Prevalence of F508 mutation in the cystic fibrosis transmembrane conductance regulator gene among cystic fibrosis patients from a Brazilian referral center

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Jornal de Pediatria, vol. 88, núm. 6, noviembre-diciembre, 2012, pp. 531-534

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Porto Alegre, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=399738193014
Introduction

Cystic fibrosis (CF) is the most frequent recessive autosomal disease in the population, especially of Caucasians. CF incidence varies according to ethnicity and, in Brazil, its prevalence is estimated to be from 1/3,500 to 1/10,000 live births, depending on the geographical area.1

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, located at the 7q31 region, cause loss of function of the CFTR protein, which in normal conditions acts as a chloride channel,2-4 leading to CF.

More than 1,900 mutations in the CFTR gene were identified, but the first to be identified and also the most frequent is the ΔF508 mutation, present in nearly 70% of CF cases, depending on the analyzed population.5 In some populations, it is possible to easily identify all mutations in

Abstract

Objective: To verify the presence of ΔF508 mutation in the cystic fibrosis transmembrane conductance regulator gene among patients with cystic fibrosis diagnosed by the sweat test for sodium and chlorine and followed at the Pediatric Pneumology Outpatient Clinic of Universidade Estadual de Campinas, Brazil, a referral center for the treatment of cystic fibrosis.

Methods: The study analyzed 167 DNA samples from cystic fibrosis patients. Patients’ genotype was determined by polymerase chain reaction, and allele and genotype frequencies of ΔF508 mutation were calculated.

Results: The genotype frequencies found for -/-, ΔF508/-, and ΔF508/ΔF508 genotypes were respectively: 43.7% (73 patients), 32.9% (55 patients), and 23.4% (39 patients). Of the 334 alleles analyzed, we observed a frequency of 201 (60.18%) alleles for the absence of ΔF508 mutation and of 133 (39.82%) for the presence of ΔF508 mutation. Hardy-Weinberg equilibrium was calculated, obtaining a chi-square value = 16.34 (p ≤ 0.001).

The study population was out of equilibrium. The expected values for -/-, ΔF508/-, and ΔF508/ΔF508 genotypes were respectively: 32.22% (60.48 patients), 47.93% (80.04 patients), and 15.86% (26.48 patients).

Conclusions: In the analyzed population, ΔF508 mutation was less prevalent than the allele without this mutation. The frequency observed in this study was similar to that from other areas in Brazil and in the world, mainly due to the predominantly Caucasian origin of the population included in the study.

the CFTR gene present in the patients; however, in mixed populations, such as the Brazilian one, it is not feasible yet. The ΔF508 mutation occurs due to a deletion of three bases in exon 10, resulting in the loss of the amino acid phenylalanine at position 508, which leads to a deficiency in CFTR folding and subsequently to a degradation in the rough endoplasmic reticulum.6

The CFTR protein promotes chlorine reabsorption in sweat glands, but in CF the CFTR is absent in the epithelium or presents qualitative or quantitative changes in its level of expression; thus, chlorine is not reabsorbed, causing high concentrations of ions in sweat. This dysfunction can affect several organs, particularly those which secrete mucus, including upper and lower airways, pancreas, biliary tract, male genitalia, intestine, and sweat glands.7 The main morbidity and mortality factor for CF is accumulation of secretions in the lungs, leading to their obstruction. Clinical presentation, disease severity, and rate of CF progression vary considerably, and some variations may occur due to presence of different combinations of mutations in the CFTR gene. Among homozygous patients for ΔF508 mutation, the severity of the pulmonary disease is variable, and the reasons for the low pulmonary correlation between genotype and phenotype are not clear; however, this mutation in homozygosity, or combined with another severe mutation, leads to the classical picture of CF.8

Considering the importance of ΔF508 mutation in CF, due to its high frequency and severity, the aim of this study was to verify the presence of ΔF508 mutation in the CFTR gene in patients diagnosed with CF by the sweat test for sodium and chlorine and followed at the Pediatric Pneumology Outpatient Clinic of Universidade Estadual de Campinas (UNICAMP), Brazil, a referral center for the treatment of CF.

Methods

We analyzed 167 peripheral blood DNA samples from patients diagnosed with CF coming from the Pediatric Pneumology Outpatient Clinic of Hospital de Clínicas of UNICAMP who showed abnormal values (above 60 mEq/L) in the sweat test for sodium and chlorine and followed at the Pediatric Pneumology Outpatient Clinic of Universidade Estadual de Campinas (UNICAMP), Brazil, a referral center for the treatment of CF.

DNA amplification to identify ΔF508 mutation was performed in the 167 samples using the polymerase chain reaction (PCR) technique, and PCR results were visualized by 12% denaturing polyacrylamide gel electrophoresis. Descriptive statistical analysis was performed by calculating allele and genotype frequencies of ΔF508 mutation in the CFTR gene for the study sample. Hardy-Weinberg equilibrium was calculated using the Online Encyclopedia for Genetic Epidemiology Studies software (2008) to verify if the genotype distribution of ΔF508 mutation is balanced. The association between the genotype frequency of ΔF508 mutation found in the study of Bernardino et al.9 and that of the present analysis was assessed with the Statistical Package for the Social Sciences v.17.0 software.10

The project was approved by the research ethics committee of the institution according to judgment #528/2008.

Results

A total of 167 samples were analyzed. Of these, the samples that had the ΔF508 mutation were amplified again to confirm the result. The results of the descriptive statistical analysis of genotypes with ΔF508 mutation are shown in Table 1. Hardy-Weinberg equilibrium was calculated, obtaining a chi-square value = 16.34 (p ≤ 0.001). The study population was out of equilibrium. The expected values for -/-, ΔF508/-, and ΔF508/ΔF508 genotypes were respectively: 32.22% (60.48 patients), 47.93% (80.04 patients), and 15.86% (26.48 patients).

Discussion

Among the 167 patients analyzed, 73 (43.7%) did not have the ΔF508 mutation; 55 (32.9%) were heterozygous; and 39 (23.4%) were homozygous, with two mutated alleles for ΔF508. All patients had CF previously diagnosed by the sweat test for sodium and chlorine.

The calculation of Hardy-Weinberg equilibrium performed in this study showed that the genotype distribution of this mutation in our sample is not in agreement with Hardy-Weinberg’s law. This may be happening because many homozygous individuals for this mutation presented with a more dramatic disease picture, having thus a higher chance of being diagnosed, while other genotypes associated with more benign mutations would have a late diagnosis. Moreover, in this population there is a strong environmental influence on the severity of clinical manifestations, which could interfere in Hardy-Weinberg equilibrium.

The allele frequency of ΔF508 mutation was 39.82% in the analyzed population. In a study conducted in São Paulo, Brazil, Okay et al.2 found an allele frequency of 44.5% for ΔF508 mutation, higher to that observed in the present study. Another study of allele frequency in Minas Gerais, Brazil, and São Paulo showed lower values than those of the present study, 21.7 and 33% respectively.11,12 In comparison, the work of Bernardino et al.,9 in which 160 samples of CF patients were analyzed for several mutations in the CFTR gene, found a genotype frequency of ΔF508 mutation of respectively 47 (29.4%), 61 (38.1%), and 52 (32.5%) for the genotypes ΔF508/ΔF508, ΔF508/-, and for patients with two unidentified mutations. Comparing our data with those from the survey of Bernardino et al.,9
Table 1 - Calculation of genotype frequency for ΔF508 mutation

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotype frequency (n)</th>
<th>Percentage of genotypes</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>-/-</td>
<td>73</td>
<td>43.7</td>
<td>43.7</td>
</tr>
<tr>
<td>ΔF508/-</td>
<td>55</td>
<td>32.9</td>
<td>76.6</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>39</td>
<td>23.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

(-) = absence of ΔF508 allele; n = sample size in absolute number of patients.

there was no statistically significant difference (p = 0.109). Anyway, the values obtained were close, and the small variation observed between the studies can be attributed to the ethnic variation in each microregion.

Because CF is a recessive autosomal genetic disease with high morbidity and mortality rates, the inclusion of testing for CF in the neonatal screening has become extremely important. Molecular analysis is essential in order to obtain an accurate diagnosis. The molecular test performed in this study is a worldwide spread exam that, combined with neonatal screening, confers better treatment conditions and allows for genetic counseling for parents before a new pregnancy occurs. Therefore, molecular analysis should begin with ΔF508 mutation, and when the patient is negative for this mutation, molecular analysis becomes complex due to the high number of existing mutations in the CFTR gene.

The molecular characterization of the disease enables genetic counseling and appropriate pulmonary surveillance, which may become even more important as therapeutic advances improve prognosis and allow for the development of new pharmaceutical methodologies that could play a role in the correction of CF phenotype, emphasizing the importance of the genotyping of each patient during diagnosis. CFTR pharmacotherapy aims to improve intracellular transportation, its expression and function; therefore, these treatments are directed to a determined class of specific mutation or to only one mutation.

In conclusion, the study population showed that ΔF508 mutation was less prevalent compared with the sum of the other mutant alleles. The frequency was found to be close to that of other regions in Brazil and in the world, mainly due to the predominantly Caucasian origin of the population included in the study. In this study, allele frequency for ΔF508 mutation was 39.82%, with 55 heterozygous patients (32.9%), showing a mutated allele for ΔF508, and 39 (23.4%) homozygous patients, with two mutated alleles for ΔF508. These data corroborate the importance of using ΔF508 mutation as a diagnostic tool and mainly as a factor to be considered for a better genetic counseling. And finally, with the development of new specific therapies for each mutation, screening for ΔF508 will be important, due to its high frequency in the population.

To determine patient’s genotype for other mutations in the CFTR gene associated with CF, the analyzed population needs to be assessed by molecular analysis for other changes in the gene using different molecular techniques, such as sequencing and other methods of gene analysis.

Acknowledgements

The authors would like to thank the staff of the Molecular Genetics Laboratory from the School of Medicine of UNICAMP for their support in this study and the interdisciplinary group of the Pediatric Outpatient Clinic of UNICAMP.

References


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