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Analysis of the ΔF508 mutation in a Brazilian cystic fibrosis population: comparison of pulmonary status of homozygotes with other patients

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Abstract

Sixty-one cystic fibrosis patients admitted for check-up or antibiotic treatment were enrolled for genetic and clinical evaluation. Genetic analysis was performed on blood samples stored on neonatal screening cards using PCR techniques to determine the presence of ΔF508 mutations. Clinical evaluation included Shwachman and Chrispin-Norman scores, age at onset of symptoms and diagnosis, spirometry, awake and sleep pulse oximetry, hyponychial angle measurement and presence of chronic *Pseudomonas aeruginosa* colonization. Eighteen patients (29.5%) were homozygous for the ΔF508 mutation, 26 (42.6%) had one ΔF508 mutation and 17 (27.9%) were noncarriers, corresponding to a 50.8% prevalence of the mutation in the whole population. Analysis by the Kruskal-Wallis test for comparison of genetic status with continuous variables or by the chi-square test and logistic regression for dichotomous variables showed no significant differences between any two groups for $\alpha = 0.05$. We conclude that genetic status in relation to the ΔF508 mutation is not associated with pulmonary status as evaluated by the above variables.

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder among Caucasians. Its incidence is around 1 per 2000 live births in most white populations (1). Since the discovery of the gene responsible for coding the protein involved, a chloride channel called cystic fibrosis transmembrane conductance regulator, many mutations causing the disease have been described. The most common among them is ΔF508, which consists of a three-base pair deletion leading to the loss of one phenylalanine at residue number 508 of the involved protein (2-4).

Many studies have been performed so far to analyze DNA from CF patients and to compare homozygotes for this mutation with heterozygotes and with carriers of two different mutations. Most results have been consistent with respect to the status of pancreatic sufficiency, showing a greater prevalence of insufficiency among homozygotes, but they have diverged as to the evaluation of pulmo-

nary involvement (5-14). For this reason, it is important to study pulmonary involvement in every CF group to search for correlations between genotype and phenotype.

In the present study we compared the pulmonary involvement of cystic fibrosis patients having or not a deletion of phenylalanine at residue 508.

Material and Methods

A cross-sectional study was conducted on CF patients seen at Hospital de Clínicas de Porto Alegre, Brazil, during their hospital stay for antibiotic treatment or check-up. The diagnosis had been previously confirmed by two sweat tests and clinical evidence of disease. The patients were invited to enter the study and their parents signed a written consent. The study was approved by the Ethics Committee of the hospital.

Sixty-one patients (38 boys and 23 girls aged 4 months to 17 years) were studied over a period of 24 months.

Blood samples (three drops) were collected on neonatal screening cards, stored in plastic bags and mailed to the Division of Genetics of Vanderbilt University for genetic analysis. Blood was then extracted and DNA amplified by the polymerase chain reaction (PCR), followed by gel electrophoresis for identification of the ΔF508 mutation.

Patients were evaluated in terms of Shwachman score as modified by Doershuk et al. (15) and Chrispin-Norman score (16), age at onset of symptoms and at diagnosis, spirometry, pulse oximetry while sleeping and awake, hyponychial angle measurement and colonization with *Pseudomonas aeruginosa.* Only patients aged 6 years or older were eligible for pulmonary function tests. Vital capacity, forced expiratory volume in one second (FEV1), and forced expiratory flow rate in the middle half of expiration (FEF 25-75%) were determined. Awake oximetry was performed with an Ohmeda 37OO pulse oximeter measuring hemoglobin saturation with the patient at rest; when saturation was evaluated during sleep, at night, mean value and percentage of total study time under 91 or 86% were obtained by transferring results from the device eight-hour memory to a personal computer. Hyponychial angle measurement was performed by the shadowgram technique that employs a light source and the projection of finger edge onto a white surface for angle measurement (17). All evaluations were made during a three-day period just prior to patient discharge, when clinical conditions were considered to be best.

The patient would be rated as chronically colonized by *P. aeruginosa* when at least three sputum samples were positive for this microorganism for at least 3 months and at the moment of evaluation, as also done by others (18).

For statistical analysis we employed SAS and SPSS programs; methods included the Kruskal-Wallis test for continuous variables or the chi-square test and logistic regression for dichotomous ones, with the level of significance set at $\alpha = 0.05$. The power of the sample size was 90%, except for spirometry which only 28 patients were able to perform (65% power).

Results

General findings

Sixty-one patients of a total of 86 under treatment at the center during the study period were included. There were 38 males and 23 females (chi-square = 3.69, P = 0.05) ranging in age from 4 months to 17.6 years. All were white (Table 1).

Eighteen patients (29.5%) had 2 ΔF508 mutations, 26 (42.6%) had one ΔF508 mutation and 17 (27.9%) were noncarriers, reflecting a 50.8% prevalence of this mutation in the population as a whole.

Comparison of the three groups showed

no difference between them for any of the variables studied (Table 2). *Pseudomonas aeruginosa* colonization was more frequent among homozygotes for the ΔF508 mutation and this difference almost reached significance (P = 0.08, logistic regression). When age was included in the regression model, the difference was shown to be related to this variable rather than to genetic group (odds ratio 1.034, 95% confidence interval 1.017-1.051) (Table 3).

Discussion

As was the case for most other studies, we were unable to show any differences in pulmonary involvement when only the ΔF508 mutation was considered (11,19-24). This is a simplification of the issue linking genotype and phenotype since many different mutations are included in the heterozygote and noncarrier groups and also because other genetic and environmental factors may play a part. Even so, we thought it would be important to perform this study because results have been variable depending on the population analyzed. Also we might find a predictable pattern of pulmonary disease among the groups studied, at least for the homozygotes, as is the case for pancreatic involvement (5-12).

Table 1 - Characteristics of the patients in the present study.

Data are reported as number and percentage. *P = 0.05 (chi-square = 3.69).

Characteristic	Number	%
Sex		
Male	38*	62.30
Female	23	37.70
Color		
White	61	100.00
Age		
<1 year	5	8.20
1-5 years	26	42.62
5-10 years	14	22.95
>10 years	16	26.23

Table 2 - Patient characteristics according to genotype.

Data are reported as mean ± SD. *P = 0.08 (logistic regression to check effect of genetic status on colonization).

	ΔΔ	NΔ	NN
Age (months)	76.06 ± 51.96	87.73 ± 60.04	66.29 ± 53.59
Age at onset of symptoms (months)	1.94 ± 4.41	1.27 ± 1.73	3.35 ± 7.19
Age at diagnosis (months)	16.78 ± 26.43	19.27 ± 27.07	27.00 ± 28.86
Shwachman score	78.67 ± 11.67	81.77 ± 10.84	78.94 ± 13.38
Chrispin-Norman score	14.11 ± 7.07	12.23 ± 8.11	14.18 ± 8.72
Hyponychial angle (^{0})	189.47 ± 8.81	188.83 ± 8.81	188.74 ± 6.90
Awake oxygen saturation (%)	96.67 ± 1.03	96.85 ± 1.57	95.12 ± 4.72
Average sleep saturation (%)	96.10 ± 1.00	95.80 ± 1.39	94.38 ± 5.29
Sleep saturation time <91% (%)	0.48 ± 0.65	2.02 ± 6.54	12.37 ± 29.72
Sleep saturation time <86% (%)	0.11 ± 0.33	0.95 ± 4.60	5.94 ± 24.24
Vital capacity (% reference)	91.42 ± 19.01	92.03 ± 28.52	78.90 ± 21.51
FEV1 (% reference)	86.15 ± 22.35	76.98 ± 30.53	66.88 ± 34.80
FEF 25-75%	61.17 ± 35.59	46.05 ± 34.97	45.20 ± 38.39
P. aeruginosa (n)	10 (55.56%)*	11 (42.31%)	4 (23.53%)*

Table 3 - Differences between genetic groups in relation to *P. aeruginosa* colonization, controlling for age (logistic regression).

Variable	Odds ratio	Confidence interval
ΔΔ (reference)	1	-
NΔ	0.8506	(0.3308-2.1874)
NN	0.4073	(0.1311-1.2655)
Age	1.034	(1.0170-1.0510)

Although some investigators have found differences between ΔF508 groups with respect to some variables of pulmonary involvement and *P. aeruginosa* colonization (11,13,25-27), we did not. When we first analyzed the results, we found a close to significant difference in bacterial colonization between homozygotes and noncarriers of the mutation. We thought that this may have happened because of the small sample size and that increasing the number of patients might give us significant results. But when age was included in the logistic regression model we found that this was a confounding variable. The lack of analysis of other variables involved may have led to divergent results by different authors. Also,

most studies do not standardize time of data collection which may oscillate significantly when patients with unstable pulmonary conditions are included in the studies.

No significant differences were found between genetic groups when only the ΔF508 mutation was analyzed. Thus, information about patient genetic status in relation to this mutation alone does not allow any conclusion with respect to pulmonary status.

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