Diagnostic contribution of molecular analysis of the cystic fibrosis transmembrane conductance regulator gene in patients suspected of having mild or atypical cystic fibrosis*

Contribuição da análise molecular do gene regulador da condutância transmembrana na fibrose cística na investigação diagnóstica de pacientes com suspeita de fibrose cística leve ou doença atípica

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Abstract

Objective: To evaluate the diagnostic contribution of molecular analysis of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in patients suspected of having mild or atypical cystic fibrosis (CF). **Methods:** This was a cross-sectional study involving adolescents and adults aged ≥ 14 years. Volunteers underwent clinical, laboratory, and radiological evaluation, as well as spirometry, sputum microbiology, liver ultrasound, sweat tests, and molecular analysis of the *CFTR* gene. We then divided the patients into three groups by the number of mutations identified (none, one, and two or more) and compared those groups in terms of their characteristics. **Results:** We evaluated 37 patients with phenotypic findings of CF, with or without sweat test confirmation. The mean age of the patients was 32.5 ± 13.6 years, and females predominated (75.7%). The molecular analysis contributed to the definitive diagnosis of CF in 3 patients (8.1%), all of whom had at least two mutations. There were 7 patients (18.9%) with only one mutation and 26 patients (70.3%) with no mutations. None of the clinical characteristics evaluated was found to be associated with the genetic diagnosis. The most common mutation was p.F508del, which was found in 5 patients. The combination of p.V232D and p.F508del was found in 2 patients. Other mutations identified were p.A559T, p.D1152H, p.T1057A, p.I148T, p.V754M, p.P1290P, p.R1066H, and p.T351S. **Conclusions:** The molecular analysis of the *CFTR* gene coding region showed a limited contribution to the diagnostic investigation of patients suspected of having mild or atypical CF. In addition, there were no associations between the clinical characteristics and the genetic diagnosis.

Keywords: Cystic fibrosis/diagnosis; Cystic fibrosis/genetics; Cystic fibrosis transmembrane conductance regulator.

Resumo

Objetivo: Avaliar a contribuição da análise molecular do gene *cystic fibrosis transmembrane conductance regulator* (*CFTR*, regulador da condutância transmembrana na fibrose cística) na investigação diagnóstica da fibrose cística em pacientes com suspeita de fibrose cística (FC) leve ou atípica. **Métodos:** Estudo transversal em adolescentes e adultos (idade ≥ 14 anos). Os voluntários foram submetidos à avaliação clínica, laboratorial e radiológica; espirometria, microbiologia do escarro, ecografia hepática, teste do suor e análise molecular do gene *CFTR*. Compararam-se as características dos pacientes divididos em três grupos, segundo o número de mutações identificadas (duas ou mais, uma e nenhuma). **Resultados:** Foram avaliados 37 pacientes com achados fenotípicos de FC, com ou sem confirmação pelo teste do suor. Houve predomínio do sexo feminino (75,7%), e a média de idade dos participantes foi de 32,5 ± 13,6 anos. A análise molecular contribuiu para o diagnóstico de FC em 3 casos (8,1%), todos esses com pelo menos duas mutações. Houve a identificação de uma e nenhuma mutação, respectivamente, em 7 (18,9%) e 26 pacientes (70,3%). Nenhuma característica clínica estudada se associou com o diagnóstico genético. A mutação p.F508del foi a mais comum, encontrada em 5 pacientes. A associação de p.V232D e p.F508del foi encontrada em 2 pacientes. Outras mutações encontradas foram p.A559T, p.D1152H, p.T1057A, p.I148T, p.V754M, p.P1290P, p.R1066H e p.T351S. **Conclusões:** A análise molecular da região codificadora do gene *CFTR* apresentou uma contribuição limitada para a investigação diagnóstica desses pacientes com suspeita de FC leve ou atípica. Além disso, não houve associações entre as características clínicas e o diagnóstico genético.

Descritores: Fibrose cística/diagnóstico; Fibrose cística/genética; Regulador de condutância transmembrana em fibrose cística.

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Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in a gene that is located on the long arm of chromosome 7 and that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[1,2]

Although CF is usually diagnosed in childhood (in the first year of life in 70% of cases), the number of cases of CF diagnosed in adolescence and adulthood has increased as a result of a higher level of clinical suspicion and the availability of diagnostic techniques.[3]

The phenotypic expression of CF varies among CF patients. The diagnosis is based on phenotypic findings, family history, newborn screening test results, and quantitative pilocarpine iontophoresis sweat test results.[4]

The sweat test is considered the gold standard for the diagnosis of CF,[5] which is confirmed by two or more determinations of sweat chloride concentrations greater than 60 mEq/L at different time points. In cases in which sweat chloride concentrations are found to be borderline or normal, additional diagnostic tests are needed, molecular analysis being performed in order to identify mutations in the *CFTR* gene.[6,7] Although the method is highly specific for CF, its sensitivity is low. This is due to the fact that the number of known mutations is quite large (over 1,900), although only a minority is included in commercially available panels.[2,8] Despite its shortcomings, analysis of mutations in the *CFTR* gene has been increasingly used as a diagnostic method for CF.

The identification of two CF-causing mutations confirms the diagnosis of atypical CF. The identification of one mutation is inconclusive. The absence of mutations means that the diagnosis of CF is highly unlikely, and differential diagnoses, such as ciliary dyskinesia and immunodeficiency, should be considered.[9]

The objective of the present study was to evaluate the contribution of molecular analysis of the *CFTR* gene coding region to the diagnostic investigation of adolescents and adults suspected of having mild or atypical CF, as well as to compare the characteristics of three groups of patients, divided according to the number of *CFTR* gene mutations identified (none, one, or two or more, the last of the three confirming the diagnosis of CF).

Methods

This was a single-center cross-sectional study. We sought to evaluate the contribution of molecular analysis of the *CFTR* gene to the diagnostic investigation of adolescents and adults suspected of having mild CF (diagnosis confirmed by sweat test results and mild lung disease without pancreatic insufficiency) or atypical CF (clinical phenotype consistent with CF without sweat test confirmation).

The study protocol was approved by the Research Ethics Committee of the *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre *Hospital de Clínicas*; Protocol no. 08-549). All of the participants gave written informed consent. Although we received donations from private companies (Roche and United Medical), those sponsors had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

The study population consisted of patients selected from among those treated at the HCPA Department of Pulmonology outpatient clinic for adolescents and adults with CF, located in the city of Porto Alegre, Brazil. We included individuals ≥ 14 years of age with phenotypic findings of CF (digital clubbing, bronchiectasis, chronic infection with typical bacteria, and exocrine pancreatic insufficiency), with or without sweat test confirmation.

We excluded patients in whom the phenotypic findings were attributable to other diseases, such as ciliary dyskinesia and primary immunodeficiency; pregnant patients; and patients who declined to participate in the study or who declined to give written informed consent.

Volunteers were selected from among patients attending the abovementioned outpatient clinic for routine medical visits between 2009 and 2010. The selected patients were interviewed by one of the members of our study group. The interviewer used a data collection form in order to gather information on the following variables: age; gender; marital status; race; family history of CF; presence of digital clubbing; and body mass index (BMI), in kg/m^2.

All of the patients underwent 2-h oral glucose tolerance tests, with the exception of those with a previous diagnosis of diabetes mellitus based on fasting blood glucose levels, in accordance with the American Diabetes Association criteria.[10,11]

The evaluation of chronic sinus disease was complemented by CT of the sinuses.

The Shwachman-Kulczycki score[12] was used in order to assess overall disease severity. This clinical scoring system is based on four different aspects (general activity, physical examination, nutrition, and chest X-ray findings). Each is scored on a 5-25 point scale, a better performance translating to a higher score. A final score of 100 points indicates optimal clinical condition.

We reviewed the bacteriological tests performed on sputum samples collected during routine clinical visits in the last 12 months. Identification of mucoid and nonmucoid *Pseudomonas aeruginosa*, methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*, *Burkholderia cepacia*, and *Haemophilus influenzae* was recorded when they were identified at least twice in sputum samples collected during routine clinical visits at intervals longer than 30 days.

Spirometry was performed in the HCPA Department of Pulmonology with a MasterScreen spirometer and the program v4.31 (Jaeger, Würzburg, Germany) in accordance with the Brazilian Thoracic Association technical acceptability criteria.[13] We recorded FVC, FEV_1, and the FEV_1/FVC ratio. Three acceptable maneuvers were performed, the values obtained on the best test being recorded. All parameters were expressed as a percentage of the predicted values for age, height, and gender.[14]

For the diagnosis of liver disease in CF, we used the ultrasound scoring system developed by Williams.[15] The scoring system is based on three ultrasound features: liver parenchyma—normal (1 point), coarse (2 points), or irregular (3 points); liver edge—smooth (1 point) or nodular (3 points); and periportal fibrosis—absent (1 point), moderate (2 points), or severe (3 points). A final score of 3 is consistent with normal liver, higher scores being suggestive of progressive liver disease. A score of 8-9 is consistent with established liver cirrhosis. This ultrasound scoring system is routinely used at the HCPA. The sonographer at the HCPA interpreted and scored the findings of routine ultrasound examinations.

Molecular analysis was performed in the HCPA Department of Genetics. A sample of 5 mL of peripheral blood was collected for DNA extraction. The *CFTR* gene coding region was amplified by polymerase chain reaction. Sequencing of the fragments was performed using the Big Dye™ Terminator v3.1 Ready Reaction Cycle Sequencing (Applied Biosystems, Foster City, CA, USA) and fluorescent dye terminator sequencing on a genetic analyzer (ABI PRISM™ 3130xl, Applied Biosystems). The analyses were performed with the DNA Sequencing Software v5.2 (Applied Biosystems). All 27 *CFTR* gene exons were sequenced. The flanking regions and exon-intron organization were also included in the sequencing. The intron 8 polymorphism was also analyzed.

For the statistical analysis, the data were processed and analyzed with the aid of the IBM SPSS Statistics software package, version 18.0 (IBM Corporation, Armonk, NY, USA).

Descriptive analysis was performed, quantitative data being presented as mean ± SD or median (interquartile range). Qualitative data were expressed as n (%).

In the statistical analysis, the patients were divided into three groups by the number of known CF-causing mutations identified (two or more, one, or none).

The three groups were compared by one-way ANOVA for continuous variables with normal distribution; by the Kruskal-Wallis test for ordinal variables or for continuous variables without normal distribution; and by the chi-square test for categorical variables, Yates' correction or Fisher's exact test being used when necessary.

The level of significance was set at 5%, and all tests were two-tailed.

Results

Between May of 2009 and November of 2010, we evaluated 37 patients with phenotypic findings of CF, with or without sweat test confirmation. None of the selected patients declined to participate in the study. All of the patients completed the study protocol.

Table 1 shows the general characteristics of the patients. Females predominated in the study sample, which consisted of 28 females (75.7%) and 9 males (24.3%). The mean age was 32.5 ± 13.6 years (range, 14-67 years). Most of the patients were White (only 1 patient was non-White), and 16 patients (43.2%) reported having relatives (first- and second-degree relatives) with a confirmed diagnosis of CF. Five patients (13.5%) had been diagnosed with exocrine pancreatic insufficiency. The mean BMI was 22.4 ± 3.85 kg/m^2 (range, 15.3-30.0 kg/m^2). Digital clubbing was found

in 21.6% of the patients. Only 3 patients (8.1%) met diagnostic criteria for diabetes mellitus, and 13 (35.1%) met diagnostic criteria for chronic sinus disease. Regarding sputum microbiology, the most commonly isolated bacterium was *P. aeruginosa* (43.2%), followed by *S. aureus* (35.1%). *H. influenzae* was identified in 7 patients (18.9%), and *B. cepacia* was isolated in 5.4% of the patients. The mean FEV_1 was 68.31 ± 25.40% of predicted, the mean FVC was 80.0 ± 19.4% of predicted, and the mean FEV_1/FVC ratio was 71.68 ± 17.34%. Sweat electrolytes were evaluated in two samples, the mean weight of the first sample being 242.00 ± 85.99 mg and the mean weight of the second sample being 228.50 ± 95.00 mg. Chloride concentrations in the first and second samples were, respectively, 54 ± 26.14 mEq/L and 51.76 ± 23.37 mEq/L.

Table 2 compares the three groups of patients (those in whom two or more mutations were identified, those in whom only one mutation was identified, and those in whom no mutations were identified) in terms of their characteristics. There were no significant differences among the groups regarding gender, age, race, or family history of CF. There were no significant differences among the three groups regarding exocrine pancreatic insufficiency, which was found in 2, zero, and 3 patients, respectively. There were also no significant differences among the groups regarding presence of digital clubbing, BMI, presence of diabetes mellitus, or presence of chronic sinus disease. Regarding sputum microbiology, we found no significant differences among the groups in terms of the identification of *P. aeruginosa*, *S. aureus*, *H. influenzae*, and *B. cepacia*. There were no significant differences among the groups regarding the Shwachman-Kulczycki score, the Williams liver score, or the presence of bronchiectasis. There were also no statistically significant differences among the three groups regarding the mean FVC and FEV_1, in % of predicted, or the mean FEV_1/FVC ratio. There were no differences among the groups in terms of sweat sodium and chloride concentrations. There were no differences among the three groups regarding normal, borderline, or abnormal sweat test results. Those results were observed, respectively, in 1, 2, and 1 of the patients in whom at least two mutations were identified; in 1, 3, and 3 of the patients in whom one mutation was identified; and in 12, 4, and 10 of the patients in whom no mutations were identified.

The mutations identified by molecular analysis are presented in Table 3. The most common mutation was p.F508del, which was found in 5 patients. The combination of p.V232D and p.F508del was found in 2 patients. In 1 patient, we identified three mutations (p.T351S and p.F508del in the first allele, and p.P1290P in the second allele). Other mutations identified were p.A559T, p.D1152H, p.T1057A, p.I148T, p.V754M, p.P1290P, and p.R1066H.

Discussion

This was a cross-sectional study evaluating patients who presented with phenotypic findings consistent with CF and who were referred to a referral outpatient clinic for the diagnosis and treatment of CF in adolescents and adults. The molecular analysis of the *CFTR* gene contributed to the definitive diagnosis of CF in 4 (10.8%) of the 37 patients evaluated. In 7 patients (18.9%), only one CF-causing mutation was identified, whereas, in 26 patients (70.3%), no mutations were identified. None of the clinical characteristics evaluated were found to be associated with the genetic diagnosis. The presence of exocrine pancreatic insufficiency tended to be more common in patients with a confirmed genetic diagnosis of CF, although the difference was not statistically significant. Of the patients with a genetic diagnosis of CF, only 1 had abnormal sweat test results. However, of the 7 patients in whom one mutation was identified, 3 had abnormal sweat test results, as did 10 of the 26 patients in whom no mutations were identified.

Clinical characteristics and reasons for referral included the presence of chronic lung disease and chronic sinus disease, bronchopulmonary colonization with germs suggestive of CF, digital clubbing, family history of CF, gastrointestinal abnormalities, and nutritional abnormalities.[2] All of the patients had previously undergone sweat tests at least twice.

In the present study, of the 4 patients with a genetic diagnosis of CF, 1 can be characterized as having mild, classic CF because that patient had late manifestations of the disease and abnormal sweat test results, whereas the remaining 3 can be characterized as having atypical CF because they presented with borderline or normal sweat electrolyte values.

The development of molecular analysis techniques allowed the identification of more than

Table 1 - General characteristics of the patients suspected of having mild or atypical cystic fibrosis.[a]

Variables	Patients (n = 37)
Gender	
Male	9 (24.3)
Female	28 (75.7)
Age, years[b]	32.5 ± 13.6
Race	
White	36 (97.3)
Non-White	1 (2.7)
BMI,[b] kg/m^2	22.4 ± 3.85
Family history of CF	16 (43.2)
Exocrine pancreatic insufficiency	5 (13.5)
Digital clubbing	8 (21.6)
Diabetes mellitus	3 (8.1)
Chronic sinus disease	13 (35.1)
Sputum bacteriology	
Pseudomonas aeruginosa	16 (43.2)
Mucoid *P. aeruginosa*	7 (18.9)
Nonmucoid *P. aeruginosa*	10 (27.0)
Staphylococcus aureus	13 (35.1)
Methicillin-sensitive *S. aureus*	12 (32.4)
Methicillin-resistant *S. aureus*	2 (5.4)
Burkholderia cepacia	2 (5.4)
Haemophilus influenzae	7 (18.9)
FEV_1, L[b]	2.20 ± 0.95
FEV_1, % of predicted[b]	68.31 ± 25.4
FVC, L[b]	2.99 ± 0.90
FVC, % of predicted[b]	80.0 ± 19.4
FEV_1/FVC[b]	71.68 ± 17.34
Sweat test[b]	
Sample 1	
Sodium, mEq/L	57.57 ± 27.34
Chloride, mEq/L	54.00 ± 26.14
Sample weight, mg	242.34 ± 85.99
Sample 2	
Sodium, mEq/L	57.19 ± 22.12
Chloride, mEq/L	51.76 ± 23.37
Sample weight, mg	228.50 ± 95.00

CF: cystic fibrosis; and BMI: body mass index. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD.

1,900 mutations in the *CFTR* gene, as can be seen in the Cystic Fibrosis Mutation Database (http://www.genet.sickkids.on.ca/cftr). Genetic mutations have been classified as severe (class I-III mutations) or mild (class IV-VI mutations) on the basis of the molecular defect of the CFTR protein.[16,17] In individuals with class I mutations, the CFTR protein is not synthesized; in those with class II mutations, the CFTR protein is improperly processed in the endoplasmic reticulum; in those with class III mutations, the CFTR protein is improperly regulated; in those with class IV mutations, the CFTR protein shows defective conductance; in those with class V mutations, CFTR protein production is defective; and in those with class VI mutations, there is rapid degradation of the CFTR protein.[18,19] Nonclassic forms of CF have been associated with mutations that reduce but do not eliminate the function of the CFTR protein.[20] In general, individuals

Table 2 - Comparison of three groups of patients (those in whom two or more mutations were identified, those in whom one mutation was identified, and those in whom no mutations were identified) in terms of their characteristics.[a]

Variables	No. of mutations			p*
	≥ 2	1	0	
	(n = 4)	(n = 7)	(n = 26)	
Gender				
Male	1 (25.0)	0 (0.0)	8 (30.8)	0.242
Female	3 (75.0)	7 (100.0)	18 (69.2)	
Age, years[b]	30.80 ± 10.90	30.00 ± 9.95	33.00 ± 14.43	0.851
Race				
White	4 (100.0)	7 (100.0)	25 (96.2)	0.805
Non-White	0 (0.0)	0 (0.0)	1 (3.8)	
BMI,[b] kg/m^2	22.9 ± 3.7	23.3 ± 2.3	22.2 ± 4.3	0.801
Family history of CF	3 (75.0)	2 (28.6)	11 (42.3)	0.322
Exocrine pancreatic insufficiency	2 (50.0)	0 (0.0)	3 (11.5)	0.570
Digital clubbing	0 (0.0)	0 (0.0)	8 (30.8)	0.115
OGTT				
Glucose intolerance	0 (0.0)	2 (28.6)	4 (15.4)	0.624
Diabetes	0 (0.0)	1 (14.3)	2 (7.7)	
Chronic sinus disease	2 (50.0)	4 (57.1)	7 (26.9)	0.266
Sputum bacteriology				
Pseudomonas aeruginosa	1 (25.0)	5 (71.4)	10 (38.5)	0.218
Mucoid *P. aeruginosa*	1 (25.0)	3 (42.9)	3 (11.5)	0.162
Nonmucoid *P. aeruginosa*	0 (0.0)	3 (42.9)	7 (26.9)	0.306
Staphylococcus aureus	3 (75.0)	3 (42.9)	7 (26.9)	0.154
Methicillin-sensitive *S. aureus*	2 (50.0)	3 (42.9)	7 (26.9)	0.530
Methicillin-resistant *S. aureus*	1 (25.0)	1 (14.3)	0 (0.0)	0.062
Burkholderia cepacia	0 (0.0)	0 (0.0)	2 (7.7)	0.639
Haemophilus influenzae	0 (0.0)	1 (14.3)	6 (23.1)	
Shwachman-Kulczycki score[c]	80 (32.5)	75 (20.0)	70 (31.3)	0.748
Liver score[c]	3 (0)	3 (0)	3 (0)	0.399
Presence of bronchiectasis	2 (50.0)	6 (85.7)	19 (73.1)	0.439
FEV_1, L[b]	3.01 ± 0.71	2.44 ± 0.70	2.20 ± 1.13	0.317
FEV_1, % of predicted[b]	89.7 ± 26.0	72.2 ± 14.7	68.2 ± 29.0	0.338
FVC, L[b]	3.7 ± 0.4	3.2 ± 0.5	2.9 ± 1.2	0.359
FVC, % of predicted[b]	95.5 ± 16.1	81.7 ± 6.2	78.5 ± 23.2	0.320
FEV_1/FVC[b]	80.3 ± 12.5	74.8 ± 14.1	73.7 ± 19.0	0.785
Sweat test[b]				
Sample 1				
Sodium, mEq/L	56.8 ± 20.3	59.14 ± 27.3	56.5 ± 28.0	0.973
Chloride, mEq/L	53.8 ± 15.7	61.3 ± 23.10	51.34 ± 27.7	0.674
Sample 2				
Sodium, mEq/L	53.3± 10.3	56.4 ± 27.8	58.1 ± 22.2	0.941
Chloride, mEq/L	49.7 ± 10.50	52.2 ± 26.0	52.2 ± 24.4	0.985
Sweat test result				
Normal	1 (25.0)	1 (14.3)	12 (46.2)	
Borderline	2 (50.0)	3 (42.9)	4 (15.4)	0.306
Abnormal	1 (25.0)	3 (42.9)	10 (38.5)	

CF: cystic fibrosis; BMI: body mass index; and OGTT: oral glucose tolerance test. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD. [c]Values expressed as median (interquartile range). *One-way ANOVA for continuous variables with normal distribution or Kruskal-Wallis test (ordinal variables or continuous variables without normal distribution); chi-square test (categorical variables), Yates' correction or Fisher's exact test being used when necessary.

Table 3 - Mutations in the groups of patients in whom at least one mutation was identified.

Groups	Mutations	
Patients	Allele 1	Allele 2
≥ 2 mutations		
1	p.T351S/p.F508del	p.P1290P
2	p.A559T	p.D1152H
3	p.V232D	p.F508del
4	p.V232D	p.F508del
1 mutation		
5	p.T1057A	
6	p.I148T	
7	p.V754M	
8	p.P1290P	
9	p.F508del	
10	p.F508del	
11	p.R1066H	

who are homozygous for class I-III mutations present with pancreatic insufficiency, diabetes, high rates of meconium ileus, early mortality, more rapid decline in lung function, more severe malnutrition, and liver disease.[16] Patients with class IV and V mutations present with milder disease, pancreatic sufficiency, and better survival.[20,21]

No mutations in the *CFTR* gene are identified in 1.0-1.5% of all patients with classic CF. In cases of nonclassic CF, various factors are important in order to establish the correct diagnosis. Our molecular analysis of the *CFTR* gene included analysis of the coding region and of the flanking regions. Mutations located in promoter regions and in distant regulatory sequences are not evaluated.[16] However, mutations in these regions are less common and would probably not have explained all of the cases in our sample.

The most common mutation is p.F508del (deletion of the amino acid phenylalanine at position 508); however, the frequency of this mutation varies across populations, being approximately 47% in Brazil.[22,23] In an analysis of Brazilian patients with CF, one group of authors showed that p.F508del and four other mutations (p.G542X, p.N1303K, p.G551D, and p.R553X) accounted for 56% of CF alleles.[24] The spectrum of CF mutations in Brazil indicates a strong European influence, being particularly similar to that in the Italian population.[25,26] In a previous study conducted at the HCPA, the p.F508del mutation was found in 48.7% of the alleles.[28]

The sensitivity of CF genotyping depends entirely on the number of mutations tested and on the ethnicity of the individuals being tested.[24] The low sensitivity of the method is generally due to the large number of known mutations and to the fact that commercially available panels include only a minority of those mutations.[2] It should be taken into consideration that our study sample consisted of patients selected from among those referred to our facility (a referral center for CF) because of the difficulty in establishing a diagnosis.

One group of authors[27] reported 6 cases of CF diagnosed in adulthood, with suppurative lung disease, preserved pancreatic function, and only one mutation identified. The authors concluded that the diagnostic spectrum of patients investigated in adulthood is different from that of those diagnosed in childhood.

One study sought to determine whether changes in the function of the CFTR protein were responsible for the full spectrum of variant CF phenotypes.[28] The authors analyzed the *CFTR* gene in 74 patients with nonclassic CF. Of those patients, 29 had two mutations in the *CFTR* gene, 15 had one mutation, and 30 had no mutations. As occurred in our study, there were no differences among patients with two, one, or no mutations in terms of their clinical characteristics and sweat chloride concentrations. The authors concluded that factors other than mutations in the *CFTR* gene can produce phenotypes clinically indistinguishable from nonclassic CF caused by CFTR protein dysfunction.

In 2004, one group of authors[29] published a study evaluating the clinical characteristics and diagnostic parameters of patients diagnosed with CF in adulthood. Of 1,051 individuals with

CF, 73 (7%) were diagnosed with the disease in adulthood. Of the 46 patients diagnosed with CF after 1990, 30 (65%) were diagnosed by sweat testing, 15 (33%) were diagnosed by mutation analysis, and 31 (67%) were diagnosed by a combination of both methods. Nasal potential difference measurements alone confirmed the diagnosis in the remaining 15 patients (33%). The authors concluded that patients with CF presenting in adulthood generally present with pancreatic sufficiency, inconclusive sweat test results, and a high prevalence of mutations that are not seen in childhood.

The present study has some limitations. The major limitation of the present study is the small sample size (in particular, the small number of cases with a confirmed diagnosis), which precludes the generalization of the results of the comparison among the three groups in terms of their clinical characteristics and limits the external validity of the study. Another limitation is that we did not perform nasal potential difference measurements, because the method was not available for use at our hospital during the study period.

The clinical implication of the present study is that it demonstrates the difficulty that specialists face in diagnosing CF in adolescents and adults clinically suspected of having the disease. Diagnostic investigation requires diagnostic tests other than the sweat test. The sweat test is not specific enough to rule out CF in such patients.

In conclusion, in this population of patients who had findings consistent with CF and who were referred to a referral program for adolescents and adults with CF for evaluation, the molecular analysis of the *CFTR* gene coding region contributed to the definitive diagnosis of CF in 3 patients (8.1%) and allowed the identification of mutations in 1 patient with mild CF, previously confirmed by sweat testing. Patients with abnormal sweat electrolytes and with no identified mutations probably have alterations that are currently unknown. None of the clinical characteristics evaluated were found to be associated with the genetic diagnosis.

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