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Incidence of cystic fibrosis in five different states of Brazil as determined by screening of p.F508del, mutation at the CFTR gene in newborns and patients

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Abstract

Cystic Fibrosis (CF) is one of the most common single-gene defects in European descent populations with an incidence of about 1 in every 2500 live births and carrier frequency of approximately 1 in 25. The most common mutation at the CF transmembrane conductance regulator (CFTR) gene is a deletion (p.F508del) of the phenylalanine codon 508; its frequency, however, is not the same throughout the world. The purpose of this paper is to document an application of a two-tier survey design in different states of Brazil, from which regional differences of the incidence of CF and frequency of CF-causing mutation(s) carriers can be for the first time estimated. We present data on genotype distributions in reference to p.F508del mutation in samples of newborns, adult controls and CF patients from five Brazilian states, in which a total of 2683 newborns born to Brazilian white parents and 500 African-Brazilians adult controls were screened, as well as 300 CF patients (262 European descents and 38 African descents) were genotyped. Our results suggest that the CF-incidence in different parts of Brazil may differ by almost 20-fold. For the five different states as a whole, nearly 48% of the CF-alleles carry the p.F508del mutation, which places the estimates of disease incidence and carrier frequencies for the Brazilian European descents as 1 in 7576 live births and 2.3%, respectively. The implications for prevention of CF and other rare Mendelian diseases through such surveys of mutation screening are discussed.

Keywords: Cystic fibrosis; p.F508del; Brazil; Incidence; Screening; Two-tier DNA

1. Introduction

Cystic fibrosis (CF) is one of the most common single-gene defect in European descent populations with an incidence of about 1 in every 2500 live births and carrier frequency of approximately 1 in 25 [1]. Over 1000 mutations at the CF transmembrane conductance regulator (CFTR) protein gene have been identified, several of which are known to have the same clinical symptoms of CF [2,3]. The most common mutation causing CF is a deletion (p.F508del) of the phenylalanine codon 508 located in the first nucleotide-binding domain (NBF1) of the CFTR protein gene. The frequency of the p.F508del mutation, however, is not the same throughout the world, nor is it an exclusively predictive indicator of the affection status for CF or carriers of CF allele. In other words, affected individuals as well as carriers of CF may not have the p.F508del mutation in their genotypes. For example, among CF patients, the frequency of p.F508del varies from 26% in Turkey and Algeria to 88%

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in Denmark [2,4,5]. 1999. In many populations, particularly where the p.F508del mutation accounts for less than 50% of the CF alleles, CF patients may often be compound heterozygous of other mutations at the CFTR gene locus [6–9]. Thus, a single-tier screening for the p.F508del mutations among newborns, without any adjustment for non-specificity of the p.F508del mutation in CF-causing allele bearers, as done in some surveys [e.g. [10]], may not provide an accurate estimate of mutation frequencies or CF incidence in populations. To make such adjustments, earlier we proposed a two-tier mutation survey design (one in a random sample of newborns and the other among CF patients) to estimate the population incidence of a rare Mendelian genetic disease and its mutant allele(s) carriers [11]. This type of survey, as shown earlier [11], is capable of detecting regional (or country-wide) differences of disease prevalence and carrier frequencies, that are important in special reference to CF and p.F508del mutation frequencies in carriers [8]. As there are now several published studies showing that in different states of Brazil, the frequency of the p.F508del and other CFTR mutations and their haplotypes distribution in Brazilian CF patients are not uniform [9,12–25], the purpose of this paper is to document an application of the two-tier survey design in different states of Brazil, from which regional differences of the incidence of CF and CF-causing mutation(s) carriers can be for the first time estimated.

We present data on genotype distributions in reference to p.F508del mutation in samples of newborns, adults and CF patients from the Brazilian states of Rio Grande do Sul (RS), Santa Catarina (SC), Parana (PR), Sao Paulo (SP), and Minas Gerais (MG), in which a total of 2683 newborns born to Brazilian white parents and 500 African-Brazilians adults were screened, as well as 300 CF patients (262 European descents and 38 African descents) were genotyped. Our results suggest that the CF-prevalence in different parts of Brazil may differ by almost 20-fold (from 1 in 32,258 live births in SP to 1 in 1587 in RS), while the differences of prevalence of carriers of CF mutation(s) is more modest (from approximately 1 in 90 in SP to 1 in 20 in RS), although this difference is not statistically significant (at 5% level). For the five different states as a whole, nearly 48% of the CF-alleles carry the p.F508del mutation, which places the estimates of disease and carrier prevalence for the Brazilian European descents as 1 in 7576 live births and 2.3%, respectively. The sampling errors of these estimates are also provided, with reference to their implications for prevention of CF and other rare Mendelian diseases through such surveys of mutation screening.

2. Materials and methods

2.1. Subject population

The populations of Brazil, because of their unique anthropological characteristics, provide some distinctive features that must be taken into consideration for any mutation-screening program for genetic diseases. Brazil is the largest country of the South American continent. Brazil has a population of approximately 170 million [26], making it the fifth most populous country in the world, and shows regional variation of ethnic composition. Portugal conquered Brazil in 1500, but various other European, Middle Eastern, and Asian immigrant groups have settled in Brazil since the middle-19th century. Africans were brought to Brazil as slaves during the sixteenth to the nineteenth century and indigenous people of Tupi and Guarani language stock were natives. Although admixture was widespread, European descendants relatively free from admixture are found primarily in the South, like in the states of Parana (PR), Santa Catarina (SC) and Rio Grande do Sul (RS) [27,28]. Intermarriage between the Portuguese and indigenous people or African slaves was relatively common mainly in the early times, and therefore the five large regions of Brazil (South, Southeast, West-Central, Northeast and North) vary in ethnic background. Today, Brazilian of European origin makes up 54% of the total population, mixed-race populations 39.9%, African-Brazilian 5.4%, and Brazilian of Asian origin 0.5%. In MG there is a high proportion of European/African mixed-race descends (38%) as compared to PR and SC (17% and 7% respectively); MG population is made up of 54% Brazilians of European origin and 7.3% of African-Brazilian.

To account for the impact of these regional differences of ethnic composition of the country, we sampled a total of 262 European-descent CF patients and 2683 white newborns. The patient samples from each state varied from 48 in SC to 57 in MG (shown in Table 1) and the newborn sample from 500 in SC to 616 in MG. In addition to patients of European

Table 1
Genotypes observed for p.F508del mutation screening in different states of Brazil

State	Among CF patients[1]				Among controls	
	+/+	+/−	−/−	Total (*m*)	+/−	Total (*n*)
Rio Grande do Sul (RS)*	15	22	16	53	13	528
Santa Catarina (SC)*	15	23	10	48	5	500
São Paulo (SP)*	14	29	11	54	3	511
Parana (PR)*	9	21	20	50	5	528
Minas Gerais (MG)*	16	22	19	57	4	616
Afro-Brazilians **	2	5	31	38	1	500
Total	71	122	107	300	31	3183
Total *	69	117	76	262	30	2683

[1,+] is the abbreviation for p.F508del mutation, so that +/+ denotes the genotypes homozygous for this mutant allele, +/− among CF patients are compound heterozygotes, whereas +/− are p.F508del carriers among controls.

m = number of CF patients typed for p.F508del mutations, and *n* = number of controls screened for ΔF508.

*European descents.

**African descents (38 patients of African ancestry from the state of Minas Gerais (MG) and 500 adult controls from state of Parana (PR).

Total with *: Excluding the patients of African ancestry from the state of Minas Gerais (MG).

origin, from MG an additional sample of 38 CF patients of African descent was also sampled to detect the presence of p.F508del mutation. We also tested for the p.F508del CFTR mutation a sample from 500 non-CF African-Brazilians adult individuals living in PR, who have been previously extensively studied by our group [29–31]. We decided to choose this sample as a African-descent control because as the criteria to distinguish between the "white" and "non-white" phenotype in this sample was as rigorous as possible and done by only one of us (Culpi) who collected the sample during a period of three years and divided it in four sub-phenotypes depending on the criteria suggested by Krieger et al. [32], Azevedo et al. [33] and Azevedo [34].

Each of these five states is served by well-established CF regional centers. Fifty four percent of the CF patients were male and the mean age was 6.7 years, ranging from 2 months to 32 years. All patients and all their parents were born in Brazil and belong to the first four generations of immigrants. The geographic origin of the CF chromosomes was ascertained by analyzing the birthplace of parents and the four grandparents. From the geographic distribution of the mutations, only unambiguous data was analyzed. From the initial MG sample of 95 CF subjects, 38 exhibited signs of African descend by the criteria suggested by Krieger et al. [32], Azevedo et al. [33] and Azevedo [34], and they have at least one African-Brazilian parent and therefore were considered African-Brazilians; fifty seven CF patients born in MG were of European descends.

2.2. Criteria for diagnosis of CF

Criteria for diagnosis included clinical findings of chronic pulmonary disease and positive sweat test. The clinical evaluation was carried out from 1990 to 2000.

General outcome variables included age at diagnosis; sweat chloride concentration (in millimoles per liter) measured by quantitative pilocarpine iontophoresis; current weight and height percentiles; the Shwachman–Kulczycki clinical score, which is a subjective assessment of activity, the physical examination, growth, and nutrition; and the chest film. Respiratory status was assessed by tests of forced vital capacity; forced expiratory volume in one second, pseudomonas colonization; and chest-film score. Pseudomonas colonization was defined as the first positive culture after a series of negative cultures (or as the first positive culture on record). Categorical data about pancreatic status (i.e., whether function was sufficient or insufficient) were requested. In addition, the patient's age at the onset of pancreatic insufficiency was requested, if applicable. The occurrence of several common complications of cystic fibrosis was recorded: history of nasal polyps; meconium ileus; distal intestinal obstruction syndrome, defined as the need for enemas, intestinal lavage, or surgery after the neonatal period; pancreatitis; diabetes mellitus, defined as insulin dependence; biliary cirrhosis; and gallbladder disease.

2.3. Criteria for inclusion as European descend or African descend

Patients were characterized as Brazilian of European origin if they were phenotypically white and both parents have European ancestry. Patients were characterized as African-Brazilians if they were not phenotypically white and at least one of the parents has African origin. The "white" and "non-white" phenotype has been assigned based on known criteria such as skin color, hair color and type, lips and nose shape, as described by Krieger et al. [32], Azevedo et al. [33] and Azevedo [34].

2.4. Blood samples and DNA analysis

Blood samples were collected by venopuncture or by fingerstick on Guthrie cards, from which DNA was directly extracted by methods as described in Raskin et al. [22,35,36]. Samples were tested for the p.F508del CFTR mutation by protocols described in these earlier reports. The genotype data, thus collected, give the genotype frequencies in relation to the p.F508del mutation in the CF patients (abbreviated as +/+, +/− and −/−, with + denoting the p.F508del mutation allele and −, all other CF mutations) that are shown in Table 1. In addition, Table 1 also shows the counts of +/− in the samples of newborns.

2.5. Statistical analysis

The proportion of the p.F508del allele among all CF patients (denoted by r) was estimated by gene count from the genotype data [11] with its standard error (S.E.) evaluated from the usual binomial distribution. From the second tier of samples of newborns, the proportion of the p.F508del allele (denoted by p), is also estimated similarly with $p = n_1/2n$, where n_1 is the counts of p.F508del heterozygotes in a sample of n newborns. The estimated p and r values are then subsequently inserted in Eqs. (8) and (9) of Chakraborty et al. [11] to estimate the incidence of CF and frequency of CF-mutation(s) carriers in the different samples. The standard errors of these estimates were evaluated by using the formulae (3) and (4) of Chakraborty et al. [11]. For the five different states as a whole, two alternative sets of estimators of the p.F508del allele among CF patients, total frequency of all CF-causing alleles, disease incidence and carrier frequencies were employed; one that uses the theory as described above [11] and the other by taking weighted average of the five different sets of estimates (with weights proportional to the inverse of their respective sampling variance [37]), the latter directly leading to a heterogeneity chi-square statistic, testing for the significance of regional differences of the sample estimates [37]. Finally, the confidence limits of the estimates were computed by the theory as described in Chakraborty et al. [11].

3. Results

Table 1 shows the raw data of the two-tier screening surveys conducted in the five states, as well as their total. The patient sample of African-descent, collected from the state of Minas Gerais (MG), and the Afro-Brazilian population sample collected from PR are listed separately, and consequently, two different totals are listed for this tier of the survey, one excluding these Afro-Brazilian subjects and the other with all subjects included. In all, 262 patients of European ancestry and 2683 newborns of the same ethnicity are included in these analyses. As expected, among the newborns, no p.F508del homozygote was observed, although nearly 48% of the CF alleles in the pooled CF patients' sample carried the p.F508del mutation.

In Table 2 we present the estimates, standard errors (S.E.), coefficient of variation (c.v.=estimate/S.E.) of the proportion (r) of the CF alleles carrying the p.F508del mutation, CF allele frequency (d), incidence of CF (d^2), and CF-carrier frequency [$2d(1-d)$] in the five states, along with the heterogeneity chi-square (χ^2) for each of the estimates. Several observations can be made from these estimates.

State-to-state variation in the proportion of the p.F508del allele among the CF patients (r) is seen in the data (from 39% in PR to 55% in SC). However, this variation does not appear to the statistically significant ($\chi^2=6.40$ with 4 df, $P\approx 0.2$), probably due to somewhat large standard errors of the state-specific estimates (between 4.7% to 5.1%). Likewise, variations of the state-specific estimates of the CF-causing alleles (d) frequency (between 0.56% in SP to 2.51% in RS), the incidence of CF (d^2, from 0.31 in SP to 6.3 in RS per 10,000 live births) and frequency of CF-carriers ($2d(1-d)$, from 1.11% in SP to 4.89% in RS) are also noticeable, but not statistically significant (at 5% level).

Table 3
Parameter estimates from p.F508del mutation screenings in the total sample from Brazil

Parameter and estimates	For the total sample of Euro-Brazilians		Afro-Brazilians
	From total data [a]	Combined estimate [b]	
Proportion of from p.F508del mutation in CF alleles (r):			
Estimate	0.487	0.486	0.118
S.E.	0.022	0.022	0.037
c.v.	0.045	0.045	0.313
CF allele frequency (d):			
Estimate	0.0115	0.0086	0.0084
S.E.	0.0022	0.0019	0.0088
c.v.	0.188	0.216	1.047
CF-homozygote frequency (d^2):			
Estimate	1.32×10^{-4}	0.50×10^{-4}	0.71×10^{-4}
S.E.	0.49×10^{-4}	0.26×10^{-4}	1.49×10^{-4}
c.v.	0.375	0.532	2.094
CF-carrier frequency [$2d(1-d)$]:			
Estimate	0.0227	0.0173	0.0167
S.E.	0.0042	0.0037	0.0174
c.v.	0.185	0.213	1.038

[a] Estimated from pooled data from five states (i.e., summed genotype frequencies).
[b] Computed from weighted averages of parameter estimates from individual state data (see Table 2).

The consequences of state-to-state variation of these estimates are also evident when the data from these five states are pooled for analysis. The results are shown in Table 3, where two different approaches are taken for estimation. In the first, the total sample (of 2683 newborns and 262 CF patients of European descent) is used to estimate each of the parameters (shown under the column of total data), and in the second, the weighted average of the state-specific estimates were computed (with weights proportional to the inverse of

Table 2
Parameter estimates from p.F508del mutation screening in different states of Brazil

Parameters and estimates	States					χ^2 (4 df)
	RS	SC	PR	SP	MG[1]	
Proportion of p.F508del mutation in CF alleles (r):						
Estimate	0.491	0.552	0.390	0.528	0.474	6.40
S.E.	0.049	0.051	0.049	0.048	0.047	
c.v.	0.099	0.092	0.125	0.091	0.099	
CF allele frequency (d):						
Estimate	0.0251	0.0091	0.0121	0.0056	0.0069	6.57
S.E.	0.0073	0.0041	0.0056	0.0032	0.0035	
c.v.	0.293	0.455	0.463	0.584	0.509	
CF-homozygote frequency (d^2) :						
Estimate	6.30×10^{-4}	0.82×10^{-4}	1.47×10^{-4}	0.31×10^{-4}	0.47×10^{-4}	3.44
S.E.	3.69×10^{-4}	0.75×10^{-4}	1.37×10^{-4}	0.36×10^{-4}	0.48×10^{-4}	
c.v.	0.586	0.911	0.927	1.167	1.018	
CF-carrier frequency [$2d(1-d)$]:						
Estimate	0.0489	0.0179	0.0240	0.0111	0.0136	6.74
S.E.	0.0140	0.0081	0.0110	0.0064	0.0069	
c.v.	0.285	0.451	0.458	0.580	0.505	

[1]The estimates for Minas Gerais (MG) are based on European descent patients only.
S.E.=its standard error evaluated from the usual biomial distribution.
c.v.=coefficient of variation (c.v.=estimate/S.E.) of the proportion (r) of the CF alleles carrying the Δ508 mutation.

Table 4
Points estimates and 95% confidence limits of CF-homozygote and carrier frequencies in Brazil, expressed as 1 in *n* individuals

State	Incidence of CF-homozygotes	Incidence of CF-carriers
Euro-Brazilians: RS	1 in 1587 (504–5005)	1 in 20 (12–36)
SC	1 in 12,195 (2044–72,690)	1 in 56 (23–135)
PR	1 in 6803 (1103–41,720)	1 in 42 (17–102)
SP	1 in 32,258 (3281–318,549)	1 in 90 (29–282)
MG	1 in 21,277 (2895–156,449)	1 in 73 (27–198)
Total [a]	1 in 7576 (3633–15,802)	1 in 44 (31–63)
Combined [b]	1 in 20,202 (8651–47,179)	1 in 58 (38–88)
Afro-Brazilians	1 in 14,085 (231–850,965)	1 in 60 (8–458)

[a] Estimated from pooled data from five states (i.e., summed genotype frequencies).
[b] Computed from weighted averages of parameter estimates from individual state data (see Table 2).

their respective sampling variance; [37]), shown under the column of combined estimate. While the estimate of the proportion (r) of the p.F508del mutation among the CF patients is nearly identical (49%) by both methods, those for the frequency (d) of all CF-causing alleles, disease incidence (d^2) and carrier frequencies [$2d(1-d)$] are higher for the total data in comparison to the weighted average of state-specific estimates. In particular, the disease incidence in the total data is almost 2.64-fold higher (1.32 per 10,000 live births as opposed to the combined estimate of 0.5 per 10,000 live births). Of course, of all the parameter estimates, the disease incidence is most poorly estimated (with a coefficient of variation of over 37% for the total data and over 53% for the combined estimate).

In Table 4 we present the state-specific as well as combined point estimates of the incidence of CF and carrier frequency and their 95% confidence intervals. The wide confidence intervals reflect the inherent errors of estimation of frequencies of rare events (distributed as a Poisson variant). Nevertheless, in the Southern states (e.g., in RS) cystic fibrosis can be as common as in Scotland and Northern Europe, with an apparent decreasing trend in the Southeastern states. Table 4 also lists the point estimates and 95% confidence interval estimates of the CF-carrier frequencies in the individual states and for the pooled sample as a whole. As expected, the rank correlation of the disease incidence (d^2) and carrier frequencies [$2d(1-d)$] is 100%, generally decreasing from South to Southeastern states. Also worth noting is that the carrier frequency estimates are in general more precise than the estimates of disease incidences.

4. Discussion

Data presented here show that a two-tier screening for the p.F508del mutation of the CFTR gene in CF patients and newborns can be used to estimate the disease incidence and carrier frequencies in five different states of Brazil. In general, our data indicate a South to Southeastern decreasing trend of disease incidence and carrier frequencies in Brazil, consistent with the ethnic composition of the populations (more people of European descent in South). The pooled data shows that in these five states, the overall frequency of CF is probably more comparable with that of Southern Europe (Italy, Spain and Portugal, and considerably less than that in Denmark, Scotland and other northern European countries [2,5].

The estimates of the prevalence of p.F508del allele among the CF chromosomes ranging between 39% in Parana to 55% in Santa Catarina (Table 2) also suggest that the predictive power of this mutation alone for detecting CF in Brazil is comparable with that of Italy and South Europe and considerably lower than that of Denmark, Scotland and Northern Germany [4].

Although we had p.F508del mutation data in 38 CF patients of African-descent from the state of Minas Gerais (shown in Table 1), we have not included that series in any subsequent analysis. However, this series shows that in individuals of African descent in MG, the prevalence of the p.F508del mutation in CF patients is considerably lower (11.8±3.7%) than that of the CF patients of European decent. Further, the genotype distribution in this sample is significantly different from that of the Hardy–Weinberg expectations (($\chi^2=5.20$ with 1 df, and $-2\ln L=3.68$; $P<0.02$, by a permutation test). We ascribe this to the admixed nature of African-Brazilians. Further, the finding of only one p.F508del mutation in 1000 CF chromosomes from Afro-Brazilians adults living in PR, suggests that this mutation is 6 times less frequent in Afro-Brazilians than in the general population of Euro-Brazilians. Since CF is rare in African populations and most of the CF-alleles in Africa, Afro-Americans, Afro-Latin Americans and in African-Brazilians patients are not due to the p.F508del mutation [9,38,45,46], and the only one p.F508del mutation in 1000 CF chromosomes from Afro-Brazilians adults living in PR was found in an adult classified as “light mulato”, that is, the phenotype closest to “white”, we conclude that the Afro-Brazilian patients probably received their p.F508del mutations during the admixture events in their families.

In this context it may be worth mentioning that our data is also consistent with the recent report of such a two-tier survey conducted in the Rio de Janeiro population of Brazil [18]. These authors estimated that the frequency of the p.F508del allele among the CF patients is 30.7% (a figure somewhat lower than our state-specific estimates, which may be due to the fact that Cabello et al. did not try to differentiate between CF patients of European descends and African descends) and the disease incidence is 1 in 3542 live births (95% CI from 1 in 340 to 1 in 36,895, applying the method of the present paper). Applying the method of the present paper, we additionally estimate that the carrier frequency as 1 in 30 individuals (95% CI as 1 in 10 to 1 in 96) in the Rio de Janeiro population. These are within the range of our state-specific estimates, supporting the consistent nature of such two-tier mutation-screening data.

We are cognizant that our results should be taken with caution, as there may be some ascertainment bias; 1) due to

technical obstacles inherent to this type of studies, such as the difficulties to determine the criteria to differentiate individuals of unmixed European descent from those who are admixed in Brazil. Even Brazilians initially considered of "European origin" are not completely free of admixture. The high admixture rates in the Brazilian population not only carries CF alleles from Brazilian descends of Europeans to African-Brazilians (such as ΔFF508) but also carries alleles from African-Brazilians to Brazilian descends of Europeans [9]. Although these difficulties to divide Brazilians based on their phenotype and ancestry obviously may have compromised the accuracy of our estimates, data arising from statewide CF newborn mandatory screening programs, recently established in SC, PR and MG states, show that our estimates fit very close to the incidence that is now being reported in these newborn screening programs [39]; 2) Some authors have questioned the validity of genotyping the CF patients by the p.F508del mutation alone (as done in the data of the present paper). For example, Feingold et al. [40] found significant departures of such genotype data from the Hardy–Weinberg expectation, suggesting the presence of ascertainment bias in relation to the severity of diseases in patient registry data. To entertain such a possibility in the present data, we performed a test of Hardy–Weinberg proportions in our samples. Either by the chi-square or likelihood ratio test [41], none of the five samples show any significant (at 5% level) departure from Hardy–Weinberg expectations of genotype frequencies in the patient series data. Thus, we conclude that ascertainment bias or misdiagnosis of our detection method for the p.F508del mutation is not an issue in the present survey. The patient series data of the Rio de Janeiro survey [18] also shows consistency with the Hardy–Weinberg expectation ($\chi^2 = 1.73$ with 1 *df*, and $-2\ln L = 1.68$; $P > 0.25$); 3) Another ascertainment bias could be introduced by the fact that the "true" p.F508del CFTR mutation frequency in patients could only be determined very early in life, such as in the neonate period after newborn screening of CF, because the p.F508del CFTR mutation could be underrepresented in a cohort of "older" patients, as CF patients homozygous for the p.F508del CFTR mutation may have a severe phenotype and could die before they are diagnosed and/or referred to regional CF centers, "lowering" the p.F508del CFTR mutation frequency in a cohort of patients. This should be at least in part true in a country such as Brazil, where CF is still largely underdiagnosed. 4) This bias may be counterbalanced by the fact that many CF patients that have mild disease are also not diagnosed in countries such as Brazil, and therefore, the p.F508del CFTR mutation frequency in a cohort of "severe" CF patients would be higher. Future data coming from the CF newborn screening programs now starting in Brazil will help to determine the strength of each of this two last ascertainment bias; 4) The fact that parameter estimates for African-descent were done by comparing patients and controls of African-descent samples coming from two different states of Brazil. As we concluded that the Afro-Brazilian patients from MG probably received their p.F508del mutations during the admixture events in their families, we believe that the p.F508del mutation frequency in Afro-Brazilian patients will vary from state to state, according to their state-specific admixture rates with Euro-Brazilians. For the same reason, we believe that the number of p.F508del mutations that will be found in future studies of African-descent controls living in other states in Brazil than PR, will be directly related to the admixture with European descent in the sample. As far as we know, no p.F508del mutation was ever reported in non-admixture Afro-Brazilians from Africa [38].

In general, the fact that less than one-half of the CF alleles are ascribed to the p.F508del mutation in South and Southeastern parts of Brazil raises some concerns about the efficiency of mutation-screening programs for CF in Brazil. As pointed out by Ten Kate [42], with 50% of the CF alleles detected as p.F508del, only one-quarter of couples at risk in Brazil can be found. Therefore, if a p.F508del-based carrier-screening program is set up in Brazil, many more couples will be detected where one member will carry the p.F508del mutation, whereas the possibility of carrying an undetected CF-causing allele in the other member cannot be ruled out. Since these couples are at significantly increased risk of having a CF child, the efficiency of a screening program with ability to detect only the p.F508del mutation will be greatly reduced. Also, if the Brazilian government decides to establish a two-tier CF newborn screening program based on testing for p.F508del all positive newborn samples measured for immunoreactive trypsin (IRT), many IRT-positive newborns will be detected carrying one copy of the p.F508del mutation, whereas the possibility of carrying an undetected CF-causing allele in the other allele cannot be ruled out, neither the possibility that they will be only asymptotic carriers, bringing up serious ethical implications in CF newborn screening [43]. Thus, the data presented in this paper, as well as previous works showing heterogeneity in the CFTR gene pool in Brazil [9,12–25], indicates that other mutations detected at the CFTR locus that are prevalent in Brazil need to be included in mutation-screening programs for prevention of CF in Brazil. In a comprehensive recent worldwide survey, this has been shown to be valid for other populations [44].

In summary, the application of the two-tier screening survey for the p.F508del mutations shows, for the first time, that in individuals of European ancestry in Brazil, the incidence of CF and carrier frequencies are comparable to those in Southern Europe. Statistical homogeneity of the estimates and the consistency with Hardy–Weinberg expectations of genotypes in the patient series suggest that the genetic structure of the populations of European descent in Brazil is by and large unaffected by population substructure. However, the individuals of African descent (in MG) probably received their CF alleles during admixture with individuals of European descent. This study also indicates that the overall ΔFF508 frequency in Brazil may be lower than the previously reported 47% in Brazilians of European

decent. Although this frequency is still valid for Brazilian CF patients of European origin (as showed by the average frequency of the five states in this study), the overall prevalence of the ΔFF508 mutation should be lower when data from other Brazilian states arise, as the contributions of the African gene pool in populations of other states of Brazil are considerable (e.g., in 18 of the 27 states of Brazil Afro-Brazilians are in majority; [26]). Considering this heterogeneity of the Brazilian population, the inter-regional migration, and the different ethnic composition of each region, one might expect that the frequency of CF mutations, as well as the incidence of CF, will vary between different regions and populations of Brazil, as well as in Latin America. Because regional differences in ethnic composition will influence both cost-benefit analyses and risk assessments, uniform policies regarding population screening of CF patients and carriers may not be appropriate in a country such as Brazil, characterized by striking ethnic diversity and regional differences in prevalent European and African ancestries.

Based on the fact that the disease is clearly underdiagnosed in Brazil and Latin America, the data presented in this work show that it is now possible to estimate the incidence of CF in different states. Further, since the majority of CFTR mutations are known in some Southern states of Brazil, we suggest that a CF neonatal screening program in the Southern states of Brazil using a two-tier approach (biochemical plus DNA test) should be immediately established, although a general newborn screening program for the entire country should be planned more carefully. But since less than one-half of the CF alleles in Brazil can be ascribed to the p.F508del mutation, it is important that other prevalent CF mutations in Brazil be included in the molecular step of the two-tier screening program that we propose.

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