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ADIPOR2 Polymorphisms in Cystic Fibrosis are Potential Modifiers of Clinical Severity

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Abstract

Objective: Cystic Fibrosis (CF) severity is determined by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene, the environment and modifier genes. The adiponectin receptor 2 (ADIPOR2) gene is one of the potential modifier genes of CF and is responsible for expressing an anti-inflammatory protein. In this context, the 315 base deletion and the 134 base insertion polymorphisms in the ADIPOR2 gene are associated with CF severity.

Methodology: A total of 169 CF patients were enrolled, and 27 CF clinical markers were analyzed.

Results: The 315 base deletion and haplotypes analyses showed an association with the patient´s age and ethnicity. For the age, the deletion in both alleles was associated with a lower age in the patients without any identified CFTR mutations (OR= 7.257; 95%CI= 1.321-44.83) or disregarding CFTR mutations (OR= 2.394; 95%CI= 1.046-5.721). For the ethnicity, the wild-type allele (315 base no deletion) showed lower risk of occurrence in Caucasians patients with two CFTR mutations identified (OR= 0.052; 95%CI= 0.002-0.382) or no CFTR mutations (OR= 0.154, 95%CI= 0.032-0.592). The same values were observed for the clustered analysis of the 315 base deletions. In the analysis of the haplotype, in the Caucasian group, the patients with two CFTR mutations were identified and demonstrated an OR of 0.076 (95%CI= 0.009-0.432) for wild-type alleles. The 134 base insertions were associated with the SpO_2 [patients without considering the CFTR genotype (p-value= 0.034) and without any identified CFTR mutations (p-value= 0.034)] and the Kanga score (no CFTR mutations identified) (p-value= 0.008). The haplotype was associated with the forced vital capacity (disregarding CFTR genotype) (p-value= 0.028).

Conclusion: The 315 base deletions and the insertion of 134 bases in the ADIPOR2 gene are potential modifiers of the severity of CF and should be considered during CFTR mutation screening.

Keywords: Adiponectin; ADIPOR2; Cystic fibrosis; CFTR; Lung disease; Polymorphisms; Modifier genes

Introduction

Cystic Fibrosis (CF) (MIM: 219700) presents as phenotypic heterogeneity of the clinical manifestations that are modulated by cystic fibrosis transmembrane regulator (CFTR) mutations [1,2], the environment and modifier genes [3-5]. Our group has studied the clinical modulation of CF by candidate modifier genes that influence drug responses, the evolution of the lung and digestive disease in CF, and CF-related comorbidities [6-14].

The adiponectin receptor 2 (ADIPOR2) gene, located in the region 12p13.3, was studied in CF and associated with the presence of meconium ileus [15]. The ADIPOR2 gene encodes an integral membrane protein, the adiponectin receptor, which regulates the expression of a diabetic hormone secreted by adipocytes and the absence of which is associated with insulin resistance [16]. Some of the functions of the ADIPOR2 protein are as follows: causing an increased expression of adiponectin, which is associated with increased insulin sensitivity; modulating inflammatory processes; modulating the fatty acid metabolism in the liver (i.e., the increased synthesis and decreased oxidation of fatty acids by the down-regulation of ADIPOR may contribute to the progression of non-alcoholic steatohepatitis); down-regulating the adiponectin receptor pathway, which could be causally implicated in decreased cardiovascular function; and reducing cancer with adiponectin/AdipoRs, at least in part, through ameliorating hyperinsulinemia, as well as through its direct effects on tumor cells via inhibition of the mammalian target of rapamycin pathway by activating AMP kinase [17].

Taking into account the expression of adiponectin receptor 2 in various tissues, especially the liver and the presence of circulating adiponectin hormone concentrations in blood that is associated with diseases, it is unclear how the signaling pathway of the adiponectin hormone can affect the meconium ileus and, in this study, the different clinical variables analyzed as CF severity. However, it is likely that the differential expression of the ADIPOR2 protein is associated with variations in the introns or promoter region of the ADIPOR2 gene, resulting in unequal phenotypic expression among CF patients. Regarding CF and meconium ileus, two mutations of copy number variation (the 315 base deletions and the insertion of 134 bases) in the ADIPOR2 gene were identified [15].

In this context, the aim of this study was to investigate the association between these two Copy Number Variations (CNVs) (the 315 base deletions and the insertion of 134 bases) in the ADIPOR2 gene and the presence of meconium ileus in CF patients.

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In addition to complete clinical characterization and association, the polymorphisms were associated with other markers of CF severity and compared considering *CFTR* mutations. The association of *ADIPOR2* polymorphisms with pulmonary inflammatory disease was made using the expression of ADIPOR2 protein in the blood and in various tissues, and the expression of the *ADIPOR2* gene was associated with the inflammatory process, a process present in the lungs of CF patients.

Methodology

This cross-sectional study was conducted between 2012 and 2013 at a university center for CF care. Two hundred and fifteen patients were selected for the study, of which 46 were excluded for not signing the consent form or because of a lack of clinical data for the statistical analysis. The CF diagnosis was confirmed if the levels of chloride in the sweat exceeded 60 mEq/L and, when possible, by *CFTR* mutation screening. CF patients, with no identified *CFTR* mutation or with one *CFTR* mutation screened, were classified as having CF. The following parameters were considered for diagnosis: (i) all of the patients had levels of chloride in their sweat that exceeded 60 mEq/L; (ii) the CF clinical symptoms were diagnosed in all of the patients as chronic obstructive pulmonary disease, bacteria in the sputum, spirometry with obstruction values for the forced expiratory volume in the first second ($FEV_1\%$), and the presence of the associated comorbidities (i.e., osteoporosis, nasal polyps, diabetes mellitus and pancreatic insufficiency); (iii) the expression of active CFTR in the epithelium determined via rectal biopsy (all of the patients included had abnormal values for biopsy, an absence of active CFTR); (iv) the nasal potential was determined in some of the patients (all of the values were altered, but the comparison was not performed due to the lack of a control standard curve and thus being an inconclusive data). By these criteria, it was possible to exclude patients with cystic fibrosis-related diseases. None of the patients were diagnosed by a neonatal screening test. Patient DNA was obtained by phenol-chloroform extraction, and 50ng/mL was used for the analysis, as evaluated by a GE Nano Vue™ Spectrophotometer (GE Healthcare Biosciences, Pittsburgh, PA, USA).

Clinical Variables

Several clinical variables were employed, including the following: Shwachman-Kulczycki, Kanga and Bhalla clinical scores [18]; body mass index (BMI) [for patients older than 19 years, the BMI = weight/(height)^2 formula was used, whereas for patients aged 0 - 5 years the WHO ANTHRO program was used and for patients aged 5 - 19 years of age the WHO ANTHRO PLUS program was used]; the patient's age (≤154 and >154 months); the time to diagnosis (≤24 and >24 months); the time of the first clinical symptoms (digestive: ≤3 and >3 months; pulmonary: ≤6 and >6 months); the time to the first colonization by *Pseudomonas aeruginosa* (≤31 and >31 months); the bacteria in the respiratory airways: mucoid *P. aeruginosa* and non-mucoid *P.aeruginosa*, *Achromobacterxylos oxidans, Burkholderia cepacia* and *Staphylococcus aureus*; and transcutaneous hemoglobin oxygen saturation (SpO_2) and spirometry variables. A positive bacterial status was determined by the presence of a chronic infection (patients in whom more than 50% of the preceding 12 months were culture positive) and intermittent infections (patients with less than 50% of cultures positive). A patient was negative for infections when the patient was considered free of bacteria (when no bacterium was grown from samples in the previous 12 months, despite a history of prior colonization) and never infected (the bacterium had never been cultured). These criteria were formulated for *P. aeruginosa*, but for our data it was used for all of the bacteria analyzed [19].

Spirometry was performed in patients older than 7 years of age with the CPFS/D spirometer (Med Graphics, Saint Paul, MN, USA), and data were recorded using thePF BREEZE software version 3.8B for Windows 95/98/NT [20]. The following variables were included: the forced vital capacity [FVC (%)]; the FEV_1 (%), the ratio between FEV_1 and FVC (%) [FEV_1/ FVC]; and the forced expiratory flow between 25 and 75% of the FVC [FEF 25-75%]. The data were analyzed considering international curve values for spirometry tests [21].

The comorbidities analyzed were nasal polyps, osteoporosis, 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 via polymerase chain reaction (PCR) for the F508del and the fragment-length polymorphism method for the G542X, R1162X, R553X, G551D, and N1303K mutations. Some of the CF mutations were identified through sequencing or a Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. A MegaBace1000° sequencer (GE Healthcare Biosciences) was used for the sequencing and MLPA. The *CFTR* genotype was used as a correction factor for the statistical analysis. All Class I, II or III mutations but not Class IV, V or VI mutations identified were included in statistical analysis. The complete sequence is in supplementary 1.

Identification of polymorphisms the 315 base deletion and the insertion of 134 bases in the *ADIPOR2* gene

Two CNVs in the *ADIPOR2* gene were analyzed. The CNV consisting of a 315 base deletion (variation #11592, also known as rs35417183) is at chr12: 1734273-1734587 in intron 2 of the *ADIPOR2* longest splice variant NM024551, and intron 1 of the shorter alternative splice variant AY424280. The insertion of 134 bases is present (variation #24961) at chr12: 1754352-1754485 in intron 3 of the long mRNA isoform. The genotyping for the 315 base deletions and the insertion of 134 bases was performed in multiplexed reactions using specific primers [ADIPOR2_315delF1 - 5´-TGA CAG CCA CCA AGG AGA TTT GGA-3´ and ADIPOR2_315delR1 - 5´-AGA CCA CGT CTT CTC CCT TCA ACA-3´;ADIPOR2_134delF1 - 5´-AGC TTG ACA AAG ACA CTG CCT ACC-3´ and ADIPOR2_134delR1 - 5´-AGC ATA ACC AGG TCC ATG TGG GAA-3´] in multiplexed PCR at standard cycling conditions (60°C for the annealing for 45 s, 1 min for the extension at 72°C). For the 315 base deletions the reaction had 991 bases (wild type) and 676 bases (deletion type); for the insertion of 134 bases, the reaction had 451 bases (wild type) and 585 bases (insertion type) [15].

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 21.0 (SPSS Inc., Chicago, IL, USA), Epi Info version 6.0 [22] and R version 2.12 (Comprehensive R Archive Network, 2011). GPower 3.0.3.1 software [23] was used to calculate the statistical power, which was required to be above 80% for analysis.

The statistical tests included the Mann-Whitney test, Kruskal-Wallis test, chi-square (χ^2) test and Fisher's exact test. The Odds Ratio

(OR) was calculated with the Mid-P exact test. To avoid spurious data caused by the performance of multiple tests, the significance level (α) was adjusted by the False Rate Discovery (FDR) correction [24]. The value of α was corrected considering the clinical marker analysis of the same group of patients and taking into account the *CFTR* mutation genotype.

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

The analyses were performed on four cohorts: (i) all patients with CF (n=169); (ii) patients with no identified *CFTR* mutation (n=40); (iii) patients with an identified mutant *CFTR* allele (Class I, II and/or III) (n=39); and (iv) Patients with two identified *CFTR* mutations (Class I, II and/or III) (n=90).

Results

The clinical data of the patients included in the study are presented in Table 1. Table 2 shows the genotype distribution described for the *ADIPOR2* gene polymorphisms and the genotypes for *CFTR* mutations (the characterization of the *CFTR* mutations identified is described in Supplementary 1). The deletion of 315 bases was not present in 51 (31.8%) patients but was found to be heterozygous in 79 (49.4%) and homozygous in 30 (18.8%) patients. Regarding the polymorphism of

insertion of 134 bases, there was no insertion in 132 patients (78.1%) and there was heterozygous insertion in 37 (21.9%).

For the variables with categorical distribution, the 315 bases deletions and the analysis of haplotypes showed a positive association. In the present study, the patient´s age and the ethnicity showed positive correlation. For age, the presence of 315 bases deletions in both alleles was associated with a lower age of the patient in the patient group without the *CFTR* mutations identified (OR= 7.257; 95% CI= 1.321 - 44.83) and in the group of patients without considering the *CFTR* mutations (OR= 2.394; 95% CI= 1.046 - 5.721). Considering the ethnicity of patients, the presence of the wild-type allele in homozygotes was associated with a lower risk of occurrence in Caucasians in groups of patients with two *CFTR* mutations identified (OR= 0.052, 95%CI= 0.002 - 0.382) and in the group disregarding *CFTR* mutations (OR= 0.154; 95% CI= 0.032 - 0.592). The same values were observed in the analysis of clustered data. In the analysis of haplotypes, in the group of patients in which two *CFTR* mutations were identified, there was an odds ratio of 0.076 (95% CI= 0.009 - 0.432) for the group 0 (wild-type alleles for both polymorphisms). The data are summarized in Table 3.

In the analysis of numerical variables, a positive association for the 134 base insertions for SpO$_2$ (Figure 1a and 1b) for the groups of patients without considering the *CFTR* genotype (p-value= 0.034) and without identified mutations (p-value= 0.034), respectively] and the Kanga score (Figure 1c - no *CFTR* mutations identified) (p-value= 0.008) was found. The SpO$_2$ was greatest in patients with wild-type

Characteristic	Total number of patients	Data distribution
Female gender	169	87 (51.5)
Age (months)	168	154; 177.13 (95%CI: 157.26 – 197.01) ± 77.71; 87 – 468
Caucasoid	169	158 (93.5)
BMI - thinness and accentuated thinness	167	34 (20.4)
One class I, II or III identified mutation		39 (23.1)
Two class I, II or III identified mutations		90 (53.3)
Age at first clinical manifestation (months)	159	2; 4.607 (95%CI: 2.775 – 6.438) ± 7.151; 0 – 39
Age at diagnosis (months)	162	16; 36.197 (95%CI: 21.528 – 50.865) ± 57.27; 0 – 379
Age at start of digestive symptoms (months)	140	3; 18.672 (95%CI: 5.189 – 32.156) ± 52.6465; 0 – 381
Age at start of pulmonary symptoms (months)	154	4; 11 (4.431 – 17.569) ± 25.649; 0 – 187
SpO$_2$ (%)	162	97; 95.164 (93.95 – 96.378) ± 4.741; 66 – 99
Bhalla score	122	8; 8.697 (7.441 – 9.772) ± 4.551; 0 – 23
Kanga score	131	18; 19.361 (17.915 – 20.806) ± 5.645; 11 – 40
Shwachman-Kulczycki score	144	65; 67.049 (63.255 – 70.843) ± 14.815; 20 – 95
FVC (%)	126	82; 82.295 (76.739 – 87.851) ± 21.693; 19 – 132
FEV$_1$ (%)	124	73; 75.131 (68.831 – 81.432) ± 24.601; 19 – 132
FEV$_1$/FVC (%)	123	86; 83.937 (80.617 – 87.317) ± 13079; 37 – 100
FEF$_{25-75}$%	124	62; 62.066 (54.110 – 70.021) ± 31.062; 8 – 137
Nasal polyps	166	32 (19.3)
Diabetes mellitus	166	30 (18.1)
Osteoporosis	166	28 (16.9)
Pancreatic insufficiency	168	136 (81)
Meconium ileus	168	25 (14.9)
Age at first isolated *P. aeruginosa* (months)	122	31; 54.066 (37.578 – 70.553) ± 64.375; 4 – 379
P. aeruginosa status[a]	169	91 (53.8)
P. aeruginosa mucoid status[a]	169	70 (41)
B. cepacia status[a]	169	17 (10.1)
A. xylosoxidans status[a]	169	23 (13.6)
S. aureus status[a]	169	134 (79.3)

Table 1: Patient characteristics (n=169). Continuous variables expressed as median; mean (95%CI) ± SD; range. Other data shown as number of patients (percentage).
[a] Based on three consecutive positive respiratory cultures.
N- Sample size; BMI:-Body mass index; SpO$_2$-Hemoglobin oxygen saturation in the blood; 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.

Gene	Region	Localization	Variation	MAF	p*
ADIPOR2 variation #11592	12p13.3	Intron 2	315 bases deletion	0.44	>0.05
ADIPOR2 variation #24961	12p13.3	Intron 3	deletion of 134 bases	0.11	>0.05
***CFTR* mutations**			N		Frequency
2183AA>G/2183AA>G			1		0.6%
3120+1G>A/3120+1G>A			1		0.6%
3120+1G>A/R1066C			1		0.6%
3120+1G>A/L206W			1		0.6%
F508del/F508del			44		26%
F508del/G542X			12		7.1%
F508del/R1162X			4		2.4%
F508del/N1303K			5		3%
F508del/R553X			3		1.8%
F508del/S549R (T>G)			1		0.6%
F508del/c.1717-1G>A			2		1.6%
F508del/1584-18672pbA>G			2		1.2%
F508del/1812-1G>A			2		1.2%
F508del/2183AA>G			2		1.2%
F508del/3272-26A>G			1		0.6%
F508del/G85E			1		0.6%
F508del/P205S			1		0.6%
F508del/R1066C			1		0.6%
F508del/duplication exon 6b – 16			1		0.6%
F508del/S4X			1		0.6%
D110H/V232H			1		0.6%
A561E/A561E			1		0.6%
622-2A>G/711+1G>T			1		0.6%
G542X/R1162X			1		0.6%
G542X/I618T			1		0.6%
G542X/2183AA>G			1		0.6%
G542X/R334W			1		0.6%
G542X/P205S			1		0.6%
G542X/R1070Q;S466X			1		0.6%
R1162X/R1162X			1		0.6%
R334W/R334W			1		0.6%
R334W/R1066C			1		0.6%
F508del/-			26		15.4%
G542X/-			5		3%
R1162X/-			1		0.6%
I507V/-			1		0.6%
TG11-5T/-			1		0.6%
V562I/-			1		0.6%
-/-			35		20.7%

Table 2 Genetic characteristics of polymorphisms analyzed and *CFTR* mutations in the cystic fibrosis population enrolled MAF- Minor Allele Frequency; ADIPOR2- Adiponectin Receptor 2; *CFTR* - Cystic Fibrosis Transmembrane Regulator. * The included polymorphisms are in Hardy-Weinberg Equilibrium.

alleles, and the kanga score values showed lower values in patients with wild-type alleles. In another analysis, the haplotype was associated with FVC (Figure 1d) without considering the *CFTR* genotype) (p-value= 0.028). The absence of polymorphisms was associated with better values of FVC.All of the p-values are described in supplementary 5 to 8 for the 134 base insertions, 315 base deletions, and 315 base deletions (clustered and haplotypes), respectively. The tables show the p-values and corrected p-values by the FRD test.

Discussion

General aspects

CF is an autosomal recessive monogenic disease but shows high phenotype variability, with individual variation in the severity of disease, especially in the pulmonary symptoms, the age of symptoms onset, the clinical course, the comorbidities, and the response to drugs. The severity of disease is also dependent on the *CFTR* mutations, environmental factors and modifier genes [3-14]. Studies to understand the observed variation have been performed, and in the present data the *ADIPOR2* gene and its variations was considered.

The *ADIPOR2* gene plays an important role in cell metabolism, encoding a receptor for adiponectin, which participates in the pathways of glucose uptake and oxidation of fatty acids. In addition, adiponectin demonstrates anti-inflammatory activity [16,17] which may play a role in the modulation of the clinical manifestations of CF.

CNVs prevalence in CF patients

In the present study, we found that the 315 base deletion in the intron 2 in *ADIPOR2* gene was present in 68.2% of patients (including

CFTR Class	Polymorphism group	Genotype	Ethnicity		Total	OR	95%CI
			Caucasian	Non-Caucasian			
MI/MI*	315 bases deletion	-/-	18	6	24	**0.052**	**0.002 – 0.382**
		-/+	44	1	45	**7.694**	**1.058 – 183.5**
		+/+	16	0	16	-	-
	Total		78	7	85		
Total*	315 bases deletion	-/-	43	8	51	**0.154**	**0.032 – 0.592**
		-/+	76	3	79	2.76	0.725 – 13.29
		+/+	30	0	30	-	-
	Total		149	11	160		
CFTR Class	Polymorphism group	Genotype	Age		Total	OR	95%CI
			< 154 months	≥ 154 months			
NMI/NMI*	315 bases deletion	-/-	4	10	24	1.503	0.298 – 7.301
		-/+	0	15	15	-	-
		+/+	5	4	9	**7.257**	**1.321 – 44.83**
	Total		9	29	38		
Total*	315 bases deletion	-/-	22	29	51	0.826	0.423 – 1.603
		-/+	37	42	79	0.819	0.438 – 1.528
		+/+	20	10	30	**2.394**	**1.046 – 5.721**
	Total		79	81	160		
CFTR Class	Polymorphism group	Genotype	Ethnicity		Total	OR	95%CI
			Caucasian	Non-Caucasian			
MI/MI#	315 bases deletion clustered	-/-	18	6	24	**0.052**	**0.002 – 0.382**
		-/+ and +/+	60	1	61	1	-
	Total		78	7	85		
Total#	315 bases deletion clustered	-/-	43	8	51	**0.154**	**0.032 – 0.592**
		-/+ and +/+	106	3	109	1	-
	Total		149	11	160		
CFTR Class	Polymorphism group	Genotype	Ethnicity		Total	OR	95%CI
			Caucasian	Non-Caucasian			
MI/MI*	Haplotype	0	12	5	17	**0.076**	**0.009 – 0.432**
		1	37	1	38	5.327	0.742 – 128.5
		2	15	0	15	-	-
		3	6	1	7	0.505	0.059 – 13.42
		4	8	0	8	-	-
	Total		78	7	85		

Table 3: Association of 315 bases deletion (variation #11592, also known as rs35417183) in *ADIPOR2* gene with thecategorical variables of cystic fibrosis patients *ADIPOR2*- Adiponectin Receptor 2; *CFTR*- Cystic Fibrosis Transmembrane Regulator; OR- Odds ratio; Ci- Confidential Interval; MI- *CFTR* Mutation Class I, II or III; MNI, no identified *CFTR* mutation or *CFTR* mutation Class IV, V or VI; +, represents the polymorphism presence; 0, only wild-type alleles; 1, insertion of 134 bases with wild-type + 315 bases deletion with one deleted allele; 2, insertion of 134 bases with wild-type + 315 bases deletion with two deleted alleles; 3, insertion of 134 bases with one inserted allele + 315 bases deletion with wild alleles; 4, insertion of 134 bases with one inserted allele + 315 bases deletion with one/two deleted alleles. * Statistical analysis performed by χ2 test; # Statistical analysis performed by Fisher's Exact Test. The positive values are in bold. The odds ratios were performed considering the best approach using the epi info software, in this case, the Mid P test was considered.

homozygous and heterozygous) and that the insertion of 134 bases in the intron 3 was observed in 21.9% of CF patients (only heterozygous). This demonstrates that the 315 base deletion polymorphism frequency was higher than the insertion of 134 bases polymorphism in the CF patients analyzed.

Clinical variables and correlation with CNVs

In the analysis of the clinical variables, we can highlight the predominance of Caucasian ethnicity (93.5%) with homogeneity between sexes (male 51.5%). There was presence of pancreatic insufficiency in 81% of the patients, infection by non-mucoid *P. aeruginosa* occurred in 53.8% of the patients, and meconium ileus occurred in 14.9% of the patients.

The association between meconium ileus and the polymorphisms analyzed in the *ADIPOR2* gene was not statistically significant when the *CFTR* mutation groups were considered. In a previous study [15],

the association of the 315 base deletions and meconium ileus presence has been described; however, as in our study, there was no association with the insertion of 134 bases. It is likely that the difference in results is related to the number and characterization of the samples because the Brazilian population is admixed. We have a high percentage of patients with meconium ileus, but in absolute number the number of patients is too low to provide multiple statistical analyses.

315 base deletions inthe *ADIPOR2* gene

For the other clinical variables analyzed, the association (p-values <0.05) between the 315 base deletions in the *ADIPOR2* gene and the ethnicity of the CF patients was observed. The prevalence of this polymorphism was higher in Caucasian individuals than in non-Caucasian patients with two *CFTR* mutations identified and when all of the patients were analyzed at the same time. This information demonstrates that the probability of a patient being Caucasoid and having this CNV is higher, despite having no direct relationship
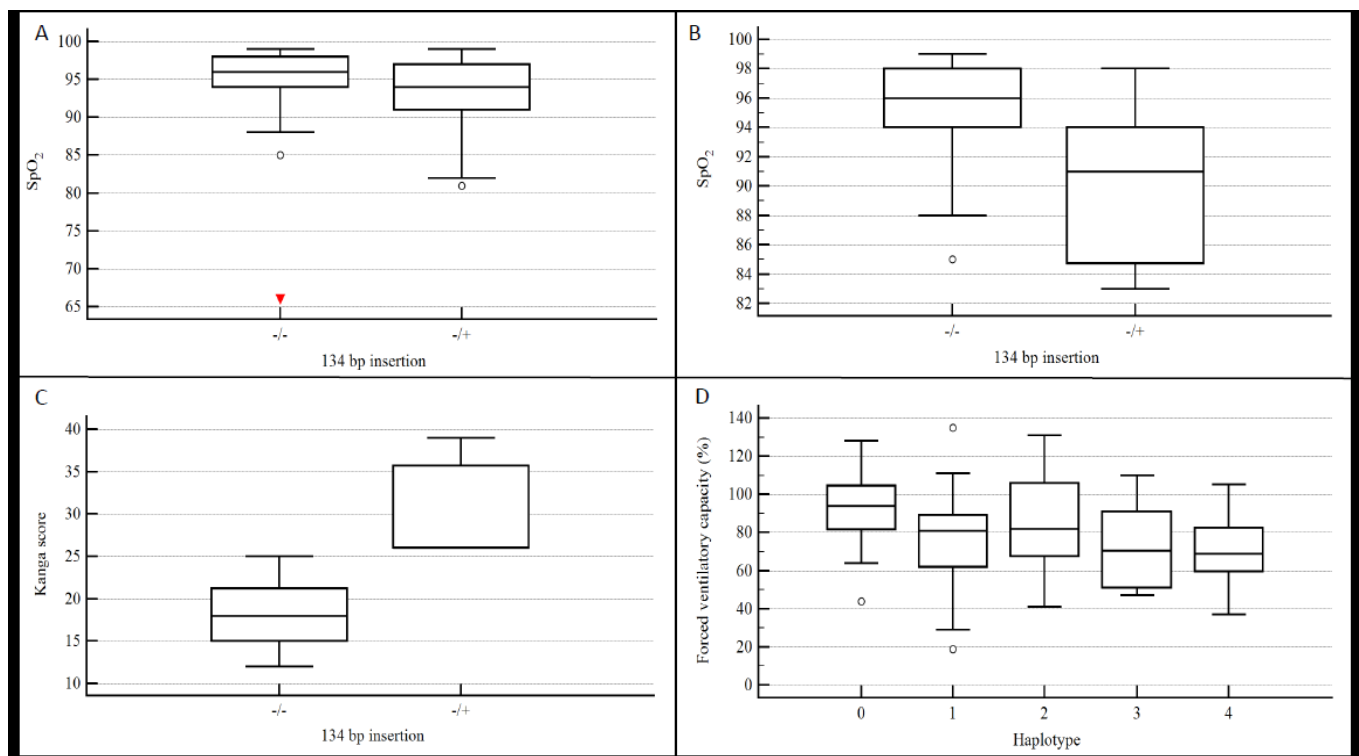
Figure 1: The association of the numerical data of cystic fibrosis patients with the insertion of 134 bases polymorphism. A. SpO2 in patients without taking into account CFTR mutations (p-value = 0.034). B. SpO2 in patients without CFTR mutations identified (p-value = 0.034). C. Kanga score in patients without CFTR mutations identified (p-value = 0.008). D. Haplotype analysis in patients without taking into account CFTR mutations considering the FVC (p-value = 0.028); 0 ≠ 1,2 and 4. CFTR= Cystic Fibrosis Transmembrane Regulator; +, represents the polymorphism presence; FVC, Forced Ventilatory Capacity; 0, only wild-type alleles; 1, insertion of 134 bases with wild-type + 315 bases deletion with one deleted allele; 2, insertion of 134 bases with wild-type + 315 bases deletion with two deleted alleles; 3, insertion of 134 bases with one inserted allele + 315 bases deletion with wild alleles; 4, insertion of 134 bases with one inserted allele + 315 bases deletion with one/two deleted alleles. For A, B and C, the Mann-Whitney test was performed. For D, the Kruskall-Wallis test was performed.

with the clinical severity of the disease. The same was observed for the haplotype analysis: the absence of polymorphisms was lower in the Caucasian patients group. It is important to highlight that the polymorphisms were in Hardy-Weinberg equilibrium in our data. For the age of the patients, there was lower age in the group of patients with both alleles with 315 base deletions. An association was found in this group of patients without the *CFTR* mutations identified, making it difficult to clarify the associations reported.

Insertion of the 134 bases in the *ADIPOR2* gene

Regarding the insertion of the 134 bases in the *ADIPOR2* gene, there was a significant association between the SpO_2 (excluding the *CFTR* mutations and in patients without identified mutations) and, on average, lower values in patients heterozygous for the deletion compared with those who did not show the polymorphism. Both the pulmonary function data and the isolated bacteria in the sputum culture were important variables related to the evolution of CF and the patient's quality of life. The insertion polymorphisms in the intron regions usually cause a reduction in gene expression. In this case, the lower expression was associated with lower SpO_2, being that this is a clinical marker of lung disease.

The Kanga score, which is considered an important marker of lung disease and is associated with pulmonary exacerbations, was associated with patients without considering genotype in the *CFTR* gene. The score was unremarkable in patients with the wild-type genotype. In this context, the insertion of 134 bases was associated with a higher Kanga score and, in this case, worse clinical manifestation.

In the present study, another important association was that between the haplotype and the FVC. The FVC values were high in patients with the wild-type alleles for the insertion of 134 bases.

Thus, the associations found with CNVs in the *ADIPOR2* gene may be helpful in future genetic research, drug development targeting and collaborating with clinical practices. Other variables could also have a positive association with the polymorphisms analyzed when a larger, less admixed population is assessed.

In conclusion, the 315 base deletions in intron 2 and the insertion of 134 bases in intron 3 of the *ADIPOR2* gene may influence the clinical severity of CF. Although there was not a statistically significant relationship between these CNVs and the presence of meconium ileus in patients with CF in our study, the other markers we analyzed showed their potential association with the severity of the disease, confirming the role of modifier assigned to the *ADIPOR2* gene.

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