Association Between the MUC5B Promoter Polymorphism and Survival in Patients With Idiopathic Pulmonary Fibrosis

Anna L. Peljto, DrPH
Yingze Zhang, PhD
Tasha E. Fingerlin, PhD
Shwu-Fan Ma, PhD
Joe G. N. Garcia, MD
Thomas J. Richards, PhD
Lori J. Silveira, PhD
Kathleen O. Lindell, PhD
Mark P. Steele, MD
Imre Noth, MD
Elissa Murphy, MS
Kevin K. Brown, MD
Max A. Seibold, PhD
Cheryl Markin, BA
Karl Kossen, PhD
Scott D. Seiwert, PhD
Kevin K. Brown, MD
Janet L. Talbert, MS
Kevin F. Gibson, MD
James E. Loyd, MD
Shwu-Fan Ma, PhD
Tasha E. Fingerlin, PhD
Yingze Zhang, PhD
Lori J. Silveira, PhD
Kathleen O. Lindell, PhD
Mark P. Steele, MD
Imre Noth, MD
Elissa Murphy, MS
Karl Kossen, PhD
Scott D. Seiwert, PhD
Kevin K. Brown, MD
Janet L. Talbert, MS
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Anna L. Peljto, DrPH

Importance Current prediction models of mortality in idiopathic pulmonary fibrosis (IPF), which are based on clinical and physiological parameters, have modest value in predicting which patients will progress. In addition to the potential for improving prognostic models, identifying genetic and molecular features that are associated with IPF mortality may provide insight into the underlying mechanisms of disease and inform clinical trials.

Objective To determine whether the MUC5B promoter polymorphism (rs35709950), previously reported to be associated with the development of pulmonary fibrosis, is associated with survival in IPF.

Design, Setting, and Participants Retrospective study of survival in 2 independent cohorts of patients with IPF: the INSPIRE cohort, consisting of patients enrolled in the interferon-γ-1b trial (n=438; December 15, 2003-May 2, 2009; 81 centers in 7 European countries, the United States, and Canada), and the Chicago cohort, consisting of IPF participants recruited from the Interstitial Lung Disease Clinic at the University of Chicago (n=148; 2007-2010). The INSPIRE cohort was used to model the association of the MUC5B genotype with survival, accounting for the effect of matrix metalloproteinase 7 (MMP-7) blood concentration and other demographic and clinical covariates. The Chicago cohort was used for replication of findings.

Main Outcomes and Measures The primary end point was all-cause mortality.

Results The numbers of patients in the GG, GT, and TT genotype groups were 148 (34%), 259 (59%), and 31 (7%), respectively, in the INSPIRE cohort and 41 (28%), 98 (66%), and 9 (6%), respectively, in the Chicago cohort. The median follow-up period was 1.6 years for INSPIRE and 2.1 years for Chicago. During follow-up, there were 73 deaths (36 GG, 35 GT, and 2 TT) among INSPIRE patients and 64 deaths (26 GG, 36 GT, and 2 TT) among Chicago patients. The unadjusted 2-year cumulative incidence of death was lower among patients carrying 1 or more copies of the IPF risk allele (T) in both the INSPIRE cohort (0.25 [95% CI, 0.17-0.32] for GG, 0.17 [95% CI, 0.11-0.23] for GT, and 0.03 [95% CI, 0.00-0.09] for TT) and the Chicago cohort (0.50 [95% CI, 0.31-0.63] for GG, 0.22 [95% CI, 0.13-0.31] for GT, and 0.11 [95% CI, 0.00-0.28] for TT). In the INSPIRE cohort, the TT genotypes (risk for IPF) were associated with improved survival compared with GG (hazard ratios, 0.23 [95% CI, 0.10-0.52] and 0.48 [95% CI, 0.31-0.72], respectively; P<.001). This finding was replicated in the Chicago cohort (hazard ratios, 0.15 [95% CI, 0.05-0.49] and 0.39 [95% CI, 0.21-0.70], respectively; P<.001). The observed association of MUC5B with survival was independent of age, sex, forced vital capacity, diffusing capacity of carbon monoxide, MMP-7, and treatment status. The addition of the MUC5B genotype to the survival models significantly improved the predictive accuracy of the model in both the INSPIRE cohort (C=0.71 [95% CI, 0.64-0.75] vs C=0.68 [95% CI, 0.61-0.73]; P=.001) and the Chicago cohort (C=0.73 [95% CI, 0.62-0.78] vs C=0.69 [95% CI, 0.59-0.75]; P=.01).

Conclusions and Relevance Among patients with IPF, a common risk polymorphism in MUC5B was significantly associated with improved survival. Further research is necessary to refine the risk estimates and to determine the clinical implications of these findings.

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DIAPATHIC PULMONARY FIBROSIS (IPF) is a chronic progressive disease with a median survival of 3 years. The prognosis is variable; patients may remain stable for several years, slowly lose lung function, progress in an intermittent step-wise fashion, or experience precipitous acute exacerbations. Although clinical and physiological parameters have modest value in predicting which patients will progress, serum biomarkers, including chemokine ligand 18, KL6, surfactant protein A (SFTPA), and SFTPD, are independently associated with outcome in IPF. Recently, elevated plasma concentrations of matrix metalloproteinase 7 (MMP-7), intercellular...
lar adhesion molecule 1, and interleukin 8 have been shown to be associated with poor outcomes in IPF, and when combined with clinical features, these plasma proteins predict mortality in IPF.8

Rare mutations in SFTPC, SFTPA2, telomerase reverse transcriptase (TERT), and telomerase RNA component (TERC)9–11 have been associated with development of pulmonary fibrosis. Recently, a common polymorphism in the promoter of a mucin gene (MUC5B) has been found to be associated with an increase in risk of developing both familial and sporadic IPF in an allele dose-dependent manner.12,13 While MUC5B expression in the lung was 14.1 times higher in patients with IPF, the MUC5B promoter polymorphism was associated with upregulation of this transcript only in unaffected participants.12

The objective of the current study was to determine whether the MUC5B (gene ID: 727897; http://www.ncbi.nlm.nih.gov/gene/727897) promoter polymorphism (rs35705950), previously reported to be associated with the development of pulmonary fibrosis, is associated with survival in IPF.

METHODS

Study Cohorts

The study was approved by the human use committees of National Jewish Health, the University of Colorado Denver, and the University of Chicago. The INSPIRE cohort (derivation cohort) consists of study participants who were enrolled (December 15, 2003–April 12, 2006) in the interferon-γ1b IPF clinical trial and provided written informed consent as part of a natural history protocol. Participants in the validation cohort were enrolled in 2007–2010 and followed up from initial evaluation until death, the time of lung transplantation, or censoring on July 9, 2012, and were treated clinically. Vital status was confirmed by the Social Security Death Index as well as by contacting each family/patient at the end of the follow-up period. To minimize any effect of population stratification, only non-Hispanic white patients were included in this study (race/ethnicity data were based on self-report). The majority of participants were observed at 3- to 4-month intervals. All diagnoses met current American Thoracic Society guideline criteria for IPF (ie, definite or probable pattern of usual interstitial pneumonia on computed tomography scan and exclusion of an alternative diagnosis).

No patient was dually enrolled in both cohorts; however, all patients in the INSPIRE cohort were included in a recent publication that demonstrated an allele dose-dependent relationship between a common polymorphism in the promoter of a mucin gene (MUC5B) and the risk of developing IPF.12,13 All analyses of both cohorts were performed anonymously. Both cohorts consisted of participants who were considered to be unrelated on the basis of self-report.

Genotyping of MUC5B rs35705950

For the INSPIRE cohort, genotypes of the MUC5B SNP rs35705950 were determined using Taqman genotyping.12,13 For the Chicago cohort, genotyping was performed using the Sequenom iPLEX-Gold assay.

MMP-7 Analysis

The plasma concentration of MMP-7 was determined by enzyme-linked immunosorbent assay in the INSPIRE cohort following manufacturer (R&D Systems) instructions and as previously reported.8

Statistical Analysis

The primary analysis tested for an association of the MUC5B polymorphism with survival using Cox proportional hazards models (Kolmogorov-type supremum tests did not indicate significant departures from the proportional hazards requirement for the MUC5B genotype variable in either the INSPIRE [P = .40] or Chicago [P = .10] cohorts). The model was developed within the INSPIRE cohort by including the MUC5B genotype and then using a stepwise backward elimination process based on the Akaike information criterion to select among potential covariates (sex, age, smoking status, baseline diffusing capacity of carbon monoxide [DLCO], baseline forced vital capacity [FVC], and interferon-γ1b treatment) for inclusion in the final model. Because of power considerations, only main effects were considered. The same model was then fit within the Chicago cohort for validation.

In a secondary analysis, the MMP-7 variable was added to the final survival model for the INSPIRE cohort to determine whether the MUC5B polymorphism was significantly associated with survival in addition to the previously reported effects of MMP-7.9 In preliminary analyses of MMP-7, evidence existed of a threshold effect on survival. Accordingly, MMP-7 was modeled as a binary variable categorized at the first quartile (≤5.7 ng/mL vs >5.7 ng/mL). An additive genetic model was used for the genotype in all survival models, consistent with previous findings.12,13 Any patient receiving a lung transplant during follow-up was censored at transplant date in the analysis.

A Wald χ2 test was used to test for association of covariates with survival, for which a 2-tailed P < .05 was considered significant. Survival analy-
Table 1. Clinical and Demographic Characteristics of the Cohorts

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>INSPIRE (n = 438)</th>
<th>Chicago (n = 148)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of follow-up, median (IQR), y</td>
<td>1.6 (1.2-2.1)</td>
<td>2.1 (1.1-3.9)</td>
<td></td>
</tr>
<tr>
<td>Deaths, No. (%)a</td>
<td>73 (17)</td>
<td>64 (43)</td>
<td></td>
</tr>
<tr>
<td>Transplant events, No. (%)</td>
<td>7 (2)</td>
<td>10 (7)</td>
<td>.003</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>115 (26)</td>
<td>35 (24)</td>
<td>.58</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>66.6 (7.54)</td>
<td>90.8 (8.75)</td>
<td>.005</td>
</tr>
<tr>
<td>Ever smokers, No. (%)</td>
<td>312 (71)</td>
<td>96 (72)</td>
<td>.93</td>
</tr>
<tr>
<td>FVC, mean (SD), % predicted</td>
<td>72.2 (12.37)</td>
<td>64.5 (18.44)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FVC, mean (SD), L</td>
<td>2.9 (0.76)</td>
<td>2.5 (0.52)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>DLCO, mean (SD), % predicted</td>
<td>47.3 (8.89)</td>
<td>46.2 (17.92)</td>
<td>.51</td>
</tr>
<tr>
<td>DLCO, mean (SD), mL/min/mm Hg</td>
<td>12.5 (4.40)</td>
<td>10.7 (4.51)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MMP-7, median (IQR), ng/mLb</td>
<td>7.8 (5.7-11.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC5B genotype, No. (%)</td>
<td></td>
<td></td>
<td>.31</td>
</tr>
<tr>
<td>GG</td>
<td>148 (34)</td>
<td>41 (28)</td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>259 (59)</td>
<td>98 (66)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>31 (7)</td>
<td>9 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DLCO, diffusing capacity of carbon monoxide; FVC, forced vital capacity; IQR, interquartile range; MMP-7, matrix metalloproteinase 7.

RESULTS

Study Cohorts

There were 438 patients from the INSPIRE cohort and 148 patients from the Chicago cohort included in the analysis (eFigure). The demographic and pulmonary function characteristics of participants in the cohorts were clinically similar; however, participants in the Chicago cohort were older and had more physiologically advanced disease compared with the INSPIRE cohort (Table 1). The Chicago cohort also had a longer period of follow-up (median, 2.1 years) than the INSPIRE cohort (median, 1.6 years), which contributed to the larger number of deaths among the Chicago participants (Table 1).

The observed similar frequencies of the MUC5B polymorphism minor allele (T) among the INSPIRE and Chicago cohorts (37% and 39%, respectively) were similar to what has been observed in IPF. The MUC5B polymorphism did not meet the expectations of Hardy-Weinberg equilibrium (HWE) (P <.001) for either cohort, indicating its likely role as a true risk allele, as previously reported.

MUC5B rs35705950 Genotype and Survival

The unadjusted 2-year cumulative incidence of death was lower among patients carrying 1 or more copies of the IPF risk allele (T) in both the INSPIRE cohort (0.25 [95% CI, 0.17-0.32] for GG, 0.17 [95% CI, 0.11-0.23] for GT, and 0.03 [95% CI, 0.00-0.09] for TT) and the Chicago cohort (0.50 [95% CI, 0.31-0.63] for GG, 0.22 [95% CI, 0.13-0.31] for GT, and 0.11 [95% CI, 0.00-0.28] for TT). In the single-variable proportional hazards model, INSPIRE participants who were heterozygous and homozygous for the minor allele (GT and TT) were at lower risk of mortality compared with participants with the GG genotype (hazard ratios [HRs], 0.52 [95% CI, 0.34-0.78] and 0.27 [95% CI, 0.12-0.60], respectively; P = .001) (Table 2 and Figure 1). A similar association of the polymorphism with survival was observed in the Chicago cohort, wherein HRs for participants with the GT and TT genotypes were 0.49 (95% CI, 0.30-0.79) and 0.24 (95% CI, 0.09-0.63), respectively (P = .004) (Table 2 and Figure 2). These associations were also significant after independent adjustments for age, sex, smoking history (ever vs never), baseline FVC, and baseline DLCO. Within the INSPIRE cohort, the HR for the MUC5B genotype also remained unchanged (and significant) after independent adjustment for interferon-γ1b treatment (treatment vs placebo); HRs for the GT and TT genotypes were 0.48 (95% CI, 0.31-0.73) and 0.23 (95% CI, 0.10-0.53), respectively (P < .001).

After including all relevant variables (sex, baseline FVC, and baseline DLCO) in a final model, the MUC5B genotype remained a statistically significant predictor of survival. The adjusted HRs for survival associated with the GT and TT genotypes were 0.48 (95% CI, 0.31-0.72) and 0.23 (95% CI, 0.09-0.63) in the INSPIRE cohort and 0.39 (95% CI, 0.21-0.70) and 0.15 (95% CI, 0.05-0.49) in the Chicago cohort (Table 2). In both cohorts, the AUC was higher for the adjusted model that included MUC5B genotype as a predictor than when MUC5B genotype was excluded (Figure 3 and Figure 4).
The increase in the C statistic value was statistically significant in both the INSPIRE (P < .001) and Chicago (P = .01) cohorts, suggesting that the MUC5B genotype improves the predictive accuracy of the model.

In the secondary analysis, the observed association between the MUC5B promoter polymorphism and survival was explored after accounting for the effect of MMP-7, which has recently been reported as prognostic in IPF. Matrix metalloproteinase 7 was significantly associated with survival when added to the final model for the INSPIRE cohort. The hazard was 2-fold higher for patients with MMP-7 levels of 5.7 ng/mL or higher than for patients with MMP-7 levels of less than 5.7 ng/mL (HR, 2.06; 95% CI, 1.05-4.07; P = .04) (Table 3). However, the HR for the MUC5B genotype remained constant and statistically significant, suggesting that the MUC5B promoter polymorphism explains a portion of the variation in survival among IPF participants beyond that explained by MMP-7 levels.

No significant association between treatment and survival was observed in the INSPIRE cohort (eTable 1), and none of the genotype groups was associated with improved survival in the treatment stratum compared with placebo. However, exploratory analyses showed evidence of a possible interaction between interferon-γ1b treatment and MUC5B genotype. When stratified by treatment group, the effect of the genotype appeared to be stronger in the treatment group than in the placebo group. For the GT and TT groups, the unadjusted HRs were 0.75 (95% CI, 0.35-1.63) and 0.56 (95% CI, 0.12-2.65), respectively, among the placebo group and the unadjusted HRs were 0.44 (95% CI, 0.27-0.71) and 0.19 (95% CI, 0.07-0.50) among the treatment group. In post hoc analyses, the interaction was not statistically significant (P = .07) when added to the final survival model (eTable 2).

DISCUSSION
These findings suggest that the common polymorphism in the promoter of MUC5B (rs35705930), previously reported to be strongly associated with the development of familial interstitial

<table>
<thead>
<tr>
<th>Model</th>
<th>INSPIRE</th>
<th>P Value</th>
<th>Chicago</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC5B genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 [Reference]</td>
<td>.001</td>
<td>1 [Reference]</td>
<td>.004</td>
</tr>
<tr>
<td>GT</td>
<td>0.52 (0.34-0.78)</td>
<td></td>
<td>0.49 (0.30-0.79)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.27 (0.12-0.60)</td>
<td></td>
<td>0.24 (0.09-0.63)</td>
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</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC5B genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 [Reference]</td>
<td>&lt;.001</td>
<td>1 [Reference]</td>
<td>.002</td>
</tr>
<tr>
<td>GT</td>
<td>0.48 (0.31-0.72)</td>
<td></td>
<td>0.39 (0.21-0.70)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.23 (0.10-0.52)</td>
<td></td>
<td>0.15 (0.05-0.49)</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>0.52 (0.32-0.83)</td>
<td>.006</td>
<td>0.53 (0.30-0.93)</td>
<td>.03</td>
</tr>
<tr>
<td>DLCO</td>
<td>0.89 (0.85-0.94)</td>
<td>&lt;.001</td>
<td>0.90 (0.83-0.98)</td>
<td>.02</td>
</tr>
<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
<td>1 [Reference]</td>
<td>&lt;.001</td>
<td>1 [Reference]</td>
<td>.06</td>
</tr>
<tr>
<td>Female</td>
<td>0.29 (0.14-0.59)</td>
<td></td>
<td>0.47 (0.22-1.02)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DLCO, diffusing capacity of carbon monoxide; FVC, forced vital capacity; HR, hazard ratio.
pneumonia and idiopathic pulmonary fibrosis,12,13 is significantly associated with improved survival in IPF. These findings are consistent with a previous report of an association between the MMP-7 variant and less severe pathological changes in familial interstitial pneumonia,16 as well as another report of slower decline in FVC for patients with IPF.17 This study is, to our knowledge, the first to demonstrate that a genetic variant is associated with survival in IPF.

While the minor (T) allele is the risk allele for development of IPF, it is also the minor allele that confers a survival advantage among patients with IPF. These results suggest that the IPF phenotype is heterogeneous and consists of at least 2 clinical subtypes that are, in part, separable by the MUC5B genotype. This would imply that the IPF phenotypes associated with other genetic and/or environmental risk factors (separate from the MUC5B promoter polymorphism) have poorer prognosis and possibly different pathogenic mechanisms. If there are several pathogenic mechanisms that lead to lung fibrosis, it is conceivable that the genotype-phenotype relationships are also unique and, consequently, give rise to specific, gene variant–directed clinical outcomes.

However, it is important to consider how and under what circumstances the promoter polymorphism of MUC5B might create a survival advantage in patients with IPF. Although individuals with the MUC5B promoter polymorphism could present earlier in the course of disease (lead time bias) or have preferential loss of sicker participants (survivor bias), these explanations are unlikely. In this study, patients with IPF with or without the MUC5B promoter polymorphism presented at similar ages and with similar degrees of lung impairment (TABLE 4). Moreover, the 2 cohorts in this manuscript are representative of physiologically mild (INSPIRE) and moderate (Chicago) stages of IPF, suggesting that the MUC5B polymorphism may be associated with outcome at different stages of disease. Previous findings indicate that patients with IPF have enhanced mucus production regardless of the MUC5B promoter genotype.12

Although the specific mechanisms that convey the observed survival advantage to patients with the MUC5B promoter polymorphism are not yet apparent, enhanced mucosal host defense, reduction in infectious complications, a beneficial drug response, and a potential dual role for MUC5B in wound repair should all be considered. Regardless of the mechanism, the results suggest that patients with IPF with the MUC5B promoter polymorphism may represent a pathogenically distinct disease entity that incorporates both a significantly higher predisposition to disease and a significantly longer survival. It is conceivable that specific pharmacologic agents may work well in a subset of patients with IPF who are separable by genomic and biochemical markers. The findings of this study suggest that there may be value in measuring the MUC5B polymorphism in future clinical therapeutic trials.

It may also be useful to assess whether future prognostic models that

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### Table 3. Survival Analysis Model in INSPIRE Cohort, Adjusted for MMP-7

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard Ratio (95% CI)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-7, ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;5.7</td>
<td>1 [Reference]</td>
<td>.04</td>
</tr>
<tr>
<td>(\geq5.7</td>
<td>2.06 (1.05-4.07)</td>
<td></td>
</tr>
<tr>
<td>MUC5B genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1 [Reference]</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>GT</td>
<td>0.46 (0.30-0.70)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.21 (0.09-0.49)</td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>0.55 (0.34-0.89)</td>
<td>.01</td>
</tr>
<tr>
<td>DLCO</td>
<td>0.89 (0.85-0.95)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Sex**

<table>
<thead>
<tr>
<th></th>
<th>1 [Reference]</th>
<th>&lt;.001</th>
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<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.25 (0.12-0.53)</td>
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</tr>
</tbody>
</table>

Abbreviations: DLCO, diffusing capacity of carbon monoxide; FVC, forced vital capacity; MMP-7, matrix metalloproteinase 7.
incorporate MUC5B and other molecular features have the potential to affect clinical care of patients. The clinical-radiographic-physiologic scoring system as well as a simpler index including pulmonary function and, most recently, a model that incorporates sex, age, FVC, and DLCO have all been found to be predictive of mortality to some extent. Additional studies have evaluated change in pulmonary function indexes over 6- to 12-month periods in IPF and have concluded that the decline in FVC is predictive of survival.20-23 Although these clinical assessments of IPF are modestly successful in correlating measures of disease severity with eventual mortality and potentially allow better staging of patients with IPF, they do not approach the predictive accuracy required to guide personalized management. In particular, these approaches cannot predict which patients, despite similar clinical presentations, will have a better or worse clinical outcome, a task that is necessary because of the variable and unpredictable course of the disease.24

Moreover, these approaches have limited utility because they focus on measures of disease severity (ie, FVC and DLCO) to predict future disease progression. Given that the addition of MUC5B resulted in relatively small gains in predictive accuracy in patients with established disease, it is premature to consider routine clinical genotyping of patients with IPF. However, there may be value in assessing scoring systems that use the potential predictive value of MUC5B combined with other genetic and molecular factors to predict prognosis in patients with subclinical and early-stage disease, before a notable decline in lung function is observed. This is especially important because there are currently no IPF pharmacological therapies approved for use in the United States, and opportunities for early genetic counseling or lung transplantation may be a patient’s only recourse. Recently, several other genetic loci have been identified as risk factors for fibrotic lung disease.26 Future studies are needed to determine whether these loci account for the apparent phenotypic heterogeneity and have added predictive value for survival in IPF that could be used to develop improved scoring systems.

Although the MUC5B polymorphism did not meet the expectations of HWE, this was not an unexpected finding and is not likely to bias the results of this analysis. Previous studies have reported similar observations among patients with IPF.12,13 In the study by Seibold et al,15 the minor allele frequency among patients with IPF was very similar to those observed in this study (38% compared with 37% for INSPIRE and 39% for Chicago), while the frequency among controls was reported to be much lower (9%) and consistent with HWE. In addition, it has been shown that these departures from HWE in the cases but not controls are consistent with the disease model wherein the minor (T) allele is associated with disease.12,27 Regardless, the findings presented here are robust to departures from HWE because a genotype-based test of MUC5B was used as opposed to an allelic test, which does assume HWE.

This study had several limitations. First, although analyses in both cohorts were well powered to detect the association of genotype with survival, the relatively low numbers of events in each cohort led to wide confidence intervals for the estimated HRs. Larger samples would be needed to obtain a more precise estimate of the HRs for the MUC5B genotype.

Second, while there was not statistically significant evidence of an interaction effect between the MUC5B genotype and interferon-γ1b treatment, it is possible that a true interaction exists that this study was not powered to detect. This would imply that the strength of the association between genotype and survival is dependent on interferon-γ1b treatment. However, if there was a true
interaction effect, replication of the strength of the association between genotype and survival within the Chicago cohort would not have been expected, since none of these patients were treated with interferon-$\gamma$1b. Further studies would be needed to determine whether an interaction effect exists between the MUC5B genotype and other clinical treatments. Nevertheless, this emphasizes the need to account for genotype in clinical trials and prediction models of IPF.

Third, this study used all-cause mortality as the primary end point, as opposed to IPF-related mortality, to maintain generalizability and comparability across cohorts since cause of death information was not complete for both cohorts. Among the INSPIRE patients, 66 of the 73 deaths were confirmed to be IPF related and 7 (2 GG and 5 GT) were possibly or definitely unrelated to IPF. This should have little effect on the results of this study because the vast majority of patients with IPF die within a relatively short period owing to the nature of the disease.

Fourth, data for additional genetic markers and serum biomarkers that have been reported to be associated with outcome in IPF were not available for these cohorts. More comprehensive predictive models may be developed by including these biomarkers and other genetic risk factors.

Idiopathic pulmonary fibrosis is etiologically heterogeneous and biologically dynamic, and sequence changes in MUC5B, transcriptional signatures in the fibrotic lung, and serum biomarkers, including chemoattractant ligand 18, KL6, SFTP A, SFTP D, MMP-7, intercellular adhesion molecule 1, and interleukin 8, are potentially predictive of disease activity and outcome in patients with IPF. Further study is necessary to refine the risk estimates and to determine the research and clinical implications of these findings.

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REFERENCES

16. Congrove GP, Groshong SD, Peljo AL, et al. The MUC5B promoter polymorphism is associated with a less severe pathological form of familial interstitial pneu...
17. Stock CJ, Sato H, Fonseca C, et al. Mucin 5B pro-
moter polymorphism is associated with idiopathic pul-
monary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. Thorax. 2013;

18. Wells AU, Desai SR, Rubens MB, et al. Idiopathic pulmonary fibrosis: a composite physiologic index de-
rived from disease extent observed by computed tomography. Am J Respir Crit Care Med. 2003;
167(7):962-969.

dimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012;156
(10):684-691.

20. du Bois RM, Weycker D, Albera C, et al. Forced vital capacity in patients with idiopathic pulmonary fi-
brosis: test properties and minimal clinically impor-
tant difference. Am J Respir Crit Care Med. 2011;
184(12):1382-1389.


22. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pul-
monary fibrosis. Am J Respir Crit Care Med. 2003;
168(5):538-542.

23. Flaherty KR, Mumford JA, Murray S, et al. Prog-
nostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J

24. King TE Jr, Pardo A, Selman M. Idiopathic pul-
monary fibrosis. Lancet. 2011;378(9807):1949-
1961.

25. Raghu G. Idiopathic pulmonary fibrosis: new

26. Fingerlin TE, Murphy E, Zhang W, et al. Gen-
ome-wide association study identifies multiple sus-
ceptibility loci for pulmonary fibrosis [published
online April 14, 2013]. Nat Genet. doi: 10.1038/ng
.2609.

27. Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Ra-
tional inferences about departures from Hardy-
76(6):967-986.

28. Boon K, Bailey NW, Yang J, et al. Molecular phe-
notypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS

29. Rosas IO, Richards TJ, Konishi K, et al. MMP1 and
MMP7 as potential peripheral blood biomarkers in id-
(4):e93.

The intellect has little to do on the road to discovery. There comes a leap in consciousness, call it intuition or what you will, and the solution comes to you and you don’t how or why.

—Albert Einstein (1879-1955)