The IFRD1 (57460C>T Polymorphism) Gene: A Negative Report in Cystic Fibrosis Clinical Severity

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Abstract

Cystic fibrosis (CF) is an autosomal recessive disease caused by more than 1,900 mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. In CF, one intriguing aspect is that patients, with same CFTR mutation, can have high clinical variability. Thus, the CFTR genotype does not seem to be the only determining factor in the clinical severity modulation. Therefore, the modifier genes and the environment must be considered. The IFRD1 (Interferon-related developmental regulator 1) gene, acts on the immune system and in the recruitment of immune cells, and consequently could be a modulator. In our data we included 88 CF patients, diagnosed by CFTR mutation screening and positive sweat test. The 57460C>T polymorphism screening in the IFRD1 gene was made by polymerase chain reaction associated to enzymatic digestion. A genotypic comparison was performed with 23 CF clinical variables. The data was analyzed by the SPSS program considering α=0.05. The patients were analyzed considering the CFTR genotype characteristic by mutation class. In our data 64.77% of patients had mutations of classes I, II or III in the CFTR gene. The IFRD1 polymorphism frequency was 28 (12.99%), 35 (75.32%) and 25 (11.69%) to the CC, CT and TT genotypes, respectively. In our study, the 57460C>T polymorphism in the IFRD1 gene was not associated with the CF clinical variables. The analysis was performed with and without consideration of the CFTR genotype, and after correction for multiple testing (Bonferroni test), no positive association was observed in both cases. Taking into account our results, in the CF patients population analyzed, there were no associations of the 57460C>T polymorphism in the IFRD1 gene with the CF clinical variables.

Keywords: Cystic fibrosis; IFRD1 gene; Genotype; Phenotype; Variability; Lung disease; Polymorphism; CFTR gene

Introduction

The cystic fibrosis (CF) is a monogenic, autosomal and recessive disease, with wide clinical variability [1-3]. Children with same expression of the gene, (ii) by altering chlorine conduction, (iii) in controlling the splicing and expression of modifier genes, conditioned by their polymorphisms, can involved in the control of infection, immunity and inflammation. The modifier genes should be considered [5-7] principally genes have a higher clinical concordance than dizygotic twins. In this case, twins, show wide clinical variability [4], however, monozygotic twins, in CF, one intriguing aspect is that patients, with same CFTR mutation, can have high clinical variability. Thus, the CFTR genotype does not seem to be the only determining factor in the clinical severity modulation. Therefore, the modifier genes and the environment must be considered. The IFRD1 (Interferon-related developmental regulator 1) gene, acts on the immune system and in the recruitment of immune cells, and consequently could be a modulator. In our data we included 88 CF patients, diagnosed by CFTR mutation screening and positive sweat test. The 57460C>T polymorphism screening in the IFRD1 gene was made by polymerase chain reaction associated to enzymatic digestion. A genotypic comparison was performed with 23 CF clinical variables. The data was analyzed by the SPSS program considering α=0.05. The patients were analyzed considering the CFTR genotype characteristic by mutation class. In our data 64.77% of patients had mutations of classes I, II or III in the CFTR gene. The IFRD1 polymorphism frequency was 28 (12.99%), 35 (75.32%) and 25 (11.69%) to the CC, CT and TT genotypes, respectively. In our study, the 57460C>T polymorphism in the IFRD1 gene was not associated with the CF clinical variables. The analysis was performed with and without consideration of the CFTR genotype, and after correction for multiple testing (Bonferroni test), no positive association was observed in both cases. Taking into account our results, in the CF patients population analyzed, there were no associations of the 57460C>T polymorphism in the IFRD1 gene with the CF clinical variables.

Our group has studied CF severity in association with modifier genes, including: MBL-2, TGF-β1, CD14 [9], GSTM1, GSTT1 [10], ACE [11], ADRB2 [12], TCFTL2 [13], COX-2 [14] and ADRA2A [15]. In our studies, the polymorphisms are associated with clinical variables including clinical markers of the pulmonary and digestive disease.

The IFRD1 (Interferon-Related Developmental Regulator 1) gene, region 7q31.1, has 13 exons, with 52 Kb, transcribed with 1,834 bases pair, and is responsible for encoding a protein with 451 amino acids [16]. The correct function of IFRD1 protein is dependent of the histone deacetylase that is expressed in the late of the neutrophils differentiation, being important in neutrophil function [17,18]. The single sequence polymorphism, rs7817 [exchanging a cytosine to thymine at position 57460], in the 3'UTR region of the IFRD1 gene, had the heterozygous genotype (CT) associated with worse lung function than the homozygous (CC and TT). Although the IFRD1 gene is located on chromosome 7, as is the CFTR gene, both genes have independent segregation [17]. In CF patients, the neutrophils are recruited continuously in the airways, causing persistent inflammatory response [19]. As the severity of the inflammatory response varies, even among patients with identical CFTR genotype, there is a need to study genes involved in the neutrophil production and maturation in CF [20]. A few studies related the IFRD1 gene as CF modifier gene, considering its ability to modulate the amplitude of the immune response of neutrophils [18-20].

In this study, we selected the IFRD1 (57460C>T) polymorphism with expression related to the immune system. The IFRD1 protein is expressed in mature neutrophils and is able to interact with the histone deacetylase enzyme [18], acting in cellular differentiation and oxidative stress. Since CF pulmonary disease is characterized by neutrophilic inflammation and oxidative stress, the IFRD1 action can exert a key role in regulating airway inflammation [17]. In this context, the aim of

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this study was to analyze the polymorphism 57460C>T in the IFRD1 gene in association with 27 clinical variables in CF patients.

Method

Patient and methods

This was a cross-sectional study conducted in a university center for CF care between 2011 and 2012. All CF patients were invited participants of the study. CF patients without data or informed consent were not included. The CF diagnosis was confirmed by two doses of sodium and chloride from the sweat with values greater than 60 mEq/L. In a patient’s cohort, the CFTR mutation was identified. No patient had diagnosis made by neonatal screening test.

Eighty eight patients were selected for the study. Patients' DNA was obtained by phenol-chloroform extraction. The DNA concentration used for analysis was 50 ng/mL, evaluated using GE NanoVue™ Spectrophotometer (GE Healthcare Biosciences, Pittsburgh, USA).

Clinical variables

The clinical variables were employed: (i) clinical scores (Shwachman-Kulczycki, Kanga and Bhalla) [21]; (ii) body mass index (BMI) for the patients older than 19 years of age the BMI= weight/height^2 formula was used; for the remaining patients: WHO ANTHRO program (children 0 - under 5 years old) and WHO ANTHRO PLUS program (children 5 - under 19 years old) were used (http://www.who.int/en/); (iii) patient age; (iv) time for the diagnosis (according to sodium and chloride dosage); (v) first clinical symptoms (digestive and pulmonary); (vi) time for the 1st colonization by Pseudomonas aeruginosa; (vii) bacteria in the respiratory airways (P. aeruginosa mucoid and no mucoid, Achromobacter xylosoxidans, Burkholderia cepacia and Staphylococcus aureus); (viii) transcutaneous hemoglobin oxygen saturation; (ix) spirometry; (x) comorbidities.

Spirometry was performed in patients older than 7 years old, using the CPFS/D spirometer (MedGraphics, Saint Paul, Minnesota, USA). Data was recorded by the PF BREEZE software version 3.8B for Windows 95/98/NT [22] and the following variables were included: forced vital capacity [FVC (%)], forced expiratory volume in the first second [FEV1, (%)] ratio between FEV1 and FVC (%), [FEV1/FVC (%)] and forced expiratory flow between 25 and 75% of the FVC [FEF25-75%].

The comorbidities included in the study were nasal polyps (diagnosed by physical examination and/or rhinoscopy), osteoporosis (diagnosed by bone densitometry), meconium ileus (diagnosed by meconium presence in the birth), diabetes mellitus type 2 (diagnosed by glucose tolerance exam) and pancreatic insufficiency (diagnosed by steatocrit).

This study was approved by the Institutional Ethics Committee from University of Campinas - Faculty of Medical Sciences (#052/2011), and all patients signed a consent form before beginning the study.

The CFTR mutation identification

The CFTR mutation identification was performed by polymerase chain reaction (PCR) (F508del) and fragment length polymorphism method (G542X, R1162X, R553X, G551D and N1303K). Some mutations in CF patients were obtained by sequencing or MLPA (Multiplex Ligation-dependent Probe Amplification) analysis: 1717-G>A and I618T. For sequencing and MLPA, we used MegaBace1000™ sequencer (GE Healthcare Biosciences, Pittsburgh, USA).

The CFTR genotype was used as a correction factor for statistical analysis. All mutations identified were included in the class I, II or III of the CFTR gene. Other identified mutations, class IV (P205S) were not included in statistical analysis.

Analysis of 57460C>T polymorphism in the IFRD1 gene

The PCR reaction for amplification of the 547 bp fragment of the IFRD1 gene was performed with bidistilled water, 10x Taq buffer with (NH4)2SO4, MgCl₂ (25 mM), dNTP (25 mM each nucleotides base), primers (0.2 pmol - sense primer: 5'-AGATAAGAGAGCACAGATGT'T-3' and antisense primer: 5'-GCTGTCCTTCAATAAATAT-3'), Taq polymerase (5U) and genomic DNA (50 ng/mL). The annealing temperature was 62°C.

After PCR, enzymatic digestion was made with the BstNI enzyme (New England Biolabs) at 60°C for 14 hours following the manufacturer’s recommendations. The reaction was analyzed on polyacrylamide gel (12%) with a voltage of 180V for 4 hours. The gel was stained in ethidium bromide solution and visualized on the Typhoon™ scanner (GE Healthcare, Pittsburgh, USA). According to fragments observed the genotype was identified, as follows: TT (444 + 113 bp), TC (444 + 326 + 118 + 103 bp) and CC (326 + 118 + 103 bp).

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) software v.21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.), OpenEpi [23] and R version 2.12 (Comprehensive R Archive Network, 2011). The statistical power calculation for the sample was performed by GPower 3.1 software [24] demonstrating statistical power above 80% for the analysis.

The data were compared using the χ² and Fisher exact test for categorical variables and the Mann-Whitney and Kruskal-Wallis tests for numerical variables.

We adopted the values of <alpha> 0.05 for all statistical analysis.

The data distribution that showed high standard deviation was analyzed by median value. The variables that were adjusted by median to short (more severe) and longtime were: patient’s age (≤ 154 and > 154 months), time for the diagnosis ≤ 24 and > 24 months), onset of the pulmonary (≤ 6 and > 6 months) and digestive symptoms (≤ 3 and > 3 months), and time for the first isolated P. aeruginosa (≤ 3 and > 3 months).

In order to avoid spurious data due to the multiple tests [25], the significance level a was adjusted by Bonferroni correction (<alpha> corrected = 0.05/number of tests).

Results

The description of the population examined in the study is shown in the table 1 for all clinical variables included in the study.

The genotypic frequency of CFTR mutations and polymorphisms are described in the table 2. The analyzed polymorphism is in Hardy-Weinberg equilibrium.

The table 3 shows the p-values, corrected and uncorrected, reported for all analyzes performed, considering all patients included in the study, and patients with two CFTR mutations identified.

The tables 4-6 shows the categorical variables and in the table 7, the numerical variables, regardless of the CFTR mutations and considering the distribution for the CFTR gene according to the presence of
Sex - masculine 48% (86)*
Caucasian 91.5% (161)*
Age 166.98 ± 11.766 months (87–468 months)*
BMI - thinness and accentuated thinness 13.6% (12)*
One Class I, II or III identified mutation 34.09% (30)*
Two Class I, II or III identified mutation 64.77% (57)*
First clinical manifestation 5.58 ± 1.222 months (0 – 39 months)*
Age at diagnosis 36.86 ± 9.368 months (1 – 379 months)*
Onset of digestive symptoms 21.74 ± 9.300 months (0 – 381 months)*
Onset of pulmonary symptoms 13.63 ± 4.543 months (0 – 187 months)*
SpO2 95.07 ± 0.812 (66 – 99)*
Bhalla score 8.53 ± 0.742 (0 – 23)*
Kanga score 18.86 ± 0.851 (11 – 40)*
Shwachman-Kulczycki score 66.98 ± 2.227 (20 – 90)*
FVC(%) 82.49 ± 3.194 (29 – 135)*
FEV1 (%) 74.67 ± 3.424 (19 – 132)*
FEV1/FVC 84.26 ± 2.034 (37 – 100)*
FEF25-75% 61.77 ± 4.461 (8 – 121)*
Nasal Polyps 12.50% (11)*
Diabetes mellitus 18.20% (16)*
Osteoporosis 14.80 % (13)*
Pancreatic insufficiency 96.90 % (85)*
Meconium ileus 17.00 % (15)*
First isolated P. aeruginosa 53.12 ± 10.557 months (4 – 379 months)
P. aeruginosa status 1 63.60 % (56)*
P. aeruginosa mucoid status 1 46.60 % (41)*
B. cepacia status 1 19.30 % (17)*
A. xylosoxidans status 1 14.80 % (13)*
S. aureus status 1 81.20 % (72)*
BMI = Body Mass Index; SpO2 = transcutaneous hemoglobin oxygen saturation; FVC = Forced Vital Capacity; FEV₁ = Forced Expiratory Volume in the first second; FEF25-75% = Forced Expiratory Flow between 25 and 75% of FVC. 1. Based on 3 Consecutive positive respiratory cultures.
*Percentage (Number of patients)
*Continuous variables expressed as mean ± SD (range)

Table 1: Clinical features of Cystic Fibrosis patients included in the study.

| Gene                                     | Chromosome position | Location                          | Variation | Genotype   | MAF   | X² | p    |
|------------------------------------------|---------------------|-----------------------------------|-----------|------------|-------|----|------|
| IFRD1, 57460C>T                         | 7q31.1              | 3' untranslated region            | C>T       | 28 (12.99%) | 35 (75.32%) | 25 (11.69%) | 0.48 | 3.65 | >0.05 |

**CFTR** mutation

| N | Frequency |
|---|-----------|
| 36 | 40.90%    |
| 10 | 11.40%    |
| 2  | 2.28%     |
| 3  | 3.42%     |
| 1  | 1.14%     |
| 1  | 1.14%     |
| 1  | 1.14%     |
| 1  | 1.14%     |
| 1  | 1.14%     |
| 28 | 31.82%    |
| 1  | 1.14%     |
| 1  | 1.14%     |

**IFRD1 = Interferon-Related Developmental Regulator 1; CFTR = Cystic fibrosis transmembrane conductance regulator; C = Cytosine; T = Thymine; ≥ = bigger than; MAF = minor allele frequency; *p = value for Hardy-Weinberg Equilibrium; N = number of patients; (-) CFTR mutation no identified. 1= IFRD1, rs7817 polymorphism is in Hardy-Weinberg Equilibrium in our sample.**

Table 2: Genotypic characteristic of IFRD1 polymorphism and CFTR mutation among Cystic Fibrosis patients.
### Variables

| Without taking CFTR mutation into account | Patients with two CFTR mutations identified |
|------------------------------------------|--------------------------------------------|
|                                          | p | p<sup>c</sup> | p | p<sup>c</sup> |
| Sex<sup>1</sup>                           | 0.765 | 1 | 0.754 | 1 |
| Race<sup>1</sup>                          | 0.882 | 1 | 0.236 | 0.472 |
| Age<sup>1</sup>                           | 0.992 | 1 | 0.925 | 1 |
| Onset of symptoms<sup>1</sup>             | 0.526 | 1 | 0.929 | 1 |
| Onset of pulmonary disease<sup>1</sup>    | 0.666 | 1 | 0.607 | 1 |
| Onset of digestive disease<sup>1</sup>    | 0.595 | 1 | 0.793 | 1 |
| Diagnosis<sup>1</sup>                     | 0.242 | 0.484 | 0.277 | 0.554 |
| BMI<sup>1</sup>                           | 0.740 | 1 | 0.859 | 1 |
| Bshalla score<sup>2</sup>                 | 0.170 | 0.340 | 0.333 | 0.666 |
| Kanga score<sup>2</sup>                   | 0.913 | 1 | 0.828 | 1 |
| Swachman-Kulczycki score<sup>2</sup>      | 0.899 | 1 | 0.446 | 0.892 |
| Nasal polyposis<sup>1</sup>               | 0.910 | 1 | 0.854 | 1 |
| Diabetes mellitus<sup>1</sup>             | 0.531 | 1 | 0.891 | 1 |
| Osteoporosis<sup>1</sup>                  | 0.502 | 1 | 0.669 | 1 |
| Meconium ileous<sup>1</sup>               | 0.750 | 1 | 0.669 | 1 |
| Insufficiency pancreatic<sup>1</sup>      | 0.331 | 0.662 | 0.436 | 0.872 |
| SpO<sub>2</sub>                           | 0.342 | 0.684 | 0.684 | 1 |
| F VC<sup>2</sup>                          | 0.036 | 0.072 | 0.100 | 0.200 |
| FEV<sub>1</sub>,%<sup>2</sup>              | 0.142 | 0.284 | 0.153 | 0.306 |
| FEV<sub>1</sub>/FVC<sup>2</sup>            | 0.838 | 1 | 0.838 | 1 |
| FEF<sub>25-75</sub>%<sup>2</sup>          | 0.517 | 1 | 0.459 | 0.918 |
| 1st P. aeruginosa<sup>1</sup>             | 0.377 | 0.754 | 0.541 | 1 |
| P. aeruginosa mucoid<sup>1</sup>          | 0.553 | 1 | 0.517 | 1 |
| P. aeruginosa no mucoid<sup>1</sup>       | 0.230 | 0.460 | 0.797 | 1 |
| A. xylosoxidans<sup>1</sup>               | 0.502 | 1 | 0.888 | 1 |
| S. aureus<sup>1</sup>                     | 0.383 | 0.766 | 0.647 | 1 |
| B. cepacia<sup>1</sup>                    | 0.344 | 0.688 | 0.269 | 0.538 |

<sup>1</sup> Categorical variables - χ<sup>2</sup> test was used.

<sup>2</sup> Numerical variables - One-way analysis of variance test was used.

**Table 3:** Clinical association of cystic fibrosis variables with IFRD1 polymorphism (rs7817) and CFTR mutation.

### IFRD1 gene

#### Without taking CFTR mutation into account

| Genotype | Sex | Total | p<sup>i</sup> | Sex | Total | p<sup>i</sup> |
|----------|-----|-------|---------------|-----|-------|---------------|
|          | Female | Male |               | Female | Male |               |
| CC       | 14 | 14 | 28 | 1 | 9 | 10 | 19 |
| CT       | 19 | 16 | 35 | 1 | 11 | 8 | 19 |
| TT       | 15 | 10 | 25 | 1 | 11 | 8 | 19 |

#### With two CFTR mutations identified

| Genotype | Race | Total | p<sup>i</sup> | Race | Total | p<sup>i</sup> |
|----------|------|-------|---------------|------|-------|---------------|
|          | Caucasian | No caucasian |               | Caucasian | No caucasian |               |
| CC       | 26 | 2 | 28 | 1 | 17 | 2 | 19 |
| CT       | 33 | 2 | 35 | 1 | 18 | 1 | 19 |
| TT       | 24 | 1 | 25 | 1 | 19 | 0 | 19 |

#### IFRD1 gene

#### Without taking CFTR mutation into account

| Genotype | Age | Total | p<sup>i</sup> | Age | Total | p<sup>i</sup> |
|----------|-----|-------|---------------|-----|-------|---------------|
|          | ≤ 154 months | > 154 months |               | ≤ 154 months | > 154 months |               |
| CC       | 18 | 10 | 28 | 1 | 13 | 6 | 19 |
| CT       | 22 | 13 | 35 | 1 | 13 | 6 | 19 |
| TT       | 16 | 9 | 25 | 1 | 12 | 7 | 19 |

#### With two CFTR mutations identified

| Genotype | First clinical manifestation | Total | p<sup>i</sup> | First clinical manifestation | Total | p<sup>i</sup> |
|----------|-----------------------------|-------|---------------|-----------------------------|-------|---------------|
|          | ≤ 3 months | > 3 months |               | ≤ 3 months | > 3 months |               |
| CC       | 19 | 9 | 28 | 1 | 12 | 7 | 19 |
| CT       | 19 | 15 | 34 | 1 | 11 | 8 | 19 |
| TT       | 17 | 8 | 25 | 1 | 11 | 8 | 19 |
### Table 4: Association between IFRD1 polymorphism with clinical variables: sex, race, age, first clinical manifestation, time for diagnosis, time for the first digestive and pulmonary clinical manifestation and body mass index.

| Genotype | Diagnosis                  | Total | p<sup>c</sup> | Diagnosis                  | Total | p<sup>c</sup> |
|----------|----------------------------|-------|---------------|----------------------------|-------|---------------|
|          | ≤ 24 months                | > 24 months |               | ≤ 24 months                | > 24 months |               |
| CC       | 16                         | 10    | 26            | 10                         | 8     | 18            |
| CT       | 21                         | 13    | 34            | 14                         | 5     | 19            |
| TT       | 20                         | 5     | 25            | 15                         | 4     | 19            |

| Genotype | First digestive manifestation | Total | p<sup>c</sup> | First pulmonary manifestation | Total | p<sup>c</sup> |
|----------|-------------------------------|-------|---------------|-------------------------------|-------|---------------|
|          | ≤ 3 months                    | > 3 months |               | ≤ 6 months                    | > 6 months |               |
| CC       | 16                            | 10    | 26            | 10                            | 8     | 18            |
| CT       | 16                            | 17    | 33            | 11                            | 8     | 19            |
| TT       | 13                            | 12    | 25            | 9                             | 10    | 19            |

| Genotype | Body mass index | Total | p<sup>c</sup> | Body mass index | Total | p<sup>c</sup> |
|----------|-----------------|-------|---------------|-----------------|-------|---------------|
|          | 0               | 1     |               | 0               | 1     |               |
| CC       | 5               | 23    | 28            | 3               | 16    | 19            |
| CT       | 4               | 31    | 35            | 2               | 17    | 19            |
| TT       | 3               | 22    | 25            | 3               | 16    | 19            |

### Table 5: Association between IFRD1 polymorphism with comorbidities: nasal polyposis, diabetes mellitus, osteoporosis, pancreatic insufficiency and meconium ileus.

| Genotype | Without taking CFTR mutation into account | Two CFTR mutation identified |
|----------|-------------------------------------------|------------------------------|
|          | Nasal polyposis                           | Osteoporosis                 |
|          | Absence                                  | Presence                     | Absence          | Presence                     |
| CC       | 25                                        | 3                            | 28               | 17                           | 2                             | 19                           |
| CT       | 30                                        | 5                            | 35               | 16                           | 3                             | 19                           |
| TT       | 22                                        | 3                            | 25               | 17                           | 2                             | 19                           |

| Genotype | Diabetes mellitus                          | Osteoporosis                 |
|----------|--------------------------------------------|------------------------------|
|          | Absence                                  | Presence                     | Absence          | Presence                     |
| CC       | 21                                        | 7                            | 28               | 15                           | 4                             | 19                           |
| CT       | 30                                        | 5                            | 35               | 16                           | 3                             | 19                           |
| TT       | 21                                        | 4                            | 25               | 15                           | 4                             | 19                           |

| Genotype | Pancreatic insufficiency                   | Meconium ileus               |
|----------|-------------------------------------------|------------------------------|
|          | Absence                                  | Presence                     | Absence          | Presence                     |
| CC       | 1                                         | 27                           | 28               | 1                             | 18                           | 19                           |
| CT       | 2                                         | 33                           | 35               | 1                             | 18                           | 19                           |
| TT       | 0                                         | 25                           | 25               | 0                             | 19                           | 19                           |

| Genotype | Meconium ileus                            |                          |
|----------|-------------------------------------------|------------------------------|
|          | Absence                                  | Presence                     |
| CC       | 22                                        | 6                            | 28               | 15                           | 4                             | 19                           |
| CT       | 30                                        | 5                            | 35               | 16                           | 3                             | 19                           |
| TT       | 21                                        | 4                            | 25               | 17                           | 2                             | 19                           |

**IFRD1** = Interferon-Related Developmental Regulator 1; **CFTR** = Cystic Fibrosis Transmembrane Conductance Regulator; **p** = p-value to statistical tests corrected by Bonferroni test; ≤ = minor than; > = bigger than; C = Cytosine; T = Thymine; 0 = thinness and accentuated thinness; 1 = overweight/obesity and eutrophy.
two mutations identified belonging to class I, II and III. Categorical variables are described in absolute frequency and numerical by mean, standard deviation, minimum and maximum value, and confidence interval. In the tables 4-7, p-corrected values are presented.

Discussion
The evolution of CF as a disease is the result of the interaction between genotype and environment. Few studies have correlated CFTR mutations, modifier genes and clinical variables in CF [2,6,7,26], a fact associated with the difficulty in obtaining: (i) sample size, (ii) patients with homogeneous treatment, and (iii) to characterize the follow-up of manifestations, thus the role of the IFRD1 protein can have influence on the CF pulmonary process, which is the basis of the pathophysiology of the CF pulmonary disease. Hence, more studies are needed [17,18,27,28].

The principal environmental factor for the clinical variability of CF is the treatment access. In our referral center, treatment is warranted for the public health system, which allows equal access for all patients included in the study, and no concerns as an additional factor in the statistical analyzes.

The IFRD1 protein expression is not restricted to neutrophils, but may also occur in epithelial cells in organs that compose the airways from the bloodstream, compared to homozygotes. However, it is still unclear how the differential expression influences and governs the defense system is still unclear.

The neutrophil regulation is important in the inflammatory process, which is the basis of the pathophysiology of the CF pulmonary manifestations, thus the role of the IFRD1 protein can have influence on the CF severity [17]. In this sense, the analysis by array for 320 CF patients divided into two groups according to clinical severity showed that IFRD1 polymorphisms could function as modulators of clinical severity [20].

Other studies [17,18] have found a relationship of 57460C polymorphism in IFRD1 gene and the severity of lung disease in children and adolescents CF patients.

In our study, we did not find this association, even considering the expression and regulation of IFRD1 in different cellular types in order to understand the complex development of lung disease, hence, more studies are needed [17,18,27,28].

Two polymorphisms (rs11771128 and rs4727770) in the IFRD1 gene were associated with CF modulation [28]. Heterozygous patients for the polymorphism had higher levels of IFRD1 in neutrophils compared to homozygotes. However, it is still unclear how the differential expression influences and governs the defense system is still unclear.

Table 6: Association between IFRD1 polymorphism with bacteria on sputum.

| IFRD1 gene | Without taking CFTR mutation into account | Two CFTR mutation identified |
|------------|-----------------------------------------|-------------------------------|
| Genotype   | First P. aeruginosa                     |                              |
|            | ≤ 31 months | > 31 months | Total | p<sup>2</sup> | First P. aeruginosa | ≤ 31 months | > 31 months | Total | p<sup>2</sup> |
| CC         | 14         | 10          | 24    | 0.754         | 10               | 6           | 16      | 1     |
| CT         | 14         | 14          | 28    |              | 7                | 8           | 15      |       |
| TT         | 14         | 6           | 20    |              | 11               | 6           | 17      |       |
| Genotype   | MPA        | Total       |
|            | Absence    | Presence    |       | p<sup>2</sup> | Absence         | Presence    | Total   | p<sup>2</sup> |
| CC         | 13         | 15          | 28    | 1             | 10               | 9           | 19      | 1     |
| CT         | 21         | 14          | 35    |              | 13               | 6           | 19      |       |
| TT         | 13         | 12          | 25    |              | 10               | 9           | 19      |       |
| Genotype   | Staphylococcus aureus                   |                              |
|            | ≤ 31 months | > 31 months | Total | p<sup>2</sup> |                           |             |         |       |
| CC         | 7          | 21          | 28    | 0.460         | 6                | 13          | 19      | 1     |
| CT         | 16         | 19          | 35    |              | 8                | 11          | 19      |       |
| TT         | 9          | 16          | 25    |              | 7                | 12          | 19      |       |
| Genotype   | Burkholderia cepacia                    |                              |
|            | ≤ 31 months | > 31 months | Total | p<sup>2</sup> |                           |             |         |       |
| CC         | 22         | 6           | 28    | 1             | 16               | 3           | 19      | 1     |
| CT         | 31         | 4           | 35    |              | 15               | 4           | 19      |       |
| TT         | 22         | 3           | 25    |              | 16               | 3           | 19      |       |

IFRD1 = Interferon-Related Developmental Regulator 1; CFTR = Cystic Fibrosis Transmembrane Conductance Regulator; p<sup>2</sup> = p-value to statistical tests corrected by Bonferroni test; C = Cytosine; T = Thymine; ≤ = minor than; > = bigger than; MPA = mucoid P. aeruginosa; NM = non-mucoid P. aeruginosa; AX = Achromobacter xylosoxidans; BC = Burkholderia cepacia; SA = Staphylococcus aureus.
| Variable | IFRD1 genotype | N  | Mean | Std. Deviation | 95% Confidential Interval | Min | Max | p² |
|----------|----------------|----|------|----------------|---------------------------|-----|-----|----|
|          |                |    |      |                | Lower Bound               |     |     |    |
|          |                |    |      |                | Upper Bound               |     |     |    |
| SpO2     | CC             | 28 | 94.54| 6.173          | 92.14                     | 96.93| 66  | 98 | 0.684 |
|          | CT             | 34 | 95.79| 2.544          | 94.91                     | 96.68| 87  | 99 | 0.340 |
|          | TT             | 25 | 96.08| 2.871          | 94.89                     | 97.27| 86  | 99 |        |
| Bhalla   | CC             | 21 | 9.76 | 3.590          | 8.13                      | 11.40| 6   | 23 |        |
|          | CT             | 22 | 7.23 | 4.503          | 5.23                      | 9.22 | 0   | 23 |        |
|          | TT             | 19 | 7.74 | 5.496          | 5.09                      | 10.39| 0   | 22 |        |
| Kanga    | CC             | 26 | 18.46| 5.770          | 16.13                     | 20.79| 10  | 36 |        |
|          | CT             | 30 | 17.97| 5.512          | 15.91                     | 20.02| 11  | 33 | 1      |
|          | TT             | 22 | 18.64| 6.730          | 15.65                     | 21.62| 12  | 40 |        |
| Shwachman- Kulczycki | CC     | 26 | 66.92| 12.496        | 61.88                     | 71.97| 40  | 85 |        |
|          | CT             | 28 | 68.75| 13.026        | 63.70                     | 73.80| 40  | 90 |        |
|          | TT             | 24 | 67.92| 17.871        | 60.37                     | 75.46| 20  | 90 |        |
| FVC%     | CC             | 22 | 72.09| 17.318        | 64.41                     | 79.77| 29  | 105| 0.072 |
|          | CT             | 22 | 87.18| 18.887        | 78.81                     | 95.56| 58  | 131|        |
|          | TT             | 16 | 84.38| 24.055        | 71.56                     | 97.19| 41  | 135|        |
| FEV₁%    | CC             | 22 | 65.36| 20.127        | 56.44                     | 74.29| 19  | 114| 0.284 |
|          | CT             | 21 | 78.67| 24.836        | 67.36                     | 89.97| 36  | 132|        |
|          | TT             | 16 | 75.19| 22.013        | 63.46                     | 86.92| 27  | 100|        |
| FEV₁/FVC | CC             | 22 | 81.95| 16.114        | 74.81                     | 89.10| 37  | 102| 1      |
|          | CT             | 21 | 83.90| 11.291        | 76.77                     | 89.04| 58  | 99 |        |
|          | TT             | 16 | 84.31| 11.780        | 78.04                     | 90.59| 59  | 99 |        |
| FEF₂⁰,₇⁵% | CC             | 22 | 53.09| 31.355        | 39.19                     | 66.99| 8   | 134|        |
|          | CT             | 21 | 63.57| 33.817        | 48.18                     | 78.96| 13  | 121| 1      |
|          | TT             | 16 | 60.44| 23.639        | 47.84                     | 73.03| 11  | 88 |        |
| SpO2     | CC             | 19 | 94.63| 7.259         | 91.13                     | 98.13| 66  | 98 |        |
|          | CT             | 18 | 95.78| 2.045         | 94.76                     | 96.79| 92  | 98 | 1      |
|          | TT             | 19 | 95.84| 3.253         | 94.27                     | 97.41| 86  | 99 |        |
| Bhalla   | CC             | 15 | 9.40 | 3.961         | 7.210                     | 11.59| 6   | 23 | 0.666 |
|          | CT             | 11 | 6.73 | 3.319         | 4.500                     | 8.96 | 0   | 10 |        |
|          | TT             | 16 | 7.94 | 5.603         | 4.950                     | 10.92| 0   | 22 |        |
| Kanga    | CC             | 18 | 18.06| 6.197         | 14.97                     | 21.14| 10  | 36 | 1      |
|          | CT             | 15 | 18.53| 6.243         | 15.08                     | 21.99| 11  | 33 |        |
|          | TT             | 16 | 19.44| 7.339         | 15.53                     | 23.35| 12  | 40 |        |
| Shwachman- Kulczycki | CC     | 18 | 70.00| 12.005        | 64.03                     | 75.97| 40  | 85 | 0.892 |
|          | CT             | 15 | 66.00| 12.845        | 58.89                     | 73.11| 45  | 90 |        |
|          | TT             | 18 | 63.89| 17.703        | 55.09                     | 72.69| 20  | 90 |        |
| FVC      | CC             | 16 | 73.31| 18.930        | 63.23                     | 83.40| 29  | 105| 0.200 |
|          | CT             | 13 | 91.23| 22.391        | 77.70                     | 104.76| 58  | 131|        |
|          | TT             | 14 | 83.93| 24.765        | 69.63                     | 96.23| 41  | 135|        |
| FEV₁     | CC             | 16 | 66.00| 21.404        | 54.59                     | 77.41| 19  | 114| 0.306 |
|          | CT             | 13 | 83.92| 28.558        | 66.67                     | 101.18| 36  | 132|        |
|          | TT             | 14 | 74.57| 22.779        | 61.42                     | 87.72| 27  | 100|        |
| FEV₁/FVC | CC             | 16 | 83.00| 17.278        | 73.79                     | 92.21| 37  | 102| 1      |
|          | CT             | 13 | 86.15| 12.694        | 78.48                     | 93.82| 58  | 99 |        |
|          | TT             | 14 | 83.86| 12.322        | 76.74                     | 90.97| 59  | 99 |        |
| FEF₂⁰,₇⁵% | CC             | 16 | 55.81| 33.293        | 38.07                     | 73.55| 8   | 134|        |
|          | CT             | 13 | 70.92| 37.604        | 48.20                     | 93.65| 13  | 121| 1      |
|          | TT             | 14 | 60.86| 25.301        | 46.25                     | 75.47| 11  | 88 |        |

*IFRD1* = Interferon-Related Developmental Regulator 1; *CFTR* = Cystic Fibrosis Transmembrane Conductance Regulator; *p²* = p-value to statistical tests corrected by Bonferroni test; C = Cytosine; T = Thymine; N = number of patients; min = minimum; max = maximum; std = standard; SpO₂ = Transcutaneous oxygen saturation; FVC = forced vital capacity; FEV₁ = forced expiratory volume in the first second; FEF₂⁰,₇⁵% = forced expiratory flow between 25 and 75% of FVC.

**Table 7:** Association between *IFRD1* polymorphism with clinical variables with numerical distribution: lung function and clinical scores.
23 variables of clinical severity. We expected that CF patients would show lower expression of the IFRD1 protein and that the results would have association with clinical variables, especially those associated with pulmonary disease. Our results differ from those of previous studies possibly because earlier studies (i) considered homogeneous populations, (ii) used fewer clinical markers, (iii) did not consider IFRD1 polymorphisms, but rather only the amount of IFRD1 protein, (iv) evaluated fewer patients.

Conclusions

We found that in our sample of CF patients, there was no association of the polymorphism 57460C in the IFRD1 gene with the disease severity. Studies considering the analysis of other polymorphisms within the same gene or other genes, as modifier gene, must be considered. However, it is still necessary to study polymorphisms achieve a better understanding of the dynamics of the clinical manifestations and clinical variability of the disease, even in individuals with the identical CFTR genotype.

Competing Interests

The authors declare that they have no competing interests.

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