Cystic fibrosis in a southern Brazilian population: characteristics of 90% of the alleles


Cystic fibrosis (CF) is a genetic disease that frequently leads to death in infancy among Europeans and their descendants. The goals of the present study were to analyze the molecular aspects of CFTR gene characterizing mutations, their frequencies, and the haplotypes formed by four CFTR gene intragenic markers, IVS8-6(T)n, IVS8CA, IVS17bTA and IVS17bCA, in a southern Brazilian population of Caucasian origin.

DNA samples from 56 non-related CF patients were analyzed using scanning techniques (single strand conformation polymorphism and denaturing gradient gel electrophoresis), restriction fragment length polymorphism and direct DNA sequencing to identify the mutations. Our results revealed a total of 25 different CF mutations representing nearly 90% of CF alleles, two being novel mutations. Microsatellite haplotypes were defined for CF and normal alleles. The mutational spectrum and the associated haplotypes described for the first time in this study should prove relevant for genetic counselling and CF population screening in Brazil. Moreover, our results suggest the presence of a major Mediterranean component in the contemporary Brazilian CF patient pool.

Key words: Brazilian CF population – CFTR gene – CFTR haplotypes – cystic fibrosis – mutation scanning

Corresponding author: Fábio Rueda Faucz, PhD, Pontifícia Universidade Católica do Paraná, PUCPR, Curitiba, Brazil. Department of Genetics, Universidade Federal do Paraná, Curitiba, Brazil. Medical and Molecular Genetics Center, IDIBELL, Hospital Durán i Reynals, Barcelona, Spain, and Genes and Diseases Programme, Center for Genomic Regulation, Biomedical Research Park, Barcelona, Spain

Received 13 March 2007, revised and accepted for publication 11 May 2007
heterogeneity in Brazilian CF patients by direct analysis of F508del and four other common mutations (G542X, N1303K, G551D and R553X). These five mutations represented 56% of CF alleles in Brazil and their frequencies varied from state to state (7, 13).

Several highly polymorphic microsatellite and diallelic markers have been described within the CFTR gene and have been used in CF genetic testing (14). Their analysis allows for prenatal and carrier diagnosis in CF families and generates CFTR haplotypes that are strongly associated with specific mutations (15, 16). In particular, haplotypes from three microsatellite markers (IVS8CA, IVS17bTA and IVS17bCA) have been described in association with specific mutations (15, 17).

Herein, we provide the updated data on the spectrum of mutations in a southern Brazilian CF population after screening the 27 exons of CFTR gene and their flanking sequences. In addition to mutation analysis, we performed for the first time in this population an association study including polymorphic intragenic markers. On the other hand, this is a blind study, parallel to that performed by Raskin et al. (7) in which 70 CFTR mutations were directly tested in a subgroup of the present series (with a total of 21 shared patients). In summary, we have improved the genetic testing in Brazilian CF families combining direct and indirect analysis and thus, increased the detection rate of CF alleles.

Materials and methods

CF families

A total of 56 non-related Brazilian CF patients and their parents, born in two southern states of Brazil [22 in the state of Santa Catarina (SC) and 34 in the state of Paraná (PR)] were selected for this study. The two states studied are highly representative of the southern Brazilian population, characterized by a European origin relatively free of admixture (18). All patients were of Caucasian origin with a male proportion of 54%. The mean age was 6.7 years and ranged from 2 months to 32 years. The age of diagnosis varied from the neonatal period to 4 years and 3 months. Ten patients with meconium ileus were diagnosed at birth. All patients had a minimum of two positive chloride sweat tests (>60 mEq/l) ranging from 60 to 154 mEq/l. Chronic lung infection was identified in 53 out of 56 CF patients. Pancreatic sufficiency was less frequent (14.3%) than insufficiency (PI; 85.7%). Infertility was determined in the two adult male patients included in this series.

Twenty-one out of 56 patients were previously analyzed for 70 common mutations (7). This blind replicated study allowed us to evaluate the two different strategies.

All patients' parents were born in Brazil. Parents' alleles were also analyzed. Their non-CF alleles were assessed as normal CFTR genes.

Detection of mutations and polymorphisms

Genomic DNA was isolated from peripheral blood lymphocytes according to standard protocols. Direct analysis of the F508del mutation was carried out in all samples (19). Single-strand conformation polymorphism (SSCP) analysis was performed for 13 exons of the CFTR gene (1, 2, 4, 6a, 6b, 7, 10, 13, 16, 17a, 19, 22 and 24) (20), and denaturing gradient gel electrophoresis (DGGE) analysis for the remaining exons (21). When abnormal band patterns were detected, direct DNA sequencing was carried out.

Microsatellites IVS8CA, IVS17bTA, and IVS17bCA were analyzed in a multiplex polymerase chain reaction (PCR) as previously described (22), with the exception of the extension temperature (from 74°C to 65°C) and the number of cycles (25 instead of 30). Migration of fragments was performed in a 6% acrylamide gel. Through segregation studies, we were able to establish the association between CF and normal alleles with their intragenic microsatellite haplotypes. For CF patients, showing homozygosity in mutations and microsatellites haplotype, special attention was given to investigating the possibility of parent's consanguinity (microsatellites haplotype, questionnaire and surname records) and gross deletions (microsatellites haplotype).

The polymorphic (T)n locus, a polythymidine tract (alleles 5T, 7T and 9T) adjacent to the acceptor splice site of intron 8 was also analyzed, using the allele specific PCR assay described by Friedman et al. (23).

Nomenclature

For mutation nomenclature, we have followed recommendations from the Cystic Fibrosis Genetic Analysis Consortium (12) and/or that recommended by the Human Genome Variation Society (HGVS) (24).

Results

CFTR mutations

We identified 25 mutations accounting for 88.4% of CF alleles. Nine mutations showed a frequency higher than 1%, F508del (45.5%), G542X (6.3%), N1303K (4.5%), G85E, R334W and R1162X (3.6%), 2183AA>G and W1282X.
Table 1. Frequencies of the CFTR mutations, their microsatellite haplotypes and IVS8-6(T)n alleles in the Brazilian CF patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon/intron</th>
<th>Chromosomes Parana State/Santa Catarina State (total)</th>
<th>%</th>
<th>Haplotypes IVS8CA, IVS17bTA, IVS17bCA (n)</th>
<th>(T)n locus (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508</td>
<td>Exon 10</td>
<td>27/24 (51)</td>
<td>45.54</td>
<td>16-7-17 (1)/16-29-14 (1)/16-31-13 (1)/17-30-13 (1)/17-31-13 (20)/17-32-13 (7) 23-31-13 (15)/23-32-14 (1)/23-46-13 (1)/25-30-13 (1)/26-31-13 (1)/unknown (1)</td>
<td>9T (44)/7T (3) unknown (4)</td>
</tr>
<tr>
<td>G542X</td>
<td>Exon 11</td>
<td>5/2 (7)</td>
<td>6.25</td>
<td>23-32-13 (1)/23-33-13 (5)/23-34-13 (1)</td>
<td>9T (7)</td>
</tr>
<tr>
<td>N1303K</td>
<td>Exon 21</td>
<td>2/3 (5)</td>
<td>4.46</td>
<td>16-30-13 (1)/23-30-13 (1)/23-31-13 (3)</td>
<td>9T (4)/7T (1)</td>
</tr>
<tr>
<td>G85E</td>
<td>Exon 3</td>
<td>2/2 (4)</td>
<td>3.57</td>
<td>16-24-13 (4)</td>
<td>7T (4)</td>
</tr>
<tr>
<td>R334W</td>
<td>Exon 7</td>
<td>1/3 (4)</td>
<td>3.57</td>
<td>16-34-13 (1)/16-48-13 (1)/17-33-13 (1)/17-41-13 (1)</td>
<td>7T (3)/unknown (1)</td>
</tr>
<tr>
<td>R1162X</td>
<td>Exon 19</td>
<td>1/3 (4)</td>
<td>3.57</td>
<td>17-31-13 (4)</td>
<td>7T (4)</td>
</tr>
<tr>
<td>2183 AA&gt;G</td>
<td>Exon 13</td>
<td>1/2 (3)</td>
<td>2.68</td>
<td>16-31-13 (2)/16-31-14 (1)</td>
<td>7T (2)/unknown (1)</td>
</tr>
<tr>
<td>W1282X</td>
<td>Exon 20</td>
<td>1/2 (3)</td>
<td>2.68</td>
<td>17-7-17 (3)</td>
<td>7T (2)/9T (1)</td>
</tr>
<tr>
<td>R553X</td>
<td>Exon 11</td>
<td>2/0 (2)</td>
<td>1.78</td>
<td>17-44-11 (1)/17-47-11 (1)</td>
<td>7T (1)/unknown (1)</td>
</tr>
<tr>
<td>S4X</td>
<td>Exon 1</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>(16-__-13) (1)</td>
<td>Unknown (1)</td>
</tr>
<tr>
<td>232del18</td>
<td>Exon 2</td>
<td>0/1 (1)</td>
<td>0.89</td>
<td>21-36-13 (1)</td>
<td>Unknown (1)</td>
</tr>
<tr>
<td>621+1G&gt;T</td>
<td>Intron 4</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>__-34-13 (1)</td>
<td>Unknown (1)</td>
</tr>
<tr>
<td>711+1G&gt;T</td>
<td>Intron 5</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-25-13 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>711+5G&gt;A</td>
<td>Intron 5</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>__-7-17 (1)</td>
<td>Unknown (1)</td>
</tr>
<tr>
<td>R347P</td>
<td>Exon 7</td>
<td>0/1 (1)</td>
<td>0.89</td>
<td>16-32-13 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>1717-1G&gt;A</td>
<td>Intron 10</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-7-17 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>1717-8G&gt;A</td>
<td>Intron 10</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-33-13 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>1812-1G&gt;A</td>
<td>Intron 11</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-31-14 (1)</td>
<td>9T (1)</td>
</tr>
<tr>
<td>A561E</td>
<td>Exon 12</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-44-13 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>E585X</td>
<td>Exon 12</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>Unknown (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>1898+1G&gt;A</td>
<td>Intron 12</td>
<td>0/1 (1)</td>
<td>0.89</td>
<td>16-45-13 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>G1069R</td>
<td>Exon 17b</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>17-30-13 (1)</td>
<td>Unknown (1)</td>
</tr>
<tr>
<td>Y1092X</td>
<td>Exon 17b</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-30-13</td>
<td>7T (1)</td>
</tr>
<tr>
<td>3849+10 kb C&gt;T</td>
<td>Intron 19</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-7-17 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>W1282G</td>
<td>Exon 20</td>
<td>1/0 (1)</td>
<td>0.89</td>
<td>16-32-14 (1)</td>
<td>7T (1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Intron 19</td>
<td>13/0 (13)</td>
<td>11.60</td>
<td>16-7-17 (1)/16-29-13 (2)/16-30-13 (1)/16-31-13 (3)/16-33-13 (1)/16-34-13 (1)/16-38-16 (1)/18-35-13 (2)</td>
<td>Unknown (13)</td>
</tr>
</tbody>
</table>

Total 112 100

*n*, the total number of chromosomes bearing each haplotype or (T)n locus; ‘unknown’, used when the haplotype/(T)n locus cannot be characterized; ‘__’, used when a specific allele of the haplotype cannot be characterized.

Numbers for microsatellites IVS8(CA)n, IVS17b(TA)n, and IVS17b(CA)n indicate the numbers of repeats.
(2.7%) and R553X (1.8%). These nine mutations represented 74.1% (83/112) of the CF alleles. The remaining 16 mutations showed frequencies lower than 1% and accounted for 14.3% of CF alleles (Table 1).

Two novel mutations were identified. One was detected by DGGE analysis and direct sequencing of exon 20 showing a substitution of T to G at nucleotide 3976, named W1282G, (p.Trp1282Gly).

The second novel mutation corresponded to an 18-bp in-frame deletion named 232del18 or c.100-117del (p.Leu34_Gln39del) (HGVS nomenclature), which was detected by SSCP analysis and direct sequencing of exon 2. The two patients with novel mutations carried the F508del mutation in trans. Both patients are women, 18 and 15 years old, respectively, with positive sweat test ranging from 93 to 110 mEq/l. They presented typical CF symptoms with PI and Pseudomonas aeruginosa colonization.

Two CF mutations were characterized in 45 out of 56 patients, leading to 34 different genotypes. Among these, homozygosity for F508del was the most frequent (n = 12). Nine patients were partially characterized (four F508del; five with several different mutations 2183AA>G, 621+1G>T, N1030K, G1069R and R553X). No mutation was identified in the two remaining patients despite the fact that both fulfilled CF clinical criteria. One presented P. aeruginosa infection, PI and two positive sweat tests (above 88 mEq/l), while the other was diagnosed earlier on with meconium ileus with other clinical findings, P. aeruginosa infection, nasal polyps and two positive sweat tests (above 75 mEq/l).

(T)n locus

This sequence in intron 8 [IVS8-6(T)n] was analyzed in 88 CF alleles. The 5T allele was undetected. The frequencies of the other two alleles were, 67% (59/88) for the 9T allele and 33% (29/88) for the 7T allele.

Microsatellite haplotypes

CFTR microsatellite haplotypes (IVS8CA, IVS17bTA and IVS17bCA) were determined in 112 CF alleles and 110 normal alleles.

Microsatellite analysis of the CF alleles allowed us to define the haplotype-mutation association. Forty-four haplotypes were identified associated with 25 different mutations and nine haplotypes with yet unknown mutations (Table 1). In addition, 48 haplotypes were found associated to the normal alleles (Table 2).

As expected, several haplotypes were found to be associated to the old mutations. Specifically, the F508del mutation was linked to 12 different haplotypes (Table 1).

Nowadays, the Brazilian population constitutes a highly heterogeneous population with a trihybrid composition (native Indians, Africans and European descendants) in which individuals of European origin represent 55% of the total (25). The population from the state of SC has the lowest admixture rate in Brazil (5.3%), with 93% of European origin. Similarly, in the state of PR, 75% of the population has European origin (25).

Consequently, CFTR molecular heterogeneity is expected. Previously, such heterogeneity was indeed identified in Brazilian CF patients of European origin by the screening of five common mutations (F508del, G542X, N1303K, G551D and R553X) (13). These mutations represented only 56% of CF alleles in Brazil and their frequencies varied from state to state (7, 26, 27).
Faucz et al.

Herein, we have identified 25 different mutations in 99 CF alleles, and nine different haplotypes associated with yet unknown mutations, supporting the high molecular heterogeneity of the population studied.

The mutation spectrum of CF in Brazil indicates strong influence of a European component; therefore, we should evaluate the European ancestry taking into account our results. Our mutational spectrum showed a certain similarity with that reported in the Italian population (F508del, 48.9%; G542X, 5.9%; N1303K, 5.9%; and 2183AA>G, 2.6%) (28). This figure could not be observed with other populations that contributed to the ethnic composition of the Brazilian population. For example, common mutations in the Portuguese population show quite different frequencies (G542X, 1.3%; N1303K, 0.7%, and 2183AA>G, 0%) (10). The fact that the mutation spectrum is closer to that found in Italian patients than in Portuguese patients is intriguing, although the Portuguese influence could be more subtle, considering that A561E, the second most common mutation in Portugal (3.2%) (10) was also found in our series (0.9%). This Italian influence in the genetic pool of southern Brazilian population has been previously documented (29).

Homozygosity for microsatellite haplotypes was detected in five patients. In all cases, the genotype was confirmed by a segregation study in each family. Parents’ consanguinity was reported in patients’ homozygous for the 2183AA>G mutation. Three other patients were homozygous for the common mutations: two with F508del and one with R1162X. The CF mutations in the fifth patient were not characterized. The segregation studies allowed us to reduce suspicion of a gross rearrangement, although not absolutely discarded in patients with unknown mutations (30).

Combining direct mutation analysis and microsatellite haplotypes, prenatal diagnosis can now be offered for almost all Brazilian CF families. It is important to note that in cases without identified CF mutations, the clinical findings must strongly support the linkage analysis for pre-natal diagnosis. In these families, genetic counselling must take into account that some CF phenotypes might not be related to CFTR pathology (31).

The duplicate analyses of 21 patients permitted us to compare our strategy with that applied by Raskin et al. (7). Firstly, all CF mutations identified by direct analysis were also detected in our study. In addition, we identified four mutations previously undetected representing an increase in detection level in both states (PR 80.9% vs 73.0% and SC 100.0% vs 94.8%). These results indicate a higher sensitivity using our strategy. Moreover, our study suggests that a strategy combining direct analysis for nine common mutations and intragenic markers allow us to take advantage to the haplotype-mutation association which could, thus, lead to specific analysis improving the mutation detection rate and limiting the CFTR gene scanning to uncharacterized CF patients.

This work has led to the characterization of almost 90% of CF mutations in a southern Brazilian population from the states of SC and PR. We now have important tools to offer genetic testing for CF families in these regions. Moreover, it is now possible to plan a Neonatal Screening Program that would allow for early diagnosis and better follow-up of CF patients. However, further comprehensive CFTR analysis is still necessary to understand the complex heterogeneity of the Brazilian CF population.

Acknowledgements

This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq and International Cystic Fibrosis (Mucoviscidosis) Association (both through a grant to Fábio Rueda Faucz); and grants Fondo de Investigaciones Sanitarias/Fondo Europeo de Desarrollo Regional (FIS/FEDER) PI050804 and ISCiii C03/07 from Spain. We thank the CF patients and their families, as well as Associação Brasileira de Apoio a Mucoviscidose (ABRAM, Brazilian CF Foundation) for their efforts in encouraging this work.

References