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Association between *MUC5B* and *TERT* Polymorphisms and Different Interstitial Lung Disease Phenotypes

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Abstract

TERT and *MUC5B* polymorphisms have been consistently associated with idiopathic pulmonary fibrosis (IPF) in recent genome-wide genetic studies. However, it remains unclear how both loci contribute to the susceptibility to different entities of sporadic interstitial lung disease (ILD). We sought to test the associations of the two polymorphisms with IPF and non-IPF ILD entities in a Caucasian population. Associations between two polymorphisms in *TERT* (rs2736100) and *MUC5B* (rs35705950) and IPF or non-IPF sporadic ILD were tested using 227 ILD patients and 689 controls. Genotypic data were also correlated with pulmonary functions measured in ILD patients. As a result, rs2736100 and rs35705950 were significantly and independently associated with ILD as a single phenotype [Odds ratio (OR)=1.29, 95% Confidence Interval (CI): 1.04–1.60, $p=2\times10^{-2}$ and OR=2.22, 95%CI: 1.69–2.92, $p=7\times10^{-9}$, respectively). When considering IPF and “other ILD” (non-IPF) separately, rs35705950 had a stronger association with IPF (OR=3.2, 95%CI: 2.21–4.63, $p=1.2\times10^{-10}$) than other ILD (OR=1.72, 95%CI: 1.22–2.42, $p=1.2\times10^{-3}$). In contrast, rs2736100 was associated with other ILD (OR=1.43, 95%CI: 1.11–1.85, $p=6.2\times10^{-3}$) but not IPF (OR=1.08, 95%CI: 0.78–1.49, $p>0.05$). Rs35705950 was significantly correlated with increased pulmonary function ($p<0.05$). It was also associated with ILD without airflow obstruction in both IPF and other ILD groups ($p<0.01$ for both), and conferred the highest risk for IPF without airflow obstruction (OR=4.46, 95%CI: 2.60–7.66, $p=4.5\times10^{-9}$). Our study suggests that while both loci confer independent risks for ILD, rs35705950 may particularly contribute

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differentially to IPF and other ILD entities. Our study further highlighted the genetic and phenotypic heterogeneity of ILD.

Keywords

interstitial lung disease; idiopathic pulmonary fibrosis; *TERT*; *MUC5B*; polymorphism

INTRODUCTION

Interstitial lung disease (ILD) encompasses a broad range of chronic lung disorders with diverse pathogenesis and complex histopathology. More specifically, idiopathic interstitial pneumonia (IIP) refers to the ILD entities without known causes. According to the current classification, there are seven major subclasses for IIP [1–3], with idiopathic pulmonary fibrosis (IPF) being the most common and severe form of IIP [4]. The etiology and pathogenesis of most ILD entities remain unknown. To date, no proven pharmacotherapy for these entities has been recognized [1, 3].

It is now clear that the development of IIP has a strong genetic basis [5]. Family-based studies have been conducted to identify genes predisposing to IIP, and causal mutations have been identified in the telomerase reverse transcriptase gene (*TERT*), the telomerase RNA component gene (*TERC*) [6–8] and surfactant proteins C (*SPC*) [9] and A2 (*SPA2*) genes [10]. Recently, a few genome-wide association studies (GWAS) have identified a number of polymorphisms confer risks to IIP [11–16]. Among these loci, two single nucleotide polymorphism (SNP), rs2736100 and rs35705950, respectively in *TERT* and *MUC5B* genes, were consistently identified in multiple independent studies [11–16].

Despite these significant studies, questions still remain unaddressed. A major controversy is whether each of IIP subphenotypes represents a different disease, or all IIP entities are actually a common disease with different manifestations [3, 4]. The recent genome-wide study suggested that the *MUC5B* locus may confer similar risk to both IPF and familial interstitial pneumonia (FIP) [12]. Given the multiple clinical manifestations in FIP patients, this finding seems to support the notion that all IIP may share a common etiological basis [12]. Indeed, recent genome-wide study using a large population of IIP patients has identified a number of polymorphisms significantly associated with IIP [15]. Meanwhile, as the most significant genetic risk factor, the *MUC5B* polymorphism was also significantly associated with sporadic ILD among a general population [17]. On the other hand, it was also found that the *MUC5B* polymorphism was not associated with interstitial pneumonia (IP) in the subjects with systemic sclerosis (SSc), although SSc-associated IP is clinically, radiologically, and histologically similar to other forms of IP [18, 19]. This indicated the genetic and phenotypic heterogeneity of ILD. In this study, we attempt to further explore this question by using sporadic ILD samples collected in the American Caucasian population by the Lung Tissue Research Consortium (LTRC). We chose the *TERT* and *MUC5B* polymorphisms as these two loci have been consistently validated to be associated with either IPF or general ILD in a few independent sample sets, whereas the relationship between these two loci and different ILD entities has never been examined in a study under

the same settings. Our study sought: 1) to test and compare the associations between IPF and other ILD entities and *TERT* and *MUC5B* polymorphisms; 2) to test the correlation between *TERT* and *MUC5B* polymorphisms and lung function measurements in ILD patients.

METHODS

Ethics statement

Samples used in this study were collected with approval of institutional review boards (IRBs) of the Lung Tissue Research Consortium (LTRC, <http://www.ltrcpublic.com>) and the University of Chicago. Written informed consent was obtained from each participant. The Purdue University IRB has approved this study. The study was carried out in compliance with the Helsinki Declaration.

Study Subjects

DNA extracted from peripheral blood of ILD patients (n=227) were obtained from the Lung Tissue Research Consortium (<http://www.ltrcpublic.com>). All patients were diagnosed with ILD in accordance with the American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias [2], with well-documented clinical data, Computed Tomography (CT) scan and pathological review of lung biopsies for all patients. Cases with known cause for the disease were excluded. The DNA samples came from patients who had been diagnosed with IPF (n=84), non-specific interstitial pneumonia (NSIP) (n=27), desquamative interstitial pneumonia (DIP) (n=9), respiratory bronchiolitis-interstitial lung disease (RB-ILD) (n=22), cryptogenic organizing pneumonia (COP) (n=10), hypersensitive pneumonitis (HP) (n=8) and uncharacterized fibrosis (UF) (n=67). Lung function records including both pre- and post-bronchodilator measurements for ILD patients and healthy donors (n=26 and 14 for pre- and post-bronchodilator measurements, respectively) were also available. These measurements included pre- and post-bronchodilator forced vital capacity (FVC) % predicted (FVCpre, FVCpost), pre- and post-bronchodilator forced expiratory volume (FEV) at 1 second % predicted (FEV1pre and FEV1post) and the FEV1/FVC ratio (FEV1/FVCpre and FEV1/FVCpost). Severity of the airflow obstruction (AO) was also assessed in a subset of the ILD patients (n=111) based on standard criteria [18] for spirometric classification of chronic obstructive pulmonary disease (COPD) with mixed pre- and post-measurements. Stage 0, 1, 2, 3 and 4 AO was defined as at risk, mild, moderate and severe COPD, respectively. Given the sample size, we defined the AO severity into non-AO (stage 0) and AO (stage >0) groups for subsequent analyses.

Control DNA samples (n=689) were collected and provided by the Translational Research Initiative in the Department of Medicine (TRIDOM) program at the University of Chicago. These samples came from patients regularly visiting the clinic, excluding individuals with any respiratory symptoms according to the ICD-9 classification. All control patients are self-reported Caucasians. Demographic as well as other covariate data for cases and controls are summarized in Table I.

Genotyping of candidate polymorphisms

The candidate SNPs rs2736100 and rs35705950 were genotyped using TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA), by using a ViiA7 Real-time PCR system (Invitrogen), both with the following program: 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Data were analyzed with ViiA 7 RUO software, respectively.

Data analysis and statistics

Statistical power of the genetic association study was calculated using the Quanto program (v1.2.4, 2009) (<http://hydra.usc.edu/GxE>), with the population risk set as 0.0003 and an assumption of log-additive inheritance mode. As a result, there was only limited power for testing the association between rs2736100 and each individual ILD subtype (power <45% for all calculations), given the moderate effect of the risk allele [odds ratio (OR) $\approx$ 1.35 based on the previous GWAS (15)]. For this reason, genetic analysis was performed by treating all ILD (labeled as “all ILD”), IPF and combined ILD entities other than IPF (labeled as “Other ILD”) as different phenotypes. Power calculation demonstrated that there were 79%, 64% and 45% of power for testing the associations between rs2736100 and these three phenotypes, respectively. Given the higher OR for the rs35705950 risk allele in previous studies, there was over 70% of power for testing the associations between rs35705950 and each of these three phenotypes for any given OR>1.5.

Deviation from the Hardy-Weinberg Equilibrium (HWE) for each SNP was separately tested in cases and controls for each group with Chi-square test with df=1. Allelic associations between SNPs and ILD or subclasses were tested using Chi-square test (CST) or Fisher's exact test (FET) when appropriate. OR and 95% confidence interval (CI) were also calculated for the association in each group. The potential interaction between the two SNPs was tested as non-random distribution of genotypes of one SNP among that of another using Chi-square test. Test of the combined dosage effect of the two risk alleles were performed using Fisher's exact test, by comparing the risk-allele carriers to the non-risk allele carriers. Correlations between SNP genotypes and lung function measurements were conducted using a linear regression model, adjusting for age (continuous), gender (nominal), smoking status (nominal [ever, never]), disease status (nominal) and/or body mass index (BMI) (continuous) when appropriate. A post-hoc nonparametric Spearman test was also used to confirm the correlation between SNP genotype and lung function measurements. For all tests, to increase the statistical power, the rare-allele homozygotes (T/T) of rs35705950 were combined with heterozygotes (G/T). Rs2736100 was tested assuming an additive model for the risk allele (A). All correlation tests were two-tailed and p=0.05 was used as the cut-off. Data were analyzed using SPSS 18.0 software package (SPSS Inc., Chicago, IL) and GraphPad Prism version 4.0 for Windows (GraphPad Software, La Jolla, CA).

RESULTS

Demographic data and environmental covariates

We compared the distribution of demographic and environmental covariates data between each ILD subset and normal lung control group. Significant differences were observed in the

distribution of age, gender and smoking status, but not BMI data. We also compared the difference in these data distribution between IPF and non-IPF “Other ILD” group. Only the distribution of age data was observed to be significantly different between these two groups ($p=0.01$) (Table I). These factors were then all included in the subsequent statistical analyses and adjusted accordingly.

Association between *MUC5B* and *TERT* polymorphisms and ILD

There was a weak deviation of HWE for rs35705950 in the IPF group ($p=0.02$), but not in the control group. This deviation was not significant after correction for multiple testing (Table II).

Both SNPs were significantly associated with ILD as a single phenotype, with rs35705950-T being a stronger risk allele (OR=2.22, 95%CI: 1.69–2.92, $p=7.04\times10^{-9}$ for rs35705950; and OR=1.29, 95%CI: 1.04–1.20, $p=1.97\times10^{-2}$ for rs2736100). However, when IPF and “other ILD” subsets were analyzed as different phenotypes, associations between each phenotype and the two SNPs were differential. As shown in Table II, rs35705950 was strongly associated with IPF (OR=3.20, 95%CI: 2.21–4.63, $p=1.16\times10^{-10}$) but relatively weakly with “other ILD” (OR=1.72, 95%CI: 1.22–2.42, $p=1.64\times10^{-3}$). In contrast, there was no statistical significance between rs2736100 and IPF (OR=1.08, 95%CI: 0.78–1.49). Instead, this SNP was significantly associated with “other ILD” as a combined group (OR=1.43, 95%CI: 1.11–1.85, $p=6\times10^{-3}$). We also compared the allele frequencies between IPF and “other ILD” groups. As a result, the frequency of rs35705950-T was significantly increased in IPF (OR=1.86, 95%CI: 1.19–2.91, $p=7.5\times10^{-3}$). With regard to rs2736100, while there was a trend of decreased A allele frequency in IPF, no statistical significant was observed (OR=0.75; 95%CI: 0.51–1.11, $p>0.05$) (Table II).

To address the question whether there is any potential interaction between the two SNPs in cases and controls, non-random distribution of the genotypes of one SNP among another SNP was tested in IPF, other ILD, all ILD and the control groups, respectively. No significant deviation was found ($p>0.05$ for all tests, data not shown). Meanwhile, we calculated the association between the rs35705950-T allele and each of the phenotypes, among the patients who were homozygous carriers (AA) for the risk allele of rs2736100. As a result, there were still significant associations between rs35705950-T and IPF (OR=4.39, 95%CI: 2.08–9.27, $p=2.7\times10^{-4}$), other ILD (OR=2.60, 95%CI: 1.42–4.78, $p=1.52\times10^{-3}$) and all ILD (OR=3.10, 95%CI: 1.83–5.25, $p=1\times10^{-5}$), suggesting that the two polymorphisms were independently associated with increased risk to ILD.

We also tested the combined dosage effect of these two risk alleles on the susceptibility to ILD. Since there were only limited samples carrying 4 risk alleles ($n=3$ and 1 in all ILD cases and controls, respectively), these samples were combined with the individuals carrying 3 risk alleles. As summarized in Table III, there was a significant correlation between the number of risk alleles and the susceptibility to the disease, with the combined effect of the two SNPs conveying similar risks to IPF, other ILD and all ILD (Table III).

Correlations between SNPs and pulmonary function

Multiple lung function measurements including pre- and post-bronchodilator % predicted FEV1, FVC, and the FEV1/FVC ratio were available in a subset of ILD and normal lung donors (see Table IV for details). In assessment of the effect of covariates, the FEV1pre and FEV1post were significantly correlated with age ($p < 10^{-4}$). Both FVCpre and FVCpost were significantly associated with age ($p < 10^{-5}$) and smoking status ($p < 0.01$). FVCpost was also associated with gender ($p < 0.05$), while both FEV1/FVCpre and FEV1/FVCpost were significantly associated with gender ($p < 0.05$) age ($p < 0.01$) and smoking status ($p < 0.05$) (data not shown). After controlling for these covariates, the ILD patients had significantly lower FEV1pre, FEV1post, FVCpre, FVCpost, but slightly higher FEV1/FVCpre than the normal lung donors ($p < 0.05$ for all tests) (Table IV). The differences were still significant when a post-hoc Spearman correlation test was performed ($p < 0.05$ for all tests, data not shown).

To assess the impact of genotypes on lung function, SNP genotypes were correlated with pulmonary functions in ILD patients by controlling for the covariates. As a result, rs35705950-T was significantly associated with higher FEV1pre, FEV1/FVCpre and FEV1/FVCpost in ILD patients ($p < 0.05$ for all tests, Table IV). Again, these associations remained significant when a post-hoc Spearman correlation test was performed ($p < 0.05$ for all tests, data not shown). No significant correlation was observed between rs2736100 genotype and any lung function measurement (data not shown).

The severity of airflow obstruction (AO) was assessed in 111 ILD patients (Table V). We first tested the correlation between the severity of AO and all covariates. There was no significant association between severity of AO (4 stages) and age, gender, BMI or smoking status ($p > 0.05$ for all tests). To test whether there was a differential association between rs35705950 and ILD with or without AO, we compared the allelic distribution between ILD patients with stage 0 obstruction (non-AO) and those with stage 1 or higher (>0) (AO), as well as between stage 0 or stage >0 and control samples. We found that rs35705950 was significantly associated with ILD without AO when compared to those with stage >0 AO (OR=2.5, 95%CI: 1.20–5.19, $p = 1.24 \times 10^{-2}$) or healthy controls (OR=3.01, 95%CI: 2.02–4.48, $p = 1.6 \times 10^{-8}$), with no association observed between ILD patients with stage >0 obstructions and controls (OR=1.21, 95%CI: 0.63–2.33). We further examined the distribution of AO status among each subset of ILD. As a result, there were more patients without AO in IPF and NSIP groups (about 80%) compared to the rest subset ($<60\%$). In all non-IPF “other ILD” group, 57% of patient on average had no significant AO ($p = 0.02$ compared to the IPF group). The rs35705950 was significantly associated with stage 0 AO in both IPF and “other ILD” group when compared to the control group ($p = 0.009$ for both), suggesting that this association was independent of disease status. When compared the allele frequency between patients with and without AO among each group, rs35705950 conferred the highest risk for IPF without AO (OR=8.85, 95%CI: 1.10–71.47, $p = 1.68 \times 10^{-2}$) (Table V).

DISCUSSION

Our study for the first time tested the associations between the *TERT* and *MUC5B* polymorphisms and different sporadic ILD entities in a Caucasian population. Although the sample size is moderate, our findings confirmed that both polymorphisms independently confer susceptibility to ILD as an overall phenotype, with the *MUC5B* locus conveying a higher risk than the *TERT* polymorphism. Our results confirmed the findings that have been previously reported in the genome-wide studies [11, 12, 14, 15]. However, the susceptibility to ILD entity is unequally attributed to these two polymorphisms: while *MUC5B* confers a higher risk to IPF than to other ILD subsets, the *TERT* polymorphism has stronger association with the ILD subsets excluding IPF. The negative association between *TERT* polymorphism and IPF could be largely attributed to the small sample size (n=84), which was reflected by the limited power pre-calculated (power =45%). Notably, although the sample size for IPF (n=84) is smaller relative to the “other ILD” group (n=143), there was still a much stronger association between *MUC5B* polymorphism and IPF. This finding indicates that a few biological pathways may be differentially involved into the pathogenesis of different ILD subtypes, although common genetic factors are also shared by all entities.

Our study demonstrated that the associations between *MUC5B* or *TERT* polymorphisms and ILD are independent in Caucasian population. There was no significant interaction between the two loci in either ILD patients or normal controls. Meanwhile, in the homozygous carriers of the *TERT* risk allele A, the *MUC5B* risk allele T is still significantly associated with ILD. When combined, the two risk alleles confer a much higher risk to ILD as an overall phenotype. This is further consistent with the differential associations between the two polymorphisms and different ILD entities. Taken together, this may reflect that the two underlying pathways are independently involved in the natural history of different subtypes of the disease. However, the mechanism of how these two pathways contribute to the disease pathogenesis is still largely unknown. Rare, germline and loss-of-function mutations in *TERT* have been identified from patients with FIP/IPF [6–8], while rs2736100 was significantly associated with germline DNA telomere length in a recent GWAS [19], consistently suggesting that decreased capacity for retaining the telomere length increases the susceptibility to adult-onset IPF. Since *TERT* is only expressed in limited types of tissues and cells e.g. proliferative stem cells of renewal tissues, this pathway was deemed to be of critical importance in maintenance of the function of bronchoalveolar epithelial and the stem cells in the lung [4, 5]. In contrast, the *MUC5B* may be more significantly involved in the disease expression and progression through a gene-environment interaction model, as mucins are important for protecting the lung from injuries by environmental factors [20, 21]. Meanwhile, multiple tissues/cells including bronchoalveolar epithelium, airway epithelium, goblet cells and submucosal glands are involved in the secretion and function of mucins in the lung [20]. Therefore, it remains to be an interesting question for continued investigation that how *TERT* and *MUC5B* are involved in the function of different pulmonary cells during the development of different ILD entities.

Our study also revealed that the *MUC5B* polymorphism was associated with better lung function and conferred the highest risk for IPF without significant airflow obstruction (AO). The results remained to be significant even after correcting demographic and environmental

factors, further indicating the phenotypic and genotypic heterogeneity in IPF and ILD. Moreover, the association between rs35705950 and the absence of AO was independent of the disease status. Previous studies hypothesized that increased secretion of mucins, e.g., 5B, may lead to bronchiolar plugging, thus producing a chronic inflammatory and toxic burden on the alveolar surface [22], which seems to be contradictory to our observation. There are a few possibilities explaining this result. First, it may suggest that IPF/ILD with or without AO are actually different diseases or entities. This may be partly supported by some lines of evidence, e.g. mucus plugging in chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) is not associated with a pulmonary fibrosis phenotype [21]. Therefore, the IPF/ILD with AO may not share the same pathogenic pathway as with those without. On the other hand, while the risk allele may be predisposed to the development of fibrosis with an unknown mechanism, the increased *MUC5B* expression in certain cells or location(s) attributed to the risk allele may be protective for the development of AO. It was observed that MUC5AC rather than MUC5B is actually the primary gel-forming mucin that is upregulated in asthma lung and significantly increased expression of MUC5AC led to airflow obstruction, while MUC5B may selectively inhibit MUC5AC production [23]. However, secretion and distribution of mucins in lung is complex with multiple cell types involved. Without systematic investigation regarding the production, storage and secretion of the MUC5B mucin as well as its distribution and function in different cells, it is difficult to corroborate these hypotheses. Therefore, while independent studies are required to further validate the findings in our study, detailed mechanistic studies are also urgently needed to delineate how the *MUC5B* allele is involved in the disease pathogenesis.

The major limitation of our study is the small sample size. It was unfortunate that there is insufficient power to test the association between the polymorphisms and each of the ILD subsets individually, e.g. NSIP, DIP, RB-ILD, etc. Due to the low prevalence of the disease, it is difficult to collect a large number of patients with well-characterized clinical evaluation. In addition, information about many potential confounding factors is not available especially in the control group, e.g. smoking status, BMI information, etc. This constrains our power in excluding the confounding effects of these factors on the genetic associations. Nevertheless, despite these limitations, our study provides important information in the field regarding the potentially distinct pathogenic mechanisms involved in different ILD phenotypes that are underlying the pathways associated with *TERT* and *MUC5B*. Our study highlights that further investigation is particularly necessary to identify additional genetic factors predisposing individuals to different ILD phenotypes.

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Abbreviations

IPF idiopathic pulmonary fibrosis

ILD	interstitial lung disease
UIP	usual interstitial pneumonia
NSIP	non-specific interstitial pneumonia
DIP	desquamative interstitial pneumonia
RB-ILD	respiratory bronchiolitis-interstitial lung disease
COP	cryptogenic organizing pneumonia
HP	hypersensitive pneumonitis
UF	uncharacterized fibrosis

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Background

TERT and *MUC5B* genes in susceptibility to pulmonary fibrosis were recently established in genome-wide genetic studies, the relationship between these two loci in contributing to different interstitial lung disease (ILD) phenotypes remains unknown.

Translational Significance

Our study for the first time observed that the *TERT* and *MUC5B* polymorphisms independently and differentially confer susceptibility to different interstitial lung disease entities, which suggests that both common and individual entity-specific genetic components for ILD may exist simultaneously. This provides important direction for future genetic studies toward the susceptibility to ILD, and forms a rationale for different treatment options for the disease.

Table 1

Demographic and covariates data associated with ILD patients and controls.

Disease status	Total number	Gender Male (N)	p	Age Mean±SD	Cigarette Smoking				p [†]	BMI Mean±SD
					Current	Ever	Never			
UIP/IPF	84	55		64.4±7.7	1	52	31			30.3±5.2
NSIP	27	10		59.1±8.4	1	15	11			30.5±6.8
DIP	9	7		54.1±7.7	3	4	2			31.3±8.7
RB-ILD	22	13		58.1±11.5	2	17	3			32.5±7.1
COP	10	7		62.2±11.9	2	6	2			29.0±6.7
HP	8	1		52.0±16	0	1	7			31.7±5.7
UF	67	40		64.8±10.4	1	45	21			29.4±5.6
Other ILD	143	78			9	88	46			30.2±6.4
Normal lung*	33	8		56.8±13.9	0	12	14			30.0±6.0
IPF vs Other ILD			-		0.01				-	-
Control	689	360		55.7±13.2	-	-	-			-

Abbreviations: SD, standard deviation; BMI, body mass index; UIP, usual interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; NSIP, non-specific interstitial pneumonia; DIP, desquamative interstitial pneumonia; RB-ILD, respiratory bronchiolitis-interstitial lung disease; COP, cryptogenic organizing pneumonia; HP, hypersensitive pneumonitis; UF, uncharacterized fibrosis. Statistical significance (p values) in the distribution of each covariate between IPF and “Other ILD” subset was shown.

* Data are available for 26 out of 33 samples.

[†] P value was calculated using CST or FET by combining the “Current” and “Ever” groups.

Table II

Association between rs35705950 and rs2736100 and ILD.

Polymorphism	IPF	Other ILD	All ILD	Control	IPF vs Other ILD
Genotype	GG	94	131	539	
	GT	44	89	139	
	TT	3	7	11	
rs35705950	G	118	233	351	1217
	T	50	53	103	161
	RAF	0.30	0.19	0.23	0.12
Allelic association*	OR	3.20	1.72	2.22	referent
	95%CI	2.21–4.63	1.22–2.42	1.69–2.92	1.19–2.91
	p	1.16×10^{-10}	1.64×10^{-3}	7.04×10^{-9}	7.5×10^{-3}
Genotype	CC	17	20	37	180
	AC	47	78	125	328
	AA	20	45	65	178
rs2736100	C	81	118	199	688
	A	87	168	255	684
	RAF	0.52	0.59	0.56	0.50
Allelic association	OR	1.08	1.43	1.29	referent
	95%CI	0.78–1.49	1.11–1.85	1.04–1.60	0.51–1.11
	p	-	6.23×10^{-3}	1.97×10^{-2}	-

Abbreviations: ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; RAF, risk allele frequency; OR, odds ratio; 95%CI, 95% Confidence Interval.

Combined dosage effect of the risk alleles.

Table III

Number of risk allele	Statistics	IPF	Other ILD	All ILD	Control
0	N	10 (referent)	14 (referent)	24 (referent)	139 (referent)
	N	24	59	83	287
1	OR	1.16	2.04		1.68
	95%CI	0.54–2.50	1.10–3.78	1.02–2.76	
	p	-	0.027	0.046	
2	N	38	50	88	214
	OR	2.47	2.32	2.38	
	95%CI	1.19–5.12	1.24–4.36	1.45–3.92	
	p	0.016	7.36×10^{-3}	4.26×10^{-4}	
3+4	N	12	20	32	40
	OR	4.17	4.96	4.63	
	95%CI	1.68–10.36	2.30–10.70	2.45–8.75	
	p	3.15×10^{-3}	4.42×10^{-5}	2.08×10^{-6}	

Abbreviations: ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; UF, uncharacterized fibrosis; OR, odds ratio; 95%CI, 95% confidence interval. Associations were tested by comparing between each of 1, 2 and 3+4 risk alleles groups and the 0 risk allele group.

Table IV

Association between rs35705950 and pulmonary function in ILD patients.

	FEV1pre		FVCpre		FEV1post		FVCpost		FEV1/FVCpre		FEV1/FVCpost	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
Total	72.23±18.92	227	71.11±19.57	227	78.01±20.05	110	77.82±19.75	110	0.78±0.11	227	0.77±0.13	110
GG	68.79±19.94	131	69.98±21.22	131	75.49±21.54	63	77.7±20.53	63	0.76±0.12	131	0.75±0.13	63
ILD	GT+TT	96	72.65±17.06	96	81.38±17.53	47	77.98±18.88	47	0.81±0.1	96	0.8±0.12	47
	r	0.16	0.004		0.07		-0.1		0.26		0.28	
	p	0.016	-		-		-		5.41×10 ⁻⁵		3.34×10 ⁻³	
Total	91.04±12.32	26	94.54±11.7	26	97.5±12.78	14	97.07±13.77	14	0.75±0.09	26	0.78±0.04	14
GG	89.14±11.96	22	94±12.41	22	97.15±13.23	13	97.23±14.32	13	0.74±0.1	22	0.78±0.04	13
Control	GT [†]	4	97.5±6.95	4	102	1	95	1	0.8±0	4	0.8	1
	r	0.34	0.04		-0.13		-0.29		0.35		0.24	
	p	-	-		-		-		-		-	
Comparison [*]	r	0.34	0.4		0.36		0.4		-0.16		-0.04	
	p	2.1×10 ⁻⁸	1.08×10 ⁻¹¹		2.79×10 ⁻⁵		2.97×10 ⁻⁶		0.012		-	

Abbreviations: FEV , forced expiratory volume; FVC, forced vital capacity; FVCpre and FVCpost, pre- and post-bronchodilator FVC% predicted; FEV1pre and FEV1post, pre- and post-bronchodilator FEVat 1 second % predicted; FEV1/FVCpre and FEV1/FVCpost, pre- and post-bronchodilator FEV1/FVC ratio; r, correlation coefficient, statistics (r and p) are shown as the results after adjusting for age, gender, smoking status and BMI.

^{*} Comparison between the total ILD and the total control group.

[†] There is only one sample in the GT group with FEV1post, FVCpost and FEV1/FVCpost data available, therefore the standard deviation was not calculated.

Table V

Association between rs35705950 and severity of airflow obstruction.

Stage of Airflow Obstruction (stage)	Genotype of rs35705950 (N)			RAF	OR*	95%CI	p	OR†	95%CI	p
	GG	GT	TT							
All	0	33	37	2	0.28	2.02–4.48	1.6×10 ⁻⁸	2.4	1.16–5.05	1.58×10 ⁻²
	>0	28	11	0	0.14	0.64–2.34		referent		
IPF	0	9	21	1	0.37	4.46	2.60–7.66	4.53×10 ⁻⁹	8.85	1.10–71.47
	>0	7	1	0	0.06	0.50	0.07–3.84	-	referent	1.68×10 ⁻²
Other ILD	0	24	16	1	0.22	2.13	1.13–3.68	9.2×10 ⁻³	1.46	0.62–3.44
	>0	21	10	0	0.16	1.45	0.72–2.92	-		-
Control	539	139	11	0.12	referent					

Abbreviations: RAF, risk allele frequency; OR, odds ratio; 95%CI, 95% confidence interval.

* Association was calculated by comparing between the stage 0 (non-AO) or stage >0 (1+2+3) (AO) in each group and controls.

† Association was calculated by comparing between the stage 0 (non-AO) and stage >0 (AO) within All, IPF and Other ILD groups.