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ORIGINAL RESEARCH

Evaluation Of *HHIP* Polymorphisms And Their Relationship With Chronic Obstructive Pulmonary Disease Phenotypes

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Purpose: We aimed to correlate three polymorphisms of the Hedgehog Interacting Protein (*HHIP*) gene with the three main phenotypes of the chronic obstructive pulmonary disease (frequent exacerbator (FE), asthma/COPD overlap (ACO), and emphysema with hyperinflation).

Patients and methods: A cross-sectional study was carried out in the Department of Pulmonology at the Rio de Janeiro State University from February 2015 to July 2018. A total of 81 patients diagnosed with COPD according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) were enrolled. The subjects were divided into three distinct groups according to their phenotypes (FE, ACO and emphysema-hyperinflation). Three polymorphisms of the *HHIP* gene that are often reported as allegedly involved in the pathogenesis of COPD were analysed: rs1828591, rs13118928, and rs6537296. Real-time PCR - TAQMAN SNP Genotyping Assay was performed. The statistical analysis was carried out with the SPSS program with a multivariate analysis with a 95% confidence interval.

Results: An increase in the frequency of the A allele of the rs13118928 *HHIP* gene polymorphism was observed in the group of subjects with COPD and emphysema-hyperinflation phenotype when compared with those in the FE phenotype (p=0.019) and subjects with ACO (p=0.04). However, the subjects with emphysema-hyperinflation phenotype presented more often the A allele (p=0.04). The genotypic analysis confirmed the difference between the emphysema-hyperinflation and ACO phenotypes, with a higher prevalence of the AA genotype in the emphysema-hyperinflation group (p=0.04). The ACO and FE phenotype subjects showed no difference in these polymorphisms. No difference was found in the frequency of the polymorphisms rs1828591 (p= 0.552) and rs6537296 (p=0.296) in the three phenotypes evaluated.

Conclusion: The presence of the A allele in the rs13118928 polymorphism of the *HHIP* gene may be related to the emphysema-hyperinflation phenotype.

Keywords: Chronic obstructive pulmonary disease, ACO, hyperinflation, exacerbator, HHIP polymorphism

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Introduction

The COPD presents some distinct phenotypes despite the same risk factors. They are clinically heterogeneous, and the identification of each subgroup seems to be important to define the therapeutic setting. The phenotypes may be related to important prognostic factors such as the presence of symptoms, a higher or lower risk of exacerbation, the response to therapy, the rate of disease progression and mortality.[1–3] Several recent studies have attempted to define phenotypes based on

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various criteria. Miravitlles et al[4] described the three main phenotypes: asthma-COPD overlap (ACO), FE and emphysema-hyperinflation.

The asthma-COPD overlap definition is highly variable. Briefly, patients with ACO have a combination of the following factors: history of asthma and/or atopy, reversibility in the bronchodilator test, notable eosinophilia in respiratory and/or peripheral secretions, high IgE, positive prick test to allergens and high concentrations of exhaled NO.[4–9] The prevalence of this phenotype is estimated at 20% of COPD patients, and it is more common among the elderly – 50% of the patients are above 50 years.[10,11] Furthermore, they commonly present a better response to corticoids.[4,12]

The exacerbator (FE) phenotype presents occasional episodes of clinical instability of the disease repeatedly within a short period of time. The FE is defined when patients present two or more exacerbations per year and the exacerbations are at least 4 weeks apart after the end of treatment or 6 weeks from the onset of symptoms in case patients have not received any treatment.[1,4] Patients who have been hospitalized in the previous year are also considered exacerbators.[4,11,13]

The emphysema-hyperinflation phenotype has an anatomical-pathological definition at the terminal bronchioles level. These patients clinical characteristics include the presence of dyspnea, exercise intolerance, signs of hyperinflation, and a tendency for low body mass index.[1,4] Hyperinflation can be detected by the chest computed tomography scans and/or a respiratory function examination with assessment of the reduced diffusing capacity for carbon monoxide (DL_{CO}). These patients seem to better benefit when treated with bronchodilator therapy involving two long-acting bronchodilators.[4,14]

The Hedgehog interaction protein is encoded by the 4q31 chromosome and participates in a series of signalling pathways that include pulmonary organogenesis and the response to lung injury in response to cigarette smoking.[15] Several studies have been carried out in an attempt to correlate polymorphisms of the Hedgehog Interacting Protein gene (*HHIP*) with the susceptibility to COPD. The reduced expression of this protein seems to predispose to COPD. Two polymorphisms seem to be related to the reduction of the triggering activity of this protein – rs6537296 and rs1542725.[16] Two other polymorphisms that tend to occur together, the rs1828591 and rs13118928, seem to facilitate the expression of an altered and non-functional protein, with a greater risk for the development of the COPD.[15,16] The SNP rs11938704 correlates significantly with the forced expiratory volume drop in the first second (FEV_1) in the COPD population.[17] However, so far, there has been no report of the association of the *HHIP* polymorphisms with a particular COPD phenotype.[17,18]

Materials And Methods

A cross-sectional study was carried out with outpatients at the Rio de Janeiro State University (UERJ). Patients enrolled in this study have had the COPD diagnosis in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018, with the spirometry revealing an obstructive ventilatory disorder (FEV_1/FVC after bronchodilator < 0.7) and a smoking history < 20 pack/year. Patients with non-controlled chronic diseases and those with previous lung diseases other than COPD were excluded from the study. The study was approved by the the local ethics committee (Ethic Committee of the Rio de Janeiro State University) – the number of this manuscript is: 39,414,414.8.0000.5259. All individuals signed a free will and information consent form before they became subjects of the study procedures, in compliance with the Helsinki Declaration.

Study Procedures

Patients diagnosed with COPD were invited to participate and answered a questionnaire to enable the collection of epidemiological and clinical data. Patients with smoking history < 20 pack/year, with an alpha1-antitrypsin deficiency history or with acute exacerbation the month before were excluded.

The patients recruited underwent a respiratory function test with a spirometry parameter analysis, a measurement of carbon monoxide diffusion capacity and an assessment of the pulmonary volumes in an HD CPL device (nSpire Health Inc., Longmont, CO, USA), following the standardization and interpretation of the American Thoracic Society, 2005. The Knudson's equations for flow and volume variables were adopted. Serum samples were obtained for the evaluation of genetic polymorphisms. The polymorphisms studied in the *HHIP* gene (rs1828591, rs13118928, and rs6537296) were evaluated by the real-time polymerase chain reaction (PCR) through the Taqman SNP Genotyping Assay. These three polymorphisms were selected because of their reported relationship with COPD development.

Subjects were divided into three phenotypes (ACO, FE and emphysema-hyperinflation) based on six parameters: history of atopy, number of exacerbations in the last 12 months, response to bronchodilator test and values of

diffusion for CO percentage, total lung volume percentage and residual volume percentage. Subjects who did not fulfil the characteristics of one phenotype or who presented characteristics related to more than one phenotype were excluded. The definition of each phenotype is described in Table 1.

Statistical Analysis

The statistical analysis was performed with the IBM-SPSS statistics 25 program. Age, sex and pulmonary function data (forced vital capacity and forced expiratory volume in the first second) are displayed as mean and standard deviation. Allelic and genotypic frequencies are displayed as absolute numbers and percentages. The differences between groups were assessed using an independent sample T student test, ANOVA and the chi-squared model, where appropriate. Statistical significance was appointed by p values of less than 0.05.

Results

A total of 81 patients with a confirmed COPD diagnosis met the criteria to become subjects included in the study and were recruited. However, 20 patients could not be evaluated because they could not be classified in 1 of the 3 phenotypes studied ($n = 6$) or because they had been included in more than one phenotype ($n = 14$). Of the 61 subjects included in the study, 21 were FE phenotypes, 26 emphysema-hyperinflation phenotypes, and 14 ACO phenotypes. Figure 1 presents the flow chart of the recruitment. There was no statistically significant difference between the groups regarding age, sex and forced vital capacity. The forced expiratory volume in the first second was statistically different because the ACO phenotype had higher values demonstrating a mild disease in our sample when compared to other phenotypes (Table 2).

When comparing these three phenotypes, there was no statistically significant difference when regarding the genotypic analysis of the rs1828591 (p= 0.552) and rs6537296 (p=0.296) *HHIP* polymorphisms. However, there was a trend for statistical difference regarding the rs13118928 (p = 0.058) polymorphism. Although not very prevalent in the 3 groups, the G allele was not found in patients in the emphysema-hyperinflation group. In the genotypic analysis, we observed a higher percentage of patients with the emphysema-hyperinflation phenotype with AA genotype (Table 3). Thus, we performed a 2 × 2 analysis to find out a possible implication of the emphysema-hyperinflation phenotype in this statistical trend.

The separate 2 × 2 analysis of the 3 phenotypes revealed a statistically significant difference in the A allele when comparing FEs with emphysema-hyperinflation (p = 0.019), in which the latter group presented this allele more frequently. The analysis between ACO and emphysema-hyperinflation also revealed a statistically significant difference in the comparison between AA × AG × GG genotypes (p = 0.04), with a higher prevalence of the AA genotype in the emphysema-hyperinflation group, and a statistically significant difference in the G allele (p = 0.04) in this same group, and it was less frequently found in patients with the emphysema-hyperinflation phenotype. No statistically significant difference was found in allelic and genotypic analysis between ACO and FEs. Therefore, the presence of the A allele may be related to the trend to develop the emphysema-hyperinflation phenotype and the presence of the G allele is possibly not related to this phenotype (Table 4).

Discussion

Much has been discussed about the different COPD phenotypes, specially the ACO, the emphysema-hyperinflation, and the FE phenotypes. In addition to these 3 phenotypes, Miravitlles et al described four other clinical phenotypes with their clinical nuances and the main therapeutic options for each one.[4] Other authors have also reported several phenotypes, in an attempt to identify a clinical spectrum to a better target therapy.[6]

Nevertheless, there are no studies that discuss a possible distinct genetic origin of each of these phenotypes,

Table 1 Characteristics Considered For Dividing Patients Into The Three Different Phenotypes

Phenotype	History Of Atopy	Number of Exacerbations In The Previous 12 Months	Bronchodilation Post Salbutamol Spray 400 mcg	DLCO % Predicted	TLC% Predicted	RV% Predicted
FE	Negative	≥ 2	Negative	> 75	< 130	< 125
ACO	Positive	< 2	Positive	> 75	< 130	< 125
Emphysema-hyperinflation	Negative	< 2	Negative	< 75	> 130	> 125

Abbreviations: ACO, asthma/COPD overlap; FE, frequent exacerbator; bronchodilator test was considered positive when an increment ≥ 12% FEV1% predicted was associated to 200 mL of the absolute baseline FEV_1, DLCO, carbon monoxide diffusion capacity; TLC%, total lung capacity % predicted; RV%, residual volume % predicted.

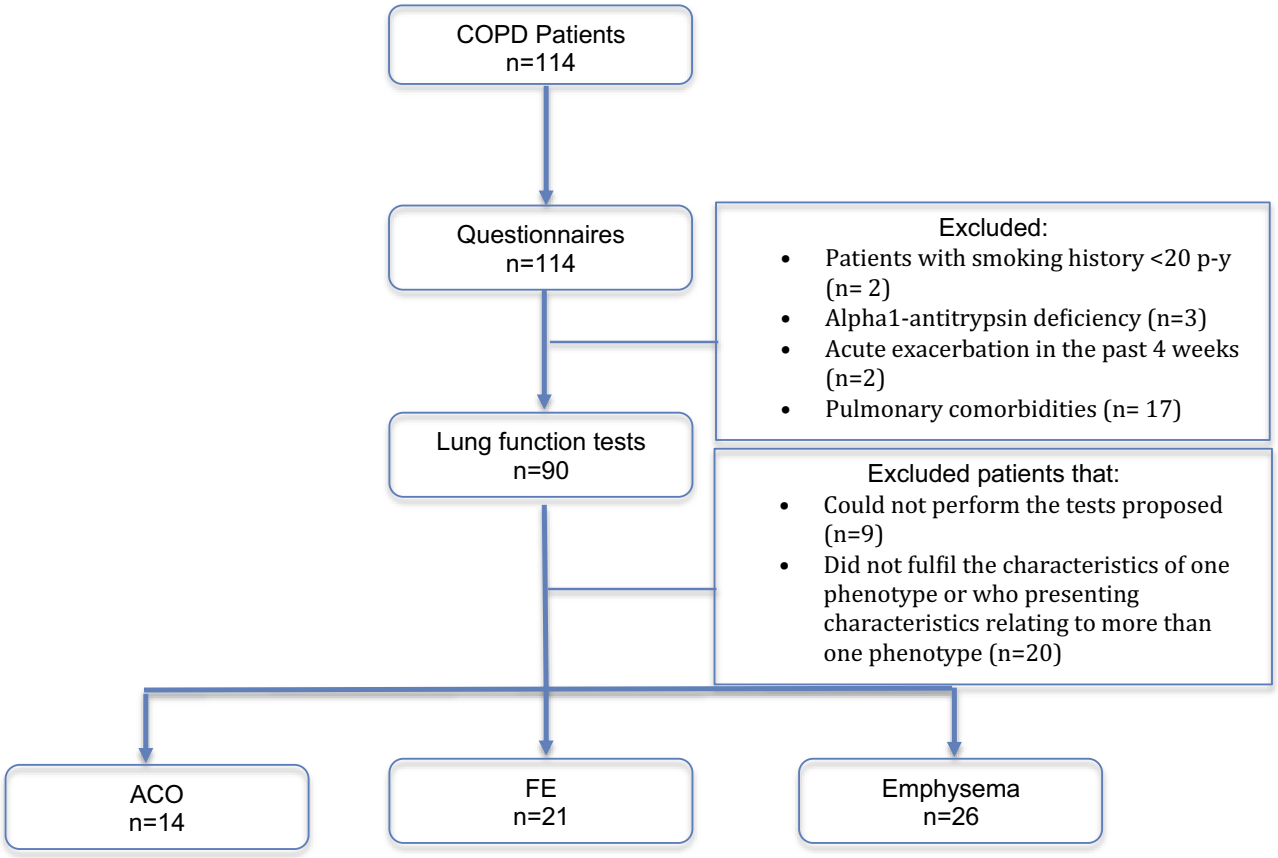

Figure 1 Recruitment flowchart.
Abbreviations: COPD, chronic obstructive lung disease; ACO, asthma/COPD overlap; FE, frequent exacerbator, pulmonary comorbidities included, tuberculosis sequelae, pulmonary fibrosis and lung cancer.

especially the three most frequent ones already cited above. The logic of this discussion is based on the fact that individuals exposed to the same risk factor – smoking – developed distinct spectra of COPD. Thus, the correlation of genetic polymorphisms and the different COPD phenotypes becomes imperative.

Several studies report polymorphisms possibly associated with the genesis of COPD, including those in the *HHIP* gene (rs1828591, rs13118928, and rs6537296).[17,18]

After a statistical analysis comparing these three polymorphisms with the three major COPD phenotypes, novel results were observed, the possible relationship of the presence of the A allele and absence of the G allele in the rs13118928 polymorphism of the *HHIP* gene with the emphysema-hyperinflation phenotype.

Our study presented some limitations, mainly regarding the determination of the phenotype within the daily basis clinical context. Some subjects were excluded from

Table 2 Subjects' Demographic And Functional Characteristics

	FE	ACO	Emphysema Hyperinflation	P-Value
Age (years, mean ± SD)	67.0 ±7.8	60.7 ± 7.8	67.0 ± 7.8	0.06
Male gender (*n*/total)	15/21	7/14	13/26	0.99
Smoking load	46.7 ± 24.5	60.2 ± 27.6	48.5±30.2	0.33
FEV_1% (mean ± SD)	58.4 ± 20.7	68.3 ± 15.3*	52.3 ± 16.3*	**0.03**
FVC% (mean ± SD)	92.0 ± 25.1	100.0 ± 19.0	91.3 ± 18.5	0.41

Notes: Used ANOVA for categorical numbers and chi-square for gender. *Multiple comparison showed difference between ACO and emphysema/hyperinflation groups (p=0.02). Bold text indicates statistical significance.
Abbreviations: ACO, asthma/COPD overlap; FE, frequent exacerbator; FEV1%, forced expiratory volume in the first second % predicted; FVC%, forced vital capacity % predicted.

Table 3 Genotypic Frequency In The Phenotypes Studied

Polymorphism	FE (*n*/%)	ACO (*n*/%)	Emphysema-Hyperinflation (*n*/%)	p-Value
***HHIP* rs 1828591**				
AA	6 (28.5)	3 (21.4)	11 (42.4)	0.552
AG	11 (34.3)	8 (57.1)	13 (50.0)	
GG	4 (19.0)	3 (21.4)	2 (7.6)	
***HHIP* rs 13118928**				
AA	8 (38.1)	4 (28.5)	16 (61.5)	0.058
AG	9 (32.1)	9 (42.8)	10 (35.7)	
GG	4 1(9.0)	1 (7.1)	0 (0.0)	
***HHIP* rs 6537296**				
AA	5 (23.8)	5 (35.7)	12 (46.1)	0.296
AG	13 (61.9)	6 (42.8)	13 (50.0)	
GG	3 (14.2)	3 (21.4)	1 (3.8)	

Abbreviations: ACO, asthma/COPD overlap; FE, frequent exacerbator; *HHIP*, Hedgehog interaction protein.

Table 4 Allelic And Genotypic Frequency In 2 × 2 Comparisons With The Rs 13118928 *HHIP* Gene Polymorphism

Polymorphism	Allele A (*n*/%)	A (p-Value)	Allele G (*n*/%)	G (p-Value)	AA x AG x GG (*n*)	AA x AG x GG (p-Value)
FE Emphysema-hyperinflation	17 (80.9) 26 (100.0)	**0.019**	13 (61.9) 10 (38.4)	0.1	AA 8/AG 9/GG 4 AA 16/AG 10/GG 0	0.1
FE ACO	17 (80.9) 13 (92.8)	0.32	13 (61.9) 10 (71.4)	0.5	AA 8/AG 9/GG 4 AA 4/AG 9/GG 1	0.4
ACO Emphysema-hyperinflation	13 (92.8) 26 (100.0)	0.16	10 (71.4) 10 (38.4)	**0.04**	AA 4/AG 9/GG 1 AA 16/AG 10/GG 0	**0.04**

Note: Bold text indicates statistical significance.
Abbreviations: ACO, asthma/COPD overlap; FE, frequent exacerbator; *HHIP*, Hedgehog interaction protein.

the sample for not meeting the criteria of a pure phenotype. Another limitation is related to the fact that the history of atopy and asthma were based on patient-based history. The number of exacerbations that had occurred in the year before the recruitment was confirmed in most cases by medical notes, but some patients had been treated in the emergency room during the episodes of exacerbation, and for this study, all episodes in which the patients had received antibiotics or systemic corticosteroids were considered exacerbations, even if they had not happened in our institution.

Conclusion

In conclusion, this study demonstrates a possible relationship between the presence of the A allele and the absence of the G allele at the rs13118928 *HHIP* polymorphism with the emphysema-hyperinflation phenotype. This data needs to be corroborated by subsequent studies, but it opens the discussion of the relationship between the COPD phenotype and genotype.

Disclosure

The authors report no conflicts of interest in this work.

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