Pulmonology 000 (xxxx) 1–11

PULMONOLOGY

www.journalpulmonology.org

ORIGINAL ARTICLE

Polymorphisms and haplotypes of *TOLLIP* and *MUC5B* are associated with susceptibility and survival in patients with fibrotic hypersensitivity pneumonitis

P.C. Mota[a,b,e], M.L. Soares[c,d,e,*], A.C. Ferreira[c,e], R.F. Santos[e,f], J.C. Rufo[g,h,i], D. Vasconcelos[c,e], A. Carvalho[b,j], S. Guimarães[b,k], F. Vasques-Nóvoa[b,l,m], C. Cardoso[a,b,e], N. Melo[a], A.T. Alexandre[a], D. Coelho[a,b,e], H. Novais-Bastos[a,b,e], A. Morais[a,b,e]

[a] *Departamento de Pneumologia, Centro Hospitalar Universitário de São João, EPE, Porto, Portugal*
[b] *Faculdade de Medicina da Universidade do Porto, Porto, Portugal*
[c] *Laboratório de Apoio à Investigação em Medicina Molecular (LAIMM), Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Portugal*
[d] *LAIMM, Núcleo de Recursos Laboratoriais, Unidade de Gestão de Conhecimento, Departamento de Recursos Comuns, Faculdade de Medicina da Universidade do Porto, Portugal*
[e] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*
[f] *Escola Superior de Saúde - Instituto Politécnico do Porto, Portugal*
[g] *Indoor Air Quality and Respiratory Health Lab, Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal*
[h] *EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal*
[i] *Center for Translational Health and Medical Biotechnology Research (T.Bio), Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal*
[j] *Departamento de Radiologia, Centro Hospitalar Universitário de São João, EPE, Porto, Portugal*
[k] *Departamento de Anatomia Patológica, Centro Hospitalar Universitário de São João, EPE, Porto, Portugal*
[l] *Departamento de Medicina Interna, Centro Hospitalar Universitário de São João, EPE, Porto, Portugal*
[m] *UnIC@RISE, Department of Surgery and Physiology, Portugal*

KEYWORDS
Hypersensitivity pneumonitis;
MUC5B;

Abstract
Introduction and Objectives: Hypersensitivity pneumonitis (HP) is an interstitial lung disease with diverse clinical features that can present a fibrotic phenotype similar to idiopathic pulmonary fibrosis (IPF) in genetically predisposed individuals. While several single nucleotide

Abbreviations: HP, Hypersensitivity pneumonitis; ILDs, Interstitial lung diseases; fHP, Fibrotic hypersensitivity pneumonitis; IPF, Idiopathic pulmonary fibrosis; SNPs, Single nucleotide polymorphisms; MUC5B, Mucin 5B; TOLLIP, Toll-interacting protein; FVC, Forced vital capacity; TLC, Total lung capacity; DLCO, Diffusing capacity of the lung for carbon monoxide; % pred, percentage of predicted; % pred, percentage of predicted; BAL, Bronchoalveolar lavage; RFLP, Restriction fragment length polymorphism; PCR, Polymerase chain reaction; dbSNP, Single nucleotide polymorphisms database; HRCT, High-resolution computed tomography; UIP, Usual interstitial pneumonia; TBLC, Transbronchial lung cryobiopsy; LD, Linkage disequilibrium; MAF, Minor allele frequency; HR, Hazard Ratio.

* Corresponding author at: Laboratório de Apoio à Investigação em Medicina Molecular, Núcleo de Recursos Laboratoriais, Unidade de Gestão de Conhecimento, Departamento de Recursos Comuns, Faculdade de Medicina da Universidade do Porto, Rua Dr. Plácido Costa, s/n - Piso 4, 4200-450 Porto, Portugal.
E-mail address: mlsoares@med.up.pt (M.L. Soares).

https://doi.org/10.1016/j.pulmoe.2024.01.002

Please cite this article in press as: P.C. Mota, M.L. Soares, A.C. Ferreira et al., Polymorphisms and haplotypes of *TOLLIP* and *MUC5B* are associated with susceptibility and survival in patients with fibrotic hypersensitivity pneumonitis, Pulmonology (2024), https://doi.org/10.1016/j.pulmoe.2024.01.002

TOLLIP;
Genetic variants;
Haplotypes;
Susceptibility;
Survival;
Fibrosis

polymorphisms (SNPs) have been associated with IPF, the genetic factors contributing to fibrotic HP (fHP) remain poorly understood. This study investigated the association of *MUC5B* and *TOLLIP* variants with susceptibility, clinical presentation and survival in Portuguese patients with fHP.

Material and Methods: A case-control study was undertaken with 97 fHP patients and 112 controls. Six SNPs residing in the *MUC5B* and *TOLLIP* genes and their haplotypes were analyzed. Associations with risk, survival, and clinical, radiographic, and pathological features of fHP were probed through comparisons among patients and controls.

Results: *MUC5B* rs35705950 and three neighboring *TOLLIP* variants (rs3750920, rs111521887, and rs5743894) were associated with increased susceptibility to fHP. Minor allele frequencies were greater among fHP patients than in controls (40.7% vs 12.1%, $P<0.0001$; 52.6% vs 40.2%, $P = 0.011$; 22.7% vs 13.4%, $P = 0.013$; and 23.2% vs 12.9%, $P = 0.006$, respectively). Haplotypes formed by these variants were also linked to fHP susceptibility. Moreover, carriers of a specific haplotype (G-T-G-C) had a significant decrease in survival (adjusted hazard ratio 6.92, 95% CI 1.73−27.64, $P = 0.006$). Additional associations were found between *TOLLIP* rs111521887 and rs5743894 variants and decreased lung function at baseline, and the *MUC5B* SNP and radiographic features, further highlighting the influence of genetic factors in fHP.

Conclusion: These findings suggest that *TOLLIP* and MUC5B variants and haplotypes may serve as valuable tools for risk assessment and prognosis in fibrotic hypersensitivity pneumonitis, potentially contributing to its patient stratification, and offer insights into the genetic factors influencing the clinical course of the condition.

Introduction

Hypersensitivity pneumonitis (HP) is an interstitial lung disease (ILD), with a heterogeneous clinical presentation and evolution, recently classified as comprising two forms — fibrotic and non-fibrotic.[1-3] HP is triggered by exposure to a variety of inciting antigens that results in inflammation, causes persistent lung injury and, in genetically predisposed individuals, leads to fibrotic disease, similar to idiopathic pulmonary fibrosis (IPF).[4,5] The differential diagnosis of these two fibrosing entities is challenging but imperative given the therapeutic and prognostic implications.[1,4,6,7] In fibrotic HP (fHP), antigen avoidance, if recognized, is the mainstay of treatment, associated with immunosuppression, when necessary.[8,9] Antifibrotics, the standard treatment for IPF, are only prescribed later in cases of HP with progressive pulmonary fibrosis.[10-12]

Several single nucleotide polymorphisms (SNPs) have been associated with IPF susceptibility and survival, such as the mucin 5B (*MUC5B*) rs35705950 promoter polymorphism and several toll-interacting protein (*TOLLIP*) SNPs.[13-17] Whether independently associated or integrating haplotypes potentially predicting altered survival,[18] these variants highlight the contribution of genetic determinants to the development and evolution of the fibrosing phenotype.

In fHP, in contrast, very little is known about predisposing genetic factors.[19] To date, no genome-wide association studies have been performed although a few studies probing candidates chosen on the basis of association with other ILDs have identified associations with disease susceptibility and outcomes in fHP.[20-22] Among those, the *MUC5B* promoter variant and telomere length were implicated as susceptibility and survival determinants in patients with chronic HP,[20,23,24] as extensively reported for IPF.[25] No study has yet focused on the involvement of the various IPF-related *TOLLIP* SNPs in the context of fHP.

The objective of this study was to determine whether the *MUC5B* promoter and neighboring *TOLLIP* polymorphisms, and respective haplotypes, are associated with susceptibility, clinical presentation and survival in patients with fHP.

Materials and methods

Participants and study design

A case-control study was conducted with 97 non-familial fHP patients followed from 2013 to 2022 in a tertiary Portuguese hospital in Porto. This group included cases enrolled in the FIBRALUNG (Fibrosing ILD Biomarkers That Rule Acceleration) project, the first national registry and biobank of ILDs.[26] A control group with 112 subjects was selected from EPIPorto,[27] a population-based cohort of healthy adults ($n = 74$), and NETDiamond,[28,29] a prospective cohort for the study of therapeutic targets in heart failure, selecting those without respiratory disease ($n = 38$).

Diagnosis of fHP was established according to international guidelines.[1,2,6] Demographic and clinical information, including lung function, bronchoalveolar lavage (BAL) as well as radiographic and histopathological data, were collected on enrolment.

The study protocol was reviewed and approved by the Ethics Committee of the Institution and performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants.

Genotyping

Individuals were genotyped for six SNPs: *TOLLIP* rs3750920, rs111521887, rs5743894, rs5743890, rs5743854, and *MUC5B* rs35705950, as described,[18] and by RFLP and real-time PCR (details in Supplementary Methods M1, Table S1).

Radiology

High-resolution computed tomography (HRCT) scans were independently reviewed by a radiologist with experience in ILD CT scan interpretation, blinded to clinical diagnosis. Detailed information on image analysis and classification scores can be found in Supplementary Methods M2.

Histopathology

Lung biopsies were scored and reviewed by an expert lung pathologist in ILDs (surgical lung biopsy or transbronchial lung cryobiopsy) blinded to the original diagnosis, using a guided pathology data collection form. Detailed information on histopathological analyses and classification scores can be found in Supplementary Methods M3.

Statistical analysis

Statistical analyses were performed in R (v4.2.0). Haplotype frequencies were estimated using Haploview v4.2. Pairwise linkage disequilibrium (LD) between SNPs was calculated using SNPAlyze v8.1.1.0 (Dynacom Co., Japan).

Continuous data are presented as means ± standard deviation or medians and interquartile range. Categorical data are presented as frequencies and percentages. Chi-squared tests were used to compare allele and genotype frequencies between patients and controls.

Overall survival was analyzed with time from diagnosis to death (in months) censured by end of follow-up or lung transplantation. Transplant-free survival considered lung transplantation equivalent to death. Kaplan-Meier plots and log-rank tests were used for survival and transplant-free survival.

Univariate and multivariate Cox proportional hazards regressions were employed to identify variables that predict survival status, with results being expressed as hazard ratio (HR) and respective 95% confidence interval (CI).

Two-sided P-values lower than 0.05 were considered statistically significant.

Results

Demographic and clinical features of the study subjects

The baseline characteristics of participants are shown in Table 1. There were no significant differences between the groups regarding gender and smoking history, although fHP patients were younger than control subjects (mean 67.5 vs 71.2 years, P = 0.002). Antigen exposure was identified in 93% of patients, with avian and molds being the most frequently reported.

All patients had radiographic signs of fibrosis. Histological confirmation of fHP diagnosis was necessary in 55.7% of patients. Most patients were initially treated with immunosuppressants with the addition of antifibrotics in cases of progressive pulmonary fibrosis.

Overall median follow-up duration, defined as time from diagnosis to death/transplant or end of study, was 24 months. Twenty-six patients (26.8%) died during follow-up.

MUC5B and *TOLLIP* associations with fHP susceptibility and survival

Genotype frequencies of the probed SNPs are distributed as shown in Table 2, all conforming to the Hardy-Weinberg equilibrium (Supplementary Table S2).

The frequency of the *MUC5B* rs35705950 minor allele T was significantly increased in fHP patients (40.7% vs 12.1%, OR=5.01, 95% CI 3.06−8.21, $P < 0.0001$). Accordingly, the genotypes of T allele carriers were significantly more frequent in the fHP group (69.1% vs 22.3 %, OR=7.77, 95 % CI 4.18-14.43, $P < 0.0001$), indicating this variant allele also confers susceptibility to fHP.

TOLLIP rs3750920, rs111521887 and rs5743894 SNPs (henceforth also referred to as T920, T887 and T894) were significantly associated with fHP. The minor allele frequencies (MAF) were significantly increased in fHP patients relative to those of controls (52.6% vs 40.2%, P = 0.011; 22.7% vs 13.4%, P = 0.013; and 23.2% vs 12.9%, P = 0.006; respectively). The overall genotypic distributions of these SNPs were also associated with fHP (P = 0.029, P = 0.049, P = 0.029), with homozygotes bearing the minor alleles at higher risk of developing the disease (significantly so for T920 TT OR=2.90, P = 0.012; and T894 CC OR=5.04, P = 0.040; and borderline significant for T887 GG, P = 0.052). Allele and genotype distributions of *TOLLIP* rs5743890 and rs5743854 SNPs did not differ significantly between patients and controls.

Kaplan-Meier survival analysis of *MUC5B* and *TOLLIP* SNPs did not show any association between individual genotypes and survival in fHP patients (Supplementary Fig. S1).

MUC5B and *TOLLIP* haplotype associations with fHP susceptibility and survival

Haplotypes defined by the *MUC5B* and three neighboring *TOLLIP* SNPs that had shown an association with susceptibility to fHP (MUC5B-T920-T887-T894 haplotype block) were reconstructed (Supplementary Table S3). Six major haplotypes were defined, of which five showed an association with susceptibility to the disease. Haplotypes T-T-C-T, T-T-G-C, and T-C-C-T were significantly more frequent, and haplotypes G-C-C-T and G-T-C-T were significantly less frequent in patients than in controls.

Pairwise LD measures indicated moderate to strong LD among the *TOLLIP* SNPs and moderate LD between these and the *MUC5B* SNP in the fHP group, while in controls linkage between tightly linked T887 and T894 and the T920 SNP was only moderate, and with the *MUC5B* SNP was considerably low (Supplementary Table S4 and Fig. S2).

Notably, Kaplan-Meier survival analysis of the haplotypes revealed a statistically significant association of haplotype G-T-G-C with reduced survival (censoring for lung transplant) (log rank test P-value = 0.006) and reduced transplant-free survival (log rank test P-value = 0.021) in fHP (Fig. 1). Multivariate Cox regression analysis showed that the presence of the G-T-G-C haplotype was independently associated with shorter survival after adjustment for age and lung function parameters (overall survival, HR 6.92, P = 0.006; transplant-free survival, HR 5.93, P = 0.009)

Table 1 Clinical and demographic characteristics of fHP patients and controls.

	fHP (*n* = 97)	Controls (*n* = 112)	p value
Gender (n,%)			0.375
Female	44 (45.4 %)	44 (39.3 %)	
Male	53 (54.6 %)	68 (60.7 %)	
Age (years)[a]	67.5 (8.7)	71.2 (8.4)	0.002
Ever smoker (n,%)	47 (48.5 %)	51 (45.9 %)	0.718
Follow up time (months)[b]	24 (16–56)		
Deaths (n,%)	26 (26.8 %)		
Transplants (n,%)	4 (4.1 %)		
Pulmonary function tests			
FVC (% pred)[a]	80.8 (17.3)		
TLC (% pred)[a]	76.9 (17.7)		
DLCO (% pred)[a]	53.9 (16.8)		
Antigen exposure (n,%)			
Avian	70 (72.2 %)		
Molds	37 (38.1 %)		
Other Organics	18 (18.6 %)		
Inorganics	21 (21.6 %)		
Unknow	7 (7.2 %)		
Multiple	46 (47.4 %)		
BAL cell count			
Total cells ($\times 10^4 mL^{-1}$)[b]	1.6 (0.7–2.8)		
Macrophages[b]	71.9 (58.7–81.4)		
Lymphocytes[b]	12 (6.6–20.1)		
Neutrophils[b]	5.4 (3.2–11.2)		
Eosinophils[b]	2.8 (1.2–7.1)		
Lung biopsy (n,%)	54 (55.7 %)		
Treatment (n,%)			
Immunosuppressants	53 (54.6 %)		
Imunosuppressants+Antifibrotics	25 (25.8 %)		
No treatment	19 (19.6 %)		
Comorbidities (n,%)			
COPD	3 (3.1 %)		
Pulmonary hypertension	7 (7.2 %)		
Lung cancer	13 (13.4 %)		
OSA	39 (40.2 %)		
GERD	21 (21.6 %)		
Metabolic conditions	29 (29.9 %)		
Cardiovascular disease	55 (56.7 %)		
Depression	20 (20.6 %)		

Values are presented as [a]mean (±standard deviation), [b]median (Q1-Q3) and number (%).
fHP, fibrotic hypersensitivity pneumonitis; COPD, chronic obstructive lung disease; OSA, obstructive sleep apnea; GERD, gastro-esophageal reflux disease;% pred, percentage of predicted normal value; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide.

(Table 3). Despite being a relatively low frequency haplotype, the difference in median survival time between non-carriers and carriers was 21 months.

Other predictors of survival

Univariate analyses did not reveal associations with gender, age, and smoking. Conversely, better pulmonary function (FVC, TLC and DLCO) associated significantly with longer survival (HR 0.97, *P* = 0.015; HR 0.98 *P* = 0.019 and HR 0.96, *P* = 0.002; respectively) (Table 3).

MUC5B and *TOLLIP* associations with pulmonary function and clinical features

The presence of at least one minor allele of *TOLLIP* SNPs T887 and T894 was associated with lower baseline FVC% pred (genotypes GG/CG vs. CC, 76.3% vs 83.6%, *P* = 0.045; and genotypes CC/CT versus TT, 75.9% vs 83.9%, *P* = 0.027; respectively), whereas for T920 it was of borderline statistical significance (79.2% vs 87.5%, *P* = 0.060) (Table 4). *MUC5B* rs35705950 and the *TOLLIP* T890 and T854 polymorphisms showed no such association (Table 4 and Supplementary Table S5).

Table 2 Genotype and allele distributions of *MUC5B* and *TOLLIP* SNPs in fHP patients and controls.

	Genotype			*P* value	Allele		*P* value
***MUC5B* rs35705950**				**< 0.0001**			**<0.0001**
	GG (%)	**GT** (%)	**TT** (%)		**G** (%)	**T** (%)	
HP	30 (30.9)	55 (56.7)	12 (12.4)		115 (59.3)	79 (40.7)	
Control	87 (77.7)	23 (20.5)	2 (1.8)		197 (87.9)	27 (12.1)	
	–	OR 6.93	OR 17.40		OR 5.01		
		(3.71−13.37)	(4.42−116.03)		(3.06−8.21)		
P*	–	**<0.0001**	**<0.0001**				
***TOLLIP* rs3750920**				**0.029**			**0.011**
	CC (%)	**CT** (%)	**TT** (%)		**C** (%)	**T** (%)	
HP	19 (19.6)	54 (55.7)	24 (24.7)		92 (47.4)	102 (52.6)	
Control	39 (34.8)	56 (50.0)	17 (15.2)		134 (59.8)	90 (40.2)	
	–	OR 1.98	OR 2.90		OR 1.65		
		(1.03−3.90)	(1.28−6.75)		(1.12−2.43)		
P*		**0.044**	**0.012**				
***TOLLIP* rs111521887**				**0.049**			**0.013**
	CC (%)	**CG** (%)	**GG** (%)		**C** (%)	**G** (%)	
HP	60 (61.9)	30 (30.9)	7 (7.2)		150 (77.3)	44 (22.7)	
Control	84 (75.0)	26 (23.2)	2 (1.8)		194 (86.6)	30 (13.4)	
					OR 1.90		
					(1.14−3.16)		
P*		0.130	0.052				
***TOLLIP* rs5743894**				**0.029**			**0.006**
	TT (%)	**TC (%)**	**CC (%)**		**T (%)**	**C (%)**	
HP	59 (60.8)	31 (32.0)	7 (7.2)		149 (76.8)	45 (23.2)	
Control	85 (75.9)	25 (22.3)	2 (1.8)		195 (87.1)	29 (12.9)	
	–		OR 5.04		OR 2.03		
			(1.01−25.13)		(1.22−3.39)		
P*		0.066	**0.040**				
***TOLLIP* rs5743890**				0.227			0.245
	CC (%)	**TC** (%)	**TT** (%)		**T** (%)	**C** (%)	
HP	0 (0.0)	25 (25.8 %)	72 (74.2)		169 (87.1)	25 (12.9)	
Control	3 (2.7)	32 (28.6 %)	77 (68.8)		186 (83.0)	38 (17.0)	
P*	–	0.985	0.985				
***TOLLIP* rs5743854**				0.668			0.371
	CC (%)	**CG** (%)	**GG** (%)		**C** (%)	**G** (%)	
HP	77 (79.4)	18 (18.6)	2 (2.1)		172 (88.7)	22 (11.3)	
Control	83 (74.1)	26 (23.2)	3 (2.7)		192 (85.7)	32 (14.3)	
P*	–	0.396	0.721				

OR, Odds Ratio (95 % Confidence Interval).
*P**, Logistic regression *P* value of each minor allele genotype vs. reference genotype (ancestral allele).
Alleles are reported in the Forward strand orientation, except rs5743854 in conformity with the dbSNP database minor allele designation.

Exposure to birds as an inciting antigen was tendentially more frequent in carriers of the G-T-G-C haplotype ($P = 0.067$). Indeed, all carriers of the haplotype were exposed to birds, whereas 30.3% of the non-carriers were not (Table 4).

Radiographic analyses showed that *MUC5B* rs35705950 minor alleles were associated with a lower total score of fibrosis ($P = 0.023$), but not to honeycombing or a pattern indicative of definite or probable UIP. No associations between radiographic features and genotype distributions were seen for the *TOLLIP* SNPs (Table 5).

BAL sample analyses revealed a tendency for a larger amount of neutrophils in patients bearing at least one of the minor alleles of T887 (G) and T894 (C) polymorphisms. Conversely, for *MUC5B* rs35705950 this trend was noted but for the ancestral allele (G). Accordingly, carriers of the G-T-G-C haplotype, which is associated with poorer prognosis, showed a

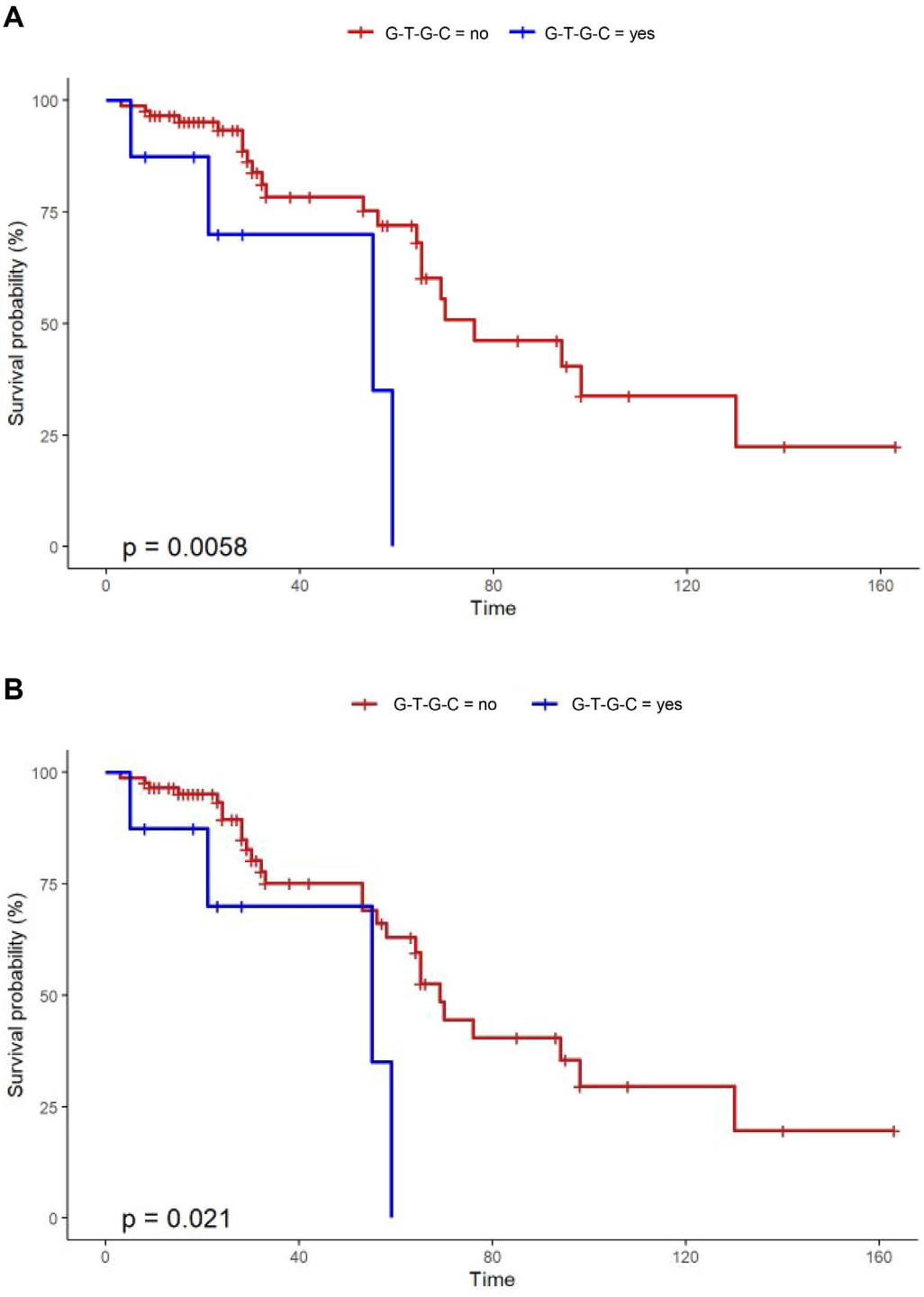

Fig. 1 Kaplan-Meier plots showing fHP patients' overall survival (A) and transplant-free survival (B) according to haplotype G-T-G-C defined by the rs35705950-rs3750920-rs111521887-rs5743894 *MUC5B-TOLLIP* block.
Time in months; P value for log-rank test.

median neutrophil amount that more than doubled that of non-carriers although not reaching statistical significance ($P = 0.085$) (Table 5).

No additional significant associations emerged between the probed SNPs or respective haplotypes and histopathological features of fibrosis (Table 5).

Discussion

This study investigated specific genetic variants as determinants of susceptibility and survival in patients with fHP. The *MUC5B* promoter rs35705950 variant and *TOLLIP* rs3750920, rs111521887 and rs5743894 variants are associated with

Table 3 Univariate and multivariate Cox proportional hazard regressions predicting patients' death.

	HR	95 % CI	p-value
Univariate analysis			
Gender	1.36	0.63 − 2.92	0.434
Age	1.05	1.00 − 1.11	0.060
Smoking	1.70	0.79 − 3.66	0.173
FVC (% pred)	0.97	0.95 − 0.99	**0.015**
TLC (% pred)	0.98	0.97 − 1.00	**0.019**
DLCO (% pred)	0.96	0.93 − 0.98	**0.002**
G-T-G-C haplotype	4.26	1.38 − 13.13	**0.012**
Multivariate analysis[*]			
Overall survival			
G-T-G-C haplotype	6.92	1.73 − 27.64	**0.006**
Age	1.08	1.00 − 1.16	**0.046**
FVC (% pred)	0.99	0.94 − 1.03	0.547
TLC (% pred)	0.99	0.96 − 1.02	0.535
DLCO (% pred)	0.97	0.94 − 1.01	0.161
Transplant-free survival			
G-T-G-C haplotype	5.93	1.55 − 22.68	**0.009**
Age	1.04	0.98 − 1.11	0.202
FVC (% pred)	0.97	0.93 − 1.01	0.206
TLC (% pred)	0.99	0.96 − 1.02	0.486
DLCO (% pred)	0.98	0.95 − 1.01	0.186

HR, Hazard Ratio; 95 % CI, Confidence Interval.
% pred, percentage of predicted normal value.
FVC, Forced Vital Capacity; TLC, Total Lung Capacity; DLCO, Diffusing Capacity of the Lung for Carbon Monoxide.
[*] Multivariate Cox regression models obtained by forward conditional stepwise selection.

susceptibility to fHP in this Portuguese cohort. Although such an association had been previously reported for *MUC5B* rs35705950,[20-22] this is the first study implicating *TOLLIP* variants as susceptibility determinants in fHP. Notably, we also found that a haplotype defined by these four variants is significantly associated with poorer survival in these patients.

The role of the *MUC5B* polymorphism in the development and progression of lung fibrosis has been extensively investigated in IPF and is considered the most influential genetic risk factor of this disease.[13-15,30] The involvement of *MUC5B* in HP was described in recent studies[20-22] with results corroborated by those presented herein. Furusawa et al identified six common IPF genetic risk variants with a positive direction of risk effect in HP cases, with the *MUC5B* variant showing the strongest association.[21]

Other common variants reportedly associated with IPF susceptibility include SNPs in the genomic region of the *TOLLIP* gene. Notwithstanding some inconsistent reports, it is generally accepted that *TOLLIP* polymorphisms play a role in IPF pathophysiology. The minor alleles of intronic SNPs rs111521887 and rs5743894 were reported to confer susceptibility to IPF, while that of the rs5743890 SNP was found to reduce such risk.[30,31] Moreover, *TOLLIP*'s exonic variant rs3750920 was associated with a favorable response to N-acetylcysteine, with carriers of the TT genotype showing a significantly reduced composite endpoint risk, including death, hospitalization, and FVC decline.[17]

Table 4 *MUC5B* and *TOLLIP* associations with demographic and clinical features.

Genotype/Haplotype	*MUC5B* rs35705950			*TOLLIP* rs3750920			*TOLLIP* rs111521887			*TOLLIP* rs5743894			G-T-G-C haplotype[*]		
	GG (*n* = 30)	GT/TT (*n* = 67)	P value	CC (*n* = 19)	CT/TT (*n* = 78)	P value	CC (*n* = 60)	CG/GG (*n* = 37)	P value	TT (*n* = 59)	TC/CC (*n* = 38)	P value	No (*n* = 89)	Yes (*n* = 8)	P value
Gender (female/male)	11 (36.7 %)	33 (49.3 %)	0.250	11 (57.9 %)	33 (42.3 %)	0.221	23 (38.3 %)	21 (56.8 %)	0.077	23 (39.0 %)	21 (55.3 %)	0.116	39 (43.8 %)	5 (62.5 %)	0.309
	19 (63.3 %)	34 (50.7 %)		8 (42.1 %)	45 (57.7 %)		37 (61.7 %)	16 (43.2 %)		36 (61 %)	17 (44.7 %)		50 (56.2 %)	3 (37.5 %)	
Age (years)	66.2 (8.7)	68.1 (8.7)	0.324	68.8 (8.7)	67.1 (8.7)	0.447	67.4 (8.7)	67.5 (8.8)	0.953	67.6 (8.6)	67.2 (8.7)	0.812	67.4 (8.9)	68.3 (5.8)	0.794
Ever smoker	17 (56.7 %)	30 (44.8 %)	0.279	9 (47.4 %)	38 (48.7 %)	0.916	31 (51.7 %)	16 (43.2 %)	0.420	30 (50.8 %)	17 (44.7 %)	0.557	44 (49.4 %)	3 (37.5 %)	0.518
Death	9 (30.0 %)	17 (25.4 %)	0.629	6 (31.6 %)	20 (25.6 %)	0.577	17 (28.3 %)	9 (24.3 %)	0.814	17 (28.8 %)	9 (28.7 %)	0.644	22 (24.7 %)	4 (50.0 %)	0.204
Pulmonary function tests															
FVC (% pred)	82.8 (16.3)	79.9 (17.7)	0.449	87.5 (14.2)	79.2 (17.6)	0.060	83.6 (14.8)	76.3 (20.1)	**0.045**	83.9 (14.7)	75.9 (19.9)	**0.027**	81.1 (16.9)	77.7 (23.1)	0.623
TLC (% pred)	76.4 (15.2)	77.2 (18.9)	0.855	80.7 (13.6)	76.0 (18.6)	0.309	77.8 (14.4)	75.4 (22.4)	0.536	77.8 (14.5)	75.4(22.1)	0.397	76.7 (17.3)	79.1 (24.3)	0.741
DLCO (% pred)	50.4 (16.8)	55.6 (16.8)	0.184	51.1 (10.2)	54.6 (18.0)	0.454	53.8 (15.4)	54.1 (19.4)	0.929	53.9 (15.6)	54.0 (19.1)	0.900	54.1 (16.2)	51.8 (24.8)	0.730
Antigen exposure															
Avian	21 (70.0 %)	49 (73.1 %)	0.750	11 (57.9 %)	59 (75.6 %)	0.122	40 (66.7 %)	30 (81.1 %)	0.124	40 (67.8 %)	30 (78.9 %)	0.232	62 (69.7 %)	8 (100 %)	0.067
Molds	10 (33.3 %)	27 (40.3 %)	0.514	10 (52.6 %)	27 (34.6 %)	0.147	21 (35 %)	16 (43.2 %)	0.417	21 (35.6 %)	16 (42.1 %)	0.519	33 (37.1 %)	4 (50.0 %)	0.471
Other organics	4 (13.3 %)	14 (20.9 %)	0.376	2 (10.5 %)	16 (20.5 %)	0.315	9 (15.0 %)	9 (24.3 %)	0.251	9 (15.3 %)	9 (23.7 %)	0.297	17 (19.1 %)	1 (12.5 %)	0.645
Inorganics	7 (23.3 %)	14 (20.9 %)	0.788	3 (15.8 %)	18 (23.1 %)	0.489	13 (21.7 %)	8 (21.6 %)	0.996	12 (20.3 %)	9 (23.7 %)	0.696	19 (21.3 %)	2 (25.0 %)	0.810

Values are presented as mean (SD) and counts as n (%).
[*] Defined by the rs35705950-rs3750920 -rs111521887- rs5743894 *MUC5B-TOLLIP* block.
FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide.

Table 5 MUC5B and TOLLIP associations with BAL, radiographic and histopathological features.

Genotype/Haplotype	*MUC5B* rs35705950			*TOLLIP* rs3750920			*TOLLIP* rs111521887			*TOLLIP* rs5743894			G-T-G-C haplotype*		
	GG (*n* = 30)	GT/TT (*n* = 67)	P value	CC (*n* = 19)	CT/TT (*n* = 78)	P value	CC (*n* = 60)	CG/GG (*n* = 37)	P value	TT (*n* = 59)	TC/CC (*n* = 38)	P value	No (*n* = 89)	Yes (*n* = 8)	P value
BAL features															
Lymphocytes	13.8 (9.6–21.1)	11.0 (6–19.2)	0.117	11.1 (5.2–13.7)	12.0 (7.0–20.2)	0.322	12.2(6.6–19.4)	11.0 (6.5–20.9)	0.615	12.2(6.6–19.4)	9.7 (6.0–20.6)	0.384	12(6.6–20.9)	8.1 (6.4–16.0)	0.377
Neutrophils	6.4 (3.4–12.1)	5.4 (3.2–11.0)	0.283	4.0 (3–8)	5.8 (3.2–11.2)	0.329	5.0 (3.2–9.9)	8.0 (3.8–12.2)	0.142	4.9 (3.2–9.4)	8.0 (4.2–13.5)	0.082	5.5 (3.2–10.5)	11.2(7.9–18.2)	0.085
Eosinophils	3.0 (1.2–12.7)	2.8 (1.4–5.8)	0.660	3.0 (0.6–9.2)	2.7 (1.4–2.6)	0.882	2.5 (1.1–5.8)	4.0 (1.6–9.3)	0.356	2.4 (1.0–5.8)	4.4 (1.7–9.9	0.210	2.8 (1.1–6.6)	4.1 (2.3–10.4)	0.332
Radiographic features															
UIP + probable UIP	5 (17.9 %)	23 (34.8 %)	0.238	4 (22.2 %)	24 (31.5 %)	0.743	16 (28.1 %)	12 (32.5 %)	0.490	16 (28.6 %)	12 (31.6 %)	0.616	25 (29.0 %)	3 (37.5 %)	0.755
Indeterminated for UIP	8 (28.6 %)	13 (19.7 %)		5 (27.8 %)	16 (21.1 %)		11 (19.3 %)	10 (27.0 %)		11 (19.6 %)	10 (26.3 %)		20 (23.3 %)	1 (12.5 %)	
Alternative diagnosis	15 (53.6 %)	30 (45.5 %)		9 (50 %)	36 (47.4 %)		30 (52.6 %)	15 (40.5 %)		29 (51.8 %)	16 (42.1 %)		41 (47.7 %)	4 (50.0 %)	
Honeycombing	13 (46.4 %)	24 (36.4 %)	0.361	8 (44.4 %)	29 (38.2 %)	0.624	25 (43.9 %)	12 (32.4 %)	0.268	24 (42.9 %)	13 (34.2 %)	0.400	35 (40.7 %)	2 (25.0 %)	0.385
Fibrosis score	9.5 (7–12)	8 (5.0–10.8)	**0.023**	8.5 (5.8–11)	8 (6–11)	0.979	9 (6.3–11)	8 (6.0–11.0)	0.482	8.5 (6.0–11.0)	8 (6.0–10.8)	0.516	8 (6.0–11.0)	10 (6.0–12.5)	0.455
Histopathological features															
Honeycombing	6 (33.3 %)	14 (38.9 %)	0.690	3 (21.2 %)	17 (42.5 %)	0.160	12 (33.3 %)	8 (44.4 %)	0.425	11 (31.4 %)	9 (47.4 %)	0.247	18 (35.3 %)	2 (66.7 %)	0.274
Area of fibrosis (%)	31.7 (16.2)	31.4 (13.1)	0.941	30.7 (17.2)	31.8 (13.0)	0.806	31.1 (14.7)	32.2 (12.9)	0.775	31.0 (14.9)	32.4 (12.6)	0.735	31.5 (14.2)	30.2 (12.4)	0.866
Fibroblast foci															
0	10 (55.6 %)	12 (33.3 %)	0.270	3(21.4 %)	19 (47.5 %)	0.206	17 (47.2 %)	5 (27.8 %)	0.390	16 (45.7 %)	6 (31.5 %)	0.600	20 (39.2 %)	2 (66.7 %)	0.561
1	3 (16.7 %)	7 (19.5 %)		4 (28.6 %)	6 (15.0 %)		6 (16.7 %)	4 (22.2 %)		6 (17.2 %)	4 (21.1 %)		10 (19.6 %)	0 (0.0 %)	
>1	5 (27.7 %)	17 (47.2 %)		7 (50.0 %)	15 (37.5 %)		13 (36.1 %)	9 (50.0 %)		13 (37.1 %)	9 (47.4 %)		21 (41.2 %)	1 (33.3 %)	
Fibrosis distribution															
Peribronchiolar	6 (33.3 %)	7 (20.0 %)	0.677	4 (28.6 %)	9 (23.1 %)	0.816	10 (28.6 %)	3 (16.7 %)	0.428	10 (29.4 %)	3 (15.8 %)	0.438	12 (24.0 %)	1 (33.3 %)	0.849
Peribronchiolar (+paraseptal or subpleural)	8 (44.4 %)	20 (57.1 %)		7 (50.0 %)	21 (53.8 %)		16 (45.7 %)	12 (66.7 %)		16 (47.1 %)	12 (63.2 %)		27 (54.0 %)	1 (33.3 %)	
Heterogeneous	3 (16.7 %)	7 (20.0 %)		2 (14.3 %)	8 (20.5 %)		7 (20.0 %)	3 (16.7 %)		6 (17.6 %)	4 (21.1 %)		9 (18.0 %)	1 (33.3 %)	
Undetermined	1 (5.6 %)	1 (2.9 %)		1 (7.1 %)	1 (2.6 %)		2 (5.7 %)	0 (0.0 %)		2 (5.9 %)	0 (0.0 %)		2 (4.0 %)	0 (0.0 %)	
Inflammatory infiltrate															
Light	14 (77.8 %)	24 (66.7 %)	0.532-	10 (71.4 %)	28 (70.0 %)	0.920	26 (72.2 %)	12 (66.7 %)	0.756	26 (74.3 %)	12 (63.2 %)	0.534	37 (72.5 %)	1 (33.3 %)	0.148
Moderate-severe	4 (22.2 %)	12 (33.3 %)		4 (28.6 %)	12 (30 %)		10 (27.8 %)	6 (33.3 %)		9 (25.7 %)	7 (36.8 %)		14 (27.5 %)	2 (66.7 %)	
Peribronchiolar metaplasia	2(1–3)	1 (1.9–2.3)	0.749	1 (0.0–2.8)	2(1.0–3.0)	0.390	2(0.8–3.0)	1 (1.0–2.0)	0.829	2(0.5–3.0)	1 (1.0–2.5)	0.623	2 (1.0–3.0)	1 (1–1)	0.353
Giant cells granulomas present	10 (55.5 %)	6 (16.7 %)	**0.003**	6 (42.9 %)	10 (25.0 %)	0.208	10 (27.8 %)	6 (33.3 %)	0.673	10 (28.6 %)	6 (31.6 %)	0.817	15 (29.4 %)	1 (33.3 %)	0.885

Values are presented mean (SD) or as median (Q1-Q3) and counts as n (%).

* Defined by the rs35705950-rs3750920 -rs111521887- rs5743894 *MUC5B-TOLLIP* block.

BAL, bronchoalveolar lavage; UIP, usual interstitial pneumonia.

Having probed five *TOLLIP* SNPs, including those above, we have uncovered a significant correlation between genotypes of *TOLLIP* rs3750920, rs111521887 and rs5743894 and susceptibility to fHP, with the minor alleles associating with higher risk. Interestingly, none of these SNPs independently associated with IPF risk in a previous study with Portuguese patients,[18] raising the possibility that unlike *MUC5B* rs35705950, *TOLLIP* can be an informative marker in a future strategy for differential diagnosis between the two fibrotic diseases, at least in the Portuguese. Several studies have implicated *TOLLIP* as playing a protective role in autophagy and apoptosis.[32,33] *TOLLIP*'s expression is decreased in IPF lungs when compared to normal lungs,[32] which has been linked to minor allele carriers of variants T887, T894 and T890, further suggesting that these could modify the course of disease.[30] In our study, the minor alleles of the former two variants tended to or associated with susceptibility (respectively) and integrated the haplotype associated with poorer prognosis in fHP. Interestingly, genotypes containing at least one minor allele of *TOLLIP* variants rs111521887 and rs5743894 were significantly associated with lower baseline FVC% pred, whereas for rs3750920 the decrease was of borderline statistical significance. In Japanese fHP patients genotyped for *TOLLIP* rs3750920 and rs5743899 (residing between T887 and T894 and not evaluated here), Katayanagi et al reported that the GG (minor allele) genotype of rs5743899 only, was associated with rapid FVC deterioration over time.[34] The relationship between rs3750920 genotypes and FVC at baseline was not stated.

The lack of association between *TOLLIP* rs5743890 and fHP risk is in line with that reported by Ley et al.[20] We are not aware of studies concerning *TOLLIP* rs5743854 in the context of fHP.

We found no association between the *MUC5B* variant and baseline FVC, in contrast with a recent study in Polish patients.[35] However, the latter reported a *MUC5B* MAF of only 17 %, with a GT/TT prevalence of 30.2 %, which contrasts strongly with those reported here and elsewhere.[20]

Ley et al reported that the *MUC5B* rs35705950 minor allele was associated with radiographic evidence of moderate to severe fibrosis and traction bronchiectasis in HP patients.[20] In our study the minor allele associated with a lower total score of fibrosis; nonetheless, HRCT fibrosis was scored differently and the median scores in both genotypic groups (GT/TT=8 and GG=9.5) already represent at least moderate fibrosis.

The use of SNP haplotypes within regions of interest previously identified by single-SNP genome-wide association studies can prove very useful in uncovering and fine-mapping genetic associations,[36] and provide better insights into the influence of genetic loci than the study of SNPs individually.[37,38] We show that four haplotypes, defined by the *MUC5B* promoter SNP and the three *TOLLIP* SNPs that associated individually with susceptibility to fHP, were also strong predictors of fHP risk. Remarkably, carriers of the haplotype G-T-G-C had a significant decrease in survival compared to non-carriers, with the median survival time differing by a remarkable 21 months. This association was confirmed by multivariate analysis adjusting for age and lung function. We had previously reported an association of *MUC5B* plus *TOLLIP*-defined haplotypes with susceptibility and survival in IPF using a similar approach,[18] suggesting that the region comprising these variants contains or segregates with an as-yet unidentified genetic determinant(s) that influence(s) the clinical course of both disorders. This, in spite of the absence of an independent association of each *TOLLIP* variant and the *MUC5B* promoter SNP with fHP survival. These results are in accordance with the report by Ley et al, who also found no independent association between *MUC5B* rs35705950 or *TOLLIP* rs5743890 and survival in patients with fHP.[20]

The G-T-G-C haplotype is also associated with a feature that can negatively impact in disease prognosis. All carriers of the haplotype had been subjected to bird exposure, an antigen that is frequently described in more severe and fibrotic forms of the disease,[39] despite the lack of an association with compromised lung function in our study.

Overall, our findings suggest that *TOLLIP* variants, as well as haplotypes (incorporating *MUC5B* rs35705950), could be of potential use for patients' risk stratification and as markers of prognostic value in fHP. Multicenter and prospective studies are needed to validate these results and to elucidate the role of both genes in the pathophysiology of hypersensitivity pneumonitis.

Funding

This work was supported by the Portuguese Society of Pulmonology (SPP) (SPP/Bolsa Novartis 2012 grant); Boehringer-Ingelheim (Unrestricted Research Grant); Fundação para a Ciência e a Tecnologia, I.P. (PTDC/MEC-RES/0158/2020 grant); and Fundação Amélia de Mello and D. José de Mello.

JCR is funded by Fundação para a Ciência e a Tecnologia through the Stimulus for Scientific Employment Individual Support (2020.01350.CEECIND).

Conflicts of interest

Patrícia Caetano Mota has received speaker fees from Boehringer-Ingelheim and research grants from Boehringer-Ingelheim and Novartis; and has participated in research with Boehringer-Ingelheim and Roche, for which her institution has been remunerated.

Hélder Novais-Bastos and António Morais have attended advisory boards for Boehringer-Ingelheim and Roche; have received speaker fees from Boehringer-Ingelheim and Roche; have participated in research with Boehringer-Ingelheim and Roche, for which their institution has been remunerated. Hélder Novais-Bastos has also received a research grant from Boehringer-Ingelheim.

The remaining authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

We are indebted to Instituto de Saúde Pública da Universidade do Porto (ISPUP) for providing samples from the EPI-Porto cohort to be used as controls; we thank the cohort management and all participants. We are grateful to Catarina Teixeira Antunes and Joana Filipa Amorim Reis for assistance in the genotyping.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.pulmoe.2024.01.002.

References

1. Raghu G, Remy-Jardin M, Ryerson CJ, et al. Diagnosis of hypersensitivity pneumonitis in adults. An official ATS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med. 2020;202(3):e36–69.
2. Fernandez Perez ER, Travis WD, Lynch DA, et al. Diagnosis and evaluation of hypersensitivity pneumonitis: CHEST guideline and expert panel report. Chest. 2021;160(2):e97–e156.
3. Santos V, Martins N, Sousa C, et al. Hypersensitivity pneumonitis: main features characterization in a Portuguese cohort. Pulmonology. 2020;26(3):130–7.
4. Vasakova M, Selman M, Morell F, Sterclova M, Molina-Molina M, Raghu G. Hypersensitivity pneumonitis: current concepts of pathogenesis and potential targets for treatment. Am J Respir Crit Care Med. 2019;200(3):301–8.
5. Nogueira R, Melo N, Novais EBH, et al. Hypersensitivity pneumonitis: antigen diversity and disease implications. Pulmonology. 2019;25(2):97–108.
6. Marinescu DC, Raghu G, Remy-Jardin M, et al. Integration and application of clinical practice guidelines for the diagnosis of idiopathic pulmonary fibrosis and fibrotic hypersensitivity pneumonitis. Chest. 2022;162(3):614–29.
7. Raghu G, Remy-Jardin M, Richeldi L, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med. 2022;205(9):e18–47.
8. Terras Alexandre A, Martins N, Raimundo S, et al. Impact of Azathioprine use in chronic hypersensitivity pneumonitis patients. Pulm Pharmacol Ther. 2020;60:101878.
9. Raimundo S, Pimenta AC, Cruz-Martins N, et al. Insights on chronic hypersensitivity pneumonitis' treatment: factors associated with a favourable response to azathioprine. Life Sci. 2021;272:119274.
10. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.
11. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet. 2011;377(9779):1760–9.
12. Flaherty KR, Wells AU, Cottin V, et al. Nintedanib in progressive fibrosing interstitial lung diseases. N Engl J Med. 2019;381(18):1718–27.
13. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;364(16):1503–12.
14. Zhang GY, Liao T, Gao WY. MUC5B promoter polymorphism and pulmonary fibrosis. N Engl J Med. 2011;365(2):178.. author reply -9.
15. Peljto AL, Selman M, Kim DS, et al. The MUC5B promoter polymorphism is associated with idiopathic pulmonary fibrosis in a Mexican cohort but is rare among Asian ancestries. Chest. 2015;147(2):460–4.
16. Bonella F, Campo I, Zorzetto M, et al. Potential clinical utility of MUC5B und TOLLIP single nucleotide polymorphisms (SNPs) in the management of patients with IPF. Orphanet J Rare Dis. 2021;16(1):111.
17. Oldham JM, Ma SF, Martinez FJ, et al. TOLLIP, MUC5B, and the response to N-acetylcysteine among individuals with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2015;192(12):1475–82.
18. Mota PC, Soares ML, Vasconcelos CD, et al. Predictive value of common genetic variants in idiopathic pulmonary fibrosis survival. J Mol Med. 2022;100(9):1341–53.
19. Blach J, Mackiewicz B. How much do we know about genetic predisposition of hypersensitivity pneumonitis? Ann Agric Environ Med. 2022;29(2):306–8.
20. Ley B, Newton CA, Arnould I, et al. The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis: an observational cohort-control study. Lancet Respir Med. 2017;5(8):639–47.
21. Furusawa H, Peljto AL, Walts AD, et al. Common idiopathic pulmonary fibrosis risk variants are associated with hypersensitivity pneumonitis. Thorax. 2022;77(5):508–10.
22. Furusawa H, Cardwell JH, Okamoto T, et al. Chronic hypersensitivity pneumonitis, an interstitial lung disease with distinct molecular signatures. Am J Respir Crit Care Med. 2020;202(10):1430–44.
23. Ley B, Torgerson DG, Oldham JM, et al. Rare protein-altering telomere-related gene variants in patients with chronic hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2019;200(9):1154–63.
24. Bouros D, Tzouvelekis A. Telomeropathy in chronic hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2019;200(9):1086–7.
25. Peljto AL, Blumhagen RZ, Walts AD, et al. Idiopathic pulmonary fibrosis is associated with common genetic variants and limited rare variants. Am J Respir Crit Care Med. 2023;207(9):1194–202.
26. https://clinicaltrials.gov/study/NCT05635032?cond=ILD&term=fibralung&rank=1.
27. Ramos E, Lopes C, Barros H. Investigating the effect of nonparticipation using a population-based case-control study on myocardial infarction. Ann Epidemiol. 2004;14(6):437–41.
28. Pintalhao M, Vasques-Novoa F, Couto-Viana B, et al. Relaxin-2, pathophysiological insights and outcomes in heart failure with preserved ejection fraction: findings from the NETDiamond cohort. Int J Cardiol. 2022;365:87–90.
29. Leite AR, Neves JS, Angelico-Goncalves A, et al. Clinical and pathophysiologic insights of free triiodothyronine/free thyroxine ratio in patients with heart failure with preserved ejection fraction: data from the NETDiamond cohort. Cardiology. 2023;148(3):239–45.
30. Noth I, Zhang Y, Ma SF, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. Lancet Respir Med. 2013;1(4):309–17.
31. Fingerlin TE, Murphy E, Zhang W, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613–20.
32. Li X, Goobie GC, Gregory AD, Kass DJ, Zhang Y. Toll-interacting protein in pulmonary diseases. abiding by the goldilocks principle. Am J Respir Cell Mol Biol. 2021;64(5):536–46.
33. Li X, Goobie GC, Zhang Y. Toll-interacting protein impacts on inflammation, autophagy, and vacuole trafficking in human disease. J Mol Med. 2021;99(1):21–31.
34. Katayanagi S, Setoguchi Y, Kitagawa S, Okamoto T, Miyazaki Y. Alternative gene expression by TOLLIP variant is associated with lung function in chronic hypersensitivity pneumonitis. Chest. 2022;161(2):458–69.
35. Lewandowska KB, Szturmowicz M, Lechowicz U, et al. The presence of T Allele (rs35705950) of the MUC5B gene predicts lower baseline forced vital capacity and its subsequent decline in patients with hypersensitivity pneumonitis. Int J Mol Sci. 2023;24(13).

36. Khankhanian P, Gourraud PA, Lizee A, Goodin DS. Haplotype-based approach to known MS-associated regions increases the amount of explained risk. J Med Genet. 2015;52(9):587—94.
37. Schrodi SJ, Garcia VE, Rowland C, Jones HB. Pairwise linkage disequilibrium under disease models. Eur J Hum Genet. 2007;15 (2):212—20.
38. Shirali M, Knott SA, Pong-Wong R, Navarro P, Haley CS. Haplotype heritability mapping method uncovers missing heritability of complex traits. Sci Rep. 2018;8(1):4982.
39. Morell F, Roger A, Reyes L, Cruz MJ, Murio C, Munoz X. Bird fancier's lung: a series of 86 patients. Medicine. 2008;87 (2):110—30.