CFTR Molecular Analysis Reveals Infrequent Allele Frequencies in Nine Cystic Fibrosis Patients from Sao Paulo State, Brazil

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Human Biology, Volume 75, Number 3, June 2003, pp. 393-398 (Article)

Published by Wayne State University Press
DOI: *https://doi.org/10.1353/hub.2003.0042*

Brief Communications

CFTR *Molecular Analysis Reveals Infrequent Allele Frequencies in Nine Cystic Fibrosis Patients from São Paulo State, Brazil*

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Abstract Although cystic fibrosis (CF) is the most common autosomal recessive disorder in whites, it is thought to be relatively rare or, alternatively, underdiagnosed in Latin America. In Brazil, different groups have shown a DF508 mutation frequency ranging from 30.7% to 50.8%. Such variation may be explained by the ethnic differences observed in this country, which is genetically very heterogeneous. We describe the molecular analysis for 32 mutations of the *CFTR* gene in nine unrelated patients with cystic fibrosis from São Paulo State, Brazil. The main observation of this study was the absence of 30 out of the 32 mutations in 12 alleles among these patients. Except for mutations DF508 and N1303K, no other mutation could be detected in any of the studied patients. In one of two alleles, a DF508 mutation was detected in four patients (22% of the total sample) and an N1303K mutation was detected in two patients (11% of the total sample). One patient was a compound heterozygote for DF508/N1303K. Although the sample studied here was small, it may be possible that our patients have infrequent alleles once these can occur at higher frequencies in selected populations and also show relevant regional differences. Additional investigations in a larger sample are currently in progress in our laboratory to confirm our results, and further studies are still needed to determine the frequencies of CF gene mutations in different regions and ethnic groups in the Brazilian population.

Cystic fibrosis (CF) is one of the most common severe autosomal recessive genetic disorders in whites. It is characterized by chronic lung disease and pancreatic insufficiency, and is still potentially fatal (Lucotte et al. 1995). The gene responsible for cystic fibrosis, the cystic fibrosis transmembrane conductance reg-

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Human Biology, June 2003, v. 75, no. 3, pp. 393–398.

KEY WORDS: *CTFR* ALLELES, BRAZIL

ulator (*CFTR*), a c-AMP dependent chloride channel, was cloned in 1989 (Kerem et al. 1989; Rommens et al. 1989), and many mutations have been detected since then by the Cystic Fibrosis Genetic Analysis Consortium (1994).

Of all the cystic fibrosis mutations, the DF508 mutation, marked by the deletion of three base pairs at codon 508 (Kerem et al. 1989), is the most frequent. It accounts for approximately 70% of worldwide chromosomes that have the *CFRT* gene. Except for this mutation, only a few alleles have a worldwide frequency of 1% or greater, although they may occur at higher frequencies in selected populations (CF Genetic Analysis Consortium 1994).

Knowledge of the spectrum of CF gene mutations in different populations enables genetic testing. Thus, for studies to be accurate they should be carried out in the geographic region where the patients were born so that risks can be calculated with more precision (Raskin et al. 1997).

Although CF is the most common autosomal recessive disorder in whites, it is thought to be relatively rare or, alternatively, underdiagnosed in Latin America (Raskin et al. 1997). In Brazil, a country of continental size, different groups have shown a DF508 mutation frequency ranging from 30.7% to 50.8% (Martins et al. 1993; Raskin et al. 1993; Parizotto et al. 1997; Maróstica et al. 1998; Cabello et al. 1999; Bernardino et al. 2000) (Table 1). Such variation may be explained by the ethnic and racial differences observed in this country, which is genetically very heterogeneous (Salzano and Freire-Maia 1967; Salzano and Pena 1987).

This study, characterizing CF patients in southeastern Brazil (São José do Rio Preto, São Paulo state), provides the molecular analysis for 32 mutations of the *CFTR* gene in nine unrelated patients with cystic fibrosis who were seen at the Genetics Service, Faculty of Medicine, São José do Rio Preto, SP, Brazil. All the patients were diagnosed with CF on the basis of typical clinical manifestations and a positive sweat chloride test (>60 mEq/L). Blood samples were collected on neonatal screening cards, stored in plastic bags, and mailed to GENETIKA Laboratory, PR, Brazil. All procedures were carried out so as to avoid contamination of the samples. DNA was extracted and amplified by the polymerase chain reaction (PCR) for the following 32 *CFTR* mutations: DF508, G542X, G551D, W1282X, 3905insT, N1303K, R117H, D1507, 3849+10kbC→T, R553X, 621+1G→T, 1717-1G→A, 1708delT, Q493X, V520F, S549N, Y1092X, R347H, R347P, R560T, R334W, Y122X, S549R, 3849+4A→G, 2789+5G→A, 711+1G→T, 1898+1G→A, R1162X, A455E, 2184delA, G85E, 3659delC, as described by Raskin et al. (1992a, b).

Table 2 shows the molecular and clinical characterization of the patients for the 32 mutations. Except for DF508 and N1303K, no other mutation could be detected in any of them. A DF508 and an N1303K mutation in one of the two alleles were detected in four patients (22% of the total sample) and in two of them (11% of the total sample), respectively. One patient was a compound heterozygote for DF508/N1303K. Mean age and sweat chloride in this group were 4.2 years (ranging from 1 to 12) and 128 mEq/L (ranging from 104 to 152), respectively. In four individuals without any mutation detected, mean age and sweat

Table 1. Frequencies of Cystic Fibrosis Mutations in Different Brazilian Reports

			Mutations					
Author	*n*	*State*	*DF508*	*N1303K*	*G542X*	*G551D*	*W1282X*	*R553X*
Raskin et al. 1993	58	São Paulo	52%	NE	NE	NE	NE	NE
Martins et al. 1993	24	São Paulo	31%	NE	NE	NE	NE	NE
Parizotto et al. 1997	60	São Paulo	31.7%	2.5%	8.3%	0	NE	0
Maróstica et al. 1998	61	Rio Grande do Sul	50.8%	NE	NE	NE	NE	NE
Cabello et al. 1999	44	Rio de Janeiro	30.7%	0	2.27%	1.14%	0	0
Raskin et al. 1999	172	5 States[a]	NE	4.4%	10.5%	0.4%	NE	1.6%
Bernardino et al. 2000	160	São Paulo	48.4%	2.5%	8.8%	NE	1.3%	0.6%
Present Study	09	São Paulo	22%	11%	0	0	0	0

Note: n = number of individuals; NE = not evaluated.

a. Brazilian states: Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, and Minas Gerais.

Table 2. Age, Sweat Chloride Values, and Clinical Signs of CF Patients Screened for 32 Mutations on the Cystic Fibrosis Gene

Patient No.	*Gender*	*Age (years)*	*Chloride (mEq/L)*	*Genotype*	*Clinical Signs*
01	M	2	104	DF/?	Pneumonia, productive cough, steatorrhea, anemia, sinusitis, dyspnea, otitis, amygdalitis, failure to thrive, bronchiectasis, vomiting, pancreatic insufficiency
02	M	12	152	DF/?	Anemia, dyspnea, bronchiectasis, vomiting, failure to thrive, dry cough, diarrhea
03	M	<1	140	DF/N1303K	Pneumonia, anemia, dry cough, gastroesophageal reflux, diarrhea, steatorrhea, failure to thrive, bronchiectasis, pancreatic insufficiency
04	F	5	142	DF/?	Pneumonia, dyspnea, vomiting, sinusitis, bronchiectasis, pancreatic insufficiency, *Staphylococcus aureus* and *Pseudomonas*, obit
05	F	<1	116	N1303K/?	Pneumonia, dyspnea, bronchiectasis, gastroesophageal reflux, vomiting, pancreatic insufficiency, hepatomegaly, atelectasia, convulsions, sepsis, obit
06	M	2	91	?/?	Pneumonia, *Streptococcus pneumoniae*, anemia, failure to thrive, dry cough, gastroesophageal reflux, vomiting, diarrhea, steatorrhea, amygdalitis
07	M	10	112	?/?	Pneumonia, dyspnea, productive cough, vomiting, steatorrhea, sinusitis, amygdalitis, pancreatic insufficiency
08	M	<1	74	?/?	Pneumonia, *Staphylococcus aureus* and *Pseudomonas*, bronchiectasis, gastroesophageal reflux, failure to thrive, steatorrhea, convulsions, pancreatic insufficiency
09	M	<1	95	?/?	Pneumonia, *Staphylococcus aureus* and *Pseudomonas*, dyspnea, dry cough, diarrhea, pancreatic insufficiency

chloride were 3.5 years (ranging from 1 to 10) and 93 mEq/L (ranging from 74 to 112), respectively. Combined studies such as clinical symptoms, sweat chloride, and PCR for 32 *CFTR* gene mutations (detecting *CFTR* gene mutations in patients numbers 1–5; Table 2) allowed confirmation of CF diagnosis in these cases, indicating the existence of undetected mutations.

The main observation of this study was the absence of 30 of the 32 mutations in 12 alleles (four patients had no mutation detected and four were compound heterozygotes: three DF508/? and one N1303K/?) among the nine patients tested. The Brazilian population is mainly a result of the three-way ethnic admixture (European, African, and Amerindian), varying proportionally in the five different official regions of the country. The European contribution to the formation of São Paulo population (southeast region) was predominantly of immigrants from Italy, Portugal, and Spain, where the frequency of DF508 mutation is lower than in the rest of Europe (Chillón et al. 1990; Novelli et al. 1990). The presence of the N1303K mutation, once common throughout Europe, is consistent with the ethnic composition above (The CF Genetic Analysis Consortium 1994). Although further investigation using a larger number of patients is needed before this observation can be confirmed, one possible explanation for the undetected mutations in our CF patients relies on the fact that some of the mutations segregating in the Latin American populations may be of African or Amerindian origin (Restrepo et al. 2000). Since we searched for CF mutations using a screening panel based on mutations identified primarily in European populations, it is unlikely that gene alterations of African and Amerindian origin can be detected. Although methods for detecting the most frequent mutations are used as reference tools, scanning methods that allow the detection of a wide range of mutations and polymorphisms should be considered, especially in highly admixed populations. Table 1 shows the different results from CF mutation analyses in the Brazilian population, where its genetically heterogeneous composition becomes clear. Raskin et al. (1997) reported the regional distribution of cystic fibrosis–linked DNA haplotypes in Brazil and demonstrated that a significant proportion of CF-bearing chromosomes had less common haplotypes, suggesting a heterogeneous distribution of CF gene mutations among Brazilians.

In conclusion, we can hypothesize that, although the sample studied here is small, it may be possible that our patients have infrequent alleles once these can occur at higher frequencies in selected populations (The CF Genetic Analysis Consortium 1994) and also show relevant regional differences (Collée et al. 1998; Raskin et al. 1999).

Molecular characterization is an important aspect of CF, and it depends on the analysis of a great number of mutations. This is particularly true in Brazil, where there is widespread ethnic mixture. Additional investigations in a larger sample are currently in progress in our laboratory to confirm our results. Further studies are still needed to determine the frequencies of CF gene mutations in different regions and in different ethnic groups in the Brazilian population. Our results contribute to the further characterization of CF in the state of São Paulo.

Received 28 July 2002; revision received 10 February 2003.

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