Screening for F508del as a first step in the molecular diagnosis of cystic fibrosis*,**

Pesquisa da mutação F508del como primeiro passo no diagnóstico molecular de fibrose cística

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Abstract

Objective: To determine the relevance of screening for the F508del mutation of the cystic fibrosis transmembrane conductance regulator gene as a first step in the genetic diagnosis of cystic fibrosis (CF) by associating the genotype with various clinical variables. **Methods:** We evaluated 180 CF patients regarding the F508del mutation. The clinical data were obtained from the medical records of the patients and from interviews with their parents or legal guardians. **Results:** Of the 180 patients studied, 65 (36.1%) did not carry the F508del mutation (group 0 [G0]), 67 (37.2%) were F508del heterozygous (G1), and 48 (26.7%) were F508del homozygous (G2). All three groups showed associations with the clinical variables. Homozygosis was associated with younger patients, younger age at CF diagnosis, and younger age at the first isolation of *Pseudomonas aeruginosa* (PA), as well as with higher prevalence of pancreatic insufficiency (PI) and non-mucoid PA (NMPA) colonization. In comparison with G1+G2 patients, G0 patients were older; first experienced clinical symptoms, digestive disease, and pulmonary disease at an older age; were older at CF diagnosis and at first PA isolation; and had a lower prevalence of PI and meconium ileus, as well as of colonization by NMPA, mucoid PA, and *Burkholderia cepacia*. In G1 patients, values were intermediate for age at CF diagnosis; age at first PA isolation, first pulmonary symptoms, and first clinical manifestations; MPA colonization; and OR for PI. **Conclusions:** The identification of F508del in 63.9% of the patients studied showed that this can be a useful tool as a first step in the genetic diagnosis of CF. The F508del genotype was associated with clinical severity of the disease, especially with the variables related to CF onset.

Keywords: Cystic Fibrosis; Cystic Fibrosis Transmembrane Conductance Regulator; Genotype; Mutation.

Resumo

Objetivo: Verificar a importância da detecção da mutação F508del no gene *cystic fibrosis transmembrane conductance regulator* como primeiro passo no diagnóstico genético de fibrose cística (FC), associando-se o genótipo com várias variáveis clínicas. **Métodos:** Foram avaliados 180 pacientes com FC quanto à mutação F508del. As variáveis clínicas foram obtidas dos prontuários médicos dos pacientes e de entrevistas com seus pais ou responsáveis. **Resultados:** Dos 180 pacientes estudados, 65 (36,1%) não apresentavam a mutação F508del (grupo 0 [G0]), 67 (37,2%) eram heterozigotos (grupo 1 [G1]), e 48 (26,7%) eram homozigotos (grupo 2 [G2]). Todos os três grupos mostraram associações com as variáveis clínicas. A homozigose associou-se a pacientes mais jovens, menor idade ao diagnóstico e menor idade no primeiro isolamento de *Pseudomonas aeruginosa* (PA), bem como maior prevalência de insuficiência pancreática (IP) e colonização por PA não mucoide (PANM). Na comparação com os pacientes G1+G2, os pacientes G0 eram mais velhos, com início de sintomas clínicos, doença digestiva e doença pulmonar mais tardio, diagnóstico tardio, PA isolada tardiamente, e menor prevalência de IP, íleo meconial e colonização por PANM, PA mucoide e *Burkholderia cepacia*. Nos pacientes G1, os valores foram intermediários para idade ao diagnóstico, idade no primeiro isolamento de PA, idade no início de doença pulmonar e de manifestações clínicas, colonização por PAM e OR para IP. **Conclusões:** A identificação de F508del em 63,9% dos pacientes estudados mostrou que ela pode ser uma ferramenta útil como primeiro passo no diagnóstico genético de FC. O genótipo F508del foi associado à gravidade clínica da doença, particularmente às variáveis relacionadas com o início da doença.

Descritores: Fibrose cística; Regulador de Condutância Transmembrana em Fibrose Cística; Genótipo; Mutação.

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Introduction

Cystic fibrosis (CF) is the most common lethal genetic disease in childhood in Caucasian populations.[1] The disease is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene, which encodes the protein of the same name.[2-4] Nearly 2,000 disease-causing mutations have been observed in the *CFTR* gene. [5] These mutations are classified in six classes according to the absence of changes or qualitative and quantitative changes in the CFTR protein.[6]

The most common *CFTR* mutation is a three-nucleotide deletion that causes the absence of amino acid 508 of the normally 1,480-amino acid protein. This mutation, which lacks a single phenylalanine codon, is commonly referred to as F508del (c.1521_1523delCTT for the DNA mutation and F508del for the mutant protein). Worldwide, the main mutation of *CFTR* gene is F508del, with a prevalence ranging from 30-80%. In Caucasian populations, the F508del mutation is found in approximately 70-88% of the alleles in CF patients.[5,7] The remaining 12-30% of the alleles comprise the other 2,000 different mutations, each of which, individually, have a very low frequency (few mutations have a worldwide frequency above 0.1%, but some can reach high prevalences in selected populations).[5,8,9]

The variability in CF severity is associated principally with genetic factors, such as modifier genes and *CFTR* mutation classes, as well as with environmental factors.[9-15] The F508del mutation is a class II mutation (causing misprocessed/misfolded CFTR proteins), and it is associated with higher clinical severity of CF.[9]

Nowadays, it is not possible to identify the full spectrum of *CFTR* mutations in most countries. Together with the newborn screening program that uses immunoreactive trypsinogen testing, the Brazilian public health system has currently been providing assistance for screening of *CFTR* gene mutations. However, because of the costs, only one mutation is screened. Therefore, studies on the screening for the F508del mutation are necessary and important because that is the only test that can be currently performed in most of the countries. In this context, the objective of the present study was to verify the importance of the screening for the F508del mutation as a first step in the genetic diagnosis of CF by associating the F508del genotype with 28 clinical variables.

Methods

This was a cross-sectional study conducted at a university center for CF between 2010 and 2011. The diagnosis of CF was confirmed by two determinations of sweat sodium and chloride (concentrations > 60 mEq/L) in all patients. We selected 215 patients for the study. Among those, 35 patients were excluded because of the lack of clinical data or of a written informed consent.

We used the phenol/chloroform method for DNA extraction, and, in all genetic analyses, DNA concentration was 50 ng/mL, determined with a spectrophotometer (NanoVue™; GE Healthcare Biosciences, Pittsburgh, PA, USA).

The determination of the F508del mutation was performed by polymerase chain reaction (PCR), using a pair of primers—sense (5'-GGC ACC ATT AAA GAA AAT ATC-3') and antisense (5'-TGG CAT GCT TTG ATG ACG C-3')—resulting in a 74-bp fragment (F508del homozygosis), a 77-bp fragment (absence of F508del), or the presence of both fragments (F508del heterozygosis). The procedure for thermal cycling consisted of initial denaturation at 94°C for 5 min, subsequent denaturation at 94°C for 1 min, annealing at 53.5°C for 1 min, and extension at 72°C for 1 min, repeated for 35 cycles, and followed by a final extension at 72°C for 10 min. The PCR contained 25 µL of a solution with 50 ng of DNA, 1 µM of each primer, 200 µM of dNTP, 1.0 mM of $MgCl_2$, 50 mM of KCl, 10 mM of Tris-HCl (pH, 8.4 at 25°C), and 1.5 U of Taq DNA polymerase. After the addition of 5 µL of glycerol-based loading buffer, 10 µL of the reaction product was applied on acrylamide gel.[16]

The determination of *CFTR* mutations was performed in the laboratory of molecular genetics of the institution using the RFLP method (G542X, R1162X, R553X, G551D, and N1303K). Some mutations were obtained by sequencing or multiplex ligation-dependent probe amplification: S4X, 2183A>G, 1717-G>A, and I618T. For both methods, we used the MegaBace1000 DNA sequencer (GE Healthcare Biosciences).[16]

Clinical data, anthropometric variables, pulmonary function results, and sputum or oropharyngeal swab culture results were collected.

The following clinical variables were investigated: clinical scores (Shwachman-Kulczycki, Kanga, and Bhalla scores)[17]; body mass index (BMI) —for the patients older than

19 years, we used the formula BMI = weight/(height)^2; for the remaining patients, we used WHO Anthro, version 3.0.1 and WHO Anthro Plus, version 1.0.2, respectively, for children under 5 years of age and for those aged 5-19 years; age (dichotomized between ≤ 154 months and > 154 months); age at diagnosis (dichotomized between ≤ 24 months and > 24 months); first digestive symptoms (dichotomized between ≤ 3 months and > 3 months); first pulmonary symptoms (dichotomized between ≤ 6 months and > 6 months); age at the first isolation of *Pseudomonas aeruginosa* (dichotomized between ≤ 30 months and > 30 months); airway colonization (mucoid *P. aeruginosa* [MPA], nonmucoid *P. aeruginosa* [NMPA], *Achromobacter xylosoxidans, Burkholderia cepacia*, and *Staphylococcus aureus*); transcutaneous SaO_2; spirometry results; and comorbidities—nasal polyps, osteoporosis, meconium ileus (MI), diabetes mellitus, and pancreatic insufficiency (PI).

All of the scores were determined by two pediatric pulmonologists, and, in case of disagreement, a third specialist was invited to review the scores in order to determine the final results.

All of the patients aged ≥ 7 years were submitted to spirometry with a CPFS/D spirometer (MedGraphics, Saint Paul, MN, USA). Data were recorded using Breeze PF, version 3.8B for Windows 95/98/NT (Medical Graphics Corp., Saint Paul, MN, USA), and the following variables were included: FVC, % of predicted; FEV_1, % of predicted; FEV_1/FVC ratio, % of predicted; and $FEF_{25-75\%}$.

The present study was approved by the Research Ethics Committee of the State University at Campinas School of Medical Sciences (Protocol no. 528/2008).

For the purposes of the statistical analysis, the variables that showed non-normal distribution (age at diagnosis, age at the first pulmonary and digestive symptoms; and age at the first isolation of *P. aeruginosa*) were categorized into two groups, using as the cutoff point the median value of each variable. The data categorized by the median were divided into two cohorts with similar sample sizes.

For the clinical evaluation of the scores, SaO_2, and spirometry tests, the analyses were performed without adjusting the data.

Bacteria isolated from the airways of the patients were used as markers according to the presence or absence of specific bacteria in three consecutive cultures within the last year.

Comorbidities were compared in terms of their presence or absence.

The statistical analyses were performed with the Statistical Package for the Social Sciences, version 17.0 (SPSS Inc., Chicago, IL, USA).

In order to avoid spurious data due to the problem of multiple testing,[18] the level of significance α was adjusted using the Bonferroni correction for three groups: G0, without the F508del mutation (patients with no F508del on the two alleles or those whose *CFTR* mutation could not be determined); G1, heterozygous F508del mutation (patients with the F508del mutation on one of the alleles, with or without another *CFTR* mutation identified); and G2, homozygous F508del mutation (patients with F508del on both alleles).

The statistical power of the sample was calculated with the freeware G*Power, version 3.0.5,[19] which showed a statistical power above 80% for the analysis performed and $\alpha = 0.05$, using a population of 159 CF patients.

The data were compared with one-way ANOVA, the Kruskal-Wallis test, the Mann-Whitney U test, and Pearson's chi-square test. For the comparisons between genotypes and variables with numerical distribution, the Kruskal-Wallis test was used for F508del genotypes, and the Mann-Whitney test was used for the F508del groups. For categorical variables, we used Pearson's chi-square test and ORs.

Results

The numerical and categorical data of the clinical variables in 180 CF patients are described in Table 1. The distribution of the patients among the groups (G0, G1, and G2) was, respectively, 65 (36.1%), 67 (37.2%), and 48 (26.7%). The population was found not to be in Hardy-Weinberg equilibrium regarding the F508del mutation ($p < 0.001$). The distribution of the patients according to *CFTR* mutation genotype is shown in Table 2.

Regarding numerical variables, SaO_2 and the Shwachman-Kulczycki scores were significantly higher in G1 and in G2 than in G0 ($p = 0.034$ and $p = 0.046$, respectively; Table 3).

Regarding the clinical categorical variables, G2 patients were associated with younger age in general ($p \leq 0.001$), younger age at CF diagnosis ($p < 0.001$), younger age at the first isolation of

Table 1 - Characteristics of the patients included in the study (n = 180).[a]

Variable	Result
Males	50.00
Age, years	17.72 ± 15.75 (0.60-24.00)
Caucasoid	91.70
Underweight and extremely underweight	22.47
Identification of one F508del allele	37.20
Identification of two F508del alleles	26.70
Age at first clinical manifestations, years	2.90 ± 8.88 (0.00-13.00)
Age at diagnosis, years	7.62 ± 13.63 (0.00-14.23)
Age at first digestive symptoms, years	3.39 ± 9.11 (0.00-12.45)
Age at pulmonary symptoms, years	2.90 ± 9.89 (0.00-13.00)
SaO_2, %	94.92 ± 4.26 (66.00-99.00)
Bhalla score	8.74 ± 5.72 (0.00-25.00)
Kanga score	18.85 ± 5.84 (10.00-40.00)
Shwachman-Kulczycki score	65.85 ± 16.77 (20.00-95.00)
FVC, % of predicted	79.29 ± 23.55 (19.00-135.00)
$FEV_{1,}$ % of predicted	71.29 ± 27.47 (17.00-132.00)
FEV_1/FVC, % of predicted	83.46 ± 15.95 (37.00-137.00)
$FEF_{25-75\%}$	59.05 ± 35.55 (7.00-150.00)
Nasal polyps	18.64
Diabetes mellitus	18.64
Osteoporosis	16.38
Pancreatic insufficiency	79.90
Meconium ileus	15.08
Age at first isolation of *Pseudomonas aeruginosa*, years	8.55 ± 14.45 (2.00-15.00)
Colonization[b]	
P. aeruginosa	56.42
Mucoid *P. aeruginosa*	42.46
Burkholderia cepacia	13.97
Achromobacter xylosoxidans	10.05
Staphylococcus aureus	78.77

[a]Values expressed as % or as mean ± SD (range). [b]Positive colonization based on three consecutive positive respiratory cultures in the past year.

P. aeruginosa (p = 0.009), and higher prevalence of PI (p = 0.001) and NMPA (p = 0.025; Table 4). In comparison with G1+G2 patients, G0 patients were older (p < 0.001), had first clinical symptoms at an older age (p < 0.001), had digestive disease at an older age (p = 0.023), had pulmonary disease at an older age (p = 0.006), were older at CF diagnosis (p < 0.001), had a lower prevalence of PI (p < 0.001), had a lower prevalence of MI (p = 0.047), were older at the first isolation of *P. aeruginosa* (p = 0.001), and had a lower prevalence of colonization by NMPA (p = 0.025), MPA (p = 0.068), and *B. cepacia* (p = 0.001; Table 4). Intermediate values were found in G1 patients: age at CF diagnosis (p < 0.001), age at the first isolation of *P. aeruginosa* (p = 0.001), age at first pulmonary symptoms (p = 0.006), age at first clinical manifestations (p < 0.001), MPA colonization (p = 0.068), and OR for PI (p < 0.001).

Table 5 shows the association of the major variables with the F508del genotype.

Discussion

The use of molecular genetics in the clinical practice has been improving, and it is considered important in various aspects related to patient care. The molecular technique is essential to the diagnosis of CF, especially in cases in which there is uncertainty, i.e., when the patient presents with CF symptoms not confirmed by sweat tests, when the onset of CF symptoms occurs in adult life, in cases of atypical CF, and when CF is caused by *CFTR* mutations belonging to classes IV, V, or VI. In CF patients with clinical variability, the genetic

Table 2 - *Cystic fibrosis transmembrane conductance regulator* genotype according to F508del mutation group.

		Patient		
F508del mutation group	*CFTR* mutation genotype	n	%	% per group
G0	-/-	43	23.9	36.1
	G542X/-	5	2.8	
	G542X/R1162X	1	0.6	
	G542X/I618T	1	0.6	
	G542X/2183A>G	1	0.6	
	G542X/2183AA→G	1	0.6	
	G542X/P205S	1	0.6	
	G542X/R334W	1	0.6	
	I507V/-	1	0.6	
	R334W/R1066C	1	0.6	
	R334W/R334W	1	0.6	
	3120+1G>A/3120+1G>A	1	0.6	
	3120+1G>A/-	1	0.6	
	TG11-5T/-	1	0.6	
	622-2A>G/711+1G>T	1	0.6	
	R1162X/R1162X	1	0.6	
	R1162X/-	1	0.6	
	D110H/V232H	1	0.6	
G1	F508del/-	40	22.2	37.2
	F508del/G542X	13	7.2	
	F508del/R1162X	5	2.8	
	F508del/N1303K	4	2.2	
	F508del/R553X	2	1.1	
	F508del/S4X	1	0.6	
	F508del/1717-1G>A	1	0.6	
	F508del/exon 6B-16 duplication	1	0.6	
	F508del/2184insA	1	0.6	
G2	F508del/F508del	48	26.7	26.7

G0: absent F508del; G1: heterozygous F508del patients; and G2: homozygous F508del patients.

Table 3 - Significantly different numerical variables in the groups studied.*

Variable	Genotype group	Median	Mean	SD	p
Shwachman-Kulczycki score	G0	95	93.38	5.935	0.046
	G1+G2	96	95.50	2.869	
SaO_2	G0	60	61.40	17.844	0.034
	G1+G2	65	67.91	15.893	

G0: absent F508del; G1: heterozygous F508del patients; and G2: homozygous F508del patients. *Mann-Whitney U test.

analysis might allow a better understanding of the disease and promote targeted therapies and better outpatient care.

Molecular tests are not available in our public health care system; however, centers linked to universities screen the major *CFTR* mutations. Among the *CFTR* mutations, screening for F508del is routinely performed in our research center in all of the patients with two sodium and chloride tests in sweat with values above 60 mEq/L. The screening for F508del is not expensive and allows the definitive diagnosis in 26.7% of the patients in our center, 37.2% of whom being identified as heterozygous. Our study showed that the identification of the F508del mutation is important in a country with great ethnic diversity, because 63.9% of our patients had at least one F508del allele. Therefore, the implementation of screening for F508del in the public health care system is necessary and should be implemented in all developing countries. The

Table 4 - Significantly different categorical variables in the groups studied.*

Group	Variable	Categorization		p	OR	95% CI	Variable	Categorization		p	OR	95% CI
	Age	≤ 154 mo	> 154 mo				Age at first clinical manifestations	≤ 3 mo	> 3 mo			
G0		18	46	< 0.001	0.238	0.121-0.457		19	37	< 0.001	0.239	0.119-0.47
G1		36	31		1.202	0.654-2.217		48	18		2.974	1.539-5.884
G2		37	11		4.754	2.263-10.53		30	18		1.366	0.689-2.749
	Age at diagnosis	≤ 24 mo	> 24 mo				Age at digestive disease	≤ 3 mo	>3 mo			
G0		15	44	< 0.001	0.157	0.007-0.315		13	27	0.023	0.362	0.165-0.77
G1		41	23		1.95	1.034-3.725		37	25		1.852	0.958-3.615
G2		36	12		3.563	1.715-7.74		26	22		1.227	0.615-2.464
	Age at pulmonary disease	≤ 6 mo	> 6 mo				PI	Presence	Absence			
G0		20	32	0.006	0.385	0.179-0.704		35	29	< 0.001	0.08	0.03-0.192
G1		44	21		2.258	1.181-4.391		62	5		4.71	1.817-14.35
G2		28	20		1.158	0.586-2.312		46	2		7.007	2.136-51.37
	MI	Presence	Absence				Age at first PA isolate	≤ 3 mo	> 3 mo			
G0		5	60	0.047	0.335	0.108-0.892		9	26	0.001	0.229	0.093-0.535
G1		13	54		1.569	0.683-3.576		30	25		1.263	0.627-2.552
G2		10	38		1.662	0.682-3.906		28	13		2.794	1.29-6.248
	MPA	Presence	Absence				NMPA	Presence	Absence			
G0		21	44	0.068	0.553	0.2731-0.984		28	37	0.025	0.438	0.233-0.814
G1		35	32		1.914	1.034-3.56		41	26		1.39	0.752-2.593
G2		20	28		0.97	0.491-1.898		32	16		1.82	0.916-3.702
	BC	Presence	Absence									
G0		4	61	0.101	0.313	0.088-0.91						
G1		12	55		1.83	0.757-4.426						
G2		8	40		1.447	0.548-3.618						

mo: months; G0: absent F508del; G1: heterozygous F508del patients; G2: homozygous F508del patients; MI: meconium ileus; PA: *Pseudomonas aeruginosa*; MPA: mucoid PA colonization; BC. *Burkholderia cepacia* colonization; PI: pancreatic insufficiency; PA: *P. aeruginosa*; and NMPA: non-mucoid PA colonization. *Chi-square test.

Table 5 - Variables significantly associated with the *cystic fibrosis transmembrane conductance regulator* genotype according to F508del mutation groups.*

Clinical variable	Genotype	G2 vs. G0+G1	G0 vs. G1+G2
	p	p	p
Sex	0.473	0.400	1
Ethnicity	0.353	0.549	0.407
Age	< 0.001	< 0.001	< 0.001
Age at first clinical manifestations	< 0.001	0.394	< 0.001
Age at diagnosis	< 0.001	0.001	0.01
Onset of digestive symptoms	0.022	0.602	< 0.001
Onset of pulmonary symptoms	0.006	0.731	0.004
BMI	0.227	0.22	0.186
Bhalla score	0.163	0.283	0.06
Kanga score	0.509	0.466	0.264
Shwachman-Kulczycki score	0.098	0.889	0.046
SaO_2	0.068	0.076	0.034
FVC, % of predicted	0.514	0.368	0.29
FEV_1, % of predicted	0.321	0.054	0.383
FEV_1/FVC, % of predicted	0.49	0.232	0.596
$FEF_{25-75\%}$	0.29	0.27	0.132
Nasal polyposis	0.521	0.516	0.842
Diabetes mellitus	0.948	1	0.842
Osteoporosis	0.236	0.255	0.139
Pancreatic insufficiency	< 0.001	0.001	< 0.001
Meconium ileus	0.047	0.238	0.016
First isolation of *Pseudomonas aeruginosa*	0.001	0.009	0.001
Colonization			
P. aeruginosa	0.025	0.092	0.012
Mucoid *P. aeruginosa*	0.068	1	0.059
Burkholderia cepacia	0.332	0.46	0.04
Achromobacter xylosoxidans	0.101	0.261	0.301
Staphylococcus aureus	0.758	1	0.572

G0: absent F508del; G1: heterozygous F508del patients; and G2: homozygous F508del patients; and BMI: body mass index. *Kruskal-Wallis one-way ANOVA (numerical data) and Pearson's chi-square test (categorical data).

identification of the F508del mutation allows improved genetic counseling.

In the state of São Paulo, newborn screening for CF has become possible by the determination of immunoreactive trypsinogen since 2010. Neonatal screening and sweat chloride/sodium determinations are free for all patients. Our clinic receives approximately US$ 30/patient from the government for the screening for *CFTR* mutations. This amount allows us to perform only the screening for the F508del mutation. The identification of additional mutations is performed by funded research projects. The main objective of the present study was to assess the importance of identifying the F508del mutation in our patients, due to the current amendment for public neonatal screening in Brazil, which provides subsidies to molecular analysis in order to identify positive cases in neonatal patients, as well as studies on new drugs for CF.

In our study, we analyzed 28 clinical variables that were associated with the F508del mutation. Associations with the F508del mutation were found mainly in the variables related to the onset of the disease. No patient was diagnosed by neonatal screening in our study. Therefore, the association between the F508del genotype and the variables related to the onset of the disease, such as age at first clinical symptoms and age at diagnosis, should be related to the clinical severity and not to the diagnosis, treatment, and follow-up of the patients.

With the inclusion of a neonatal screening program for CF in the state of São Paulo in 2010,

screening for F508del has become important as a means of predicting the clinical manifestations of CF, enabling a better monitoring of the patients in our health care clinic.

The patients in G0 presented with a lower risk for early clinical manifestations of CF and a protective factor for some of the variables studied (age, age at CF diagnosis, first clinical symptoms, digestive and pulmonary diseases, MI, and age at the first isolation of *P. aeruginosa*). In addition, there was a protective factor against MPA, NMPA, and *B. cepacia* colonization, which is an important risk factor for pulmonary disease. Corroborating the literature, PI was less common in G0 than in G1/G2 in our study.

The patients in G2 were younger, were diagnosed with CF at a younger age, were younger at the first isolation of *P. aeruginosa*, and were more commonly diagnosed with PI.

Some of the variables studied were significantly different in G1 than in G0 and G2 (age at first clinical manifestation, age at the onset of pulmonary disease, and MPA colonization). In addition, PI and age at CF diagnosis showed intermediate results.

In the analysis for gene clusters regarding variables with numerical data, patients in G1 and G2 presented with significantly higher Shwachman-Kulczycki scores and SaO_2. Higher values for these variables are associated with less severe disease; however, those patients were younger, and this is associated with the variation in the Shwachman-Kulczycki score and SaO_2.

When measuring the risk factors for long-term survival in a group of older CF patients (> 40 years of age), one group of authors reported that the residual activity of CFTR was not a factor associated with increased life expectancy but with other factors, such as BMI.[20] The greater importance of the F508del mutation and its identification is associated with the onset of the illness. In the present study, markers of initial severity of the pathophysiology were more evidently associated with the F508del genotype than were other clinical variables.[20] Thus, we believe that the genotype has a greater importance in the onset of disease and that the environment progressively becomes a higher risk factor with increasing age. In addition, we believe that survival selection is related to the class of mutation in the *CFTR* gene.

Another fact which underscores the importance of screening for F508del is that this mutation has been the most commonly studied, and the use of new drugs has been focused on patients with this mutation, which could favor their treatment. The study of correctors of F508del-CFTR depends on the use of pharmacological chaperones that stabilize the protein in its native state, of target cells using proteostasis regulators in order to enhance the folding efficiency of the protein, or of both at the same time. Although stabilizing and folding correctors of F508del-CFTR have been developed, we need to know the entire mechanism of action of these drugs before using them in our clinical practice. Current efforts to identify correctors, based largely on phenotype screens, have not been successful in identifying highly efficient molecules.[21,22] Although there are numerous defects in the CFTR protein, some of them might be liable to correction. New treatments are aimed at correcting defective CFTR proteins.[22]

Despite the advances in the scientific knowledge on CF, not much is known about the management of the disease, and many controversies are still present.[23] Much remains to be learned about the mechanism that involves the expression of the CFTR protein associated with the F508del mutation,[24] because F508del acts in multiple steps in the biogenesis of CFTR.[25]

Currently, the study of the genetic variation in CF using molecular technology allows new therapeutic possibilities and provides knowledge about the unknown factors of the severity of the disease.[9] As the prevalence of F508del is higher than that of other *CFTR* mutations with clinical importance described, this is the main factor to be analyzed as a first step in the molecular diagnosis of CF. Mutation analysis in a predominantly Caucasian population might provide improvements in diagnosis, genetic counseling, use of new drugs that are still under study, less expensive molecular analysis, monitoring and targeting outpatients, and promoting molecular diagnosis in individuals with a positive neonatal screening test for CF even with the low subsidy provided. There should be an understanding and an association between the outpatient clinic and the research laboratory in order to promote better patient monitoring.[8]

We currently have priority areas for the study of CF: to explore the pathogenic mechanisms of

early pulmonary disease; to improve newborn screening; to develop a spectrum of early lung disease biomarkers that reflect the pathophysiology, the clinical course, and the response to treatment; to explore the role of genetics/genomics in the pathogenesis of the disease; to define the microbiological events in early lung disease; and to elucidate the changes in remodeling, inflammation, and repair mechanisms in the pulmonary disease.[26] Much has yet to be done in this context, and the determination of a point mutation might bring benefits as important as those brought by the identification of other *CFTR* mutations.

In our study, a univariate analysis was performed. A multivariate logistic regression model could have been used; however, a larger sample of CF patients would be necessary in order to adjust for age and other factors simultaneously. For instance, a 40-year-old patient is significantly more likely to be colonized than a 5-year-old patient. In our study, we directly analyzed the influence of the F508del genotype on CF patients.

A huge ethnic diversity is present in Brazil, and, therefore, it might be disadvantageous for some subjects to be tested for F508del only. However, and not surprisingly, our findings were similar to those in the literature, and the screening for only one CF mutation was able to demonstrate the genetic diagnosis in one third of the patients. Another third of the patients presented with at least one allele with this mutation. The screening for F508del is important, particularly in developing countries and in countries with limited resources.

In conclusion, the identification of F508del and its association with the clinical severity of the disease allowed a better understanding of its influence on the clinical manifestations in CF patients. The association with variables related to the onset of the disease highlights the importance of using the screening for this mutation at the time of diagnosis and after positive neonatal screening for CF. In the future, the use of new drugs designed to one particular genotype will be associated with molecular analysis. Due to its high prevalence in the CF population, F508del should be analyzed primarily, mainly in developing countries. The genetic counseling of parents and patients is better carried out with the knowledge of the mutation associated with disease. Outpatient care can be better performed, especially considering the importance of F508del in association with CF severity variables, such as the isolation of bacteria that cause chronic pulmonary infection. In summary, the identification of F508del promotes genetic counseling, management, monitoring, diagnosis, and the use of new drugs. We believe that genetic laboratories worldwide should only initially consider the screening for F508del in patients with two altered sweat sodium/chloride tests.

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