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Low Frequency of the $\Delta F508$ Mutation of the *CFTR* Gene in a Highly Admixed Population in Bahia, Brazil

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Abstract Cystic fibrosis (CF) is the most common autosomal recessive disease in the European (Caucasian) population, with an incidence of 1:2,000 to 1:8,000. The $\Delta F508$ mutation (66%) is predominant among more than 1,300 different mutations of the *CFTR* gene. The population of the state of Bahia, in northeastern Brazil, is highly admixed (mainly African and Portuguese descendants), and so far, no study has been carried out to assess the molecular basis of CF in this population. We determined the $\Delta F508$ mutation frequency in 503 individuals from the general population of Salvador, the capital of the state of Bahia, and in 144 CF patients from several cities in Bahia. In the general population samples we found 4 individuals heterozygous for the $\Delta F508$ mutation (allele frequency of 0.4%). This frequency was lower than that found in the state of Rio de Janeiro, in southeastern Brazil, and similar to that reported for the state of Paraná, in the far south. In the CF patients we found 9 heterozygous individuals and 8 homozygous individuals (allele frequency of 8.68%) for the $\Delta F508$ mutation. This frequency is considerably lower than the average frequency of CF in the world population and in the Brazilian CF population of European ancestry (47%). These data could be explained by the intense admixture among the population in Bahia, and they suggest a heterogeneous molecular basis for CF in this area of Brazil.

Cystic fibrosis (CF) is the most common autosomal recessive disease in the European (Caucasian) population, with an occurrence rate of 1 of every 2,000 live births. The clinical features of CF appear predominantly in the respiratory and digestive systems (Noone et al. 1994).

In 1989 the gene responsible for CF, the cystic fibrosis transmembrane conductance regulator (*CFTR*), was cloned. The *CFTR* gene is located on chromosome 7q31, expands for 250 kb, and contains 27 exons. It codifies a protein of 1,480 amino acids. So far, more than 1,300 mutations have been identified, and

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many of them are specific to a certain population, family, and/or geographic region (Kerem et al. 1989). The $\Delta F508$ mutation (p.F508 del), the most common *CFTR* gene mutation, is a 3-bp deletion that leads to the deletion of the amino acid phenylalanine at position 508 of exon 10 (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989).

Several CF mutation screening studies have been carried out in general populations of several countries in South America. In Uruguay the $\Delta F508$ allele frequency was 0.4% (4/1,000 chromosomes) (Cardoso et al. 2004). In Bogota, Colombia, in a study of 130 non-CF individuals, 2 heterozygotes were detected, corresponding to an allele frequency of 0.77% (Keyeux et al. 2003). In Chile this allele frequency was 2% (2/100 chromosomes) (Vera et al. 2006). In Brazil the $\Delta F508$ mutation was analyzed in 291 individuals from the general population of Rio de Janeiro, in southeastern Brazil, and only 3 heterozygotes were identified, corresponding to an allele frequency of 0.51% (Cabello et al. 1999). In the state of Paraná, in southern Brazil, the $\Delta F508$ mutation was also studied in a sample of 270 non-CF individuals of Italian origin, and only 2 mutant alleles were found, giving an allele frequency of 0.37% (Pereira et al. 1999).

According to data from the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) (CFGAC 2005), the $\Delta F508$ mutation world average frequency in CF chromosomes is 66%. Tsui and Buchwald (1992) presented data in which the $\Delta F508$ mutation accounted for 50–88% of CF occurrences in western European countries.

In Latin American CF patients the $\Delta F508$ mutation average frequency is 45%, varying from 29% in Chile to 62% in Argentina (Rios et al. 1994; Visich et al. 2002). In Brazil an average frequency of 47% was found for patients of European origin (Raskin et al. 1993). In Rio de Janeiro the frequency of the $\Delta F508$ mutation was 30.68% (Cabello et al. 1999). Up to now, the lowest frequency in Brazil (22.7%) has been found in the state of Pará in northern Brazil (Araújo et al. 2005).

This study is the first screening for the $\Delta F508$ mutation in both the general population of Salvador and CF patients from Bahia who attend the Bahia Cystic Fibrosis Reference Center.

Materials and Methods

Patients. A cross-sectional study was carried out in the general population of Salvador, capital of the state of Bahia, located in northeastern Brazil. The city's population is approximately 2.5 million inhabitants and is composed of people mostly of African and Portuguese descent (Azevedo et al. 1982). The samples were collected during a large ongoing sanitation project in Salvador, called Projeto Bahia Azul. The sampling method has been described in detail elsewhere (Teixeira et al. 2002). In short, the study population was drawn from a spatial sample of 30 neighborhoods throughout the city. The neighborhoods were specifically selected from census districts and were stratified according to the level of sanitation coverage and income to include a wide range of living conditions. We randomly

selected 503 anonymous samples from the larger sample (2,149 individuals) to determine the frequency of the $\Delta F508$ mutation. These 503 individuals probably had the same characteristics as the general population. The sample size was calculated using Epi Info (version 3.2.2) and the $\Delta F508$ mutation allele frequency in the general population of Rio de Janeiro (Cabello et al. 1999). Informed consent was obtained from the individuals.

Salvador's Cystic Fibrosis Reference Center was set up in 1991 and has records of 271 families with registered CF cases. After obtaining formal consent from either patients or the individuals responsible for them, we collected blood samples from 144 unrelated CF patients and from 184 family members. The patients were widely distributed across 40 towns in the state of Bahia. However, most of them (85 patients) were from Salvador. The median and average age of these patients was 9.6 years and 4 years, respectively (range: 2 months to 78 years). All the patients had been diagnosed with CF based on the Consensus Conference and Consensus Statement on the diagnosis of CF (Rosenstein and Cutting 1998; Leigh 2004).

This study was approved by the Gonçalo Moniz Research Center/Oswaldo Cruz Foundation Ethics Board (no. 78/2005).

Methods. Genomic DNA was extracted from total blood using standard methods (Lahiri and Nurnberger 1991). A PCR amplification of a 98-bp fragment from exon 10 of the *CFTR* gene was performed using primers and conditions described by Kerem et al. (1989). The amplification was carried out with an initial 6-min denaturation at 94°C and 2-min annealing at 64°C, followed by 35 cycles of 1 min at 72°C, 1 min at 94°C, and 45 s at 64°C, and a final extension of 10 min at 72°C. The $\Delta F508$ mutation was detected by electrophoresis of the PCR product in an 8% polyacrylamide gel (29:1 acrylamide:bis-acrylamide) at 150 V for 3 hr and stained with ethidium bromide. The size fragment distribution showed 98 bp for wild-type homozygous subjects, 95 bp for mutant homozygous subjects, and 95-bp and 98-bp bands for heterozygous subjects.

Results

Among the general population sample of 503 individuals, we found 4 heterozygous individuals for the $\Delta F508$ mutation, corresponding to an allele frequency of 0.4%.

As for the 144 CF patients from the Bahia Reference Center, we found the $\Delta F508$ mutation in 25 chromosomes (allele frequency, 8.68%), and we identified the following genotypes: 8 mutant homozygous, 9 heterozygous, and 137 with no mutation in any of the alleles.

Discussion

The $\Delta F508$ mutation frequency of 8.68% in CF patients from the state of Bahia was much lower than the 66% world average frequency reported. This low

frequency may be due to the high degree of admixture in this population, which is composed mainly of African descendants. The only study to present a lower frequency than this one was carried out in South Africa in 2001 (Goldman et al. 2001). Goldman studied 12 black CF patients and did not find any $\Delta F508$ mutation alleles. In Brazil the average frequency of the $\Delta F508$ mutation is 47%, varying from 22.7% to 55.2% among the different states and geographic regions, depending on ancestry differences in the studied populations (Streit et al. 2003; Raskin and Fauez 2001; Raskin et al. 1999; Cabello et al. 1999, 2001; Araújo et al. 2005).

The 0.4% allele frequency found in the chromosomes of the general population of Salvador is lower than that found in the populations of Rio de Janeiro, Brazil, and Colombia, but it is similar to the frequencies observed in Paraná, Brazil, where the population is composed mainly of individuals of Italian ancestry, and in Uruguay. The low frequency of the $\Delta F508$ mutation among the general population of Salvador might also explain the low frequency of the mutant allele among our CF patients. This result is compatible with the high rate of admixture of the population, suggesting a heterogeneous molecular basis of CF in Bahia.

Since 2001 the Brazilian Neonatal Screening Program (GM/MS no. 822, June 6, 2001) has included the immunoreactive trypsinogen test to screen newborns for CF. The molecular confirmation of positive cases is based on the presence of the $\Delta F508$ mutation. However, because of the large differences in the distribution of the $\Delta F508$ mutation in the Brazilian regions, this CF genetic screening should always be done taking the different mutation rates between regions into account.

Considering the great contribution of Portuguese and African ancestry in the population of the state of Bahia, our findings show the importance of investigating other mutations already detected in these regions (Azevedo et al. 1982). It is possible that the $c.3120 + 1G \rightarrow A$ mutation, of African origin, might be found in our region (Goldman et al. 2001).

The low frequency of the $\Delta F508$ mutation found in CF patients from Bahia confirms the molecular heterogeneity of this disease. We are currently carrying out studies into *CFTR* gene mutations to establish a more accurate genotype of CF patients from Bahia.

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