Polymorphisms in the glutathione pathway modulate cystic fibrosis severity: a cross-sectional study

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Abstract

Background: Cystic fibrosis (CF) clinically manifests with various levels of severity, which are thought to be modulated by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), modifier genes, and the environment. This study verified whether polymorphisms in modifier genes associated with glutathione (GSH) metabolism influence CF severity.

Methods: A cross-sectional study of 180 CF patients was carried out from 2011 to 2012. We analyzed CFTR mutations, polymorphisms (GSTM1 and GSTT1 deletions, GSTP1 + 313A > G, GCLC-129C > T, and GCLC-3506A > G) in modifier genes and CF clinical severity as assessed by 28 clinical and laboratory variables.

Results: Significant associations were found between modifier gene polymorphisms and particular phenotypes or genotype changes. These included GCLC-129C > T with a higher frequency of the Pseudomonas aeruginosa mucoid to CC genotype (p = 0.044), and GCLC-3506A > G with a higher frequency of the no-mucoid P. aeruginosa (NMPA) to AA genotype (p = 0.012). The GSTT1 deletion was associated with a higher frequency of the NMPA to homozygous deletion (p = 0.008), GSTP1 + 313A > G with a minor risk of osteoporosis (p = 0.036), and patient age ≤ 154 months (p = 0.044) with the AA genotype. The Bhalla score was associated with GCLC-3506A > G (p = 0.044) and GSTM1/GSTT1 deletion polymorphisms (p = 0.02), while transcutaneous hemoglobin oxygen saturation levels were associated with GSTT1 deletions (p = 0.048).

Conclusion: CF severity is associated with polymorphisms in GSH pathways and CFTR mutations.

Keywords: Cystic fibrosis, CFTR, GSH, GCLC, GST, Genotype, Phenotype, Modifier genes

Background

Cystic fibrosis (CF) presents with broad phenotypic variability, even in patients with identical mutations in the causative gene, cystic fibrosis transmembrane conductance regulator (CFTR) [1]. Explanations for this include environmental factors [2], medical management [3], nutritional status [4], emotional maladjustments [5], socioeconomic status [3], CFTR mutations [1], and modifier genes [1,3,6]. In this context, CF modifier genes have been studied with the aim of increasing chlorine transport and/or controlling pulmonary inflammation and infection [6-9].

Our group studied CF severity in association with several modifier genes including polymorphisms in the genes: MBL-2, TGF-β1, CD14 [10], ACE [11], ADRB2 [12], TCF7L2 [13], ADRA2A [14], COX-2 [15] and IFRD1 [16]. These polymorphisms were associated with clinical variables including lung and digestive disease.

Glutathione (GSH) is a tripeptide composed of L-cysteine, L-glutamic acid, and glycine. It is a crucial part of the intracellular defense system, which protects the epithelium against the injuries and inflammation [17] common to CF that are caused by oxidation [18]. As polymorphisms can alter the GSH metabolic pathway, genetic variations of this pathway have previously been studied in association with CF [19-21].
The glutathione S-transferase (GST) family of enzymes comprises proteins with distinct genetic origins that form a detoxification system, which protects the human body against electrophilic compounds and oxidative stress [22]. The GST protein is responsible for combining compounds that cause oxidative stress with GSH. It is therefore possible that GST polymorphisms are involved in CF severity [18,22], especially with regard to pulmonary disease.

Genetic variants of the GST genes include glutathione S-transferase mu 1 (GSTM1) located on chromosome 1p13.3, and glutathione S-transferase theta 1 (GSTT1) on chromosome 22q11.23 [23], which both exhibit polymorphic deletions [22,24]. The null GST allele does not encode a GST protein, so homozygous genotypes are associated with increased CF clinical severity [25,26]. The glutathione S-transferase pi gene (GSTPI) on chromosome 11q13 [23] is associated with xenobiopic metabolism and susceptibility to cancer and other diseases [22]. Its most commonly studied polymorphism is an A → G base exchange at the +313 position (substituting isoleucine by valine at codon 105) [27].

The glutamate-cysteine ligase, catalytic subunit gene (GCLC) on chromosome 6p12 [23] encodes the catalytic subunit of glutamate-cysteine ligase (GCL), which is the first limiting enzyme in GSH synthesis [28]. The GCL holoenzyme is a heterodimer of approximately 104 kDa composed of catalytic-GCLC and regulatory-GCLR subunits [18]. The -129C > T and -3506A > G polymorphisms of GCLC are located in the promoter region and are responsible for reduced production of GSH [18,28].

Of these genes, GSTPI is associated with hepatic disease [19] and infection [20], GSTM1 with greater CF clinical severity [21], GSTT1 with no CF clinical variables, while GCLC has not been previously studied in relation to CF. However, as the action of the GSH protein is closely related to that of CFTR [29], it is conceivable that GCLC and GST polymorphisms influence CF severity [19-21,26,30]. This study therefore aimed to determine whether genetic polymorphisms in the GSH metabolic pathway are associated with CF severity under different phenotypes of the disease.

Methods
This cross-sectional study was conducted in a university center for CF care between 2011 and 2012. Two hundred and fifteen patients were selected for the study, of which 35 were excluded for not signing the consent form or because of a lack of clinical data for statistical analysis. CF diagnosis was confirmed if levels of chloride in the sweat exceeded 60 mEq/L and by CFTR mutation screening when possible. CF patients, with no identified CFTR mutation or with one CFTR mutation screened, were classified as CF disease, considering: (i) all patients had levels of chloride in the sweat exceeded 60 mEq/L; (ii) CF clinical symptoms were diagnosed in all patients as: chronic obstructive pulmonary disease, bacteria in sputum, spirometry with obstruction values for forced expiratory volume in the first second (FEV1(%)), associated comorbidities (i.e. osteoporosis, nasal polyps, diabetes mellitus and pancreatic insufficiency); (iii) the dosage of active CFTR in epithelium via rectal biopsy was performed - all patients included had abnormal values for biopsy – absence of active CFTR was found; (iv) nasal potential was realized in some patients - all values were changed – but the comparison was not performed, taking into account a control standard curve, being an inconclusive data. By this method was possible to exclude Cystic Fibrosis Related Diseases.

No patients were diagnosed by a neonatal screening test. Patient DNA was obtained by phenol-chloroform extraction and 50 ng/mL was used for analysis as evaluated by a GE NanoVue® Spectrophotometer (GE Healthcare Biosciences, Pittsburgh, PA, USA).

Clinical variables
Several clinical variables were employed, including Swachman-Kulczycki, Kanga and Bhalla clinical scores [31]; body mass index (BMI) [for patients older than 19 years, the BMI = weight/(height)^2 formula was used, while remaining patients used the WHO ANTHRO program (children 0–5 years of age) or the WHO ANTHRO PLUS program (children 5–19 years of age)]; patient’s age (≤154 and >154 months); time to diagnosis (≤24 and >24 months); time of first clinical symptoms (digestive: 3 ≤ 3 months; pulmonary: 3 ≤ 6 months); time to first colonization by Pseudomonas aeruginosa (≤31 and >31 months); bacteria in the respiratory airways: mucoid P. aeruginosa and no mucoid P. aeruginosa, Achromobacter xylosoxidans, Burkholderia cepacia and Staphylococcus aureus - the positive status was evaluated considering chronic infection patients in whom more than 50% of the preceding 12 months was culture positive) + intermittent infection (patients with less than 50% of cultures positive). A patient was negative considering as free of bacterium (when no bacterium was grown from samples in the previous 12 months, despite a history of prior colonization) + never infected (patients in whom the bacterium) has never been cultured, i.e. this consensus was formulated for P. aeruginosa, but in our data was used for all bacteria [32]; transcutaneous hemoglobin oxygen saturation (SpO2) and spirometry variables.

Spirometry was performed in patients older than seven years of age with the CPFS/D spirometer (MedGraphics, Saint Paul, MN, USA) and data were recorded using the PF BREEZE software version 3.8B for Windows 95/98/NT [33]. The following variables were included: forced vital capacity [FVC(%)]; forced expiratory volume in the first second [FEV1(%)], the ratio between FEV1 and FVC(%) [FEV1/FVC(%)]; and forced expiratory flow between 25
and 75% of the FVC [FEF_{25-75}%]. The data was analyzed
considering international curves values for spirometry
Tests [34,35].

The comorbidities analyzed were nasal polyps, osteopor-
osis, meconium ileus, diabetes mellitus, and pancreatic insufficiency. This study was approved by the Institutional Ethics Committee from the Faculty of Medical Sciences, University of Campinas (#528/2008), and all included patients or their parents signed a consent form before beginning the study.

**CFTR mutation identification**

*CFTR* mutation identification was performed by polymerase chain reaction (PCR) for F508del and the fragment-length polymorphism method for G542X, R1162X, R553X, G551D, and N1303K mutations. Some CF mutations were identified by sequencing or Multiplex Ligation-dependent Probe Amplification (MLPA) analysis: S4X, 2183A > G, 1717-G > A, and I618T. A MegaBace1000® sequencer (GE Healthcare Biosciences) was used for sequencing and MLPA.

The *CFTR* genotype was used as a correction factor for statistical analysis. All class I, II or III mutations, but not class IV mutations (P205S and R334W), identified were included in statistical analysis.

**Identification of polymorphisms associated with GSH metabolic pathway genes**

Polymorphism identification was carried out using PCR
analysis. For *GSTM1* and *GSTT1* genes, a multiplex PCR reaction was performed using the *CYP1A1* gene as an internal amplification control [36]. *GCLC*-129C > T, -3506A > G [18,28] and *GSTP1* +313A > G [27] polymorphisms were identified by PCR followed by enzymatic digestion.

**Statistical analysis**

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 21.0 (SPSS Inc., Chicago, IL, USA), Epi Info version 6.0 [37] and R version 2.12 (Comprehensive R Archive Network, 2011). GPower 3.0.3.1 software [38] was used to calculate the statistical power, which was required to be above 80% for analysis.

Statistical tests included the analysis of variance (ANOVA) and the chi-square (χ²) test (Odds Ratio -OR) for *GSTM1* and *GSTT1*, and the t-test and Fisher’s exact test for *GCLC*-129C > T, *GCLC*-3506A > G and *GSTP1* +313A > G polymorphisms. To avoid spurious significance level (α), the significance level (α) was corrected by the Bonferroni correction (α_corrected = 0.05/number of tests → 0.05/4 = 0.0125). The value of α was corrected considering clinical marker analysis of the same group of patients, taking into account, the *CFTR* mutation genotype.

Data distribution showing a high standard deviation was analyzed in groups distributed according to median value. Variables that were adjusted by median to short (more severe) and longtime were patient’s age, time to diagnosis, onset of pulmonary and digestive symptoms, and time to the first isolation of *P. aeruginosa*.

Analyses were performed of four cohorts: (i) all patients with CF (n = 180); (ii) patients with no identified *CFTR* mutation (n = 44); (iii) patients with an identified mutant *CFTR* allele (Class I, II and/or III) (n = 51); and (iv) patients with two identified *CFTR* mutations (Class I, II and/or III) (n = 85). For (ii) and (iii) groups, a second analysis was performed. In this case, patients with pancreatic sufficiency (PS) were excluded. Patients with mutations Class I, II and III for *CFTR* gene have severe disease, strongly associated with pancreatic insufficiency (PI). Excluding PI patients was a method to associated different *CFTR* mutation groups with no atypical CF – associated less severe mutation (Class IV, V and VI). After exclusion, we have in (ii) and (iii) groups, respectively, 35 and 43 CF patients.

**Results and discussion**

One of the most intriguing aspects of CF is that patients with the same *CFTR* genotype can present with phenotypic differences [40]. At our CF center, all patients receive free medication provided by the state, have a similar socioeconomic status, share similar Class I, II and/or III mutations, receive support from the Cystic Fibrosis Association (http://www.fibrocis.org.br/), and there are no severe cases of malnutrition. This therefore makes our sample more phenotypically homogeneous for studies involving gene modulation characteristics.

Variations in CF severity can be associated with a modifier gene, such as those associated with oxidative stress [19-21,26,30] that are related to chronic obstructive pulmonary disease (COPD) [18]. The COPD pathophysiology is similar, in some aspects, to CF in that it involves cellular responses, inflammatory mediators, and oxidative stress [41]. However, there is no mention in the scientific literature of GCLC polymorphisms as clinical modulators of CF severity, and is necessary new studies to illuminate about GST genes and CF severity.

One of the main functions of GSH is to detoxify xenobiotics and their metabolites, and this function is dependent on GST proteins. The *GSTM* gene family has been linked with several diseases [22], as *GSTM1, GSTT1* and *GSTP1* polymorphisms were found to be associated with cancer, drugs, chemotherapy resistance [42], and respiratory diseases such as asthma [30]. For example, expression of the variant form of *GSTP1* (where isoleucine is substituted for valine at codon 105) results in lower enzymatic activity, which is a risk
factor for the development of cancer and pulmonary diseases such as CF [43].

The effects of GSTM1, GSTT1 and GSTP1 polymorphisms on spirometry were previously investigated in 1,940 children (aged 8–11 years) [44]. The null GSTM1 genotype was associated with a decrease in annual FVC(%) and FEV1(%) gain; likewise, homozygosity for the GSTP1 allele was linked with slower spirometric gain for the same markers. The GSTM1 and GSTP1 genotypes therefore appear to be associated with spirometric evolution, and could increase the severity of diseases of pulmonary obstruction, depending on the genotype and gene combination.

Table 1 shows the GCLC, GSTM1, GSTT1 and GSTP1 polymorphism distribution according to genotype in the present study. The -129C > T polymorphism in the promoter region of GCLC stimulates different responses to oxidative stress by decreasing GSH production and reducing cellular antioxidant capacity [45]. In the present study, it was associated with a higher frequency of the mucoid P. aeruginosa to CC genotype for GCLC-129C > T polymorphism in patients with one CFTR mutation identified (Table 2; \( p = 0.044 \)). This association may be related to the lower GCLC protein expression in CC genotypes, which reduces circulating GSH levels. The T allele is also associated with increased GSH expression, as described in protein expression studies on cardiovascular disease [45]. In the cited literature, we only found one study that associated the GCLC polymorphism with CF severity. In this previous study, the GAG micro-satellite polymorphism was analyzed in 440 CF patients, and CFTR mutations of lower gravity and highest number of GAG repeats in the GCLC gene were associated with higher values of FEV1(%) [20].

The GCLC-3506A > G polymorphism is not in Hardy-Weinberg equilibrium as shown in Table 3, which also shows the complete genotypic characteristics of GCLC, GSTM1, GSTT1, and GSTP1 polymorphisms and CFTR mutations in CF patients with regard to chromosomal position, polymorphism location within the gene, and minor allele frequency. GCLC-3506A > G was associated with a higher frequency of the no mucoid P. aeruginosa to AA genotype, and with a lower frequency of the no mucoid P. aeruginosa to AG + GG genotype group in patients with one CFTR mutation identified (Table 2; \( p = 0.012 \)) and higher Bhalla score values (without taking CFTR mutation into account; \( p = 0.044 \)).

The Bhalla score is associated with an impairment of the pulmonary parenchyma structure and higher values characterize major changes in thoracic tomography. Unexpectedly, we also found that the greatest expression of the A allele in the GCLC-3506A > G polymorphism did not protect against no mucoid P. aeruginosa colonization. However, protection against lung deterioration was evident when we considered the Bhal score. This score was also associated with GSTM1/GSTT1 deletions (\( p = 0.02 \)), with a lower frequency of heterozygous compared with homozygous deletions. Moreover, the GSTT1 deletion was found to be associated with SpO2 values (\( p = 0.048 \); Table 4).

GCLC haplotype analysis for GCLC-129C > T and GCLC-3506A > G showed association for A. xylosoxidans and CC + AA genotypes (OR = 17.9; CI95% = 2.781-411.6; Table 5).

The present study found that the AA genotype of the GSTP1 + 313A > G polymorphism was associated with a low risk of osteoporosis (\( p = 0.036 \); with two CFTR mutations identified) as a protective factor and with young

Table 1 Distribution of GCLC, GSTM1, GSTT1 and GSTP1 polymorphisms

| Gene   | Polymorphism   | Genotypes (N analyzed and %) | Grouping (N analyzed and %) | Total |
|--------|----------------|-------------------------------|-----------------------------|-------|
| GCLC  | -129C > T²     | CC (80.11%)                  | 145 (80.11%)                | 181 (100%) |
|        |                | CT (16.02%)                  | 29 (16.02%)                 |       |
|        |                | TT (3.87%)                   | 7 (3.87%)                   |       |
|        | -3506A > G³    | AA (65.75%)                  | 119 (65.75%)                | 181 (100%) |
|        |                | AG (30.94%)                  | 56 (30.94%)                 |       |
|        |                | GG (3.31%)                   | 6 (3.31%)                   |       |
| M1    | Deletion²      | -                             | 73 (40.33%)                 | 181 (100%) |
| T1    | Deletion³      | -                             | 108 (59.67%)                |       |
| M1/T1 | Deletion      | -/−                          | 63 (34.81%)                 | 181 (100%) |
|       |                | +/-                          | 118 (65.19%)                |       |
| GSTP1 | +313A > G⁴    | AA (54.14%)                  | 98 (54.14%)                 | 181 (100%) |
|        |                | AG (40.88%)                  | 74 (40.88%)                 |       |
|        |                | GG (4.98%)                   | 9 (4.98%)                   |       |

GSTM1, Glutathione S-transferase Mu; GSTT1, Glutathione S-transferase Theta 1; GSTP1, Glutathione S-transferase Pi 1; GCLC, Glutamate-cysteine ligase, catalytic subunit; N, Sample size; −, Null allele; +, Expressed allele.

The statistical association, taking into account the CFTR mutation groups, with the polymorphisms distribution was by p-values in the table: \( 0.880 (\text{GCLC-129C} > \text{T}) \); \( 0.075 (\text{GCLC-3506A} > \text{G}) \); \( 0.969 (\text{M1}) \); \( 0.088 (\text{T}) \); \( 0.329 (\text{GSTP1} + 313A > \text{G}) \).
The role of the \( \text{GSTP1} \) polymorphism in CF hepatic disease has previously been analyzed [19]. The authors noted that CFTR protein expression was limited in liver epithelium; however, recent discoveries indicate that CFTR modulates the transport of GSH, creating a dysfunction in the antioxidant defense [47]. Of the liver detoxifying enzymes, GST plays a major role in protection against oxidative stress. The impact of \( \text{GSTM1} \) and \( \text{GSTP1} \) was also previously assessed in 106 CF patients where it was verified that the frequency of the GG genotype for the \( \text{GSTP1} \) + 313A > G polymorphism was significantly higher in CF patients with hepatic disease. This genotype was associated with an eight-fold increase in hepatic disease risk in patients younger than six years of age. These findings suggest that the identification of this polymorphism may have prognostic and awareness values for the treatment of CF patients with hepatic disease.

Considering the importance of the glutathione transport versus CFTR protein-mediated, patients with residual CFTR protein expression would have better performance in the extracellular oxidative stress response being favorable for the passage of GSH to the outside by residual CFTR activity. However, CF patients with two mutations screened in \( \text{CFTR} \) gene have principally alternate routes for the passing of GSH. Even taking into account that the most of GSH is transferred to the external environment via CFTR, in cases of residual CFTR (mutations Class IV, V and VI) would be modified slightly in relation to the GSH activity, since it is known that under 5% of CFTR expression occurs in pancreatic 

### Table 2 Polymorphisms in modifier genes associated with categorical variables of cystic fibrosis severity

| CFTR group | Polymorphism | Genotype | Variable | p\textsuperscript{5} | OR | CI (5–95%) |
|------------|--------------|----------|----------|----------------------|----|-----------|
| One CFTR mutation identified | GCLC-129C > T | Presence | PAM |  |  |  |
|  |  | Absence | Total |  |  |  |
| CC | 25 | 17 | 42 | 0.044 | 11.27 | 1.6–272.6 |
| CT + TT | 1 | 8 | 9 | - | - | - |
| GCLC-3506A > G | PANM | Presence | Absence | Total |  |  |
| AA | 28 | 9 | 37 | 0.012 | 7.408 | 1.905–33.43 |
| AG + GG | 4 | 10 | 14 | - | - | - |
| No mutation identified | GSTT1 gene deletion | PANM | Presence | Absence | Total |  |  |
| Not expressed | 13 | 9 | 21 | 0.008 | 7.895 | 2.095–34.96 |
| Expressed | 4 | 23 | 27 | - | - | - |
| One CFTR mutation identified + IP | GSTM1 gene deletion | Digestive symptoms | < 6 months | ≥ 6 months |  |  |
| Not expressed | 3 | 12 | 15 | 0.032 | 0.134 | 0.023–0.606 |
| Expressed | 14 | 7 | 21 | - | - | - |
| Two mutations identified | GSTP1 + 313A > G | Osteoporosis | Presence | Absence | Total |  |  |
| AA | 2 | 42 | 44 | 0.036 | 0.141 | 0.028–0.687 |
| AG + GG | 9 | 26 | 35 | - | - | - |
| Without taking \( \text{CFTR} \) mutation into account | Age (months) | ≤ 154 | > 154 | Total |  |  |
| AA | 58 | 39 | 97 | 0.044 | 2.198 | 1.208–4.037 |
| AG + GG | 33 | 49 | 82 | - | - | - |

Statistical analysis was performed by Fisher’s exact test. \( \text{CFTR} \), Cystic fibrosis transmembrane regulator; \( \text{GCLC} \), Glutamate-cysteine ligase catalytic subunit; \( \text{GSTM1} \), Glutathione S-transferase mu 1; \( \text{GSTT1} \), Glutathione S-transferase theta 1; \( \text{GSTP1} \), Glutathione S-transferase Pi 1; PI, Pancreatic insufficiency; PANM, Pseudomonas aeruginosa mucoid; PAM, Pseudomonas aeruginosa no mucoid; \( p \textsuperscript{5} \), P-value corrected by Bonferroni test; OR, Odds ratio; CI, Confidence interval.

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presence of PI patients enables better grouping of patients and optimizes the response of the associations found in our study.

Most studies analyzing the GSTP1 gene related it to cancer and other diseases [22,30,44,48]. For example, the AA genotype of the GSTP1 +313A > G polymorphism was shown to offer protection against asthmatic symptoms [22]. Indeed, the GSTP1 polymorphism was not previously found to affect pulmonary function in CF patients [30]. In an analysis of different genes involved in GST, there were no differences in GST activity and antioxidant levels observed between CF patients and controls. However, GST activity was lower in P. aeruginosa-infected CF children with severe clinical symptoms, as was the frequency of the GSTP1 +313A > G polymorphism AA genotype in uninfected (75%) compared with infected (33%) children [21]. It is possible that GST activity and GSTP1 genotype play an important role in P. aeruginosa infection in CF patients. In support of this, the G allele of the GSTP1 gene appears to be associated with an increased risk of severe pulmonary disease [21]. However, in a previous investigation into GSTM1 and GSTP1 polymorphisms in patients with CF and COPD, no significant associations were found between GSTM1 activity and pulmonary disease severity. An analysis of genotypic combinations for GSTM1 and GSTP1 polymorphic loci showed that changes in GSTP1 activities

| Table 3 Genotyping of GCLC, GSTM1, GSTT1, and GSTP1 polymorphisms and CFTR mutations |
|------------------------------------------|------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Gene | Chromosomal position | Location | Polymorphism | MAF | HWE | p-value* |
|------|----------------------|----------|---------------|-----|-----|---------|
| GCLC, rs17883901 | 6p12 | Promoter region | C > T | 0.12 | 9.97 | <0.005 |
| GCLC, rs137852340 | 6p12 | Promoter region | A > G | 0.19 | 0.04 | >0.05 |
| GSTP1, rs1695 | 11q13 | Exon | A > G | 0.25 | 1.11 | >0.05 |
| GSTM1 | 1p13.3 | Deletion | | | | |
| GSTT1 | 22q11.23 | Deletion | | | | |

**CFTR mutation** | **N** | **Frequency** |
|-----------------|--------|---------------|
| F508del/F508del | 57     | 31.67%         |
| F508del/G542X    | 12     | 6.67%          |
| F508del/R1162X   | 5      | 2.78%          |
| F508del/N1303K   | 4      | 2.22%          |
| F508del/R553X    | 1      | 0.56%          |
| F508del/S4X      | 1      | 0.56%          |
| F508del/1717-1G > A | 1 | 0.56% |
| GS42X/R1162X     | 1      | 0.56%          |
| GS42X/I618T      | 1      | 0.56%          |
| GS42X/2183A > G  | 1      | 0.56%          |
| R1162X/R1162X    | 1      | 0.56%          |
| F508del/-        | 45     | 25.00%         |
| GS42X/-          | 5      | 2.78%          |
| R1162X/-         | 1      | 0.56%          |
| −/−               | 44     | 24.45%         |

| MAF, Minor allele frequency; HWE, Hardy Weinberg Equilibrium; *P-value for Hardy-Weinberg Equilibrium; N, Number of patients; −, No identified CFTR mutation. |

| Table 4 Polymorphisms in modifier genes associated with numerical variables of cystic fibrosis severity |
|------------------------------------------------------------------------------------------------|
| **CFTR group** | **Variable** | **Polymorphism** | **Genotype** | **N** | **Mean** | **SD** | **SEM** | **p-value corrected** |
|----------------|--------------|------------------|--------------|------|----------|--------|--------|----------------------|
| Without taking CFTR mutation into account | Bhalla scorea | GCLC-3506A > G | AA | 94     | 19.70   | 6.007  | 0.620  | 0.044                |
| | | | AG + GG | 43 | 17.00 | 5.033 | 0.768 |
| No mutation identified | SpO2a | GSTT1 deletion | Not expressed | 15 | 96.13 | 2.232 | 0.576 | 0.048 |
| | | | Expressed | 29 | 93.17 | 5.245 | 0.974 |
| Bhalla scoreb | GSTM1/GSTT1 deletions | −/− | 4 | 14.75 | 1.258 | 0.629 | 0.02 |
| | | | +/- and +/+ | 21 | 6.900 | 6.610 | 1.442 |
| | | | ++ | 9 | 15.33 | 7.533 | 2.511 |

*aUsing Student’s t-test; bUsing analysis of variance. |

**CFTR**, Cystic fibrosis transmembrane regulator; SpO2, Hemoglobin oxygen saturation in the blood; −, Null allele; +, Expressed allele; N, Number of patients.
produced adverse effects in patients with COPD. Although GSTM1 gene deletions may not themselves be implicated in pathogenesis, they may aggravate the disease in combination with GSTP1 polymorphisms. Perhaps the strongest performance for the GSTP1 gene in CF may result from the primary expression of this GST in the airways [48].

The present study showed that the homozygous deletion in GSTT1 was a no mucoid P. aeruginosa risk factor in the no CFTR mutation group (p = 0.008; Table 2) and a protective factor for low values of SpO2. GSTT1 expression is likely to act in the inflammatory response of the pulmonary parenchyma. As chronic airway infection by no mucoid P. aeruginosa is associated with greater clinical severity [49], the GSTT1 polymorphism may be associated with the presence of P. aeruginosa through different mechanisms, including a low antioxidant response leading to further pulmonary degradation and the formation of a favorable environment for no mucoid P. aeruginosa colonization or infection.

The mechanism of gene action that determines which bacteria can colonize the lungs of CF patients is not fully understood. Similarly, it is also unclear which microorganisms are risk factors for the disease. Therefore, confirmation of a gene acting as modulator of an important metabolic pathway, such as GSH, may open up novel ways to identify the genetic factors that determine the severity of pulmonary disease. Future pharmacogenetic studies could then use this knowledge to provide new CF therapies.

Many previous studies have revealed that polymorphisms of GSTM1 and GSTT1 are associated with cancer [22,24,50,51], but few have been conducted in CF. Fifty-three children with CF were studied by Hull and Thomson [26], of which 26 with the GSTM1 null allele had a significantly lower Shwachman-Kulczycki score. This supports the hypothesis that inflammation in CF contributes to tissue injury. Indeed, GSTM1 null alleles can be a risk factor for pulmonary diseases in individuals with a reduced ability to deal with oxidants. There is also evidence that a high level of oxidative stress in the lungs of CF patients is caused by the release of reactive oxygen species by neutrophils [26]. In the present study, we found that expression of only one allele of GSTM1 and GSTT1 polymorphisms was associated with a low Bhalla score in patients with no CFTR mutation identified.

An interesting aspect was the high frequency of PS patients. The presence of PS occurred at exactly 20% of the sample. However, there was no difference distribution between the groups of patients with CF taking into account CFTR mutations groups (p = 0.621). Patients with two mutations identified in CFTR gene had 22.36% (19/85) of PS, values close to the other groups of patients [one identified mutation and no mutation identified with, respectively, 15.7% (8/51) and 20.5% (9/44)].

| CFTR group | PI taking into account | Haplotype | Genotype | Variable | p<sup>c</sup> | OR | CI (5–95%) |
|------------|------------------------|-----------|----------|----------|-------------|----|------------|
| Two mutation identified | No | GCLC-129C > T + GCLC-3506A > G | CC + AA | Presence | 10 | 25 | 36 | 17.9 | 2.781-411.6 |
| | | | CC + (AG or GG) | Absence | 1 | 30 | 31 | 0.024 | 0.149 | 0.007-0.959 |
| | | | (CT or TT) + GG | Total | 0 | 13 | 13 | - | - |
| | | | TT + GG | - | 0 | 5 | 5 | - | - |

Statistical analysis was performed by χ<sup>2</sup> test. CFTR, Cystic fibrosis transmembrane regulator; GCLC, Glutamate-cysteine ligase catalytic subunit; PI, Pancreatic insufficiency; AX, Achromobacter xylosoxidans; p<sup>c</sup>, P-value corrected by Bonferroni test; OR, Odds ratio; CI, Confidence interval.

| Pancreatic status | Groups | N | Mean (months) | Standard deviation | Confidential interval | Minimum (months) | Maximum (months) | p-value | Osteoporosis (N/%) | p-value |
|-------------------|--------|---|--------------|--------------------|-----------------------|------------------|------------------|---------|-------------------|---------|
| No mutation identified | 44 | 211.75 | 217.501 | 145.62 | 277.88 | 20 | 932 | 8 (19%) |
| Pancreatic insufficiency | One CFTR mutation identified | 51 | 201.18 | 165.050 | 154.76 | 247.60 | 11 | 782 | 0.854 | 9 (17.6%) |
| Two mutation identified | 84 | 220.06 | 188.643 | 179.12 | 261.00 | 7 | 1274 | 12 (14.3%) | 0.761 |
| Pancreatic insufficiency | One CFTR mutation identified | 35 | 221.57 | 216.17 | 147.31 | 295.83 | 25 | 932 | 7 (21.2%) |
| Two mutation identified | 43 | 198.81 | 171.31 | 146.09 | 251.54 | 11 | 782 | 0.940 | 5 (11.6%) | 0.345 |

CFTR, Cystic fibrosis transmembrane regulator; N, number of patients.
The PI is an important clinical marker of CF and is considered associated with the severity of disease and severe CFTR mutations (Class I, II and/or III). Studies considering populations of patients with CF, as performed by the Cystic Fibrosis Foundation give the prevalence of PI ranging from 5-10%. In our study, the high prevalence of PI may be associated with: (i) presence of higher frequency of mutations Class IV, V and/or VI, (ii) presence of modifier genes acting on the symptom of the disease, (iii) high miscegenation could be a protective factor for PI, (iv) environmental factor as an unknown protector.

The PI was used in statistical analysis as factor correction for no determination of CFTR mutation in CF groups with no or one CFTR mutation screened. After the patient exclusion to statistical analysis, all the previous positive associations were negative, except for GSTM1 null allele. The null allele was associated as protector factor for onset of digestive symptoms (OR = 0.134; CI = 0.023-0.606; Table 2).

One important aspect considered was the age. Before the statistical analysis, the age was considered between the CFTR mutations groups (p = 0.854). The same occurred for CFTR mutations groups + insufficiency pancreatic (p = 0.940) (Table 6). No positive association was find considering age.

The divergent immune response is associated with multiple factors that denote the CF complexity such as the multigenic response, environmental influences, and interaction between airway microorganisms [49,52]. Clinically severe patients may have high initial inflammatory response, characterizing CF as a disease where inflammation occurs prior to infection [53]. Polymorphisms in genes that are involved in inflammation may be a risk factor for early severity of the disease [1], and patients with airways colonized by bacteria suffer early clinical deterioration and high levels of airway inflammation [54].

For the same population, a first study taking into account the same polymorphisms and clinical variables was performed. The previous data analyzed the genetic interaction among GST and GCLC polymorphisms, CFTR mutations and clinical markers. The data showed an interaction of GSTM1 and GSTT1 genes deletion, GSTP1 + 313A > G, and CFTR mutations (p = 0.008) and Bhalla clinical score by multifactor dimensionality reduction test. The Bhalla score is a computed tomography, which measures pulmonary involvement, therapeutic effects and selection of patients for transplantation, which detects anatomical changes of the lung parenchyma. 

In the present study, we studied a CF population with complex clinical characteristics. By considering the different possible groupings of polymorphisms and clinical variables (Table 7) in relation to the CFTR gene, we performed various association studies. Supplementary data for GCLC-129C > T, GCLC-3506A > G, GSTM1 gene deletion, GSTT1 gene deletion, GSTM1/GSTT1 gene deletions and GSTP1 + 313A > G are shown in Tables 8, 9, 10, 11, 12, 13 and 14. Further multicenter studies should be conducted to verify the influence of modifier genes in different CFTR genotypes.

Study limitations: (i) CFTR mutation with no complete screening; (ii) short population of CF patients; (iii) spirometry test performed by transversal method and did no performed longitudinally; (iv) no measure of GSH activity or GST and GCLC proteins, taking into account the sample collection limitation in our center and time to process

| Characteristic                      | Male gender | 50% (90) |
|------------------------------------|-------------|----------|
| Age (months)                       | 212 ± 15.75 (7-288) |
| Caucasoïd                          | 91.75%      |
| BMI - thiness and accentuated thiness | 22.2% (40) |
| One class I, II or III identified mutation | 28.3% (51) |
| Two class I, II or III identified mutations | 47.2% (85) |
| Age at first clinical manifestation (months) | 35 ± 8.88 (0-156) |
| Age at diagnosis (months)          | 87 ± 13.63 (0-170.76) |
| Age at start of digestive symptoms (months) | 40.6 ± 9.11 (0-149.4) |
| Age at start of pulmonary symptoms (months) | 34.8 ± 9.88 (0-1156) |
| SpO2(%)                            | 94.92 ± 4.26 (66-99) |
| Bhalla                             | 8.74 ± 5.72 (0-25) |
| Kanga                              | 18.85 ± 5.84 (10-40) |
| Shwachman-Kulczycki                | 65.85 ± 16.77 (20-95) |
| FVC(%)                             | 79.29 ± 23.55 (19-135) |
| FEV1(%)                            | 71.29 ± 27.467 (17-132) |
| FEV1/FVC(%)                        | 83.46 ± 15.95 (37-137) |
| FEF25–75,%                         | 59.05 ± 35.55 (7-150) |
| Nasal polyps                       | 18.33% (33) |
| Diabetes mellitus                  | 18.33% (33) |
| Osteoporosis                       | 16.11% (29) |
| Pancreatic insufficiency           | 80.0% (144) |
| Meconium ileus                     | 15.00% (27) |
| Age at first isolated P. aeruginosa (months) | 102.6 ± 14.45 (24-180) |
| P. aeruginosa status               | 56.67% (102) |
| P. aeruginosa mucoid status        | 42.22% (76) |
| B. cepacia status                  | 13.88% (25) |
| A. xylosidans status               | 10.00% (18) |
| S. aureus status                   | 78.88% (142) |

Continuous variables expressed as mean ± SD (range). Other data shown as percentage (number of patients). *Based on three consecutive positive respiratory cultures. N, Sample size; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF25–75, Forced expiratory flow between 25 and 75% of FVC.
Table 8 GCLC-129C > T polymorphism associated with CF clinical variables as distributed by CFTR mutation

| Variable                           | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected |
|------------------------------------|------------------------------------------|------------------------------|--------------------------------------|------------------------------|--------------------------------------|---------------------------------|---------|------------|---------|------------|---------|------------|---------|------------|
| Gender*                            | 0.577                                    | 1                            | 1                                    | 1                            | 1                                    | 0.024                          | 0.096                           | 0.698   | 0.249      | 0.418   | 0.996      |
| Age†                               | 0.348                                    | 1                            | 1                                    | 0.667                        | 1                                    | 1                              | 1                              | 1       | 0.168      | 0.764   |
| Onset of symptoms*                 | 0.162                                    | 0.648                        | 1                                    | 1                            | 0.409                                | 1                              | 1                              | 0.650   | 0.583      | 1       |
| Onset of pulmonary disease*        | 0.142                                    | 0.568                        | 1                                    | 0.354                        | 1                                    | 0.710                          | 1                              | 0.660   | 1          | 1       |
| Onset of digestive disease*        | 1                                        | 1                            | 0.405                                | 1                            | 0.715                                | 1                              | 0.412                          | 1       | 0.764      | 1       |
| Diagnosis*                         |                                          |                              |                                      |                              |                                      |                                |                                 |         |            |         |
| BMI†                               | 0.626                                    | 1                            | 0.47                                 | 1                            | 0.023                                | 0.092                          | 0.851                          | 0.090   | 0.360      | 0.834   |
| Bhalla score*                      | 0.277                                    | 1                            | 0.45                                 | 1                            | 0.632                                | 1                              | 0.687                          | 1       | 0.192      | 0.768   |
| Kanga score*                       | 0.917                                    | 1                            | 0.532                                | 1                            | 0.041                                | 0.164                          | 0.405                          | 1       | 0.043      | 0.767   |
| Shwachman-Kulczycki score*         | 0.811                                    | 1                            | 0.66                                 | 1                            | 0.555                                | 1                              | 0.332                          | 1       | 0.066      | 0.264   |
| Nasal polyposis*                   | 0.811                                    | 1                            | 1                                    | 1                            | 0.951                                | 1                              | 0.951                          | 1       | 0.905      | 1       |
| Diabetes mellitus*                 | 0.306                                    | 1                            | 0.299                                | 1                            | 0.333                                | 1                              | 0.286                          | 1       | 0.286      | 1       |
| Osteoporosis*                      | 0.792                                    | 1                            | 0.576                                | 1                            | 0.651                                | 1                              | 0.147                          | 0.588   | 0.727      | 1       |
| Meconium ileus                     | 0.063                                    | 0.252                        | 0.267                                | -                            | -                                    | 0.328                          | 1                              | 0.036   | 1          | 1       |
| Pancreatic insufficiency*          | 0.384                                    | 1                            | 0.296                                | 1                            | 0.078                                | 0.312                          | 0.124                          | 0.496   | 0.864      | 1       |
| SpO2*                              | 0.822                                    | 1                            | 0.828                                | 1                            | 0.127                                | 0.508                          | 0.922                          | 1       | 0.506      | 0.597   |
| FVC(%)†                            | 0.598                                    | 1                            | 0.310                                | 1                            | 0.160                                | 0.640                          | 0.983                          | 1       | 0.510      | 0.820   |
| FEV1(%)†                           | 0.109                                    | 0.436                        | 0.386                                | 1                            | 0.873                                | 1                              | 0.820                          | 1       | 0.170      | 0.680   |
| FEV1/FVC                           | 0.048                                    | 1                            | 0.044                                | 0.176                        | 1                                    | 0.082                          | 1                              | 0.767   | 0.537      | 1       |
| First P. aeruginosa*               | 0.133                                    | 0.532                        | 1                                    | 1                            | 0.691                                | 1                              | 0.695                          | 1       | 0.361      | 1       |
| P. aeruginosa mucoid*              | 0.534                                    | 1                            | 1                                    | 1                            | 0.266                                | 1                              | 0.680                          | 1       | 0.391      | 1       |
| P. aeruginosa no mucoid*           | 0.261                                    | 1                            | 0.093                                | 0.372                        | 1                                    | 1                              | 1                              | 1       | 1          | 1       |
| A. xylosidans*                     | 0.656                                    | 1                            | 1                                    | 1                            | 1                                    | 1                              | 1                              | 1       | 1          | 1       |
| S. aureus*                         | 0.318                                    | 1                            | 1                                    | 1                            | 1                                    | 1                              | 1                              | 1       | 1          | 1       |

*Fisher’s exact test used for categorical variables; †Student’s t-test used for numerical variables. P-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; PI, Pancreatic insufficiency; GCLC, Glutamate cysteine ligase catalytic subunit; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
Table 9 GCLC-3506A > G polymorphism in association with CF clinical variables as distributed by CFTR mutation

| Variable                                      | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|-----------------------------------------------|------------------------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|---------------------------------|
|                                               | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected |
| Gender*                                       | 0.753    | 1 | 0.532 | 1 | 1 | 1 | 0.149 | 0.596 | 0.510 | 1 | 0.824 | 1 |
| Age†                                          | 0.057    | 0.228 | 0.710 | 1 | 1 | 0.245 | 0.980 | 0.541 | 1 | 1 | 1 | 0.339 | 1 |
| Onset of symptoms‡                           | 1 | 1 | 1 | 1 | 1 | 0.420 | 1 | 0.731 | 1 | 0.078 | 0.312 | 0.812 | 1 |
| Onset of pulmonary disease‡                 | 0.507    | 1 | 1 | 1 | 1 | 0.152 | 0.608 | 0.727 | 1 | 0.302 | 1 | 0.816 | 1 |
| Onset of digestive disease‡                 | 0.865    | 1 | 0.646 | 1 | 0.408 | 1 | 1 | 1 | 0.158 | 0.632 | 1 | 1 |
| Diagnosis‡                                   | 0.335    | 1 | 0.419 | 1 | 1 | 1 | 0.330 | 1 | 1 | 1 | 1 | 1 |
| BMI‡                                         | 1 | 1 | 1 | 1 | 0.281 | 1 | 0.704 | 1 | 0.709 | 1 | 0.785 | 1 |
| Bhalla score‡                                 | 0.35     | 1 | 0.830 | 1 | 0.468 | 1 | 1 | 0.169 | 0.676 | 0.833 | 1 | 0.495 | 1 |
| Kanga score§                                 | 0.011    | 0.044 | 0.734 | 1 | 0.788 | 1 | 0.067 | 0.268 | 0.588 | 1 | 0.027 | 0.108 |
| Shwachman-Kulczycki score§                  | 0.091    | 0.364 | 0.725 | 1 | 0.223 | 0.892 | 0.034 | 0.136 | 0.545 | 1 | 0.159 | 0.636 |
| Nasal polyposis§                             | 0.688    | 1 | 0.251 | 1 | 0.555 | 1 | 0.692 | 1 | 1 | 1 | 0.083 | 0.332 |
| Diabetes mellitus§                           | 0.688    | 1 | 1 | 1 | 1 | 0.419 | 1 | 0.217 | 0.868 | 1 | 1 |
| Osteoporosis§                                | 0.133    | 0.532 | 1 | 1 | 0.068 | 0.272 | 0.25 | 1 | 0.630 | 1 | 0.335 |
| Meconium ileus§                              | 1 | 1 | 1 | 1 | 0.304 | 1 | 1 | 1 | 0.417 | 1 | 1 |
| Pancreatic insufficiency§                    | 0.698    | 1 | 0.180 | 0.720 | - | - | 0.376 | 1 | - | - | 1 |
| SpO2§                                        | 0.033    | 0.132 | 0.234 | 0.936 | 0.142 | 0.568 | 0.548 | 1 | 0.134 | 0.536 | 0.149 | 0.596 |
| FVC(%)§                                      | 0.412    | 1 | 0.944 | 1 | 0.061 | 0.244 | 0.036 | 0.144 | 0.755 | 1 | 0.955 | 1 |
| FEV(%)§                                      | 0.166    | 0.664 | 0.877 | 1 | 0.094 | 0.376 | 0.030 | 0.120 | 0.381 | 1 | 0.577 | 1 |
| FEV/FVC§                                     | 0.054    | 0.216 | 0.912 | 1 | 0.403 | 1 | 0.050 | 0.200 | 0.247 | 0.988 | 0.111 |
| FEF25–75%§                                   | 0.061    | 0.244 | 0.934 | 1 | 0.177 | 0.708 | 0.029 | 0.116 | 0.577 | 1 | 0.272 | 1 |
| First P. aeruginosa§                         | 0.350    | 1 | 0.453 | 1 | 0.433 | 1 | 0.716 | 1 | 0.015 | 0.060 | 0.799 | 1 |
| P. aeruginosa mucoid§                        | 0.152    | 0.608 | 1 | 1 | 0.443 | 1 | 0.064 | 0.256 | 0.178 | 0.712 | 0.371 | 1 |
| P. aeruginosa no mucoid§                     | 0.057    | 0.228 | 1 | 1 | 0.243 | 0.972 | 0.003 | 0.012 | 0.023 | 0.092 | 0.351 | 1 |
| A. xylosoxidans§                             | 0.187    | 0.748 | 0.066 | 0.044 | 0.176 | 0.565 | 1 | 1 | 0.343 | 1 |
| S. aureus§                                   | 0.849    | 1 | 0.708 | 1 | 1 | 0.376 | 1 | 0.024 | 0.096 | 0.394 | 1 |
| B. cepacia§                                  | 0.246    | 0.984 | 1 | 1 | 0.471 | 1 | 0.082 | 0.328 | 0.394 | 1 |

*Fisher’s exact test used for categorical variables; †Student’s t-test used for numerical variables. P-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; GCLC, Glutamate cysteine ligase catalytic subunit; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
| Variable                              | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|--------------------------------------|-------------------------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|-------------------------------|
|                                      | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected |
| Gendera                             | 0.285   | 1           | 0.302   | 1           | 0.505   | 1           | 0.424   | 1           | 0.479   | 1           | 0.223   | 0.892      |
| Ageb                                | 0.437   | 1           | 0.639   | 1           | 0.382   | 1           | 0.601   | 1           | 0.777   | 1           | 0.625   | 1          |
| Onset of symptomsa                  | 0.098   | 0.392       | 0.407   | 1           | 0.421   | 1           | 0.143   | 0.572       | 0.180   | 0.720       | 0.036   | 0.144      |
| Onset of pulmonary diseasea         | 0.802   | 1           | 0.091   | 0.364       | 0.163   | 0.652       | 0.424   | 1           | 0.321   | 1           | 0.980   | 1          |
| Onset of digestive diseasea         | 0.640   | 1           | 0.237   | 0.948       | 0.311   | 1           | 0.692   | 1           | 0.318   | 1           | 0.452   | 1          |
| Diagnosisa                          | 0.334   | 1           | 0.620   | 1           | 0.637   | 1           | 0.715   | 1           | 0.613   | 1           | 0.218   | 0.872      |
| BMIa                                | 0.620   | 1           | 0.376   | 1           | 0.510   | 1           | 0.848   | 1           | 0.754   | 1           | 0.665   | 1          |
| Bhalla scoreb                       | 0.942   | 1           | 0.808   | 1           | 0.158   | 0.632       | 0.830   | 1           | 0.324   | 1           | 0.311   | 1          |
| Kanga scoreb                        | 0.879   | 1           | 0.884   | 1           | 0.805   | 1           | 0.753   | 1           | 0.745   | 1           | 0.822   | 1          |
| Shwachman-Kulczycki scoreb          | 0.985   | 1           | 0.416   | 1           | 0.151   | 0.604       | 0.538   | 1           | 0.837   | 1           | 0.981   | 1          |
| Nasal polyposisb                    | 0.582   | 1           | 0.347   | 1           | 0.285   | 1           | 0.457   | 1           | 0.631   | 1           | 0.986   | 1          |
| Diabetes mellitusb                  | 0.255   | 1           | 0.858   | 1           | 0.917   | 1           | 0.201   | 0.804       | 0.337   | 1           | 0.383   | 1          |
| Osteoporosisb                       | 0.562   | 1           | 0.829   | 1           | 0.108   | 0.432       | 0.339   | 1           | 0.036   | 0.144       | 0.713   | 1          |
| Meconium ileusa                     | 0.420   | 1           | 0.389   | 1           | 0.379   | 1           | 0.130   | 0.520       | 0.080   | 0.320       | 0.619   | 1          |
| Pancreatic insufficiencya           | 0.159   | 0.636       | 0.398   | 1           | -       | -           | 0.167   | 0.668       | -       | -           | 0.601   | 1          |
| SpO2b                               | 0.506   | 1           | 0.201   | 0.804       | 0.128   | 0.512       | 0.422   | 1           | 0.525   | 1           | 0.278   | 1          |
| FVC(%)b                             | 0.498   | 1           | 0.216   | 0.864       | 0.121   | 0.484       | 0.738   | 1           | 0.901   | 1           | 0.499   | 1          |
| FEV1(%)b                            | 0.668   | 1           | 0.214   | 0.856       | 0.201   | 0.804       | 0.479   | 1           | 0.731   | 1           | 0.769   | 1          |
| FEV1/FVCb                           | 0.615   | 1           | 0.592   | 1           | 0.671   | 1           | 0.407   | 1           | 0.686   | 1           | 0.373   | 1          |
| FEF25–75%b                          | 0.643   | 1           | 0.326   | 1           | 0.531   | 1           | 0.548   | 1           | 0.942   | 1           | 0.851   | 1          |
| First P. aeruginosa                  | 0.147   | 0.588       | 0.125   | 0.500       | 0.341   | 1           | 0.146   | 0.584       | 0.027   | 0.108       | 0.264   | 1          |
| P. aeruginosa mucoida                | 0.559   | 1           | 0.316   | 1           | 0.366   | 1           | 0.569   | 1           | 0.285   | 1           | 0.160   | 0.640      |
| P. aeruginosa no mucoida            | 0.319   | 1           | 0.263   | 1           | 0.352   | 1           | 0.276   | 1           | 0.082   | 0.328       | 0.347   | 1          |
| A. xylosoxidansb                     | 0.327   | 1           | 0.018   | 0.072       | 0.013   | 0.052       | 0.687   | 1           | 0.646   | 1           | 0.006   | 0.024      |
| S. aureusb                          | 0.843   | 1           | 0.677   | 1           | 0.931   | 1           | 0.049   | 0.196       | 0.032   | 0.128       | 0.466   | 1          |
| B. cepacia                         | 0.461   | 1           | 0.734   | 1           | 0.974   | 1           | 0.150   | 0.600       | 0.243   | 0.972       | 0.671   | 1          |

*Fisher’s exact test used for categorical variables; Student’s t-test used for numerical variables. P-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; GCLC, Glutamate cysteine ligase catalytic subunit; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
| Variable                | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|-------------------------|-------------------------------------------|-----------------------------|-------------------------------------|-----------------------------|--------------------------------------|-------------------------------|
|                         | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected |
| Gendera                 | 0.171   | 0.684      | 0.764   | 1           | 0.075   | 0.300      | 0.267   | 1           | 0.537   | 1           | 0.110   | 0.440      |
| Ageb                    | 1       | 1          | 0.498   | 1           | 0.736   | 1           | 0.579   | 1           | 1       | 1          | 0.629   | 1           |
| Onset of symptomsa      | 0.268   | 1           | 0.459   | 1           | 0.721   | 1           | 1       | 1           | 1       | 1          | 0.742   | 0.639      |
| Onset of pulmonary diseasea | 0.424 | 1           | 0.008   | 0.272      | 1       | 1           | 1       | 1           | 0.742   | 1           | 0.639   | 1           |
| Onset of digestive diseasea | 0.409 | 1           | 0.665   | 1           | 1       | 1           | 1       | 1           | 0.008   | 0.032      | 0.635   | 1           |
| Diagnosisa              | 1       | 1          | 0.059   | 0.236      | 0.729   | 1           | 0.149   | 0.596      | 1       | 1          | 1       | 1           |
| BMId                    | 0.462   | 1           | 0.503   | 1           | 0.115   | 0.460      | 0.725   | 1           | 1       | 1          | 0.169   | 0.676      |
| Bhalla scoreb           | 0.86    | 1           | 0.11    | 0.44       | 0.059   | 0.236      | 0.050   | 0.200      | 0.692   | 1           | 0.879   | 1           |
| Kanga scoreb            | 0.982   | 1           | 0.693   | 1           | 0.367   | 1           | 0.822   | 1           | 0.480   | 1           | 0.784   | 1           |
| Shwachman-Kulczycki scoreb | 0.501 | 1           | 0.449   | 1           | 0.884   | 1           | 0.123   | 0.492      | 0.777   | 1           | 0.568   | 1           |
| Nasal polyposisa        | 0.331   | 1           | 0.136   | 0.544      | 0.610   | 1           | 1       | 1           | 0.407   | 1           | 0.765   | 1           |
| Diabetes mellitusc      | 0.560   | 1           | 1       | 1           | 1       | 1           | 0.703   | 1           | 1       | 1          | 0.169   | 0.676      |
| Osteoporosisa           | 0.217   | 0.868      | 0.435   | 1           | 0.377   | 1           | 0.173   | 0.633      | 1       | 1          | 1       | 1           |
| Meconium ileusa         | 1       | 1           | 1       | 1           | 0.640   | 1           | 0.726   | 1           | 1       | 1          | 0.776   | 1           |
| Pancreatic insufficiencya | 1       | 1           | 0.765   | 1           | -       | -           | 1       | 1           | -       | -           | 1       | 1           |
| SpO2c                   | 0.187   | 0.748      | 0.012   | 0.048      | 0.500   | 1           | 0.780   | 1           | 0.652   | 1           | 0.645   | 1           |
| FVC(%)d                 | 0.990   | 1           | 0.741   | 1           | 0.020   | 0.08       | 0.538   | 1           | 0.307   | 1           | 0.967   | 1           |
| FEV1(%)d                | 0.827   | 1           | 0.623   | 1           | 0.030   | 0.012      | 0.786   | 1           | 0.972   | 1           | 0.943   | 1           |
| FEV1/FVCd               | 0.915   | 1           | 0.749   | 1           | 0.532   | 1           | 0.918   | 1           | 0.244   | 0.976      | 0.597   | 1           |
| FEF 25–75%(e)           | 0.853   | 1           | 0.718   | 1           | 0.197   | 0.788      | 0.819   | 1           | 0.255   | 1           | 0.847   | 1           |
| First P. aeruginosaid   | 0.724   | 1           | 0.744   | 1           | 0.473   | 1           | 0.056   | 0.224      | 1       | 1           | 0.312   | 1           |
| P. aeruginosa mucoidid  | 0.092   | 0.368      | 0.729   | 1           | 0.289   | 1           | 1       | 1           | 0.541   | 1           | 0.107   | 0.428      |
| P. aeruginosa no mucoidd | 0.879 | 1           | 0.754   | 1           | 0.292   | 1           | 0.776   | 1           | 1       | 1          | 0.629   | 1           |
| A. xylosidovansd        | 0.619   | 1           | 0.537   | 1           | 1       | 1           | 0.35    | 1           | 0.511   | 1           | 0.52    | 1           |
| S. aureusd              | 0.362   | 1           | 0.175   | 0.700      | 0.391   | 1           | 1       | 1           | 0.776   | 1           | 1       | 1           |
| B. cepaciae             | 0.371   | 1           | 0.116   | 0.464      | 0.313   | 1           | 0.703   | 1           | 1       | 1          | 1       | 1           |

*aFisher’s exact test used for categorical variables; bStudent’s t-test used for numerical variables. P-values < 0.05 denote clinical association (bold). CFTR, Cystic fibrosis transmembrane regulator; GSTM1, Glutathione S-transferase mu 1; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
Table 12 GSTT1 deletion polymorphism in association with CF clinical variables as distributed by CFTR mutation

| Variable                             | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|--------------------------------------|-------------------------------------------|-----------------------------|-------------------------------------|-----------------------------|-------------------------------------|-------------------------------|
|                                      | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected | p-value | p-corrected |
| Gendera                              | 0.211   | 0.844       | 0.778   | 1           | 0.378   | 1           | 0.115   | 0.338       | 0.215   | 0.860       | 0.081   | 0.324       |
| Ageb                                 | 0.043   | 0.166       | 0.750   | 1           | 0.505   | 1           | 0.083   | 0.332       | 0.531   | 1           | 0.795   | 0.795       |
| Onset of symptomsa                    | 1       | 1           | 0.305   | 1           | 1       | 1           | 0.202   | 0.808       | 0.746   | 1           | 0.600   | 1           |
| Onset of pulmonary diseasea          | 0.620   | 1           | 0.721   | 1           | 0.292   | 0.916       | 0.521   | 1           | 0.746   | 1           | 0.796   | 1           |
| Onset of digestive diseasea          | 0.863   | 1           | 0.390   | 1           | 1       | 1           | 0.344   | 1           | 0.179   | 0.716       | 1       | 1           |
| Diagnosisa                           | 0.745   | 1           | 1       | 1           | 0.303   | 1           | 0.068   | 0.272       | 0.537   | 1           | 0.779   | 1           |
| BMIa                                 | 0.447   | 1           | 0.747   | 1           | 0.103   | 0.412       | 0.295   | 1           | 0.728   | 1           | 0.537   | 1           |
| Bhalla scoreb                        | 0.485   | 1           | 0.824   | 1           | 0.134   | 0.536       | 0.322   | 1           | 0.634   | 1           | 0.185   | 0.740       |
| Kanga scoreb                         | 0.737   | 1           | 0.743   | 1           | 0.421   | 1           | 0.093   | 1           | 0.321   | 1           | 0.767   | 1           |
| Shwachman-Kulczycki scoreb           | 0.734   | 1           | 0.984   | 1           | 0.013   | 0.052       | 0.653   | 1           | 0.925   | 1           | 0.393   | 1           |
| Nasal polyposisa                     | 0.313   | 1           | 1       | 1           | 1       | 1           | 0.062   | 0.248       | 0.685   | 1           | 1       | 1           |
| Diabetes mellitusb                   | 0.158   | 0.632       | 0.115   | 0.460       | 0.398   | 1           | 0.450   | 1           | 0.071   | 0.284       | 0.764   | 1           |
| Osteoporosisb                        | 1       | 1           | 1       | 1           | 0.085   | 0.340       | 1       | 1           | 0.230   | 0.920       | 0.718   | 1           |
| Meconium ileus                      | 0.276   | 1           | 0.077   | 0.308       | 0.658   | 1           | 1       | 1           | 0.445   | 1           | 0.335   | 1           |
| Pancreatic insufficiencya            | 0.847   | 1           | 0.561   | 1           | -       | -           | 1       | 1           | -       | -           | 0.557   | 1           |
| SaO2c                                | 0.988   | 1           | 0.740   | 1           | 0.170   | 0.680       | 0.595   | 1           | 0.333   | 1           | 0.703   | 1           |
| FVC(%)b                              | 0.268   | 1           | 0.086   | 0.344       | 0.154   | 0.616       | 0.464   | 1           | 0.412   | 1           | 0.623   | 1           |
| FEV1(%)b                             | 0.310   | 1           | 0.167   | 0.668       | 0.029   | 0.116       | 0.564   | 1           | 0.597   | 1           | 0.636   | 1           |
| FEV1/FVCb                            | 0.404   | 1           | 0.288   | 1           | 0.017   | 0.068       | 0.692   | 1           | 0.676   | 1           | 0.424   | 1           |
| FEF25–75%b                           | 0.687   | 1           | 0.390   | 1           | 0.027   | 0.108       | 0.686   | 1           | 0.829   | 1           | 0.959   | 1           |
| First P. aeruginosaa                 | 0.472   | 1           | 1       | 1           | 0.724   | 1           | 0.320   | 1           | 0.713   | 1           | 0.085   | 0.340       |
| P. aeruginosa mucoida                | 0.433   | 1           | 0.747   | 1           | 1       | 1           | 0.393   | 1           | 0.753   | 1           | 1       | 1           |
| P. aeruginosa no mucoida             | 0.876   | 1           | 0.002   | 0.008       | 0.489   | 1           | 0.133   | 0.266       | 0.541   | 1           | 1       | 1           |
| A. xylosoxidansb                     | 0.437   | 1           | 1       | 1           | 0.338   | 1           | 0.623   | 1           | 0.151   | 0.604       | 0.296   | 1           |
| S. aureusb                          | 0.705   | 1           | 1       | 1           | 0.658   | 1           | 0.325   | 1           | 0.735   | 1           | 1       | 1           |
| B. cepaciaa                          | 1       | 1           | 1       | 1           | 0.177   | 0.708       | 0.699   | 1           | 0.407   | 1           | 0.215   | 0.860       |

*Fisher's exact test used for categorical variables; †Student's t-test used for numerical variables. P-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; GSTT1, Glutathione S-transferase theta 1; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
### Table 13 GSTM1/GSTT1 deletion polymorphism in association with CF clinical variables as distributed by CFTR mutation

| Variable                          | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|----------------------------------|------------------------------------------|-----------------------------|--------------------------------------|------------------------------|--------------------------------------|------------------------------|
|                                  | p-value                | p-corrected | p-value                | p-corrected | p-value                | p-corrected | p-value                | p-corrected | p-value                | p-corrected | p-value                | p-corrected |
| Gendera                         | 0.036                   | 0.144    | 0.943                   | 1           | 0.601                   | 1           | 0.369                   | 1           | 0.114                   | 0.456    | 0.014                   | 0.056    |
| Age                             | 0.331                   | 1         | 0.647                   | 1           | 0.496                   | 1           | 0.054                   | 0.216 | 0.149                   | 0.596    | 0.908                   | 1         |
| Onset of symptomsa              | 0.300                   | 1         | 0.996                   | 1           | 0.895                   | 1           | 0.579                   | 1           | 0.854                   | 1         | 0.049                   | 0.196    |
| Onset of pulmonary diseasea     | 0.588                   | 1         | 0.359                   | 1           | 0.431                   | 1           | 0.776                   | 1           | 0.267                   | 1         | 0.559                   | 1         |
| Onset of digestive diseasea     | 0.626                   | 1         | 0.480                   | 1           | 0.581                   | 1           | 0.433                   | 1           | 0.458                   | 1         | 0.051                   | 0.204    |
| Diagnosisa                      | 0.520                   | 1         | 0.207                   | 0.282 | 0.490                   | 1           | 0.710                   | 1           | 0.510                   | 1         | 0.992                   | 1         |
| BMIb                            | 0.283                   | 1         | 0.954                   | 1           | 0.717                   | 1           | 0.252                   | 1           | 0.998                   | 1         | 0.596                   | 1         |
| Bhalla scoreb                   | 0.088                   | 0.352    | **0.005**               | **0.02** | 0.915                   | 1           | 0.381                   | 1           | 0.218                   | 0.872    | 0.481                   | 1         |
| Kang scoreb                     | 0.885                   | 1         | 0.443                   | 1           | 0.216                   | 0.864 | 0.912                   | 1           | 0.261                   | 1         | 0.455                   | 1         |
| Shwachman-Kulczycki scoreb      | 0.627                   | 1         | 0.144                   | 0.576 | 0.087                   | 0.348 | 0.387                   | 1           | 0.104                   | 0.416    | 0.195                   | 0.780    |
| Nasal polyposisa                | 0.098                   | 0.392    | 0.483                   | 1           | 0.699                   | 1           | 0.467                   | 1           | 0.362                   | 1         | 0.102                   | 0.408    |
| Diabetes mellitusc              | 0.259                   | 1         | 0.240                   | 0.96 | 0.790                   | 1           | 0.992                   | 1           | 0.555                   | 1         | 0.334                   | 1         |
| Osteoporosissc                  | 0.204                   | 0.816    | 0.501                   | 1           | 0.525                   | 1           | 0.427                   | 1           | 0.187                   | 0.748    | 0.386                   | 1         |
| Meconium ileus                  | 0.683                   | 1         | 0.266                   | 1           | 0.348                   | 1           | 0.517                   | 1           | 0.905                   | 1         | 0.626                   | 1         |
| Pancreatic insufficiencya        | 0.065                   | 1         | 0.791                   | 1           | -                      | -           | 0.975                   | 1           | -                      | -         | 0.653                   | 1         |
| SpO2d                           | 0.449                   | 1         | 0.021                   | 0.084 | 0.557                   | 1           | 0.616                   | 1           | 0.774                   | 1         | 0.786                   | 1         |
| FVC(%e)                         | 0.576                   | 1         | 0.518                   | 1           | 0.859                   | 1           | 0.928                   | 1           | 0.475                   | 1         | 0.758                   | 1         |
| FEV1,%(e)                       | 0.778                   | 1         | 0.182                   | 0.728 | 0.977                   | 1           | 0.799                   | 1           | 0.827                   | 1         | 0.657                   | 1         |
| FEV1/FVC                         | 0.178                   | 1         | 0.007                   | 0.028 | 0.265                   | 1           | 0.789                   | 1           | 0.395                   | 1         | 0.593                   | 1         |
| FEF25–75%(f)                    | 0.881                   | 1         | 0.014                   | 0.056 | 0.751                   | 1           | 0.719                   | 1           | 0.370                   | 1         | 0.382                   | 1         |
| First P. aeruginosaa            | 0.045                   | 1         | 0.686                   | 1           | 0.295                   | 1           | 0.019                   | 0.076 | 0.299                   | 1         | 0.123                   | 0.492    |
| P. aeruginosa mucoida           | 0.134                   | 0.536    | 0.118                   | 0.472 | 0.492                   | 1           | 0.575                   | 1           | 0.940                   | 1         | 0.337                   | 1         |
| P. aeruginosa no mucoida        | 0.487                   | 1         | 0.051                   | 0.204 | 0.682                   | 1           | 0.167                   | 0.668 | 0.190                   | 0.760    | 0.847                   | 1         |
| A. xylosoxidasa                 | 0.541                   | 1         | 0.779                   | 1           | 0.663                   | 1           | 0.079                   | 1           | 0.537                   | 1         | 0.995                   | 1         |
| S. aureusa                      | 0.660                   | 1         | 0.243                   | 0.972 | 0.829                   | 1           | 0.716                   | 1           | 0.181                   | 0.724    | 0.667                   | 1         |
| B. cepacia                      | 0.861                   | 1         | 0.142                   | 0.568 | 0.054                   | 1           | 0.759                   | 1           | 0.145                   | 0.580    | 0.640                   | 1         |

*Fisher’s exact test used for categorical variables; bStudent’s t-test used for numerical variables. p-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; GSTM1, Glutathione S-transferase mu 1; GSTT1, Glutathione S-transferase theta 1; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.

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Table 14 GSTP1 + 313A > G polymorphism in association with CF clinical variables as distributed by CFTR mutation

| Variable | Without taking CFTR mutation into account | No CFTR mutations identified | No CFTR mutations identified with PI | One identified CFTR mutation | One identified CFTR mutation with PI | Two identified CFTR mutations |
|----------|------------------------------------------|-----------------------------|-------------------------------------|-------------------------------|------------------------------------|-----------------------------|
| Gendera | p-value 0.550 p-corrected 1 | p-value 0.396 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.267 p-corrected 1 | p-value 0.763 p-corrected 1 | p-value 0.184 p-corrected 0.736 |
| Ageb    | p-value 0.011 p-corrected 0.444 | p-value 0.750 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.051 p-corrected 0.204 | p-value 1 p-corrected 1 | p-value 0.058 p-corrected 0.232 |
| Onset of symptomsa | p-value 0.876 p-corrected 1 | p-value 0.473 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.531 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 |
| Onset of pulmonary diseasea | p-value 0.754 p-corrected 1 | p-value 0.729 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.757 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 |
| Onset of digestive diseasea | p-value 0.516 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.761 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 |
| Diagnosisa | p-value 0.644 p-corrected 1 | p-value 0.694 p-corrected 1 | p-value 0.185 p-corrected 0.740 | p-value 0.561 p-corrected 1 | p-value 0.763 p-corrected 1 | p-value 0.441 p-corrected 1 |
| BMIA   | p-value 0.856 p-corrected 1 | p-value 0.331 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.488 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 |
| Bhalla scoreb | p-value 0.098 p-corrected 0.392 | p-value 0.187 p-corrected 1 | p-value 0.671 p-corrected 1 | p-value 0.491 p-corrected 1 | p-value 0.098 p-corrected 0.392 | p-value 0.392 p-corrected 1 |
| Kang scoreb | p-value 0.716 p-corrected 1 | p-value 0.867 p-corrected 1 | p-value 0.604 p-corrected 1 | p-value 0.407 p-corrected 1 | p-value 0.416 p-corrected 1 | p-value 0.300 p-corrected 1 |
| Shwachman-Kulczycki scoreb | p-value 0.554 p-corrected 1 | p-value 0.984 p-corrected 1 | p-value 0.121 p-corrected 0.484 | p-value 0.73 p-corrected 1 | p-value 0.198 p-corrected 0.792 | p-value 0.170 p-corrected 0.680 |
| Nasal polyposisa | p-value 0.848 p-corrected 1 | p-value 0.306 p-corrected 1 | p-value 0.601 p-corrected 1 | p-value 0.412 p-corrected 1 | p-value 0.562 p-corrected 1 | p-value 1 p-corrected 1 |
| Diabetes mellitusa | p-value 0.336 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.703 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.582 p-corrected 1 |
| Osteoporosisa | p-value 0.159 p-corrected 0.318 | p-value 0.715 p-corrected 0.953 | p-value 1 p-corrected 1 | p-value 0.345 p-corrected 1 | p-value 0.009 p-corrected 0.036 | p-value 1 p-corrected 1 |
| Meconium ileusa | p-value 0.403 p-corrected 1 | p-value 1 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.457 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.161 p-corrected 0.644 |
| Pancreatic insufficiencya | p-value 0.581 p-corrected 1 | p-value 0.393 p-corrected 1 | p-value 0.187 p-corrected 0.748 | p-value 0.703 p-corrected 1 | p-value - p-corrected - | p-value 0.578 p-corrected 1 |
| SpO2b | p-value 0.967 p-corrected 1 | p-value 0.839 p-corrected 1 | p-value 0.230 p-corrected 0.920 | p-value 0.156 p-corrected 0.624 | p-value 0.157 p-corrected 0.628 | p-value 0.346 p-corrected 1 |
| FVC(%)b | p-value 0.441 p-corrected 1 | p-value 0.407 p-corrected 1 | p-value 0.279 p-corrected 1 | p-value 0.849 p-corrected 1 | p-value 0.315 p-corrected 1 | p-value 0.626 p-corrected 1 |
| FEV1(%)b | p-value 0.338 p-corrected 1 | p-value 0.467 p-corrected 1 | p-value 0.923 p-corrected 1 | p-value 0.907 p-corrected 1 | p-value 0.221 p-corrected 0.884 | p-value 0.451 p-corrected 1 |
| FEV1/FVCb | p-value 0.295 p-corrected 1 | p-value 0.265 p-corrected 1 | p-value 0.218 p-corrected 0.872 | p-value 0.575 p-corrected 1 | p-value 0.771 p-corrected 1 | p-value 0.439 p-corrected 1 |
| FEF25–75%b | p-value 0.146 p-corrected 0.584 | p-value 0.498 p-corrected 1 | p-value 0.261 p-corrected 1 | p-value 0.505 p-corrected 1 | p-value 0.379 p-corrected 1 | p-value 0.291 p-corrected 1 |
| First P. aerugi
osa | p-value 0.025 p-corrected 0.140 | p-value 1 p-corrected 1 | p-value 0.473 p-corrected 1 | p-value 0.056 p-corrected 0.224 | p-value 1 p-corrected 1 | p-value 0.203 p-corrected 0.812 |
| P. aerugi
osa mucoida | p-value 0.289 p-corrected 1 | p-value 0.331 p-corrected 1 | p-value 0.505 p-corrected 1 | p-value 0.782 p-corrected 1 | p-value 0.760 p-corrected 1 | p-value 0.653 p-corrected 1 |
| P. aerugi
osa no mucoida | p-value 1 p-corrected 1 | p-value 0.548 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.776 p-corrected 1 | p-value 0.760 p-corrected 1 | p-value 0.482 p-corrected 1 |
| A. xylo
oxidans | p-value 0.806 p-corrected 1 | p-value 0.196 p-corrected 0.784 | p-value 1 p-corrected 1 | p-value 0.350 p-corrected 1 | p-value 0.488 p-corrected 1 | p-value 0.755 p-corrected 1 |
| S. aureua | p-value 0.721 p-corrected 1 | p-value 0.507 p-corrected 1 | p-value 1 p-corrected 1 | p-value 0.743 p-corrected 1 | p-value 0.185 p-corrected 0.740 | p-value 0.565 p-corrected 1 |
| B. cepacia | p-value 0.667 p-corrected 1 | p-value 0.196 p-corrected 0.784 | p-value 1 p-corrected 1 | p-value 0.703 p-corrected 1 | p-value 0.698 p-corrected 1 | p-value 0.404 p-corrected 1 |

*Fisher’s exact test used for categorical variables; Student’s t-test used for numerical variables. P-values < 0.05 denote clinical association (bold).

CFTR, Cystic fibrosis transmembrane regulator; GSTP1, Glutathione S-transferase pi 1; PI, Pancreatic insufficiency; BMI, Body mass index; SpO2, Hemoglobin oxygen saturation in the blood; FVC, Forced vital capacity; FEV1, Forced expiratory volume in the first second; FEF, Forced expiratory flow between 25 and 75% of vital capacity.
all data. Study highlights the data by: (i) one CF center collection – considering an admixed population, the CF patients from one center minimizes miscegenation factors. Another fact, is the similar environmental and the same access to treatment; (ii) high number of clinical markers evaluated provides better association and characterization of modifier genes action; (iii) complete CF diagnosis performed by different methods.

**Conclusions**

Our results show that, although a monogenic disease, CF is heavily influenced in its clinical characteristics, evolution and severity by polymorphisms in modifier genes. Nevertheless, there is still a long way before the dynamics of polymorphisms in genes active in the GSH metabolic pathway and involved in detoxification in CF are fully understood.

Another fact is the prevalence of PS and PI that should be considered in all studies in the future, being associated with different phenotype and genotype.

**Abbreviations**

CF: Cystic fibrosis; CFTR: Cystic fibrosis transmembrane regulator; GCLC: Glutamate-cysteine ligase, catalytic subunit; GST: Glutathione S-transferase; GSTM1: Glutathione S-transferase mu 1; GSTT1: Glutathione S-transferase theta 1; CD4: Cystic fibrosis transmembrane regulator; BMI: Body mass index; WHO: World health organization; MLPA: Multiplex ligation-dependent probe amplification; PCR: Polymerase chain reaction; SSPE: Statistical package for social science for windows; PS: Pancreatic sufficiency; PI: Pancreatic insufficiency; COPD: Chronic obstructive pulmonary disease.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

FALM contributed to the study conception and design, acquired, analyzed and interpreted the data, drafted the manuscript and revised it for intellectual content. CSB carried out the molecular genetic studies and interpreted the data, drafted the manuscript and revised it for intellectual content. FALM contributed to the study conception and design, acquired, analyzed and interpreted the data, drafted the manuscript and revised it for intellectual content. JDR approved the manuscript for publication. All authors read and approved the final manuscript.

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