ORIGINAL ARTICLE

Overlap of Genetic Risk between Interstitial Lung Abnormalities and Idiopathic Pulmonary Fibrosis

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Abstract

Rationale: Interstitial lung abnormalities (ILAs) are associated with the highest genetic risk locus for idiopathic pulmonary fibrosis (IPF); however, the extent to which there are unique associations among individuals with ILAs or additional overlap with IPF is not known.

Objectives: To perform a genome-wide association study (GWAS) of ILAs.

Methods: ILAs and a subpleural-predominant subtype were assessed on chest computed tomography (CT) scans in the AGES (Age Gene/Environment Susceptibility), COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease [COPD]), Framingham Heart, ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points), MESA (Multi-Ethnic Study of Atherosclerosis), and SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) studies. We performed a GWAS of ILAs in each cohort and combined the results using a meta-analysis. We assessed for overlapping associations in independent GWASs of IPF.

Measurements and Main Results: Genome-wide genotyping data were available for 1,699 individuals with ILAs and 10,274 control subjects. The *MUC5B* (mucin 5B) promoter variant rs35705950 was significantly associated with both ILAs ($P = 2.6 \times 10^{-27}$) and subpleural ILAs ($P = 1.6 \times 10^{-29}$). We discovered novel genome-wide associations near *IPO11* (rs6886640, $P = 3.8 \times 10^{-8}$) and *FCF1P3* (rs73199442, $P = 4.8 \times 10^{-8}$) with ILAs, and near *HTRE1* (rs7744971, $P = 4.2 \times 10^{-8}$) with subpleural-predominant ILAs. These novel associations were not associated with IPF. Among 12 previously reported IPF GWAS loci, five (*DPP9*, *DSP*, *FAM13A*, *IVD*, and *MUC5B*) were significantly associated ($P < 0.05/12$) with ILAs.

Conclusions: In a GWAS of ILAs in six studies, we confirmed the association with a *MUC5B* promoter variant and found strong evidence for an effect of previously described IPF loci; however, novel ILA associations were not associated with IPF. These findings highlight common genetically driven biologic pathways between ILAs and IPF, and also suggest distinct ones.

Keywords: genetics; genome-wide association study; interstitial lung abnormalities; idiopathic pulmonary fibrosis; SNP

Idiopathic pulmonary fibrosis (IPF), the most common and severe form of interstitial lung disease (ILD) (1), is a disorder of lung scarring that is reported to affect one in 200 adults over the age of 65 (2), and results in a high rate of mortality (3, 4). A strong genetic basis for PF has been demonstrated in studies of familial aggregation (5), as well as in genome-wide linkage and association studies that have provided replicable evidence for associations between

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At a Glance Commentary

Scientific Knowledge on the Subject: Individuals with interstitial lung abnormalities (ILAs) exhibit a clinical syndrome similar to that observed in patients with idiopathic pulmonary fibrosis (IPF), including physiologic decrements, radiologic progression, accelerated lung function decline, and an increased risk of death. ILAs are associated with the most common and highest genetic risk locus in IPF, the *MUC5B* (*mucin 5B*) promoter polymorphism rs35705950. However, the extent to which there is additional overlap in the genetic risk of ILAs and IPF is not known.

What This Study Adds to the Field: In a genome-wide association study of ILAs, we confirmed findings at the *MUC5B* locus and identified three novel loci for ILAs and subpleural-predominant ILAs. These novel loci were not associated with IPF. Additionally, among 12 distinct, previously identified IPF GWAS loci, we identified 11 directionally consistent associations with ILAs, of which 7 were at least nominally significant and 5 (near *DPP9*, *DSP*, *FAM13A*, *IVD*, and *MUC5B*) were significantly associated after adjustment for multiple testing.

common genetic variants and IPF (6–11). Most consistently, IPF has been associated with increased copies of a common variant (rs35705950) in the promoter of the *MUC5B* (mucin 5B) gene (6, 8–11), a finding that may explain up to 30% of the risk of the disease (6).

Due to the severity of physiologic decrements and the high rate of mortality at the time of diagnosis, investigators have recently made efforts to identify IPF and other forms of PF in their earliest stages (12–14). These efforts include chest computed tomography (CT) image characterization of research participants who have not received a diagnosis but have classified imaging features that are suggestive not only of IPF specifically (15–17) but also of the broader set of ILDs (termed interstitial lung abnormalities [ILAs]) (13). Evidence supporting a correlation between some research participants with ILAs who have not received a diagnosis and patients with IPF include a shared association with increased copies of the *MUC5B* promoter variant (12, 18). However, the extent to which research participants with ILAs who have not received a diagnosis and patients with IPF share common or unique genetic etiologies remains unclear.

We hypothesized that comparisons between research participants with and without ILAs would identify findings of genetic association shared with those identified in patients with IPF and, based on the diversity of ILA phenotypes (18) and ILDs in general (19), unique associations. To test this hypothesis, we genotyped common genome-wide single-nucleotide variants and imputed additional genotypes from reference panels, and then tested for association with visually assessed ILAs and the subpleural-predominant ILA subtype in populations of research participants from six unique cohorts. Based on the results, we performed further comparisons to examine the overlap of top genetic associations in research participants with ILAs (and subpleural-predominant ILAs) with genetic associations previously reported in patients with IPF (8). Some of the results of this study have been previously reported in the form of an abstract (20).

Methods

Study Population

The protocols for participant enrollment and phenotyping in the FHS (Framingham Heart Study) and the AGES (Age Gene/Environment Susceptibility)-Reykjavik, COPDGene (Genetic Epidemiology of COPD), ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points), MESA (Multi-Ethnic Study of Atherosclerosis)-Lung Study, and SPIROMICS (Subpopulations and Intermediate Outcome Measures in COPD Study) studies have been described previously (12–14, 21–23). Approval from the appropriate ethical/regulatory bodies was obtained for each cohort, and informed consent was obtained from all individuals. More detailed cohort information, including cohort-specific methods, can be found in the online supplement.

ILA Characterization

ILA was characterized in all of the cohorts by visual assessments of chest CT scans. In FHS, AGES, COPDGene, and ECLIPSE, CT scans were evaluated for ILAs via a sequential reading method by up to three readers (radiologists and pulmonologists) who were blind to all participant-specific information, as previously described (24). ILAs in these cohorts were defined as nondependent changes affecting >5% of any lung zone. The abnormalities included ground-glass or reticular abnormalities, diffuse centrilobular nodularity, multiple nonemphysematous cysts, traction bronchiectasis, and honeycombing. Chest CT scans with either focal or unilateral ground-glass attenuation, focal or unilateral reticulation, or patchy ground-glass abnormalities were indeterminate for ILAs (12, 13). In MESA, ILAs were assessed by a radiologist using the above criteria, as previously described (25). In SPIROMICS, ILAs were classified as present or absent according to the presence or absence of bilateral, nondependent, peripheral (but not necessarily subpleural) ground-glass and/or reticular opacities and/or honeycombing (*see* the online supplement for further details).

Based on prior data on the genetics of ILAs (18), and to provide consistency of subtyping across cohorts, an additional ILA subset was created that excluded ILAs limited to participants with centrilobular nodules alone (13) and included all participants with ILAs with predominantly subpleural imaging findings (subpleural predominant).

Genotyping and Imputation

Details regarding genotyping in each cohort can be found in the online supplement. The genotypes of individuals from European-ancestry studies were imputed via the Michigan Imputation Server using minimac3 with the Haplotype Reference Consortium (HRC v1.1) reference panel (26). Individuals of African American ancestry in COPDGene and MESA were imputed using the 1000 Genomes Project (Phase 3, version 5) (27). The *MUC5B* promoter polymorphism (rs35705950) was poorly imputed (imputation $R^2 < 0.5$) in

the AGES study, so instead we used direct genotyping data, which were available for 3,209 individuals.

Genome-Wide Association Study and Meta-analysis

Given the case–control imbalance, we performed Firth bias-corrected logistic regression (28, 29) in each ancestry subset of each study, adjusting for age, sex, pack-years of smoking, and ancestry-based principal components as appropriate for each study. In the FHS, to allow application of the Firth bias-corrected logistic regression, we selected a subset of unrelated participants for analysis, preferentially choosing ILA cases. Summary statistics from individual studies, including chromosome and position (hg19), effect allele and other allele oriented to the + strand, effect allele frequency, and imputation quality were uploaded to a secure site at the Brigham and Women's Hospital/Channing Division of Network Medicine.

The summary statistics from each study were assessed using EasyQC (30) version 10.1. Quality control assessments included allele frequency comparisons with either a Haplotype Reference Consortium or 1000 Genomes reference panel, SE versus sample size checking, and quantile-quantile plot visualization. Variants with an imputation quality metric of <0.5, a minor allele count of <10 (using the effective sample size or the number of cases adjusted for imputation quality when appropriate), were set to missing. Variant names were all normalized to hg19 chromosome and position. Only the highest-frequency alternate allele was retained for multiallelic variants.

After completing the summary statistical quality control process, we performed an inverse-variance–weighted, fixed-effects meta-analysis in METAL (version 2011-03-25) (31, 32) for both the ILA and subpleural-predominant ILA analyses. In a set of secondary analyses, we performed a meta-analysis restricted to ILA and subpleural ILA results from European-ancestry subpopulations, and a smoking-stratified (ever-smokers compared with never-smokers) meta-analysis of our genome-wide significant variants. Only variants that were present in at least half of the cohort subpopulations in each meta-analysis were further evaluated. Genome-wide significance for all associations was considered to be $P < 5 \times 10^{-8}$. To identify distinct results at each locus in the European-ancestry subpopulations, we used genome-wide complex trait analysis–conditional and joint analysis (33, 34) on all results with $P < 5 \times 10^{-6}$, using the default distance of 10 Mb. COPDGene non-Hispanic whites (the largest representative population) were the reference population for the genome-wide complex trait analysis–conditional and joint analysis.

Overlap of ILA Genetic Loci with IPF, High-Attenuation Areas, Smoking Behaviors, and Connective Tissue Disease

We evaluated the overlap of top ILA-associated genetic variants with IPF in two ways: *1*) lookup of IPF genome-wide association study (GWAS) loci from the National Human Genome Research Institute–European Bioinformatics Institute (NHGRI-EBI) GWAS Catalog (35) (downloaded June 4, 2018) in our ILA GWAS results; *2*) lookup of our top ILA-associated variants ($P < 5e{-}7$ with either ILAs or subpleural ILAs in a European-ancestry subpopulation) with IPF in a recent European-ancestry IPF GWAS and meta-analysis (*see* online supplement) (11). In the lookup of prior IPF GWAS variants in our results, we restricted the lookup to 12 distinct IPF GWAS loci (reported results are for the variant that demonstrated the greatest statistical significance at each locus). Significance for association of IPF GWAS variants with ILAs or subpleural-predominant ILAs was set to $P < 0.05/12$. Additional analyses using logistic regression conditioning on the *MUC5B* promoter polymorphism (rs35705950) were done to assess for independence of the multiple SNPs previously identified at the 11p15 locus. To evaluate the overlap between ILAs and high-attenuation areas (HAAs), which have been associated with early or subclinical ILD and future ILAs (14, 25), we performed a lookup of the previously reported genome-wide significant variants associated with HAAs (36). To evaluate the overlap between ILAs and smoking behaviors, we performed a lookup of the previously reported genome-wide significant variants associated with smoking behaviors (37). To assess a potential overlap of the genetic susceptibility to ILAs (and subpleural-predominant ILAs) with connective tissue disease (CTD)–associated ILD and sarcoidosis, we searched the NHGRI-EBI GWAS Catalog (35) for genome-wide significant SNPs in European-ancestry association studies related to rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, inflammatory myopathies, and systemic sclerosis. We assessed the *P* value for association of the genome-wide significant rheumatologic disease SNPs with ILAs and subpleural-predominant ILAs. We used Bonferroni *P* values as a significance threshold to correct for multiple testing (number of genome-wide significant SNPs) within each unique trait.

Expression Quantitative Trait Loci Lookups of Top GWAS Variants

We assessed whether our significant ILA genetic risk variants were expression quantitative trait loci (eQTLs) in the lung and blood using multiple available datasets, including GTEx lung and blood eQTLs (38, 39), Westra and colleagues' blood eQTLs (40), Hao and colleagues' lung eQTLs (41), Jansen and colleagues' Netherlands Study of Depression and Anxiety/Netherlands Twin Register conditional blood eQTLs (42), and eQTL Consortium blood eQTL meta-analysis data (43). Only *cis*-eQTLs were assessed, and significant associations were determined using the adjusted *P* values reported in each available eQTL dataset.

Results

We performed a GWAS and meta-analysis of 1,699 participants with ILAs and 10,274 control subjects in six cohorts, and the subjects in each study were stratified into subpopulations according to European, African, and Hispanic ancestry. We performed a secondary GWAS and meta-analysis using the subset of 1,287 subpleural-predominant ILA cases (Figure 1). The baseline characteristics of each cohort and subpopulation, stratified by ILA status, are included in Table 1 (for the characteristics of participants with ILAs limited to the subpleural-predominant subtype, *see* Table E1 in the online supplement). Similar to what was found in prior studies, participants with ILAs tended to be older (15) and generally had greater exposure to tobacco smoke than those without ILAs.

Genome-Wide Association

We identified three genome-wide significant variants associated with ILAs, including one

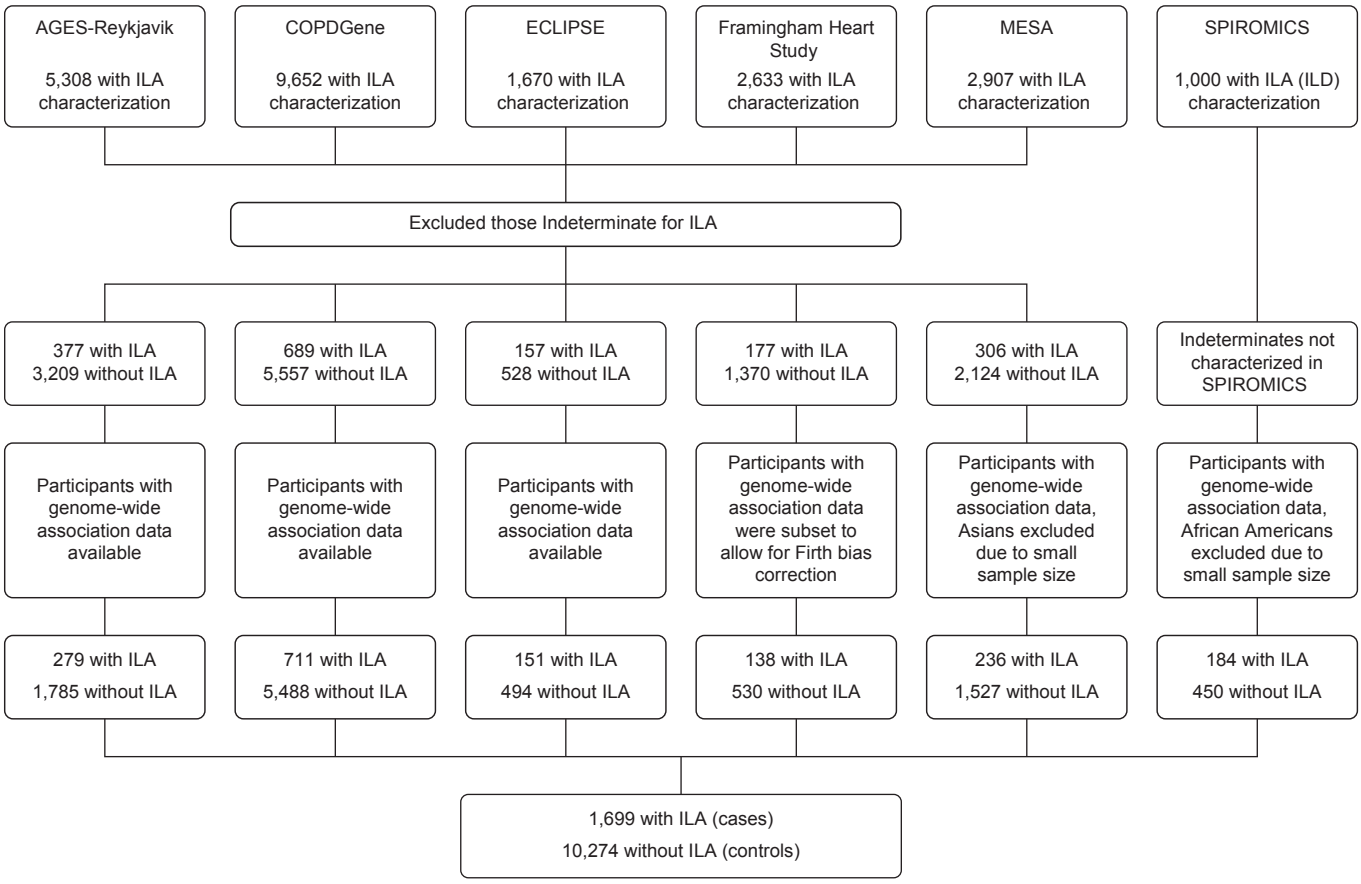

Figure 1. Flowchart depicting the participants included and excluded from the genome-wide association analysis by cohort and interstitial lung abnormality (ILA) status. AGES = Age Gene/Environment Susceptibility; COPD = chronic obstructive pulmonary disease; COPDGene = Genetic Epidemiology of COPD; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-Points; ILD = interstitial lung disease; MESA = Multi-Ethnic Study of Atherosclerosis; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.

at 11p15, at the known *MUC5B* promoter polymorphism, rs35705950 (odds ratio [OR], 1.97; 95% confidence interval [CI], 1.74–2.22; $P = 2.6 \times 10^{-27}$), as well as two novel loci: rs6886640 at 5q12, near *IPO11* (OR, 1.28; 95% CI, 1.18–1.41; $P = 3.8 \times 10^{-8}$), and rs73199442 at 3q13, near the long noncoding RNA *FCF1P3* (OR, 1.68; 95% CI, 1.39–2.02; $P = 4.8 \times 10^{-8}$) (Table 2 and Figures 2, E1, and E2). In the subpleural-predominant ILA analysis, in addition to the association with the *MUC5B* variant rs35705950 (OR, 2.22; 95% CI, 1.93–2.55; $P = 1.6 \times 10^{-29}$), we identified a novel genetic association at the 6q15 locus with rs7744971, near *HTR1E* (OR, 1.32; 95% CI, 95% CI 1.19–1.45; $P = 4.2 \times 10^{-8}$) (Table 2 and Figure 2). The ILA risk variant at 3q13.1 (rs73199442) was missing in the African- and Hispanic-ancestry subpopulations (due to low minor allele frequency), but it showed a consistent direction of effect in all European-ancestry subpopulations (*see* forest plots in Figure 2). Similar results were noted in meta-analyses of ILAs and subpleural-predominant ILAs limited to individuals of European ancestry; however, the 5q12 locus was not significantly associated with ILAs, and the 6q15 locus had genome-wide significance in association with both ILAs and subpleural-predominant ILAs (Tables E2 and E3). For each variant that demonstrated genome-wide significance, we tested for genotype-by-smoking (ever-smokers compared with never-smokers) interactions. There was no evidence of a significant interaction between smoking status and any of the four genome-wide significant variants (Table E4 and Figure E3). To assess whether these novel ILA risk loci overlapped with IPF, we attempted to replicate our genome-wide significant associations with ILA and subpleural ILA associations in a European-ancestry GWAS and meta-analysis of 2,668 patients with IPF and 8,591 control subjects (11) (*see* the online supplement). Aside from the known overlap at rs35705950 (IPF *P* value = 1.2×10^{-203}), none of our top ILA loci were significantly associated with IPF (Table 2).

Assessment of Replication for Prior IPF, HAAs, Smoking Behaviors, and CTD Genetic Loci

We examined the overlap of ILA and subpleural-predominant ILA genetic associations with 12 previously reported, distinct IPF GWAS loci from the NHGRI-EBI GWAS Catalog. There was a substantial enrichment of the 12 IPF GWAS loci in our ILA association results. Five SNPs near *DPP9*, *DSP*, *FAM13A*, *IVD*, and *MUC5B* were significantly associated ($P < 4.2 \times 10^{-3}$) with ILAs, and two additional SNPs at *MAPT* and *LRRC34* were nominally significant ($P < 0.05$, but did not meet the

Table 1. Baseline Characteristics of the Participants, Stratified by Interstitial Lung Abnormality Status, in Each Cohort

	AGES-Reykjavik		COPDGene				ECLIPSE		Framingham Heart Study		MESA						SPIROMICS	
			Non-Hispanic Whites		African Americans						Non-Hispanic Whites		Hispanics		African Americans			
	No ILA (*n* = *1,785*; *86%*)	ILA (*n* = *279*; *14%*)	No ILA (*n* = *3,771*; *88%*)	ILA (*n* = *497*; *12%*)	No ILA (*n* = *1,717*; *89%*)	ILA (*n* = *214*; *11%*)	No ILA (*n* = *494*; *77%*)	ILA (*n* = *151*; *23%*)	No ILA (*n* = *530*; *79%*)	ILA (*n* = *138*; *21%*)	No ILA (*n* = *675*; *85%*)	ILA (*n* = *116*; *15%*)	No ILA (*n* = *385*; *88%*)	ILA (*n* = *54*; *12%*)	No ILA (*n* = *467*; *88%*)	ILA (*n* = *66*; *12%*)	No ILA (*n* = *450*; *71%*)	ILA (*n* = *184*; *29%*)
Age, yr, mean (SD)	76 (5)	77 (5)	61 (9)	64 (9)	54 (7)	55 (8)	62 (7)	64 (8)	58 (11)	71 (11)	69 (9)	75 (10)	67 (9)	74 (9)	69 (9)	74 (8)	65 (8)	68 (8)
Sex, F, *n* (%)	1,078 (60)	125 (45)	1,814 (48)	226 (45)	701 (41)	116 (54)	166 (34)	40 (26)	278 (53)	67 (49)	344 (51)	60 (52)	209 (54)	29 (54)	247 (53)	41 (62)	206 (46)	83 (45)
Body mass index, mean (SD)	27 (4)	27 (5)	29 (6)	29 (6)	29 (7)	29 (7)	27 (6)	26 (5)	28 (5)	28 (5)	28 (5)	28 (6)	30 (6)	30 (6)	30 (6)	30 (5)	27 (5)	29 (5)
Pack-years smoking, median (IQ)	2 (0–26)	21 (0–51)	40 (29–56)	45 (34–63)	34 (22–46)	35 (24–47)	45 (33–62)	43 (29–61)	0 (0,12)	8 (0–23)	14 (3–34)	15 (2–33)	3 (0–11)	13 (2–28)	11 (3–24)	18 (6–33)	41 (30–60)	50 (37–65)
Smoking status, *n* (%)																		
Current	211 (12)	46 (16)	1,426 (38)	263 (53)	1,377 (80)	178 (83)	189 (38)	69 (46)	33 (6)	11 (8)	43 (6)	8 (7)	20 (5)	6 (11)	50 (11)	8 (12)	126 (28)	59 (32
Former	756 (42)	162 (58)	2,345 (62)	234 (47)	340 (20)	36 (17)	305 (62)	82 (54)	238 (45)	74 (54)	322 (48)	68 (59)	158 (41)	25 (46)	206 (44)	38 (58)	294 (65)	119 (65)
Never	818 (46)	71 (26)	—	—	—	—	—	—	259 (49)	53 (38)	310 (46)	40 (35)	207 (54)	23 (43)	211 (45)	20 (30)	30 (7)	6 (3)
History of COPD, *n* (%)	—	—	1,527 (40)	171 (34)	380 (22)	57 (27)	494 (100)	151 (100)	46 (9)	18 (13)	129 (22)	21 (21)	62 (17)	11 (23)	98 (24)	14 (25)	290 (64)	116 (63)

Definition of abbreviations: AGES = Age Gene/Environment Susceptibility; COPD = chronic obstructive pulmonary disease (defined as FEV_1/FVC ratio <70 on spirometry); COPDGene = Genetic Epidemiology of COPD; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-Points; ILA = interstitial lung abnormalities; IQ = interquartile interval; MESA = Multi-Ethnic Study of Atherosclerosis; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.

Missing data: MESA non-Hispanic whites COPD status: no ILA—76, ILA—17; MESA African Americans COPD status: no ILA—58, ILA—9; Framingham Heart Study body mass index—1; AGES-Reykjavik body mass index—1.

Table 2. Genome-Wide Significant Variants Associated with Interstitial Lung Abnormalities and Subpleural-Predominant Interstitial Lung Abnormalities, and Replication in an Idiopathic Pulmonary Fibrosis Cohort

Chromosome/ Location	Position	rsID	Risk Allele	Risk Allele Frequency	Nearest Gene	ILA vs. No ILA		Subpleural ILA vs. No ILA		Replication in IPF Cohort	
						Odds Ratio* (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
3q13	106571023	rs73199442	T	0.06	*FCF1P3*	1.68 (1.39–2.02)	5×10^{-8}	1.61 (1.31–1.99)	7×10^{-6}	0.98 (0.85–1.12)	0.73
5q12	62172476	rs6886640	G	0.62	*IPO11*	1.28 (1.18–1.41)	4×10^{-8}	1.27 (1.14–1.40)	8×10^{-6}	1.06 (0.99–1.14)	0.11
6q15	87737841	rs7744971	G	0.28	*HTR1E*	1.26 (1.16–1.37)	1×10^{-7}	1.32 (1.19–1.45)	4×10^{-8}	1.01 (0.94–1.09)	0.75
11p15	1241221	rs35705950	T	0.11	*MUC5B*	1.97 (1.74–2.22)	3×10^{-27}	2.22 (1.93–2.55)	2×10^{-29}	4.84 (4.37–5.36)	1×10^{-203}

Definition of abbreviations: CI = confidence interval; ILA = interstitial lung abnormality; IPF = idiopathic pulmonary fibrosis.
*Odds ratios are per copy of the risk allele.

threshold for significance after adjustment for multiple testing) in association with ILAs (Tables 3 and E5). All but one of the 12 IPF GWAS SNPs had a consistent direction of risk effect in IPF and ILAs. Despite the smaller sample size in the subpleural-predominant ILA association analysis, IPF genetic risk loci were generally more strongly associated (larger ORs and smaller *P* values) with the risk of subpleural ILAs than with the risk of ILAs.

We assessed the top 21 loci reported in a GWAS of several HAA phenotypes (36) in our ILA and subpleural GWAS results, and found no overlap of genetic loci between HAAs and ILAs; however, the direction of effect between the risk of HAAs and risk of ILAs and subpleural ILAs was generally consistent (Table E6).

To evaluate the overlap in genetic susceptibility to ILAs and smoking behaviors, we performed a lookup of our four genome-wide significant ILA variants in a recent GWAS of four smoking behaviors: smoking initiation, age of smoking initiation, smoking cessation, and cigarettes per day (37) The *P* value was >0.05 for association of the top four ILA SNPs with any smoking behavior (Table E7). We also assessed the genome-wide significant loci reported in the smoking GWAS (37). After correction for multiple testing, there was no significant overlap of smoking behavior SNPs with ILAs or subpleural-predominant ILAs (Table E8).

In a search for CTD–associated ILD and sarcoidosis-associated SNPs in the NHGRI-EBI GWAS Catalog, we found 357 SNPs associated with 17 traits reported in 39 publications. No SNPs were associated with ILAs. Only one CTD SNP (rs13389408, intronic to *STAT4* on chromosome 2) met the threshold for Bonferroni significance in association with subpleural ILAs (OR, 1.3; 95% CI, 1.1–1.5; $P = 9.7 \times 10^{-4}$). The SNP rs13389408 was discovered in a meta-analysis of "systemic seropositive rheumatic diseases" (including systemic sclerosis, systemic lupus erythematosus, and idiopathic inflammatory myopathies) (44).

Logistic Regression Conditioning on *MUC5B* at 11p15

Our conditional analysis did not identify any conditionally distinct signals at 3q13, 5q12, or 6p15. However, a previous GWAS of IPF reported variant associations in *TOLLIP* (rs5743894, rs5743890, and rs111521887) that—despite proximity to *MUC5B* in the 11p15 region—were reported to be independent of the rs35705950 association with IPF due to minimal linkage disequilibrium ($R^2 < 0.2$) (9). In the COPDGene study non-Hispanic white and African American participants, we performed a meta-analysis of the association of previously reported *TOLLIP* SNPs with ILAs and subpleural ILAs, and conditioned each *TOLLIP* SNP association on the *MUC5B* rs35705950 genotype. When each *TOLLIP* SNP association was adjusted for the rs35705950 genotype, the *TOLLIP* SNPs' effect sizes and strengths of association were diminished (Table E9). These data suggest that the *TOLLIP* SNP associations with ILAs and subpleural-predominant ILAs in COPDGene are not independent of *MUC5B* rs35705950.

eQTL Assessments for Identified Loci

We sought to determine whether our four genome-wide significant ILA- and subpleural ILA–associated variants have been reported as lung or blood eQTLs. The ILA risk variants at 5q12 (rs6886640) and 3q13 (rs73199442) were not reported as lung or blood eQTLs in any of the examined data. The *MUC5B* promoter polymorphism (rs35705950) T allele was associated with increased expression of *MUC5B* in the lung (q value = 3.99×10^{-9}) in the GTEx lung eQTL data, but not in Hao and colleagues' lung eQTL data (41). Furthermore, the rs35705950 T allele was associated with decreased expression of *CD151* in blood in the eQTL Consortium *cis*-eQTL data (43). The subpleural ILA 6q15 variant rs7744971 risk allele (G) was significantly associated with decreased blood expression of *AKIRIN2*, *SLC35A1*, *C6orf164*, and *RP1-102H19.6*, and increased blood expression of *ZNF292* in the eQTL Consortium *cis*-eQTL data (43). In the Netherlands Study of Depression and Anxiety/Netherlands Twin Register Conditional eQTL Catalog (42) the rs7744971 G allele was also associated with decreased *AKIRIN2* expression as well as with decreased *C6orf162* expression in blood.

Discussion

Our study, which presents the first GWAS of visually assessed ILAs, included 1,699 participants with ILAs and 10,274 control subjects, and has several notable findings. First, we provide the most comprehensive data to date demonstrating the links between genetic association findings in patients with IPF and research participants with ILAs. For example, these findings provide at least nominal, and directionally consistent, evidence for an association between ILAs and most of the common genetic variants that have previously been demonstrated to be associated with IPF (7–11). In addition, our results provide genome-wide significant evidence for association with two new genetic risk loci for ILAs overall (3q13 and 5q12) and one loci for subpleural-predominant ILAs specifically (6q15). These new loci do not show evidence of association with IPF, and although these data could represent

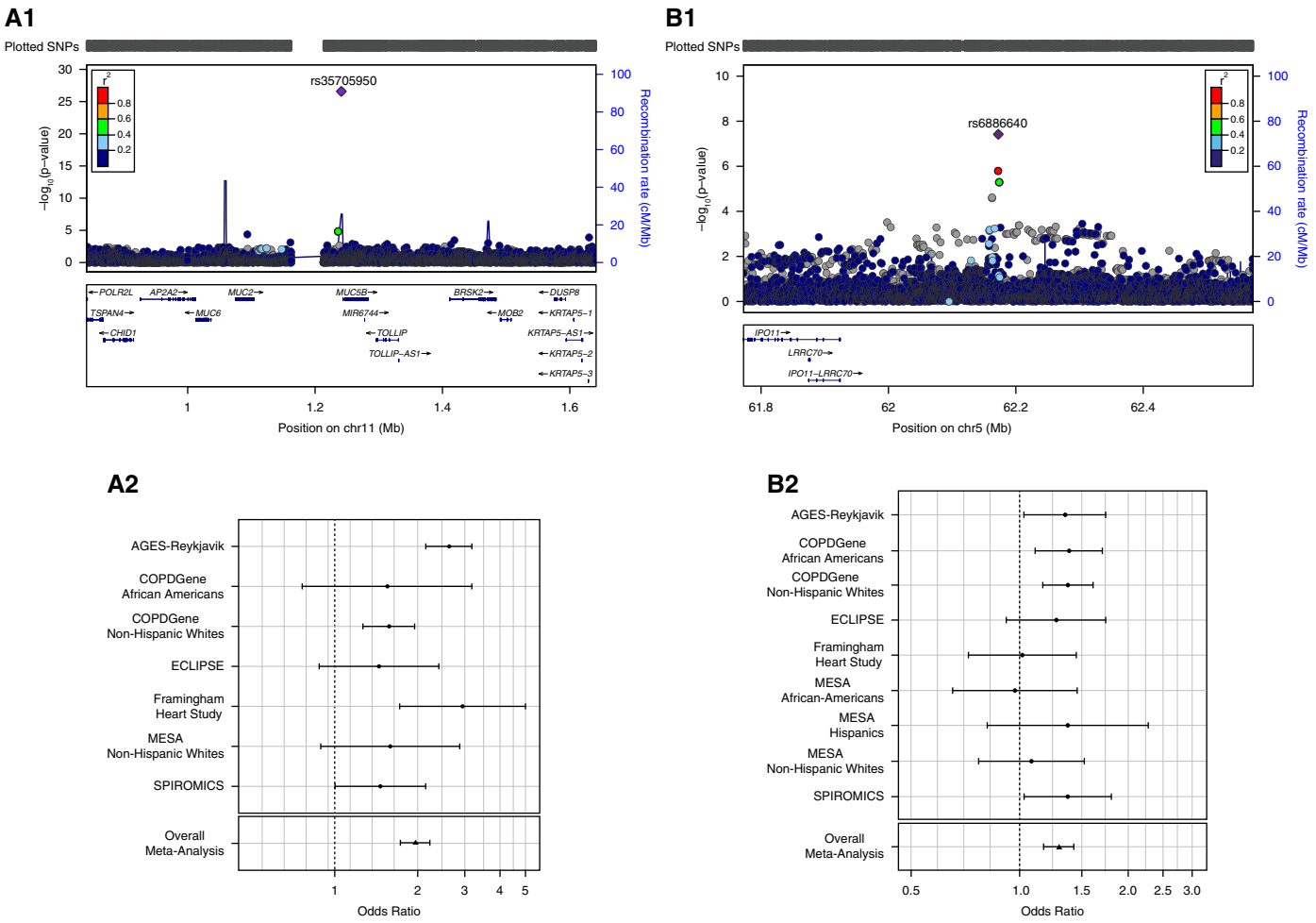

Figure 2. Locus zoom and forest plots for the genome-wide significant loci associated with interstitial lung abnormalities (ILAs) and subpleural-predominant ILAs. (*A*) Comparison of participants with and without ILAs. A1 is a locus zoom plot demonstrating the genome-wide significant association at rs35705950 (nearest gene *MUC5B*); A2 is a forest plot demonstrating the results in each individual cohort and the overall meta-analysis, with the *x*-axis on the log odds scale. (*B*) Comparison of participants with and without ILAs. B1 is a locus zoom plot demonstrating the genome-wide significant association at rs6886640 (nearest gene *IPO11*); B2 is a forest plot demonstrating the results in each individual cohort and the overall meta-analysis, with the *x*-axis on the log odds scale. (*C*) Comparison of participants with and without ILAs. C1 is a locus zoom plot demonstrating the genome-wide significant association at rs73199442 (nearest gene *FCF1P3*); C2 is a forest plot demonstrating the results in each individual cohort and the overall meta-analysis, with the *x*-axis on the log odds scale. (*D*) Comparison of participants with subpleural-predominant ILAs and those without ILAs. D1 is a locus zoom plot demonstrating the genome-wide significant association at rs7744971 (nearest gene *HTR1E*); D2 is a forest plot demonstrating the results in each individual cohort and the overall meta-analysis, with the *x*-axis on the log odds scale. AGES = Age Gene/Environment Susceptibility; COPD = chronic obstructive pulmonary disease; COPDGene = Genetic Epidemiology of COPD; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points; MESA = Multi-Ethnic Study of Atherosclerosis; SPIROMICS = Subpopulations and Intermediate Outcome Measures in COPD Study.

false-positive associations, they may also be consistent with some cases of ILA representing early stages of diverse forms of ILD, associated with genetic risk factors distinct from IPF.

Multiple lines of evidence now demonstrate a shared genetic risk between some research participants with ILAs who have not received a diagnosis and patients with clinically diagnosed IPF. Similar to what was found in all prior GWASs of IPF that included the gain-of-function *MUC5B* promoter variant (8–11), our study demonstrates that the *MUC5B* promoter variant rs35705950 has the most significant association with ILAs. In addition, among 12 loci that previously showed genome-wide evidence for association with IPF (7–11) in at least one study, we present evidence for directionally consistent associations in 11, and 5 of these were significant after adjustment for multiple testing ($P < 0.05/12$). More specifically, this study provides support for the fact that common genetic variants in seven genomic regions are associated with early and/or mild stages of PF in addition to their known association with more advanced stages of disease. In support of the latter statement is the fact that most of these genetic association findings were stronger when the ILA phenotype was limited to those with subpleural

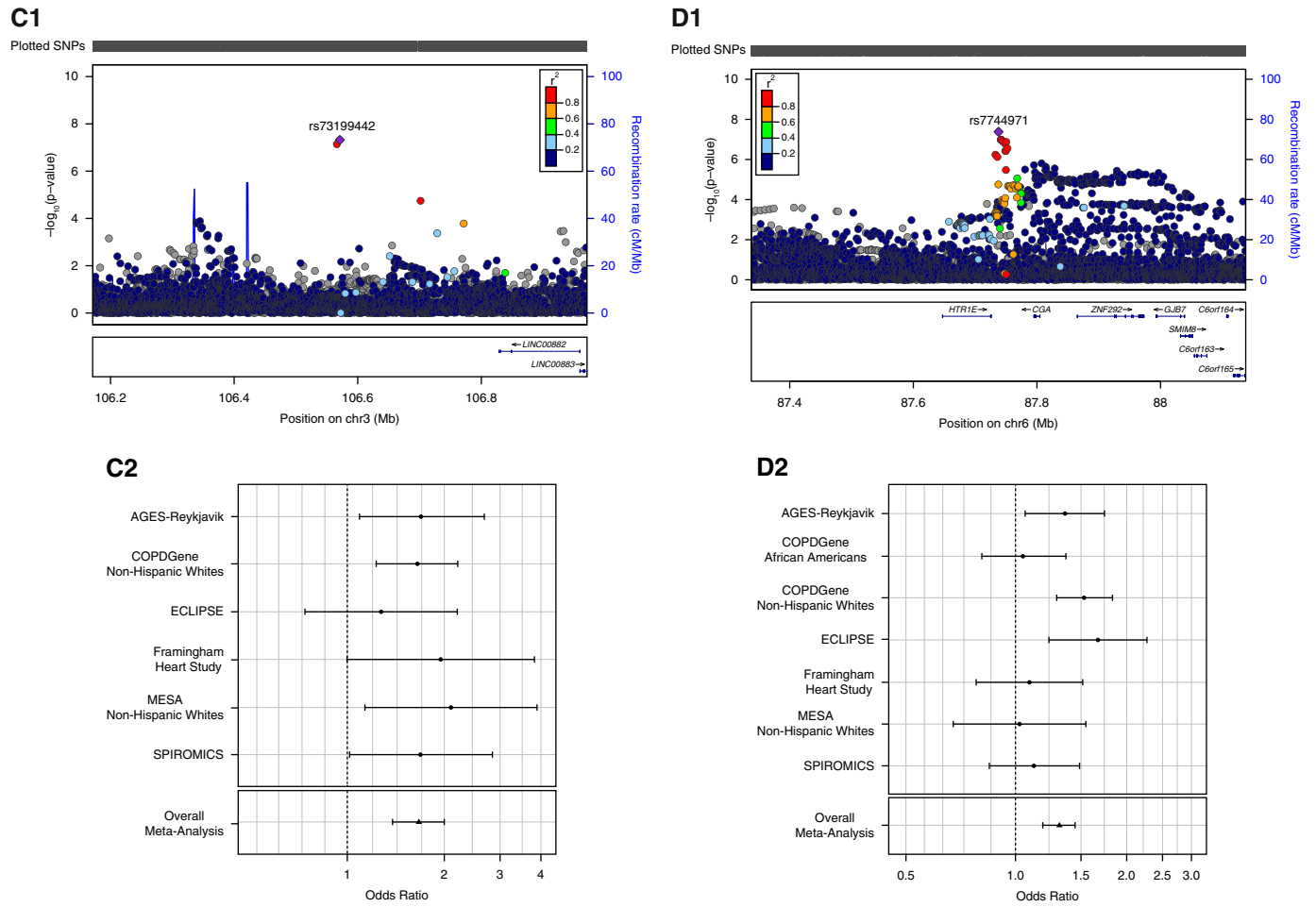

Figure 2. (Continued).

reticular involvement (an imaging phenotype we have previously demonstrated to be associated with subpleural fibrosis) (16).

Our study adds to the growing body of evidence that demonstrates that increasing copies of the gain-of-function minor allele of the *MUC5B* promoter variant (rs35705950) increase the risk of IPF (6, 8–11), ILA (12, 18), and other forms of PF (44), perhaps as the result of an increased expression *MUC5B* in the distal airspaces (6, 45–47). Additionally, our conditional logistic regression analyses are consistent with prior analyses (8) that demonstrated that there do not appear to be additional distinct 11p15 genetic variants associated with either ILA or IPF, after accounting for the effects of the *MUC5B* promoter genotype (rs35705950). Recent studies in mice demonstrated that *Muc5b* overexpression led to impaired mucociliary clearance and the persistence of PF in response to bleomycin challenge, which could be mitigated by targeted mucolytic agents (48). Future studies will be needed to determine whether intervention in individuals with early stages of *MUC5B*-associated interstitial changes (12, 18) could help to prevent progression to more advanced forms of PF.

Although our study demonstrates that genetic risk factors are shared by patients with IPF and some research participants with ILAs, it also identifies genetic loci in some research participants with ILAs that are distinct. These results may be consistent with the fact that ILAs identify diverse imaging features associated with ILDs in general (24), and it is possible that genetic associations that reflect more diverse pathobiologic and clinical processes than are found in patients with IPF contribute to some of the interstitial imaging findings in these unique diseases (49). For example, given the fact that many of these cohorts include large populations of smokers, some of these findings could be consistent with a genetic risk of developing smoking-related interstitial fibrosis (50). Although our results do not provide statistically significant evidence of a genotype-by-smoking interaction, the power for these analyses was limited, so this interaction should be evaluated in future studies. In addition, there is some evidence to suggest that the genetic risk of developing ILD in some CTDs may be distinct from the processes that lead to IPF (51–53). Until our findings can be tested in sufficiently large cohorts of these other important populations of individuals with ILD, they should be viewed as preliminary.

The association between the minor allele (G) of the SNP rs7744971 on chromosome 6q14 and subpleural ILAs deserves some mention, as the G allele of this SNP was previously demonstrated to be

Table 3. Association of 12 Previously Identified Idiopathic Pulmonary Fibrosis Genome-Wide Association Loci with Interstitial Lung Abnormalities and Subpleural Interstitial Lung Abnormalities

Chromosome/ Location	rsID	IPF Risk Allele	Nearest Gene	Studies	IPF Odds Ratio (95% CI)	ILA vs. No ILA		Subpleural ILA vs. No ILA		Direction of Effect Consistent with Prior Reports
						Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	
4q22	rs2609255	G	*FAM13A*	(8)	1.29 (1.18–1.42)	1.18 (1.07–1.29)	5×10^{-4}	1.22 (1.09–1.35)	3×10^{-4}	Yes
6p24	rs2076295	G	*DSP*	(8, 11)	1.44 (1.35–1.54)	1.14 (1.05–1.2)	0.001	1.18 (1.08–1.29)	3×10^{-4}	Yes
11p15	rs35705950	T	*MUC5B*	(8)	2.43 (2.13–2.77)	1.97 (1.74–2.22)	3×10^{-27}	2.22 (1.93–2.55)	2×10^{-29}	Yes
15q15	rs2034650	A	*IVD*	(8)	1.30 (1.19–1.41)	1.08 (0.99–1.17)	0.07	1.15 (1.05–1.26)	0.003	Yes
19p13	rs12610495	G	*DPP9*	(8)	1.29 (1.18–1.41)	1.14 (1.03–1.26)	0.01	1.23 (1.10–1.37)	2×10^{-4}	Yes
3q26	rs6793295	C	*LRRC34*	(8)	1.30 (1.19–1.42)	1.06 (0.97–1.15)	0.20	1.12 (1.01–1.24)	0.03	Yes
17q21	rs1981997	G	*MAPT*	(8)	1.41 (1.28–1.56)	1.16 (1.03–1.30)	0.01	1.19 (1.05–1.36)	0.009	Yes
5p15	rs2736100	A	*TERT*	(7, 8)	2.11 (1.61–2.78)	1.03 (0.95–1.12)	0.44	1.06 (0.96–1.16)	0.23	Yes
10q24	rs11191865	A	*OBFC1*	(8)	1.25 (1.15–1.35)	1.03 (0.95–1.12)	0.46	1.03 (0.94–1.13)	0.56	Yes
13q34	rs1278769	G	*ATP11A*	(8)	1.27 (1.14–1.39)	1.04 (0.95–1.15)	0.37	1.04 (0.94–1.16)	0.45	Yes
15q25	rs62025270	A	*AKAP13*	(11)	1.27 (1.18–1.37)	1.09 (0.99–1.20)	0.08	1.07 (0.96–1.20)	0.23	Yes
7q22	rs4727443	C	*LOC100128334/ LOC105375423*	(8)	1.30 (1.19–1.41)	0.95 (0.87–1.03)	0.19	0.93 (0.84–1.02)	0.12	No

Definition of abbreviations: CI = confidence interval; ILA = interstitial lung abnormality; IPF = idiopathic pulmonary fibrosis.
For each locus, only the SNP is reported.

an eQTL that is expected to result in decreased expression of the gene *AKIRIN2* (42). Basic research has indicated that Akirin-2, which is known to be expressed in the lung (54), is a critical factor in the innate immune system, which helps to regulate inflammatory gene transcription (55) and B-cell activation (56). Although Akirin-2 is required for embryonic development (57), selective knockdown of *akirin2* in myeloid cells in mice was shown to result in impaired inflammatory cytokine production in macrophages in response to Toll-like receptor stimulation (57). It is believed that some of the effect of Akirin-2 is mediated through its important function as a bridge between some transcription factors (e.g., NF-κB [57] and Twist [58], which have also been implicated in the development of pulmonary inflammation [59] and fibrosis [60]) and gene transcription. Future work to confirm the role of this variant in expression of *AKIRIN2*, and to test the role of Akirin-2 deficiency in some forms of ILD mediated by pulmonary inflammation may be warranted.

In addition to the lack of statistically significant replication for the novel loci presented here, our study has some other limitations. First, even though our study included nearly all available cohorts in which ILA characterization and genetic testing have been performed, larger sample sizes may still be required to more adequately characterize the genetic risk of ILA. Second, we cannot exclude the possibility that the generalizability of our findings may be limited by the omission of some participants from these analyses. Third, although we were able to demonstrate genetic associations with ILA and subpleural-predominant ILA, the sample size may have limited our ability to detect associations with additional specific radiologic features and patterns. In addition, we cannot rule out the possibility that intercohort differences in the methods used for ILA characterization or subclassification could have introduced phenotypic heterogeneity, thus influencing our power to detect genetic associations. Efforts to develop standards for ILA characterization across different research populations could help to minimize this concern. Finally, although our findings demonstrate that the genetic risk of ILA in research participants overlaps with the genetic risk of IPF, it is important to note that we do not know the extent to which the genetic risk of IPF is shared with the risk of other forms of PF (e.g., ILD in the setting of rheumatoid arthritis) (45) or could result in a milder or less progressive form of PF.

In conclusion, our study demonstrates that although the *MUC5B* promoter variant is the dominant variant that is common between ILAs and IPF, there are nominally significant associations between ILAs and the majority of genetic loci that were previously associated with IPF. In addition, our findings provide evidence of novel genetic loci associated with ILAs, but not with IPF. These findings provide further evidence that the *DPP9*, *DSP*, *FAM13A*, *IVD*, and *MUC5B* loci may be important in the risk of both early and later stages of PF. Our findings also suggest that ILA characterization may help to identify the genetic risk of developing imaging abnormalities that may represent an early stage of other diverse forms of ILD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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