Clinical and Genetic Associations of Objectively Identified Interstitial Changes in Smokers

Samuel Y. Ash, MD, MPH; Rola Harmouche, PhD; Rachel K. Putman, MD, MPH; James C. Ross, PhD; Alejandro A. Diaz, MD, MPH; Gary M. Hunninghake, MD, MPH; Jorge Onieva Onieva, MSc; Fernando J. Martinez, MD; Augustine M. Choi, MD; David A. Lynch, MD; Hiroto Hatabu, MD, PhD; Ivan O. Rosas, MD; Raul San Jose Estepar, PhD; and George R. Washko, MD; for the COPDGene Investigators

BACKGROUND: Smoking-related lung injury may manifest on CT scans as both emphysema and interstitial changes. We have developed an automated method to quantify interstitial changes and hypothesized that this measurement would be associated with lung function, quality of life, mortality, and a mucin 5B (*MUC5B*) polymorphism.

METHODS: Using CT scans from the Genetic Epidemiology of COPD Study, we objectively labeled lung parenchyma as a tissue subtype. We calculated the percentage of the lung occupied by interstitial subtypes.

RESULTS: A total of 8,345 participants had clinical and CT scanning data available. A 5% absolute increase in interstitial changes was associated with an absolute decrease in FVC % predicted of 2.47% ($P < .001$) and a 1.36-point higher St. George's Respiratory Questionnaire score ($P < .001$). Among the 6,827 participants with mortality data, a 5% increase in interstitial changes was associated with a 29% increased risk of death ($P < .001$). These associations were present in a subgroup without visually defined interstitial lung abnormalities, as well as in those with normal spirometric test results, and in those without chronic respiratory symptoms. In non-Hispanic whites, for each copy of the minor allele of the *MUC5B* promoter polymorphism, there was a 0.64% ($P < .001$) absolute increase in the percentage of lung with interstitial changes.

CONCLUSIONS: Objective interstitial changes on CT scans were associated with impaired lung function, worse quality of life, increased mortality, and more copies of a *MUC5B* promoter polymorphism, suggesting that these changes may be a marker of susceptibility to smoking-related lung injury, detectable even in those who are healthy by other measures.

CHEST 2017; 152(4):780-791

KEY WORDS: CT scanning; emphysema; interstitial lung abnormalities; mortality; pulmonary fibrosis

ABBREVIATIONS: COPDGene = Genetic Epidemiology of COPD; ILA = interstitial lung abnormality; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; *MUC5B* = mucin 5B; SGRQ = St. George's Respiratory Questionnaire

AFFILIATIONS: From the Division of Pulmonary and Critical Care Medicine (Drs Ash, Putman, Diaz, Hunninghake, Rosas, and Washko),

Department of Medicine, Boston, MA; the Laboratory of Mathematics in Imaging (Drs Harmouche, Ross, and San Jose Estepar and Mr Onieva Onieva), Department of Radiology (Dr Hatabu), Brigham and Women's Hospital, Boston, MA; the Department of Medicine (Drs Martinez and Choi), Weil Cornell Medical College, New York, NY; and the Department of Radiology (Dr Lynch), National Jewish Health, Denver, CO.

Smoking-related lung disease may manifest as a variety of parenchymal diseases, including emphysema and fibrosis.^{1,2} These features frequently coexist, especially in advanced disease.³⁻⁵ Visual assessments of CT scans of smokers' lungs have revealed subtle radiologic patterns suggestive of fibrosis, some of which have been termed *interstitial lung abnormalities* (ILA).^{6,7} These abnormalities are associated with mortality, as well as with a specific single nucleotide polymorphism (rs35705950) in the promoter region of the gene encoding mucin 5B (*MUC5B*), suggesting that in some cases they may indicate early or mild idiopathic pulmonary fibrosis (IPF).⁸⁻¹² However, ILA are only a subset of the broad range of the nonemphysematous patterns of lung injury evident on the CT scans of smokers, and a more comprehensive assessment of interstitial changes may be required to determine a person's susceptibility to injury from long-term tobacco smoke exposure.¹³

We have developed an automated tool that detects and quantifies interstitial and emphysematous features on the lung CT scans of smokers.^{14,15} We hypothesized that interstitial features measured with this tool would be a marker of smoking-related lung injury, and, as such, would be associated with clinically significant measures such as lung function, quality of life, and mortality. In addition, given the ability of this method to detect visually identified ILA we hypothesized that these features would be associated with the presence of the *MUC5B* promoter polymorphism. Finally, we suspected that visual, spirometric, and clinical-based assessments alone may underestimate disease susceptibility. Therefore, we sought to determine whether the clinical and genetic associations with objective interstitial changes were present in those without visual ILA, in those with normal spirometric test results, and in those without chronic respiratory symptoms.

Methods

We performed our study by using CT scanning and clinical and genetic data from the Genetic Epidemiology of COPD (COPDGene) Study, which has been described in detail elsewhere.^{15,16} Briefly, 10,300 smokers between the ages of 45 and 80 years, with a history of at least 10 pack-years, were enrolled and underwent baseline testing, including an extensive interview, volumetric high-resolution CT scanning of the chest, and spirometric testing. COPDGene excluded Hispanics from the study, and the only two races represented were white and black. Genotyping of the *MUC5B* promoter polymorphism (rs35705950) was performed (TaqMan Genotyping Assays; Applied Biosystems) as previously described.^{8,10} Participants were excluded if their predominant lung condition was either bronchiectasis or interstitial lung disease (ILD). All participants provided written informed consent, and the overall study was approved by the institutional review boards at all of the participating centers (details available in the online supplement). This specific study was approved by the Partners Human Research Committee, protocol number 2016P001562/BWH.

The objective CT scanning analysis performed in this study has been detailed previously and is described in detail in the online supplement.^{14,15} Briefly, two experts trained the tissue subtype classification tool by placing 33,865 fiducials in 138 randomly selected CT scans on radiographic features unique to each disease subtype: normal, interstitial (reticular, honeycombing, centrilobular nodule, linear scar, nodular, subpleural line, ground glass), and emphysematous (centrilobular and paraseptal). (Note that panlobular emphysema was not identified in the training cases likely because patients with α_1 -antitrypsin disease were not represented in the cohort.) These training points were used to develop tissue classification vectors for each disease subtype, and de novo regions of lung were then classified based on their similarity to these vectors. Using this method, we assigned normal, emphysematous, or interstitial labels to every portion of the lung parenchyma with the results stratified by lung zone (upper, middle, and lower) (Figs 1 and 2). The aggregate of these subtype volumes summed to the total lung volume at CT scanning for each patient, and the subtype volumes were expressed as a percentage of total lung volume (ie, percentage normal, percentage emphysematous, and percentage interstitial).

The visual analysis of COPDGene CT scans for ILA has been described elsewhere.^{7,12,13,15} An individual's CT scan was determined to have ILA if there were nondependent ground-glass or reticular abnormalities, diffuse centrilobular nodularity, nonemphysematous cysts, honeycombing, or traction bronchiectasis affecting more than 5% of any lung zone.¹³ Individuals who had focal or unilateral ground-glass attenuation, focal or unilateral reticulation, and patchy ground-glass abnormalities (present in less than 5% of the lung) were considered to have indeterminate findings.

Statistical Analysis

We had no a priori hypothesis for the normal range of the percentage of lung occupied by interstitial features. Therefore, for all of the primary analyses, the percentage of interstitial features was evaluated as a continuous variable. Except where stated, in both the primary and subgroup analyses, effect sizes are given per a 5% absolute increase in the percentage of lung occupied by interstitial changes. This scaling was selected because it roughly approximated the SD of

Drs Ash and Harmouche contributed equally to this work.

Drs San Jose Estepar and Washko contributed equally to this work.

FUNDING/SUPPORT: This study was funded by the National Institutes of Health [Grants 5-T32-HL007633-30 (S. Y. A., R. K. P.), R01-HL107246 (R. H., J. O. O., R. S. J. E., G. R. W.), R01-HL116933 (R. H., J. C. R., J. O. O., R. S. J. E., G. R. W.), R01-HL111024 (G. M. H.), P01-HL114501 (A. M. C., I. O. R., G. R. W.), and R01-HL089856 (J. C. R., D. A. L., R. S. J. E., G. R. W.)]; and Boehringer Ingelheim Pharmaceuticals, Inc. (G. R. W.).

CORRESPONDENCE TO: Samuel Y. Ash, MD, MPH, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, 75 Francis St, PBB, CA-3, Boston, MA 02115; e-mail: syash@partners.org

Copyright © 2017 American College of Chest Physicians. Published by Elsevier Inc. All rights reserved.

DOI: <http://dx.doi.org/10.1016/j.chest.2017.04.185>

Figure 1 – Axial CT scan images including objective labeling of sample cases without interstitial lung abnormalities (A, B) and with interstitial lung abnormalities (C, D).

interstitial changes in the entire cohort and could be kept stable across subgroups (as opposed to the use of subgroup-specific SDs or quartiles). Secondary analyses, including the analysis of the percentage of interstitial features as a dichotomous variable and by quartiles, and stratification by smoking status can be found in the online supplement.

Univariate and multivariable linear regression were used to evaluate the associations among spirometric test results, St. George's Respiratory Questionnaire (SGRQ) scores, and percentage of interstitial changes, as well as for the association between the number of minor *MUC5B* alleles and interstitial changes.^{17,18} All linear regression effect sizes are stated as absolute, not relative,

Figure 2 – Distribution of the percentage of lung occupied by objectively identified interstitial changes in the cohort for the whole lung (A), for the upper lung zone (B), for the middle lung zone (C), and for the lower lung zone (D). Upper graphs show box plot of distribution with outliers denoted individually by dots. IQR = interquartile range.

differences. Because of known differences in prevalence, genetic analyses were stratified by race.⁸ Univariate and multivariable Cox regression were used to evaluate the relationship between interstitial changes and mortality. All of the variables were assessed using the Schoenfeld residuals method, and none violated the proportional hazards assumption.¹⁹ Details regarding the acquisition of mortality data are available in the online supplement.

The multivariable analyses of SGRQ score, *MUC5B*, and mortality were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. The multivariable analyses of spirometric measures were adjusted for the same covariates with the exception of FEV₁ % predicted.

Subgroup analyses were performed in the following radiographic subsets: subgroup A, which included those without ILA, as well as those with indeterminate findings (ie, this subgroup excluded only those with ILA), and subgroup B, which included only those without ILA (ie, this subgroup excluded both those with indeterminate findings and those with ILA). In addition, subgroup analyses were also performed in those with normal spirometric test results (subgroup C) and in those who did not have chronic respiratory symptoms (subgroup D). Details regarding the subgroup definitions are available in the online supplement (e-Table 1). The reported *P* values are two sided; *P* values less than .05 were considered to indicate statistical significance, and all analyses were performed using software (SAS 9.4 or JMP 12; SAS Institute).

Results

A total of 8,345 participants had both clinical and objective imaging data available for analysis. Baseline characteristics of the entire cohort and subgroups are shown in Table 1. The distributions of interstitial changes in the cohort by lung zone are shown in Figure 2.

As shown in Table 2, in both the adjusted and unadjusted analyses, increasing amounts of interstitial features were associated with lower FEV₁ % predicted, lower FVC % predicted, higher FEV₁/FVC ratio, and a worse quality of life as measured with the SGRQ score. Similar findings were present across subgroups with the

TABLE 1] Baseline Characteristics of the Cohort

Characteristic	Entire Cohort	Subgroup A ^a	Subgroup B ^b	Subgroup C ^c	Subgroup D ^d
No. of participants	8,345	7,472	4,812	4,030	4,426
Age, y	59.9 ± 9.1	59.6 ± 9.0	58.9 ± 8.8	56.8 ± 8.5	59.4 ± 9.1
Sex female, No. (%)	3,814 (45.7)	3,385 (45.3)	2,169 (45.1)	1,938 (48.1)	1,932 (43.7)
Race, No. (%)					
White	5,742 (68.8)	5,091 (68.1)	3,363 (69.9)	2,386 (59.2)	3,103 (70.1)
Black	2,603 (31.2)	2,381 (31.9)	1,449 (30.1)	1,644 (40.8)	1,323 (29.9)
Clinical characteristic					
BMI	28.4 ± 5.9	28.4 ± 6.0	28.2 ± 5.9	28.8 ± 5.7	28.0 ± 5.2
Pack-years	44.3 ± 24.9	44.2 ± 24.8	42.6 ± 23.3	37.2 ± 20.3	39.4 ± 21.6
Currently smoking, No. (%)	4,248 (50.9)	3,812 (51.0)	2,410 (50.1)	2,344 (58.2)	2,170 (49.0)
FEV ₁ % predicted	78.0 ± 26.6	77.7 ± 26.8	79.4 ± 27.0	98.2 ± 11.4	89.7 ± 18.8
FVC % predicted	89.6 ± 17.9	89.4 ± 18.0	90.3 ± 17.8	97.5 ± 11.3	95.4 ± 14.1
FEV ₁ /FVC ratio	0.658 ± 0.164	0.656 ± 0.166	0.664 ± 0.168	0.785 ± 0.050	0.723 ± 0.113
SGRQ score	26.2 ± 22.7	26.3 ± 22.8	24.6 ± 22.5	16.4 ± 17.7	10.9 ± 11.6
Objective analysis					
Percentage of interstitial features	5.8 ± 4.5	5.5 ± 4.1	4.3 ± 3.1	5.6 ± 4.6	5.4 ± 4.3
Percentage of emphysematous features	10.1 ± 17.0	10.4 ± 17.3	10.4 ± 17.6	2.3 ± 4.5	4.6 ± 9.4
Visual analysis					
Proportion with ILA, No. (%)	516 (6.2)	...	...	243 (6.0)	246 (5.6)

Data are presented as mean ± SD unless otherwise indicated. ILA = interstitial lung abnormality; SGRQ = St. George's Respiratory Questionnaire.

^aSubgroup A: Individuals visually determined to have no ILA but including those who have indeterminate findings.

^bSubgroup B: Only those individuals visually determined not to have ILA and not to have indeterminate findings.

^cSubgroup C: Individuals with normal spirometric test results.

^dSubgroup D: Individuals without chronic dyspnea or bronchitis.

TABLE 2] Spirometric and Quality-of-Life Associations With Interstitial Features for All Participants

Measure	Unadjusted			Adjusted		
	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
FEV ₁ % predicted	−1.65	−2.29 to −1.02	< .001	−2.65	−3.15 to −2.14	< .001
FVC % predicted	−2.09	−2.52 to −1.27	< .001	−2.47	−2.88 to −2.06	< .001
FEV ₁ /FVC ratio	0.004	0.000-0.008	.035	−0.004	−0.007 to −0.001	.005
SGRQ score	2.80	2.26-3.34	< .001	1.36	0.92-1.81	< .001

All associations are absolute changes per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted, with the exception of the spirometric measures, which were not adjusted for FEV₁ % predicted. See Table 1 legend for expansion of abbreviation.

exception of the FEV₁/FVC ratio and SGRQ score in certain subgroups (Tables 3-6).

A total of 6,827 participants had mortality data available for analysis. As shown in Table 7, in the adjusted analyses, for every 5% increase in the percentage of lung occupied by interstitial changes there was a 29% increased risk of death (95% CI, 21-38; $P < .001$) in all participants. This association was similar across all subgroups.

Finally, and as shown in Table 8, in non-Hispanic whites, for each copy of the minor allele of the *MUC5B* promoter polymorphism (rs35705950), there was a higher percentage of lung with interstitial changes in

both the unadjusted and adjusted analyses. This association was present in those without visual ILA (subgroup A), in those with normal spirometric test results (subgroup C), and in those without respiratory symptoms (subgroup D), but not in those who did not have ILA or indeterminate findings (subgroup B) (Tables 9-12). No such association was seen in black participants.

Discussion

In a large cohort of current and former smokers, we found that objectively detected interstitial features were associated with reduced lung function, worse quality of

TABLE 3] Spirometric and Quality-of-Life Associations With Interstitial Features for Subgroup A^a

Measure	Unadjusted			Adjusted		
	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
FEV ₁ % predicted	−2.21	−2.95 to −1.47	< .001	−3.35	−3.93 to −2.76	< .001
FVC % predicted	−2.45	−2.95 to −1.95	< .001	−2.89	−3.37 to −2.41	< .001
FEV ₁ /FVC ratio	0.003	−0.002 to 0.007	.234	−0.007	−0.010 to −0.004	< .001
SGRQ score	3.15	2.52-3.78	< .001	1.17	0.65-1.69	< .001

All associations are absolute changes per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted, with the exception of the spirometric measures, which were not adjusted for FEV₁ % predicted. See Table 1 legend for expansion of abbreviation.

^aSubgroup A: Individuals visually determined to have no ILA but including those who have indeterminate findings.

TABLE 4] Spirometric and Quality-of-Life Associations With Interstitial Features for Subgroup B^a

Measure	Unadjusted			Adjusted		
	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
FEV ₁ % predicted	−4.49	−5.72 to −3.26	< .001	−4.83	−5.78 to −3.89	< .001
FVC % predicted	−4.02	−4.83 to −3.21	< .001	−4.09	−4.85 to −3.32	< .001
FEV ₁ /FVC ratio	−0.005	−0.013 to 0.002	.171	−0.010	−0.016 to −0.005	< .001
SGRQ score	4.02	3.00-5.04	< .001	0.806	−0.027 to 1.639	.058

All associations are absolute changes per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted, with the exception of the spirometric measures, which were not adjusted for FEV₁ % predicted. See Table 1 legend for expansion of abbreviation.

^aSubgroup B: Only those individuals visually determined not to have ILA and not to have indeterminate findings.

life, and increased mortality. In addition, for each copy of the minor allele of the *MUC5B* promoter polymorphism (rs35705950), more of the lung was affected by interstitial features. These associations were present even in participants who did not have visually defined ILA, in those with normal lung function, and in those without chronic respiratory symptoms.

There has been increased recognition that smoking-related lung injury may have important clinical associations, even when patients do not have a disease formally diagnosed. For instance, smokers with clinical symptoms but preserved lung function have more

respiratory exacerbations and activity limitation, and they may have increased mortality.²⁰⁻²² In addition, the visual presence of ILA in smokers and former smokers is associated with increased mortality and loss of lung function.^{12,23}

From an objective imaging standpoint, it has been known for some time that emphysema, measured as the percentage of lung occupied by low-density tissue, is associated with respiratory disease severity.²⁴⁻²⁶ However, it has become increasingly clear that high-density lung tissue on CT scans, which may indicate inflammation or fibrosis, is also associated with cigarette

TABLE 5] Spirometric and Quality-of-Life Associations With Interstitial Features for Subgroup C^a

Measure	Unadjusted			Adjusted		
	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
FEV ₁ % predicted	−1.72	−2.10 to −1.34	< .001	−1.78	−2.17 to −1.38	< .001
FVC % predicted	−1.97	−2.34 to −1.59	< .001	−1.96	−2.35 to −1.57	< .001
FEV ₁ /FVC ratio	0.003	0.002-0.005	< .001	0.002	−0.002 to −0.003	.079
SGRQ score	2.53	1.94-3.12	< .001	1.05	0.46-1.64	.001

All associations are absolute changes per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted, with the exception of the spirometric measures, which were not adjusted for FEV₁ % predicted. See Table 1 legend for expansion of abbreviation.

^aSubgroup C: Individuals with normal spirometric test results.

TABLE 6] Spirometric and Quality-of-Life Associations With Interstitial Features for Subgroup D^a

Measure	Unadjusted			Adjusted		
	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	Change per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
FEV ₁ % predicted	−2.10	−2.75 to −1.46	< .001	−2.25	−2.85 to −1.66	< .001
FVC % predicted	−1.93	−2.41 to −1.45	< .001	−2.04	−2.53 to −1.55	< .001
FEV ₁ /FVC ratio	−0.001	−0.005 to −0.003	.490	−0.003	−0.006 to 0.000	.086
SGRQ score	1.02	0.62-1.41	< .001	0.31	−0.07 to 0.70	.113

All associations are absolute changes per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted, with the exception of the spirometric measures, which were not adjusted for FEV₁ % predicted. See Table 1 legend for expansion of abbreviation.

^aSubgroup D: Individuals without chronic dyspnea or bronchitis.

smoke exposure and worse clinical outcomes. For instance, Lederer et al²⁷ and Podolanczuk et al²⁸ have shown that the percentage of high-attenuation areas on CT scans of the lungs is associated with cigarette smoke exposure, spirometric restriction, and increased mortality. In addition to these densitometric approaches to image analysis, local histogram-based techniques have also been used to attempt to detect and quantify smoking-related lung disease. These techniques use the local properties of tissue density measured at CT scanning to characterize small regions of lung tissue as a

particular tissue subtype. We have demonstrated that a local histogram-based and distance-based method can identify both emphysema and ILA on the CT scans of smokers, and several studies have shown that interstitial changes measured using a similar approach are associated with measures of disease severity in those with known ILD.^{14,15} For example, Bartholmai et al²⁹ showed that the quantity of reticular changes objectively identified using a local histogram approach was correlated with pulmonary function test measures and exercise capacity in patients with ILD, and Iwasawa

TABLE 7] Mortality and Interstitial Features

	Unadjusted			Adjusted		
	HR per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value	HR per 5% Absolute Increase in the Percentage of Lung Occupied by Interstitial Features	95% CI	P Value
All participants	1.20	1.13-1.27	< .001	1.29	1.21-1.38	< .001
Subgroup A ^a	1.17	1.09-1.26	< .001	1.27	1.16-1.39	< .001
Subgroup B ^b	1.16	1.01-1.33	.039	1.20	1.02-1.42	.031
Subgroup C ^c	1.21	1.05-1.39	.009	1.25	1.07-1.46	.004
Subgroup D ^d	1.23	1.09-1.40	.001	1.26	1.11-1.44	.001

All HRs are per 5% absolute increase in the percentage of lung occupied by interstitial features. Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. HR = hazard ratio.

^aSubgroup A: Individuals visually determined to have no interstitial lung abnormalities but including those who have indeterminate findings.

^bSubgroup B: Only those individuals visually determined not to have interstitial lung abnormalities and not to have indeterminate findings.

^cSubgroup C: Individuals with normal spirometric test results.

^dSubgroup D: Individuals without chronic dyspnea or bronchitis.

TABLE 8] Associations Between the Number of Minor Alleles With a Single Nucleotide Polymorphism (rs35705950) in the Promoter Region of the Gene Encoding *MUC5B* and the Percentage of Lung Occupied by Interstitial Features for All Participants

Race/Ethnicity	Unadjusted		Adjusted	
	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value
Non-Hispanic White	0.64	< .001	0.60	< .001
Black	0.56	.265	0.40	.404

Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. *MUC5B* = mucin 5B.

et al^{30,31} and Maldonado et al³² used similar approaches and found associations with mortality in cohorts of patients with known IPF.

In this study, we found that interstitial features detected and quantified by means of a local histogram- and distance-based method were associated with respiratory disease severity and mortality. These associations were present even in those who were healthy by other metrics, such as visual CT scanning analysis, spirometric test results, and symptoms, suggesting that this method may identify individuals at increased risk for disease who are classified as healthy by means of other screening methods. In addition, a specific polymorphism in the promoter region of *MUC5B* (rs35705950) known to be associated with ILA and IPF was also associated with interstitial features measured using our technique, suggesting that in some cases these findings may indicate early fibrotic disease.^{8,33} However, in our study, even slight increases in the volume of interstitial features were associated with lower lung function, worse quality of life, and higher mortality, associations unlikely to be entirely explained by early fibrotic lung disease. For

instance, those in the second quartile of the percentage of lung with interstitial changes had worse outcomes than those in the first quartile (e-Tables 6 and 7). One potential explanation for these findings is that interstitial changes detected using this method reflect both early fibrosis and, more broadly, susceptibility to lung injury. This argument is bolstered by the interaction between this measure and smoking status.

Although there was no statistical evidence of effect modification for all clinical associations, in general, interstitial features in current smokers were associated with a smaller effect on outcomes than were those in former smokers (e-Tables 2 and 3). Thus, it may be that the presence of these changes in current smokers is, to some extent, a short-term response to tobacco smoke, whereas in former smokers it indicates susceptibility to long-term injury. By combining this approach with clinical characteristics such as smoking status, we may be able to determine which patients are most susceptible to disease and most likely to benefit from new treatment options. In addition, although longitudinal studies are needed to determine whether these features are

TABLE 9] Associations Between the Number of Minor Alleles With a Single Nucleotide Polymorphism (rs35705950) in the Promoter Region of the Gene Encoding *MUC5B* and the Percentage of Lung Occupied by Interstitial Features for All Participants for Subgroup A^a

Race/Ethnicity	Unadjusted		Adjusted	
	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value
Non-Hispanic White	0.34	.004	0.30	.007
Black	0.40	.415	0.21	.641

Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. See Table 8 legend for expansion of abbreviation.

^aSubgroup A: Individuals visually determined to have no ILA but including those who have indeterminate findings.

TABLE 10] Associations Between the Number of Minor Alleles With a Single Nucleotide Polymorphism (rs35705950) in the Promoter Region of the Gene Encoding *MUC5B* and the Percentage of Lung Occupied by Interstitial Features for All Participants for Subgroup B^a

Race/Ethnicity	Unadjusted		Adjusted	
	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value
Non-Hispanic White	0.19	.074	0.17	.087
Black	0.25	.630	0.18	.722

Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. See Table 8 legend for expansion of abbreviation.

^aSubgroup B: Only those individuals visually determined not to have ILA and not to have indeterminate findings.

associated with lung function decline and mortality over time, if that were the case, then these features could be used as a surrogate end point to monitor disease progression and response to therapy.

Our study has several limitations. These include the use of a single cohort to develop and apply the objective analysis tool and the fact that, although most of the statistical associations were strong, the effect sizes were in some instances relatively small. That said, we believe that subsequent work that combines this tool with other objective imaging features such as body composition and bronchiectasis, as well as with visual radiographic and clinical prediction tools, may result in combinations of findings or scoring systems that have greater relative effects on outcomes.³⁴⁻³⁶ In addition, for the primary analyses we purposefully chose to analyze our measure of interstitial changes as a continuous variable because we had no a priori hypothesis of a “normal” amount of such changes. Although this allows for a more nuanced measurement of disease severity, it makes the application of this measure to a clinical scenario, in which disease is often defined as present or absent, more challenging. A sample of an approach to

dichotomization, and clinical associations by quartile are available in e-Tables 4-7.

Further work is needed to define better what a normal range is for these features, but other continuous biomarkers of risk have overcome similar challenges. For instance, those in the highest quartile of interstitial features in this study had an 84% higher risk of death than did those in the lowest quartile (95% CI, 1.45-2.32; $P < .001$) (e-Table 7), which is similar to the risk for death related to coronary heart disease among those in the highest quartile of serum cholesterol compared with that for those in the lowest quartile (hazard ratio, 1.5-2.3) in the Seven Countries Study.³⁷ Thus, similar to cardiac disease risk factors that are useful for risk stratification but that do not replace a cardiologist, this imaging tool is not a more sensitive version of a pulmonologist’s or radiologist’s eye, but rather an adjunct that may be useful for identifying individuals who may be at higher risk for disease.

Finally, there were several outliers in the objective analysis: participants whose CT scans were characterized as having extremely high percentages of interstitial or emphysematous features. As detailed in the online

TABLE 11] Associations Between the Number of Minor Alleles With a Single Nucleotide Polymorphism (rs35705950) in the Promoter Region of the Gene Encoding *MUC5B* and the Percentage of Lung Occupied by Interstitial Features for All Participants for Subgroup C^a

Race/Ethnicity	Unadjusted		Adjusted	
	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value
Non-Hispanic White	0.74	< .001	0.75	< .001
Black	−0.41	.537	−0.42	.495

Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. See Table 8 legend for expansion of abbreviation.

^aSubgroup C: Individuals with normal spirometric test results.

TABLE 12] Associations Between the Number of Minor Alleles With a Single Nucleotide Polymorphism (rs35705950) in the Promoter Region of the Gene Encoding *MUC5B* and the Percentage of Lung Occupied by Interstitial Features for All Participants for Subgroup D^a

Race/Ethnicity	Unadjusted		Adjusted	
	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value	Additional Percentage of Lung Occupied by Interstitial Features per Minor Allele of <i>MUC5B</i> Polymorphism	P Value
Non-Hispanic White	0.51	.001	0.55	<.001
Black	0.41	.553	0.64	.325

Multivariable analyses were adjusted for age, sex, race, clinical center, current smoking status, pack-years, BMI, percentage of lung occupied by emphysematous changes as determined by means of the objective detection tool, and FEV₁ % predicted. See Table 8 legend for expansion of abbreviation.
^aSubgroup D: Individuals without chronic dyspnea or bronchitis.

supplement, visual review of these cases revealed that most were participants who had a high BMI or other cause for significant noise in their imaging (e-Table 8). Future work involving both additional training of the detection algorithm and the use of denoising methods will be needed to overcome this issue.^{38,39}

Conclusions

We have developed and applied an objective analysis tool that quantifies interstitial changes on CT scans to a large cohort of smokers without known interstitial disease. Objective interstitial changes defined by this method were associated with reduced lung function, worse quality of life, and higher mortality, as well as a higher number of copies of a specific polymorphism in the promoter region of *MUC5B*. Additional work is needed to replicate these findings in other cohorts to help define a normal range of these findings and to determine whether they are associated with change in lung function over time or with other biomarkers.

In addition, although the shared association between objectively measured interstitial changes visible by using this technique and a specific polymorphism in the promoter region of *MUC5B* known to be associated with both ILA and IPF is intriguing, additional work is needed to understand better the potential biological mechanisms by which these objective findings might be linked to pulmonary fibrosis or other ILDs. Also, because it is unclear whether these findings indicate only very early ILD or some other measure of disease susceptibility, we do not yet know whether their presence indicates that an individual patient should be treated; what effect, if any, that treatment may have on these findings; and whether changes in these findings indicate changes in disease activity or severity over time. Finally, further work is needed in larger cohorts to determine whether similar findings exist in patients who are healthy in all respects (ie, patients without symptoms who have normal spirometric test results and no visual evidence of ILA).

Acknowledgments

Author contributions: S. Y. A. and G. R. W. take full responsibility for the content of this manuscript including the data and analysis. S. Y. A., R. H., J. C. R., R. S. J. E., and G. R. W. contributed to the study concept and design. All authors contributed to the acquisition, analysis, or interpretation of data. All authors contributed to the drafting of the manuscript. All authors contributed to the intellectual content. S. Y. A., R. K. P., A. A. D., and G. R. W. contributed to the statistical analysis. G. M. H., A. M. C., D. A. L., R. S. J. E., and G. R. W. obtained funding. S. Y. A., R. H., J. C. R., J. O. O., and R. S. J. E. contributed to administrative, technical, or material support. R. S. J. E. and G. R. W. supervised the study. The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following: A. A. D. reports receiving travel and accommodations from the COPD Foundation and speaker fees from Novartis. G. M. H. reports consulting for Gerson Lehrman Group, Medina LLC, and PatientsLikeMe and is on a scientific advisory board for Genentech. F. J. M. reports grants from GlaxoSmithKline (during the conduct of the study) and National Institutes of Health; nonfinancial support from Biogen Stromedix and Gilead Sciences; personal fees, nonfinancial support, and other from Boehringer Ingelheim Pharmaceuticals, Inc.; nonfinancial support and other from Centocor; and personal fees from Academic CME; Adept; Afferent; American Thoracic Society; Amgen; Annenberg; AstraZeneca; Axon Communications; Bayer; BioScale; California Society of Allergy, Asthma and Clinical Immunology; Clarion; CME Incite; Columbia University; ConCert; Continuing Education; Falco; Forest; Genentech; GlaxoSmithKline; Haymarket Communications; Ikaria/Bellerophon; Informa; Integritas; inThought; Janssen; Johnson & Johnson; Kadmon; Lucid; Methodist Hospital; Miller Medical; National Association For Continuing Education; Novartis; Nycomed/Takeda; Paradigm; Pearl; PeerView Network (outside the submitted work); PeerVoice; Pfizer; Potomac; Prime; Roche; Sunovion; Theravance; Unity Biotechnology; UpToDate; Veracyte; WebMD; and Western Society of Allergy, Asthma & Immunology. D. A. L. reports grants from the National Heart, Lung, and Blood Institute; personal fees from Boehringer Ingelheim Pharmaceuticals, Inc., Genentech/Roche, and Parexel; and research support from Veracyte. H. H. reports grant funding from Aze Inc., Canon Inc., and Toshiba Medical Systems Ltd. and is on an advisory board for Toshiba Medical Systems Ltd. G. R. W. reports other support from Genentech, GlaxoSmithKline, Janssen, and Pulmonx. None declared (S. Y. A., R. H., R. K. P., J. C. R., J. O. O., A. M. C., I. O. R., R. S. J. E.).

Role of sponsors: Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) and the National Institutes of Health had no role in the design, analysis or interpretation of the results in this study; BIPI was given the opportunity to review the manuscript for medical and scientific accuracy as it relates to BIPI substances, as well as intellectual property considerations.

Collaborators: A list of COPDGene Investigators can be found in the Supplemental Materials section of the online article.

Additional information: The e-Appendix and e-Tables can be found in the Supplemental Materials section of the online article.

References

1. Kirchner J, Goltz JP, Lorenz F, Obermann A, Kirchner EM, Kickuth R. The "dirty chest": correlations between chest radiography, multislice CT and tobacco burden. *Br J Radiol*. 2012;85(1012):339-345.
2. Reid L, Simon G. Pathological findings and radiological changes in chronic bronchitis and emphysema. *Br J Radiol*. 1959;32(377):291-305.
3. Grubstein A, Bendayan D, Schactman I, Cohen M, Shitrit D, Kramer MR. Concomitant upper-lobe bullous emphysema, lower-lobe interstitial fibrosis and pulmonary hypertension in heavy smokers: report of eight cases and review of the literature. *Respir Med*. 2005;99(8):948-954.
4. Kitaguchi Y, Fujimoto K, Hanaoka M, Kawakami S, Honda T, Kubo K. Clinical characteristics of combined pulmonary fibrosis and emphysema. *Respirology*. 2010;15(2):265-271.
5. Cottin V, Cordier JF. The syndrome of combined pulmonary fibrosis and emphysema. *Chest*. 2009;136(1):1-2.
6. Doyle TJ, Hunninghake GM, Rosas IO. Subclinical interstitial lung disease: why you should care. *Am J Respir Crit Care Med*. 2012;185(11):1147-1153.
7. Washko GR, Hunninghake GM, Fernandez IE, et al. Lung volumes and emphysema in smokers with interstitial lung abnormalities. *N Engl J Med*. 2011;364(10):897-906.
8. Hunninghake GM, Hatabu H, Okajima Y, et al. *MUC5B* promoter polymorphism and interstitial lung abnormalities. *N Engl J Med*. 2013;368(23):2192-2200.
9. Putman RK, Rosas IO, Hunninghake GM. Genetics and early detection in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2014;189(7):770-778.
10. Seibold MA, Wise AL, Speer MC, et al. A common *MUC5B* promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-1512.
11. Doyle TJ, Washko GR, Fernandez IE, et al. Interstitial lung abnormalities and reduced exercise capacity. *Am J Respir Crit Care Med*. 2012;185(7):756-762.

12. Putman RK, Hatabu H, Araki T, et al. Association between interstitial lung abnormalities and all-cause mortality. *JAMA*. 2016;315(7):672-681.
13. Washko GR, Lynch DA, Matsuoka S, et al. Identification of early interstitial lung disease in smokers from the COPDGene Study. *Acad Radiol*. 2010;17(1):48-53.
14. Castaldi PJ, San Jose Estepar R, Mendoza CS, et al. Distinct quantitative computed tomography emphysema patterns are associated with physiology and function in smokers. *Am J Respir Crit Care Med*. 2013;188(9):1083-1090.
15. Ash SY, Harmouche R, Ross JC, et al. The objective identification and quantification of interstitial lung abnormalities in smokers [published online ahead of print December 15, 2016]. *Acad Radiol*. <http://dx.doi.org/10.1016/j.acra.2016.08.023>.
16. Regan EA, Hokanson JE, Murphy JR, et al. Genetic Epidemiology of COPD (COPDGene) study design. *COPD*. 2010;7(1):32-43.
17. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. *Respir Med*. 1991;85(suppl B):25-31.
18. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation: the St. George's Respiratory Questionnaire. *Am Rev Respir Dis*. 1992;145(6):1321-1327.
19. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika*. 1982;69(1):239-241.
20. Woodruff PG, Barr RG, Bleeker E, et al. Clinical significance of symptoms in smokers with preserved pulmonary function. *N Engl J Med*. 2016;374(19):1811-1821.
21. Stavem K, Sandvik L, Erikssen J. Breathlessness, phlegm and mortality: 26 years of follow-up in healthy middle-aged Norwegian men. *J Intern Med*. 2006;260(4):332-342.
22. Stavem K, Sandvik L, Erikssen J. Can global initiative for chronic obstructive lung disease stage 0 provide prognostic information on long-term mortality in men? *Chest*. 2006;130(2):318-325.
23. Araki T, Putman RK, Hatabu H, et al. Development and progression of interstitial lung abnormalities in the Framingham Heart Study. *Am J Respir Crit Care Med*. 2016;194(12):1514-1522.
24. Muller NL, Staples CA, Miller RR, Abboud RT. "Density mask": an objective method to quantitate emphysema using computed tomography. *Chest*. 1988;94(4):782-787.
25. Ash SY, Washko GR. Controversies in COPD: the value of CT scanning. *Eur Respir Soc Monogr*. 2015;69:121-133.
26. Coxson HO, Rogers RM, Whittall KP, et al. A quantification of the lung surface area in emphysema using computed tomography. *Am J Respir Crit Care Med*. 1999;159(3):851-856.

27. Lederer DJ, Enright PL, Kawut SM, et al. Cigarette smoking is associated with subclinical parenchymal lung disease: the Multi-Ethnic Study of Atherosclerosis (MESA)-lung study. *Am J Respir Crit Care Med*. 2009;180(5):407-414.
28. Podolanczuk AJ, Oelsner EC, Barr RG, et al. High attenuation areas on chest computed tomography in community-dwelling adults: the MESA study. *Eur Respir J*. 2016;48(5):1442-1452.
29. Bartholmai BJ, Raghunath S, Karwoski RA, et al. Quantitative computed tomography imaging of interstitial lung diseases. *J Thorac Imaging*. 2013;28(5):298-307.
30. Iwasawa T, Ogura T, Sakai F, et al. CT analysis of the effect of pirfenidone in patients with idiopathic pulmonary fibrosis. *Eur J Radiol*. 2014;83(1):32-38.
31. Iwasawa T, Asakura A, Sakai F, et al. Assessment of prognosis of patients with idiopathic pulmonary fibrosis by computer-aided analysis of CT images. *J Thorac Imaging*. 2009;24(3):216-222.
32. Maldonado F, Moua T, Rajagopalan S, et al. Automated quantification of radiological patterns predicts survival in idiopathic pulmonary fibrosis. *Eur Respir J*. 2014;43(1):204-212.
33. Peljto AL, Zhang Y, Fingerlin TE, et al. Association between the *MUC5B* promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. *JAMA*. 2013;309(21):2232-2239.
34. Beijers RJ, van den Borst B, Newman AB, et al. A multidimensional risk score to predict all-cause hospitalization in community-dwelling older individuals with obstructive lung disease. *J Am Med Dir Assoc*. 2016;17(6):508-513.
35. McDonald ML, Diaz AA, Ross JC, et al. Quantitative computed tomography measures of pectoralis muscle area and disease severity in chronic obstructive pulmonary disease: a cross-sectional study. *Ann Am Thorac Soc*. 2014;11(3):326-334.
36. Martinez-Garcia MA, de la Rosa Carrillo D, Soler-Cataluna JJ, et al. Prognostic value of bronchiectasis in patients with moderate-to-severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2013;187(8):823-831.
37. Verschuren WM, Jacobs DR, Bloemberg BP, et al. Serum total cholesterol and long-term coronary heart disease mortality in different cultures: twenty-five-year follow-up of the seven countries study. *JAMA*. 1995;274(2):131-136.
38. Kim HJ, Li G, Gjertson D, et al. Classification of parenchymal abnormality in scleroderma lung using a novel approach to denoise images collected via a multicenter study. *Acad Radiol*. 2008;15(8):1004-1016.
39. Colom M, Buades A, Morel JM. Nonparametric noise estimation method for raw images. *J Opt Soc Am A Opt Image Sci Vis*. 2014;31(4):863-871.