A985G	0.0041 (7)	0.0057 (5)	0.0024 (2)
	G985G	-	-	-
Alleles	985A	0.9980	0.9972	0.9988
	985G	0.0020	0.0028	0.0012

N corresponds to the number of the population individuals.

All A985G heterozygous individuals were at the newborn stage or under 12 years old. These were divided into six states in the country, with no possibility of calculating the existence of any predominance of genotypes among the Brazilian federal units (Table 2).

Table 2. Profile of subjects with heterozygote genotype for A985G mutation.

Individuals	Gender	Age*	Hometown
1	Female	5 years old	Paraíba
2	Female	16 days old	Pernambuco
3	Male	19 days old	Ceará
4	Male	17 days old	Amapá
5	Male	6 days old	Amapá
6	Male	27 days old	Rio de Janeiro
7	Male	30 days old	Distrito Federal

*Age on the date of molecular test collection.

National geographic distribution

We assessed 231 individuals in Brazil's Center West region, 438 in the Northern region, 260 in the Northeast, 709 in the Southeast, and 84 in the South, for whom genotypic frequencies were calculated (Table 3). There was no statistical difference in genotypic frequencies in Brazilian geographic regions (P = 0.268). Heterozygous individuals belonged to the States of Rio de Janeiro (1), Amapá (2), Ceará (1), Paraíba (1), Pernambuco (1), and the Federal District (1) (Table 2). The allelic frequency was not calculated by geographical region, due to large stratification of samples of the heterozygote genetic profile.

Table 3. A985G mutation genotypic frequency in MCAD gene, according to Brazilian federal region.

Federal region	N (% of total population)	Wild homozygous A985A (N)	Heterozygosity A985G (N)
North	438 (25.43)	0.9954 (436)	0.0046 (2)
Northeast	260 (15.10)	0.9885 (257)	0.0115 (3)
Center West	231 (13.42)	0.9957 (230)	0.0043 (1)
Southeast	709 (41.17)	0.9986 (708)	0.0014 (1)
South	84 (4.88)	1 (84)	-

N corresponds to the number of individuals in relation to the total population.

DISCUSSION

The clinical manifestations of the metabolic syndrome of MCAD deficiency usually begin in the first days of life, during which the risk of fatality is significant, and the recommendation to undergo laboratory screening tests is made. Through this study, it was observed that, in Brazil, there is a substantial request for molecular tests in time to take the necessary preventive measures in cases of mutant genotype results, since 84.3% of the population analyzed consists of newborns. However, performing molecular screening after the first 28 days of life is also valid, since several cases of late manifestations have been reported (Goodman et al., 2002; Olsen et al., 2004).

The autosomal inheritance profile of MCAD deficiency associated with the lack of sexual selective pressure on their respective genotypes justify the non-existence of statistically significant differences in A985G genotypic and allelic frequencies between females and males.

The lack of statistical differences in genotypic frequencies among the country geographical regions (P = 0.268) can be explained by the great diversity of the Brazilian population or the need to enlarge the sample, to represent significantly the population of each of these regions. The latter also made it impossible to estimate if, statistically, any Brazilian federal unit has an increased number of A985G heterozygotes compared to the others.

The importance of these records for the Brazilian population is due to the fact that Brazil is one of the most heterogeneous populations in the world, whose genetic variability is based on indigenous, European and African populations, besides the constant internal migration (Parra et al., 2003). Consequently, the Brazilian regions have different pedigrees, characterized by plurality and diversity in their origins, which is reflected in the genetic parameters of various hereditary diseases, bringing genetic diversity to the population along with the need for well-conducted studies to help elucidate the puzzles of these diseases.

Data from several countries show that MCAD disability caused by G985 mutation is more common in West Europe countries (Gregersen et al., 1993). Two studies conducted in the United Kingdom showed a frequency from 1 in 6400 to 1 in 13,400 (Matsubara et al., 1991; Blakemore et al., 1991). It is noteworthy that the results of this study cannot be accurately extrapolated to Brazil, due to the large mixing of the Brazilian population (Parra et al., 2003).

In our study, the mutant allele frequency was found to be 0.002. Assuming that the population is in Hardy-Weinberg equilibrium, we can estimate that MCAD disability incidence due to A985G mutation is 1 in 250,000 individuals.

So far in Brazil, there have been no reports of molecular data on A985G mutation in the MCAD gene. In this sense, this study is very important in determining, in unprecedented way, this mutation incidence in the Brazilian population. Due to the absence of the 985GG genotype, the frequency of 1:246 of A985G heterozygous genotype in the country, and the low estimated incidence of homozygous individuals for the mutant allele (1:250.000), it is appropriate to say that this mutation genotyping gains more relevance in cases of a family history of MCAD deficiency and sudden death syndrome in children, and in cases of suspected changes in the metabolism of medium-chain fatty acids, either through clinical signs or laboratory evidence of an acylcarnitine biochemical profile. The molecular test is also important for genetic counseling involving 985GG and A985G individuals and consanguineous marriages. In any of these situations, it is essential that the methodology used is quick, effective and of high predictive value, features present in the molecular analysis of A985G mutation in the MCAD gene. We hope that this study can assist in the selection of tests, according to the profile of the mentioned population, and that it can offer clinical benefits for the health of Brazilians.

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