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COX-2 GENE POLYMORPHISMS: GENETIC DETERMINANTS OF CYSTIC FIBROSIS COMORBIDITIES

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Abstract- Cystic fibrosis (CF) has clinical variability associated with *CFTR* genetic mutations, environmental factors and modifier genes. Genes associated with the inflammatory process are important for understanding CF variability as well as identifying new therapies and prognostic factors. Because of the importance of *COX-2* in inflammation, *COX-2* polymorphisms were studied in a CF population and compared with 27 clinical variables with consideration of the *CFTR* mutations. There were 106CF patients included in the study, and the rs20417 and rs5275 *COX-2* gene polymorphisms were analyzed using the RFLP technique. The polymorphism -8473C>T was associated with nasal polyposis in the CC+CT genotype (OR=5.552, IC=1.318-38.37) without taking the *CFTR* mutations into account. The haplotype analysis showed an association with nasal polyposis (NP) and diabetes mellitus (DM). NP was associated with the GG/TT haplotype (OR=0.1056, IC=0.004-0.642) in patients without taking the *CFTR* mutations into account. DM was associated with the GC/TC haplotype, (OR=6.164, IC=1.719-23.83) in patients with two identified *CFTR* mutations. The association of *COX-2* polymorphisms with CF was not clear. In the literature, only one study considering these polymorphisms and CF was found, and that study did not include CF comorbidities in the clinical variables. Therefore, our study provides the first evidence showing an association between the *COX-2* gene and the CF comorbidities of NP and DM. In recent years, the prognosis and life expectancy of CF patients has improved, and the comorbidities have increased as a result. A better understanding of the complex aspects involved in CF comorbidities could provide a better treatment, primarily in older CF patients.

Keywords- Cystic fibrosis, *COX-2* gene, *CFTR* gene, diabetes mellitus, nasal polyposis

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Introduction

Cystic fibrosis (CF) has clinical variability associated with mutations in the Cystic Fibrosis Transmembrane Regulator (*CFTR*) gene, environmental factors and modifier genes [1-3]. Our group has previously conducted studies to understand the genetic modulators of CF clinical severity [4-8] and has recently studied the *COX-2* gene as a modifier of CF clinical severity.

The *COX-2* gene (cyclo-oxygenase 2; known as PTGS2= prostaglandin-endoperoxide synthase 2) is located in the genomic region 1q31.1, has 10 exons and encodes a protein with 604 amino acids. The COX-2 protein regulates the conversion of free arachidonic acid into prostaglandin (PGH2), which leads to prostanoid formation [9]. Prostanoids control homeostasis and inflammation in response to infection or trauma [10]. There are two COX isoforms; the COX-1 isoform is expressed constitutively in most cells, and the COX-2 isoform is expressed in response to local inflammatory processes [11].

The *COX-2* gene is immediately induced in response to the action of cytokines and mitogenic factors [12]. Pathological conditions can increase *COX-2* expression; however, inhibitors that attenuate chronic inflammation can reduce its expression [13]. Although the COX-2 protein can be regarded as a pro-inflammatory molecule, there is evidence that it can be a target for the treatment of chronic inflammatory diseases, and studies have shown that COX-2 can be anti-inflammatory, antifibrotic and antirhombic [14].

The *COX-2* gene has a 5´UTR that regulates its expression 15,16], and polymorphisms in this region are important in the inflammatory response. The COX-2 protein plays a role in the immune system by causing vasodilation and promoting cell migration into the inflamed tissues. Polymorphisms related to COX-2 synthesis can lead to changes in the immune system and consequently alter the CF phenotype [17]. A study with 94 homozygous F508del patients compared some CF clinical variables with *COX-1* and *COX-2* polymorphisms. Two polymorphisms [-765G>C (rs20417 - promoter region) and 8473T>C (rs5275 - 3'UTR)] in the *COX-2* gene were found to be CF severity modulators[17] and acted by decreasing the protein concentration and COX-2 mRNA degradation.

High levels of COX-2 can have a negative impact on CF due to the

synthesis of prostaglandin, which causes inflammation and results in the deterioration of airways. Thus, genetic variants that increase the expression of *COX-2* are associated with inflammation and severe lung disease.

Considering the importance of COX-2 in the inflammatory process, the objective of this study was to compare *COX-2* polymorphisms with 27 clinical variables in a CF population with consideration for *CFTR* mutations.

Material and Methods

The cross-sectional study was conducted in a university center for CF care between 2011 and 2012. The CF diagnosis was confirmed by sodium and chloride measurements greater than 60 mEq/L in the sweat. *CFTR* mutations were also identified in the patient cohort. None of the patients were diagnosed by a neonatal screening test.

One hundred six patients were selected for the study. Patients without data for statistical analysis and those who did not sign the consent form were excluded from the study.

Patient DNA was obtained by a phenol-chloroform extraction. The DNA concentration was evaluated using a GE NanoVue™ Spectrophotometer (GE Healthcare Biosciences, Pittsburgh, USA), and 50 ng/mL was used for the analysis.

Clinical Variables

The following clinical variables were considered in this study: Shwachman-Kulczycki, Kanga and Bhalla scores [18]; body mass index (BMI) [for patients older than 19 years, the formula BMI=weight/(height)^2 was used; for the remaining patients, the WHO ANTHRO program (children <5 years old) and the WHO ANTHRO PLUS program (children 5 to 19 years old) were used]; age (groups: ≤154 and >154 months); time to diagnosis using two altered sodium values (groups: ≤24 and >24 months); time of the first clinical symptoms (digestive: ≤3 and >3 months; pulmonary: ≤6 and >6 months); time to the first colonization by *Pseudomonas aeruginosa* (≤31 and >31 months); the presence of bacteria in the respiratory airways (*P. aeruginosa* mucoid and no mucoid, *Achromobacter xylosoxidans, Burkolderia cepacia* and *Staphylococcus aureus*); transcutaneous hemoglobin oxygen saturation (SpO2); spirometry variables.

The spirometry was performed in patients older than 7 years old using the CPFS/D spirometer (MedGraphics, Saint Paul, Minnesota, USA). Data were recorded using the PF BREEZE software version 3.8B for Windows 95/98/NT, and the following variables were included: forced vital capacity [FVC (%)]; forced expiratory volume in the first second [FEV_1(%)]; the ratio between FEV_1 and FVC (%) [FEV_1/FVC(%)]; forced expiratory flow between 25 and 75% of the FVC [FEF_{25-75}%].

The following comorbidities were analyzed: nasal polyps, osteoporosis, meconium ileus, diabetes mellitus, and pancreatic insufficiency.

CFTR Mutation Identification

The *CFTR* mutations were identified by polymerase chain reaction (PCR) for F508del and the fragment-length polymorphism method for the G542X, R1162X, R553X, G551D, and N1303K mutations.

The following CF mutations were identified by sequencing or MLPA (Multiplex Ligation-dependent Probe Amplification) 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 the statistical analysis. All *CFTR* gene mutations identified were included in class I, II or III. The other identified mutations, which were included in class IV (P205S and R334W), were not considered in the statistical analysis. More details are provided in [Table-2].

Analysis of the -765G>C and 8473T>C polymorphisms in the *COX-2* gene

PCR amplification of the 157 and 177 base pair fragments for the -765G>C and 8473T>C *COX-2* gene polymorphisms, respectively, was performed with bidistilled water, 10x Taq Buffer containing KCl, $MgCl_2$ (25 mM), dNTPs (25 mM of each nitrogenous base), 0.2 pmol primers (5'- ATT CTG GCC ATC GCC GCT TC -3' and 5'- CTC CTT GTT TCT TGG AAA GAG ACG -3' for the -765G>C polymorphism, and 5'- GAA ATT TTA AAG TAC TTT TGA T- 3'and 5'- CTT TTA CAG GTG ATT CTA CCC -3′ for the 8473T>C polymorphism) [19,20], Taq polymerase (5U) and genomic DNA (50 ng/mL).

The annealing temperature was 62°C for the -765G>C and 58°C for the 8473T>C *COX-2* gene polymorphisms.

After PCR amplification, the enzymatic digestion was performed following the manufacturer's recommendations with the *BstU*I enzyme (New England BioLabs, Massachusetts, USA) at 60°C for 14 hours for the -765G>C polymorphism and the *Bcl*I (New England BioLabs, Massachusetts, USA) at 50°C for 14 hours for the 8473T>C polymorphism.

The reaction products were resolved on a polyacrylamide gel (12%) with a voltage of 180 V for 4 hours. The gel was stained with an ethidium bromide solution and visualized on the Typhoon™ scanner (GE Healthcare, Wisconsin, USA).

According to the fragments observed, the following genotypes were identified: (i) -765G>C polymorphism: GG (134 + 23 base pairs), GC (157 + 134 + 23 base pairs), CC (157 base pairs); (ii) 8473T>C polymorphism: TT (177 base pairs), TC (177 + 156 + 21 base pairs), CC (156 + 21 base pairs).

Statistical Analysis

The statistical analysis was performed using the software Statistical Package for Social Sciences (SPSS) v.17.0 (version 17, SPSS Inc., Chicago, IL), Epi Info v.6.0 [21] and R version 2.12 (Comprehensive R Archive Network, 2011).

The statistical power calculation for the sample was performed using the GPower 3.1.2 software [22], which demonstrated a statistical power above 80% for the analysis.

The data were compared using the c^2 and Fisher exact tests for categorical variables and the Mann-Whitney and Kruskal-Wallis tests for numerical variables according to the data distribution.

The -765G>C and 8473T>C polymorphisms in the *COX-2* gene were compared directly with the variables shown in [Table-2]. Subsequently, the haplotype analysis was performed considering the following genotype groups: 0 (GG/TT-34 CF patients), 1 (GC/TC-31 CF patients) and 2 (GG/TC+GG/CC+GC/TTGC/CC-31 CF patients) [Table-3].

As a result of the high standard deviation in the distribution of the patient data, several variables (including the patient's current age, age at diagnosis, onset of pulmonary and digestive symptoms and the time before the first *P. aeruginosa* isolated) were categorized into two classes: shorter time (relating to more severe variables)

and longer time (relating to less severe variables).

To control for the multiple statistical tests [23], the significance level α was adjusted by the Bonferroni correction method ($\alpha_{corrected}$=0.05/ number of tests).

The analyses were performed for the following cohorts: (1) all CF patients (106 patients) and (2) patients with two class I, II or III mutations in the *CFTR* gene (66 patients). The second group was necessary to determine the influence of modifier genes associated with the clinical variation in CF. In this group, all patients had two known *CFTR* mutations, and these mutations were not additional factors that determined the clinical variation among the CF patients.

The Hardy-Weinberg equilibrium was calculated using the OEGE software (Online Encyclopedia for Genetic Epidemiology studies - http://www.oege.org/software/hardy-weinberg.html).

This study was approved by the Institutional Ethics Committee of the University of Campinas, Faculty of Medical Sciences (#528/2008), and all patients signed a consent form prior to beginning the study.

Results

The clinical variables for the population examined this study are shown in [Table-1].

The genotypic frequency of *CFTR* mutations and *COX-2* polymorphisms are described in [Table-2]. All of the polymorphisms analyzed in this study were in Hardy-Weinberg equilibrium except for the -765G>C polymorphism in the *COX-2* gene.

[Table-3] shows the haplotype distribution for the *COX-2* polymorphism under consideration in this study. There were high frequencies of the GG/TT and GC/TC haplotypes in the data. To analyze the association between the haplotype and the CF variables, we considered the following three groups: (i) GG/TT, (ii) GC/TC and (iii) other possible combinations.

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

Feature	
Genre - masculine	47.2% (50)#
Caucasian	94.3% (100)#
Age	156.99 ± 10.62 months (7-512 months)*
BMI - thinness and accentuated thinness	15.1% (16)#
One Class I, II or III identified mutation	36.79% (39)#
Two Class I, II or III identified mutation	62.26% (66)#
First clinical manifestation	8.59 ± 2.57 months (0-228 months)*
Age at diagnosis	36.98 ± 6.73 months (0-379 months)*
Onset of digestive symptoms	15.53 ± 4.43 months (0-381 months)*
Onset of pulmonary symptoms	14.43 ± 3.18 months (0-228 months)*
SpO2	95.64 ± 0.378 (66-99)*
Bhalla score	7.65 ± 0.503 (0-23)*
Kanga score	18.25 ± 0.586 (10-40)*
Shwachman-Kulczycki score	68.82 ± 1.465 (20-95)*
FVC (%)	82.49 ± 2.14 (29-135)*
FEV_1 (%)	75.32 ± 2.79 (19-132)*
FEV_1/FVC (%)	84.21 ± 1.71 (37-137)*
FEF_{25-75}%	62.32 ± 3.80 (8-138)*
Nasal Polyps	17.9% (19)#
Diabetes mellitus	18.9% (20)#
Osteoporosis	13.2% (14)#
Pancreatic insufficiency	94.3% (100)#
Meconium ileus	18.9% (20)#
First isolated P. aeruginosa	51.20 ± 7.64 months (2-383 months)
P. aeruginosa status [1]	61.3% (65)#
P. aeruginosa mucoid status [1]	47.2% (50)#
B. cepacia status [1]	18.9% (20)#
A. xylosoxidans status [1]	14.2% (15)#
S. aureus status [1]	80.2% (85)#

BMI = body mass index; % = percentage; SpO2 = Transcutaneous Hemoglobin Oxygen Saturation; FVC = forced vital capacity; FEV_1 = forced expiratory volume in the first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of FVC. 1. Based on 3 consecutive positive respiratory cultures.
*# Percentage (Number of patients)**
Continuous variables are expressed as the mean ± SD (range).

Table 2- Genotypic characteristics of the COX gene polymorphisms and CFTR gene mutations among the Cystic Fibrosis patients

Gene	Chromosome position	Location	Variation	Genotype			MAF	c^2	p*
				G/G	G/C	C/C			
COX2, rs20417	1q25.2-q25.3	promoter region	G>C	58 (10.24%)	44 (69.28%)	1 (20.48%)	0.22	5.52	<0.05[1]
				T/T	T/C	C/C			
COX2, rs5275	1q25.2-q25.3	3' untranslated region	T>C	41 (12.99%)	50 (75.32%)	8 (11.69%)	0.33	1.84	0.051
CFTR mutation genotype		N			Frequency				
F508del/F508del		41			38.68%				
F508del/G542X		12			11.32%				
F508del/R1162X		2			1.89%				
F508del/N1303K		4			3.77%				
F508del/R553X		1			1.14%				
F508del/S4X		1			0.94%				
F508del/1717-1G>A		1			0.94%				
F508del/2184insA		1			0.94%				
G542X/R1162X		1			0.94%				
G542X/I618T		1			0.94%				
F508del/duplication of exons 6b to 16		1			0.94%				
G542X/P205S		1			0.94%				
G542X/R334W		1			0.94%				
F508del/-		34			32.07%				
G542X/-		2			1.89%				
R1162X/-		1			0.94%				
-/-		1			0.94%				

*COX-2 = Cyclooxygenase-2; CFTR = Cystic fibrosis transmembrane conductance regulator; C = Cytosine; T = Thymine; G = Guanine; < = minor than; MAF = minor allele frequency; % = percentage; *p = value for Hardy-Weinberg Equilibrium; N = number of patients; (-) CFTR mutation not identified. 1= The COX-2 rs20417 polymorphism is not in Hardy-Weinberg Equilibrium in our samples.*

Table 3- Haplotype groups for the COX-2 polymorphisms rs20417 (-765G>C) and rs5275 (8473T>C)

Haplotype[1]	Frequency[2]	Genotype combination[3]	Haplotype group[4]
0	34 (%)	GG/TT	0
1	16 (%)	GG/TC	2
2	2 (%)	GG/CC	2
3	7 (%)	GC/TT	2
4	**31 (%)**	**GC/TC**	**1#**
5	6 (%)	GC/CC	2
6	0 (0%)	CC/TT	2
7	0 (0%)	CC/TC	2
8	0 (0%)	CC/CC	2

1. Haplotype groups for the COX-2 polymorphisms. The number codification was used to show the different groups of COX-2 polymorphism combinations. 2. Haplotype frequency - number of patients (percentage). 3. Genotype groups of the rs20417 and rs5275 COX-2 polymorphisms, respectively. 4. Haplotype group statistical analysis. # The heterozygous group (4) for the rs20417 and rs5275 COX-2 polymorphisms was the prevalent group, and the homozygous GG/TT (1).
COX-2 = Cyclooxygenase-2; C = Cytosine; T = Thymine; G = Guanine.

The associations between the *COX-2* polymorphisms and the categorical variables with p< 0.05 after the Bonferroni correction are shown in [Table-4]. The -8473C>T polymorphism was associated with nasal polyposis for the CC+CT genotype (OR=5.552, IC=1.318 -38.37), without accounting for the *CFTR* mutation. The haplotype analysis also showed an association with nasal polyposis and diabetes mellitus. Nasal polyposis was associated with the GG/TT genotype (OR=0.1056, IC=0.004-0.642) in patients, without accounting for the *CFTR* mutation. The GC/TC haplotype was associated with diabetes mellitus (OR=6.164, IC=1.719-23.83).

In [Table-5] (supplementary data), the corrected and uncorrected p-values are reported for all of the analyses performed, including the analysis of all of the patients included in the study and the patients with two mutations identified in the *CFTR* gene.

All of the data regarding the variables with p<0.05 and the haplotype distributions are shown in [Fig-1].

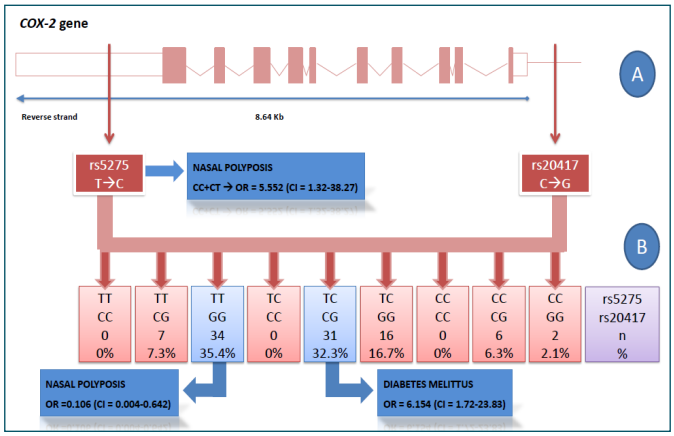

Fig. 1(A)- The *COX-2* gene structure. The exons are represented as rectangles, and the introns are represented as lines. A total of 10 exons can be observed. The locations of the rs20417 (-765G>C) and rs5275 (8473T>C) polymorphisms in the *COX-2* gene are indicated by bolts. **(B)-** The haplotype could be observed in all possible combinations and clinical associations observed in our sample. There was association with nasal polyposis and diabetes mellitus. For the association with nasal polyposis, the *CFTR* mutation was not taken into account. For the association with diabetes mellitus, all cystic fibrosis patients had two *CFTR* mutation identified in class I, II and III. *COX-2* = Cyclooxygenase-2; *CFTR* = Cystic Fibrosis Transmembrane Regulator; OR = odds ratio; CF = confidential interval; n = number of patients; C = cytosine; G = guanine; % = percentage.

Table 4- Comorbidities associated with the rs20417 (-765G>C) and rs5275 (8473T>C) COX-2 gene polymorphisms

Polymorphism 8473 in the *COX-2 gene*	Nasal polyposis		Total	c^2	p^c	OR	CI (5-95%)
	Presence	Absence					
CC+CT	13	2	15	**6.5091**	**0.044**	**5.552**	**1.318-38.27**
TT	45	39	84			-	-
Haplotype without taking the *CFTR mutation into account*	Nasal polyposis						
	Presence	Absence					
0	1	33	34			**0.1056**	**0.004-0.642**
1	8	23	31	**8.3241**	**0.032**	2.847	0.902-9.168
2	6	25	31			1.487	0.448-4.696
Haplotype in patients with two *CFTR mutation identified*	Diabetes mellitus						
	Presence	Absence					
0	2	22	24			0.236	0.0323-1.075
1	8	10	18	**8.4201**	**0.03**	**6.164**	**1.719-23.83**
2	3	18	21			0.538	0.107-2.139

COX-2 = Cyclooxygenase-2; CFTR = Cystic fibrosis transmembrane conductance regulator; C = Cytosine; T = Thymine; p^c = p-value corrected; OR = odds ratio; CI = confidence interval; % = percentage; 0 = GG/TT genotype, respectively, to rs20417 and rs5275 COX-2 gene polymorphisms (34 cystic fibrosis patients); 1 = GC/TC genotype for the rs20417 and rs5275 polymorphisms in the COX-2 gene, respectively (31 cystic fibrosis patients); 2 = GG/TC + GG/CC + GC/TT + GC/CC genotypes for the rs20417 and rs5275 COX-2 gene polymorphisms, respectively (31 cystic fibrosis patients).
The p (p-value) was corrected using the Bonferroni test (p^c)
1. c^2 and Fisher exact tests were used in all analyses according to the data distribution.
p-values with positive associations are in bold.

Discussion

CF is a disease with high clinical variability. Studies aimed at understanding the clinical variability of CF have primarily considered three variables: (i) *CFTR* mutations, (ii) the environment and (iii) modifier genes [1-3]. Modifier genes have been shown to be important factors for this lung disease. Previous studies considering polymorphisms in genes associated with immune system and the repercussions on lung function have demonstrated the impacts of

these genes on the clinical severity of CF [1-8].

The *COX-2* protein is associated with inflammation, which is one of the most important characteristics of CF [11,14,17,24]. There are two important polymorphisms in the *COX-2* gene: -765G>C and 8473T>C. The guanine substitution to cytosine in the -765 position occurs in the promoter region and is associated with a lower gene expression (approximately 30%) [24]. This may be due to the presence of a binding site for Sp1, which promotes transcription [25], and other proteins such as Sp3, which competes for the same site [26]. The -765G>C polymorphism is associated with cancer [27-30], and other polymorphisms in the promoter region are not associated with inflammation [31]. In contrast, the 8473T>C polymorphism occurs in the 3´UTR and alters the genetic expression by affecting the mRNA stability and protein synthesis, and in this context, this polymorphism can alter the COX-2 concentration [32,33]. The 8473T>C polymorphism was shown to be associated with the sarcoidosis [28] and carcinogenesis processes [34]. Sarcoidosis has a high inflammatory response [28]. Studies have indicated that *COX-2* polymorphisms in the promoter sequence are capable of modifying genetic expression and consequently changing the immune response in inflammatory diseases and carcinogenesis [24,35].

In CF, it has been hypothesized that high COX-2 levels can negatively impact the disease severity due to increased prostaglandin production, which intensifies the inflammatory response. Inflammation is a major cause of rapid airway deterioration in CF patients. A decrease in the concentration of active COX-2 is associated with a reduction in inflammation because prostaglandin synthesis is reduced. Consequently, lung damage would be reduced by the presence of the -765C and 8473C allelic variants [17,36].

The -765G>C and 8473T>C polymorphisms were previously associated with the 94 CF F508del homozygous genotype [17]. The -765C and 8473C allelic variants were associated with a reduction in the protein concentration. In this study, heterozygous genotypes were associated with higher FEV_1% and minor *P. aeruginosa* frequency values, but these associations were not found in all age groups, which could reflect the effects of chronic, progressive and age-dependent CF severity.

In our study, the -765G>C polymorphism did not follow a Hardy-Weinberg equilibrium, and this could be due to the clinical variation. The 8473T>C polymorphism was associated with nasal polyposis and the haplotype analysis with nasal polyposis and diabetes mellitus.

Table 5- Association of the rs20417 and rs5275 COX-2 gene polymorphisms with the clinical variables in the Cystic Fibrosis patients distributed by CFTR mutation and haplotype analysis of the COX-2 gene

Variables	-765				8473				Haplotype			
	Without taking the *CFTR mutation into account*		Two *CFTR identified mutations*		Without taking the *CFTR mutation into account*		Two *CFTR identified mutations*		Without taking the *CFTR mutation into account*		Two *CFTR identified mutations*	
	p	p^c	p	p^c	p	p^c	p	p^c	p	p^c	p	p^c
Sex[1]	1	1	0.223	0.446	1	1	0.617	1	0.792	1	0.271	
Race[1]	1	1	1	1	1	1	0.618	1	0.994	1	0.76	1
Age[1]	0.54	1	1	1	0.394	0.788	1	1	0.091		0.929	1
Onset of symptoms[1]	0.532	1	0.609	1	0.28	0.56	0.435	0.87	0.967	1	0.826	1
Onset of pulmonary disease[1]	0.837	1	0.217	0.434	0.676	1	0.798	1	0.866	1	0.589	1
Onset of digestive disease[1]	0.837	1	1	1	0.097	0.194	0.073	0.146	0.561	1	0.525	1
Diagnosis[1]	1	1	0.783	1	0.665	1	0.581	1	0.801	1	0.952	1
BMI[1]	0.278	0.556	1	1	0.578	1	0.741	1	0.983	1	0.256	0.512
Bhalla score[2]	0.495	0.99	0.042	0.084	0.747	1	0.564	1	0.812	1	0.368	0.736
Kanga score[2]	0.957	1	0.346	0.692	0.267	0.534	0.711	1	0.679	1	0.77	1
Shwachman-Kulczycki score[2]	0.645	1	0.177	0.354	0.923	1	0.628	1	0.709	1	0.146	0.292
Nasal polyposis[1]	0.798	1	1	1	0.022	0.044	0.092	0.184	0.016	0.032	0.096	0.192
Diabetes *mellitus*[1]	0.199	398	0.075	0.15	0.439	0.878	0.116	0.232	0.62	1	0.015	0.03
Osteoporosis[1]	0.773	1	0.282	0.564	1	1	1	1	0.726	1	0.876	1
Meconium ileous[1]	1	1	1	1	0.196	0.392	0.028	0.056	0.194	0.388	0.184	0.368
Insufficiency pancreatic[1]	0.39	0.78	0.495	0.99	0.14	0.28	0.495	0.99	0.219	0.438	0.373	0.746
SpO2[2]	0.901	1	0.377	0.754	0.203	0.406	0.561	1	0.631	1	0.522	1
FVC(%)[2]	0.696	1	0.382	0.764	0.611	1	0.684	1	0.617	1	0.291	0.522
FEV_1(%)[2]	0.569	1	0.362	0.724	0.683	1	0.629	1	0.534	1	0.263	0.526
FEV_1/FVC[2]	0.684	1	0.814	1	0.658	1	0.823	1	0.718	1	0.971	1
FEF_{25-75}%[2]	0.568	1	0.95	1	0.472	0.944	0.697	1	0.705	1	0.727	1
1st *P. aeruginosa*[1]	0.659	1	0.787	1	0.253	0.506	0.783	1	0.793	1	0.828	1
P. aeruginosa mucoid[1]	0.319	0.638	0.082	0.164	1	1	0.618	1	0.297	0.594	0.37	0.74
P. aeruginosa no mucoid[1]	0.839	1	0.797	1	0.532	1	0.442	0.884	0.826	1	0.918	1
A. xylosoxidans[1]	0.783	1	1	1	0.778	1	1	1	0.464	0.929	0.353	0.706
S. aureus[1]	0.129	0.258	0.765	1	0.439	0.878	1	1	0.194	0.388	0.903	1
B. cepacia[1]	0.798	1	0.765	1	0.615	1	0.55	1	0.99	1	0.422	0.844

COX-2 = Cyclooxygenase-2; BMI = body mass index; % = percentage; SpO2 = Transcutaneous Hemoglobin Oxygen Saturation; FVC = forced vital capacity; FEV_1 = forced expiratory volume in the first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of FVC; P. aeruginosa = Pseudomonas aeruginosa; A. xylosoxidans = Achromobacter xylosoxidans; S. aureus = Staphylococcus aureus; B. cepacia = Burkolderia cepacia.

The p (p-value) was corrected using the Bonferroni test (p^c)

1. c^2 and Fisher exact tests were used in all analyses according to the data distribution.

2. Mann-Whitney and Kruskal-Wallis tests were used in all analysis according to the data distribution.

p-values with positive associations are in bold.

Nasal polyposis and diabetes mellitus are important CF comorbidities, being present in approximately 17.9 and 18.9% of the CF patients in our center, respectively. The comorbidity associations found in this study are important, but we did not find any previous studies regarding the association between the *COX-2* gene and the CF comorbidities or the genetic factors associated with CF nasal polyposis. To the best of our knowledge, diabetes mellitus associated with CF is also associated with the deterioration of endocrine signaling by the pancreas. Genes associated with inflammation can be risk factors for some of the present comorbidities, and in this case, we showed an association between the *COX-2* polymorphism and nasal polyposis and diabetes mellitus. Interestingly, an association between the haplotype and comorbidities was observed in this study, which demonstrates that an analysis of only one polymorphism can mask the true associations between clinical severity and modifier genes.

In conclusion, the associations between *COX-2* polymorphisms and CF are not clear. In the literature, only one study assessing the associations between the polymorphisms and CF was found, and that study did not consider CF comorbidities or other clinical variables. Thus, the study described here is the first evidence for an association between the *COX-2* gene and nasal polyposis and diabetes mellitus CF comorbidities. Modifiers genes are important factors in CF with respect to the lung disease, but we need to also consider other clinical aspects as the comorbidities of CF. In recent years, the prognosis and life expectancy of CF patients have improved, and as a result, the comorbidities have increased. A better understanding of the complex aspects of CF comorbidities can provide better treatments, particularly in older CF patients. In the future, a large population should be studied with class I, II and III *CFTR* mutations, and new gene polymorphisms should be considered with haplotypes and clinical variables, including comorbidities.

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