

CYSTIC FIBROSIS MUTATIONS R1162X AND 2183AA→G IN TWO SOUTHERN BRAZILIAN STATES

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ABSTRACT

We screened 79 southern Brazilian patients with cystic fibrosis for the rare cystic fibrosis mutations R1162X and 2183AA→G. Forty-nine patients were born in the State of Paraná (PR) and 30 in the State of Santa Catarina (SC). Two 2183AA→G alleles were found among the SC patients and one among the PR patients. Six R1162X alleles were found among the SC patients and one among the PR patients. Fourteen percent of the alleles found among patients of Italian origin were R1162X, and 7% were 2183AA→G mutations. These mutations, together with Δ F508, were also studied in a sample of 270 normal non-related subjects of Italian origin who have been born in PR. In this sample we found two Δ F508 alleles and one 2183AA→G allele. Δ F508, R1162X and 2183AA→G frequencies were not statistically different from those observed in Italy. Our results demonstrate that it is important to include these mutations in southern Brazilian surveys of cystic fibrosis patients, especially when they are of Italian descent.

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal autosomal recessive genetic disease among Caucasians. It occurs in approximately one in 2500 live-births in populations of European ancestry, and the frequency of heterozygotes is estimated at one in 25 Caucasians (Romens *et al.*, 1989; Riordan *et al.*, 1989; Kerem *et al.*, 1989).

The incidence of CF in the Brazilian population is unknown. The incidence of CF is expected to have regional differences due to the various patterns of racial mixture, immigration and selection pressure (Raskin *et al.*, 1993, 1997a,b).

The CF gene, that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein, is located on the long arm of chromosome 7, at position 7q31. It has a length of 250 kb and contains 27 exons. This is involved in active ion transport through the apical membrane of epithelial cells (Riordan *et al.*, 1989; Tsui and Buchwald, 1992).

The Δ F508 3-bp deletion is the most frequent CF mutation. However, more than 1000 different mutations of the CFTR gene have been reported (Mercier *et al.*, 1994; Zielenski and Tsui, 1995; CFGAC, 1999). The frequency of Δ F508 among Brazilian CF patients is about 47% (Raskin *et al.*, 1993). This frequency varies among states, being 53% in Minas Gerais, 52% in São Paulo, 49% in Rio Grande do Sul, 44% in Paraná and 27% in Santa Catarina. Presence of Δ F508 in Brazil is much higher than in the United States or England (CFGAC, 1999). As expected, the frequencies

found in Portugal, Spain and Italy are similar to those found in Brazil, due to the colonization of Brazil by southern Europeans (Raskin *et al.*, 1993). CF haplotype studies also suggest that a high number of CF mutations are present in Brazilian CF patients (Raskin *et al.*, 1997a,b).

Other authors have reported frequencies of the Δ F508 in Brazil: 50.8% in Rio Grande do Sul (Marostica *et al.*, 1998), 35% in Rio de Janeiro (de Miranda *et al.*, 1993) and 33% in São Paulo (Martins *et al.*, 1993), but these studies generally involved only a small number of patients.

In northeastern Italy the three most frequent CF mutations are Δ F508 (48%), R1162X (10%) and 2183AA→G (9%) (Bonizzato *et al.*, 1995; Rendine *et al.*, 1997). The R1162X and 2183AA→G mutations are rare in other regions, with respective world frequencies of 0.8 and 0.3%.

R1162X is a nonsense mutation characterized by the substitution of cytosine for thymine (C→T) at position 3616 of the DNA in exon 19. As a consequence, an amino acid arginine is substituted by a stop codon at position 1162 (CFGAC, 1993).

2183AA→G is a frameshift mutation characterized by the substitution of adenine for guanine (A→G) at DNA position 2183 and the deletion of an adenine at position 2184 in exon 13 (R domain) (Bozon *et al.*, 1994; CFGAC, 1994).

The Brazilian Caucasian population is not ethnically homogeneous. While in the northern states the Portuguese contribution prevails, different waves of European immigration have caused a higher ethnic diversity in the southern regions (Salzano and Freire-Maia, 1967).

Italian immigration to Brazil consisted mainly of inhabitants of the Veneto region, which accounted for 48% of the Italian immigrants. In certain southern Brazilian regions, this percentage has been as high as 90%, which is the case for rural centers in the States of Paraná, PR and Santa Catarina, SC (Hutter, 1987). Considering the relatively low frequency of the Δ F508 mutation in SC and PR

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(Raskin *et al.*, 1993), we decided to screen Brazilians for R1162X and 2183AA→G.

MATERIAL AND METHODS

Characterization of the sample

The CF patient sample consisted of 79 families, 49 from PR and 30 from SC. Blood samples were collected from the patient, the father and mother. Average patient age was 7.05 ± 6.59 years and ranged from two months to 32 years at the time of blood collection. We also examined 270 normal individuals of Italian descent by both maternal and paternal lines. Average subject age was 52.02 ± 17.44 years and ranged from 16 to 89 years at the time of blood collection.

The data was analyzed by a chi-square test, with Yates correction when necessary.

Methodology and molecular analysis

DNA extraction was performed by the phenol-chloroform method (Sambrook *et al.*, 1989) modified by Lahiri and Nurnberger Jr. (1991). The extracted DNA was amplified by polymerase chain reaction (PCR), digestion with restriction enzymes, when necessary, and polyacrylamide gel electrophoresis. Methodology for these mutations were essentially as described in the references: the $\Delta F508$ mutation (Raskin *et al.*, 1992); the R1162X mutation (Gasparini *et al.*, 1992); the 2183AA→G mutation (Bozon *et al.*, 1994).

RESULTS AND DISCUSSION

Sample of normal subjects of Italian descent

Among the 540 alleles analyzed we found two

$\Delta F508$ alleles, one 2183AA→G allele and no R1162X allele. These alleles were all found in heterozygous individuals.

In northeastern Italy, the frequency of the $\Delta F508$ mutation is 48% (Bonizzato *et al.*, 1995). Since our sample consisted of northern Italian descendants, we expected to find about five mutant alleles among the 270 normal individuals. The observed difference was not statistically significant ($P > 0.2$).

The frequency of the 2183AA→G mutation in northeastern Italy is 9% of the CF alleles. Thus, we should find approximately one 2183AA→G allele in every 500 alleles in normal subjects of Italian descent. We found one heterozygote (0.2%), a frequency not significantly different from that observed in northern Italy ($P > 0.6$).

Sample of patients from Paraná and Santa Catarina

Seven R1162X alleles among the 158 alleles analyzed were found. This frequency varied significantly when the patients were classified by state of birth. Among patients born in PR, only one R1162X allele was found among the 98 analyzed, while among those born in SC, six alleles were found among the 60 examined. In the same sample we found three 2183AA→G. One 2183AA→G allele was found in PR and two in SC, in one homozygote (Table I).

The heterogeneity observed in the patient sample in comparison to the homogeneity observed in the control sample may be a consequence of the selection of the control group. They were of Italian descent, free from mixture, at least as far as we know. However, the patient sample was intensely mixed, since they were not selected by ethnic origin, but for having CF and for being born in PR or SC. All patients who had the R1162X and the 2183AA→G

Table I - Clinical symptoms in patients who are carriers of the mutations studied.

Patient	Age	State	Pat. chrom.	Mat. chrom.	Mat. ancestry	Pat. ancestry	Meconium ileus	Pancreatic function	Pulmonary function symptoms	Sweat test (mEq/l)
SHS	10	PR	$\Delta F508$	R1162X	Father I Mother I	Father G Mother P	No	PI	Moderate	65 / 80
KMR	11	SC	R1162X	U	Father P Mother I	Father I Mother I	No	PS	Mild	100 / 112
ALA	15	SC	R1162X	$\Delta F508$	Father I Mother U	Father I Mother I	No	PI	Moderate	96 / 108
AG	5	SC	R1162X	R1162X	Father I Mother I	Father I Mother I	Yes	PI	Mild	72 / 98
CT	3*	SC	R1162X	R1162X	Father I Mother I	Father I Mother I	No	PI	Moderate	111 / 120
MM	10	PR	2183AA→G	U	Father I Mother I	Father I Mother I	No	PI	Mild	100 / 126
JCM	20	SC	2183AA→G	2183AA→G	Father I Mother I	Father I Mother I	No	PI	Moderate	86 / 108

PR - Paraná State; SC - Santa Catarina State; PI - pancreatic insufficiency; PS - pancreatic sufficiency; Pat - paternal; Mat - maternal; I - Italian; G - German; P - Portuguese; U - unknown. Sweat tests, at least two positive tests. * Obit.

mutations were also of Italian descent (Table I). When patients were classified into three familial categories (Italian/Italian (I x I); Italian/other (I x O); other/other (O x O)), we found the following frequencies for R1162X (I x I = 14% (N = 26); I x O = 2% (N = 32) and O x O = 0% (N = 40) and for 2183AA→G (I x I = 7%; I x O and O x O = 0%).

There was no significant difference when the northeastern Italian frequency of the R1162X mutation was compared to the frequency found among all patients (I x I) from both states (14.29%) ($P > 0.2$) and among (I x O) PR patients (3%) ($P > 0.1$). However, the frequency observed among (I x I) SC patients (37%) was significantly higher ($P < 0.001$) than that found in northeastern Italy.

The frequency of the 2183AA→G mutation (7.15%), among all the patients (I x I) from both states, was not significantly different from that observed in northeastern Italy (9.3%), ($P > 0.4$). If the comparison is made for each state separately, the differences are not statistically significant ($P > 0.2$ for PR and $P > 0.6$ for SC).

Genotype x phenotype in the patient sample

In relation to R1162X carriers, our results agree with previous studies (Rolfini and Cabrini, 1993). Their study proposed that the truncated CFTR protein could be partially functional in pulmonary tissues. Therefore, only mild or moderate pulmonary disease would occur. Probably this happens because when this mutation is present, the protein still contains the regulatory domain, the first nucleotide binding site and both transmembrane domains (Table I).

The phenotypes of the 2183AA→G carriers did not agree with Bozon *et al.* (1994) and Bonizzato *et al.* (1995), who suggested that this mutation could lead to severe symptoms. However, it did agree with Castaldo *et al.* (1996), who believe that this mutation causes only moderately severe disease (Table I).

The heterogeneity observed in the patients was discussed by Wolf (1997), who explained that genetic heterogeneity may occur when different mutations converge to give a similar phenotype, and phenotypic heterogeneity occurs when mutations are within the same gene, and diverge to give different phenotypes. Although many examples of this heterogeneity have been cited, the mechanisms involved are still poorly understood.

Anyane-Yeboah and Heggarty (1998) discussed the discordant expression of CF mutations in siblings. They suggested that there are factors other than mutations of the CFTR gene which account for the discrepant CF phenotype in siblings.

One possible explanation is interaction of modifier genes. Rozmahel *et al.* (1996) have identified modifier genes in CFTR-deficient mice. Zielenski *et al.* (1998) continued the study of Rozmahel. They mapped the CF-modifier (CFM) loci in the human genome and tested these CFM in patients. Their data showed a strong association

between the markers in the 19q13 region and meconium ileus status in CF sibpairs, but the association was not so strong for pulmonary function.

In summary, we found no significant differences between samples of normal subjects of Italian origin from PR and SC and the northeastern Italian population in terms of the CF mutations $\Delta F508$, R1162X and 2183AA→G. In the patient sample, the overall frequency of R1162X and 2183AA→G mutations did not differ significantly from those found in northeastern Italy in subjects of Italian ancestry, except for the R1162X mutation, whose frequency was higher in SC than in northeastern Italy. The R1162X and 2183AA→G mutations are associated with mild or moderate lung disease and pancreatic insufficiency. Our data suggest that the R1162X and 2183AA→G mutations should be included as part of the mutation pool to be analyzed in CF patients of northeastern Italian descent. This suggestion applies also for other countries with a substantial Italian immigration.

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RESUMO

Realizou-se a análise de 79 pacientes provenientes do Sul do Brasil para duas mutações raras da fibrose cística (CF), R1162X e 2183AA→G; dentre estes pacientes, 49 eram nascidos no Estado do Paraná (PR) e 30 eram nascidos no Estado de Santa Catarina (SC). Para a mutação 2183AA→G, dois alelos foram detectados entre os pacientes de SC e um alelo nos pacientes de PR. Para a mutação R1162X, seis alelos foram detectados entre os pacientes de SC e um alelo entre os pacientes do PR. Quando estes pacientes foram classificados de acordo com a origem étnica, 14% dos alelos detectados entre os pacientes de origem italiana eram portadores da mutação R1162X e 7% da mutação 2183AA→G. Estas mutações, juntamente com a mutação $\Delta F508$, também foram analisadas em uma amostra de 270 indivíduos normais de origem italiana não-consangüíneos, os quais eram nascidos no Estado do PR. Nessa amostra foram detectados dois alelos $\Delta F508$ e um alelo 2183AA→G. As frequências das mutações $\Delta F508$, R1162X e 2183AA→G não mostraram desvio estatístico significativo daquelas frequências observadas no norte da Itália. Nossos resultados demonstram que é importante incluir estas mutações no conjunto de mutações a serem pesquisadas nos pacientes com FC do sul do Brasil, especialmente quando estes pacientes tiverem origem italiana.

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