

## ONLINE FIRST

# 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

James E. Loyd, MD

Kevin F. Gibson, MD

Max A. Seibold, PhD

Kevin K. Brown, MD

Janet L. Talbert, MS

Cheryl Markin, BA

Karl Kossen, PhD

Scott D. Seiwert, PhD

Elissa Murphy, MS

Imre Noth, MD

Marvin I. Schwarz, MD

Naftali Kaminski, MD

David A. Schwartz, MD

IDIOPATHIC PULMONARY FIBROSIS (IPF) is a chronic progressive disease with a median survival of 3 years.<sup>1</sup> The prognosis is variable; patients may remain stable for several years, slowly lose lung function, progress in an intermittent stair-step fashion, or experience precipitous acute exacerbations.<sup>2-4</sup> Although clinical and physiological parameters have modest value in predicting which patients will progress,<sup>5</sup> serum biomarkers, including chemokine ligand 18, KL6, surfactant protein A (SFTPA), and SFTPD, are independently associated with

**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 (rs35705950), 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- $\gamma$ 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 and GT 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;  $P < .002$ ). 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.

JAMA. 2013;309(21):2232-2239

Published online May 21, 2013. doi:10.1001/jama.2013.5827

www.jama.com

outcome in IPF.<sup>6,7</sup> Recently, elevated plasma concentrations of matrix metalloproteinase 7 (MMP-7), intercellu-

**Author Affiliations** are listed at the end of this article.  
**Corresponding Author:** David A. Schwartz, MD, University of Colorado, 12631 E 17th Ave, B178, Aurora, CO 80045 (david.schwartz@ucdenver.edu).

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.<sup>8</sup>

Rare mutations in *SFTPC*, *SFTPA2*, telomerase reverse transcriptase (*TERT*), and telomerase RNA component (*TERC*)<sup>9-11</sup> 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.<sup>12,13</sup> While *MUC5B* expression in the lung was 14.1 times higher in patients with IPF, the *MUC5B* promoter polymorphism was associated with up-regulation of this transcript only in unaffected participants.<sup>12</sup>

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- $\gamma$ 1b IPF clinical trial and provided written informed consent.<sup>14</sup> INSPIRE cohort participants were treated with concomitant agents if they met strict criteria for progression of disease.<sup>14</sup> Participants with available genetic and clinical follow-up data from the INSPIRE study were included in this study. Participants in the derivation set were followed up from randomization until death or study termination (a maximum of approximately 3 years after randomization). Vital status was assessed through a combination of pa-

tient/family/caregiver contacts and use of death registries (where permitted). Despite early termination of the study, assessable data (including vital status) were obtained for 99% of study patients, including all of the 438 patients in the current analysis.<sup>14</sup>

The Chicago cohort (validation cohort) consists of IPF participants who were recruited from the Interstitial Lung Disease Clinic at the University of Chicago 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.<sup>12,13</sup> 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.<sup>12,13</sup> 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.<sup>8</sup>

### 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- $\gamma$ 1b 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.<sup>8</sup> 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  $\geq 5.7$  ng/mL). An additive genetic model was used for the genotype in all survival models, consistent with previous findings.<sup>12,13</sup> Any patient receiving a lung transplant during follow-up was censored at transplant date in the analysis.

A Wald  $\chi^2$  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

Characteristics	INSPIRE (n = 438)	Chicago (n = 148)	P Value
Period of follow-up, median (IQR), y	1.6 (1.2-2.1)	2.1 (1.1-3.9)	
Deaths, No. (%) <sup>a</sup>	73 (17)	64 (43)	
Transplant events, No. (%)	7 (2)	10 (7)	.003
Female, No. (%)	115 (26)	35 (24)	.58
Age, mean (SD), y	66.6 (7.54)	69.0 (8.75)	.005
Ever smokers, No. (%)	312 (71)	96 (72)	.93
FVC, mean (SD), % predicted	72.2 (12.37)	64.5 (18.44)	<.001
FVC, mean (SD), L	2.9 (0.76)	2.5 (0.82)	<.001
DLCO, mean (SD), % predicted	47.3 (8.89)	46.2 (17.92)	.51
DLCO, mean (SD), mL/min/mm Hg	12.5 (4.40)	10.7 (4.51)	<.001
MMP-7, median (IQR), ng/mL <sup>b</sup>	7.8 (5.7-11.0)		
<i>MUC5B</i> genotype, No. (%)			
GG	148 (34)	41 (28)	.31
GT	259 (59)	98 (66)	
TT	31 (7)	9 (6)	

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

<sup>a</sup>Number of deaths not directly comparable across cohorts because of different lengths of follow-up time.

<sup>b</sup>MMP-7 data unavailable for Chicago cohort.

ses were performed using SAS, version 9.3 (SAS Institute Inc), and the Cox proportional hazards models were applied using the SAS PHREG procedure.

The area under the receiver operating characteristic curve (AUC) was calculated at 100-day intervals for the final, adjusted model in each cohort (with and without the *MUC5B* genotype covariate). In addition, the integral of time-specific AUC measures (C statistic) was calculated as an overall measure of the predictive value of these nested models.<sup>15</sup> We used a bootstrapping procedure with 10 000 samples to calculate 95% confidence intervals for the C statistic within each cohort. We used a permutation test to assess the difference between C statistic values for the nested models in each cohort. For this purpose, we used 10 000 permutations of the genotype values, with respect to the rest of the data set, to calculate empirical P values. All C statistic calculations were performed using R, version 3.0.0, packages “risksetROC” and “boot.”

## 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.<sup>12,13</sup> 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.<sup>12,13</sup>

### *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- $\gamma$ 1b 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, 0.10-0.52) 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 integral of the time-specific AUC (C statistic) was higher for the adjusted model including the *MUC5B* genotype compared with the adjusted model that excludes the genotype (C = 0.71 [95% CI,

0.64-0.75] and C=0.68 [95% CI, 0.61-0.73], respectively) for the INSPIRE cohort. This result was replicated in the Chicago cohort (C=0.73 [95% CI, 0.62-0.78] and C=0.69 [95% CI, 0.59-0.75], respectively). 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.<sup>8</sup> 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- $\gamma$ 1b 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

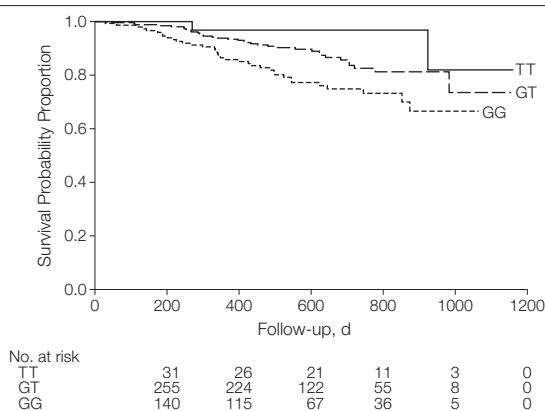
*MUC5B* (rs35705950), previously reported to be strongly associated with the development of familial interstitial

**Table 2.** Survival Analysis Models of *MUC5B*

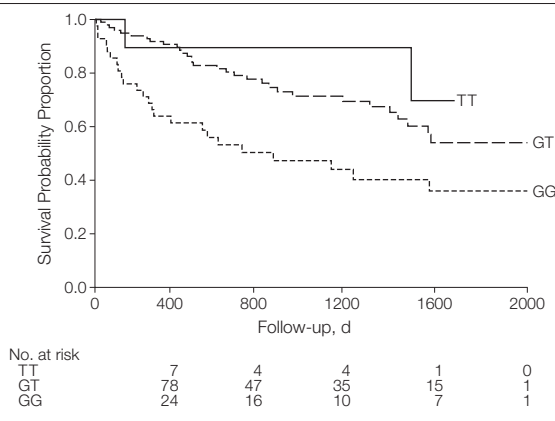
Model	INSPIRE		Chicago	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Univariable				
<i>MUC5B</i> genotype				
GG	1 [Reference]	.001	1 [Reference]	.004
GT	0.52 (0.34-0.78)		0.49 (0.30-0.79)	
TT	0.27 (0.12-0.60)		0.24 (0.09-0.63)	
Adjusted				
<i>MUC5B</i> genotype				
GG	1 [Reference]	<.001	1 [Reference]	.002
GT	0.48 (0.31-0.72)		0.39 (0.21-0.70)	
TT	0.23 (0.10-0.52)		0.15 (0.05-0.49)	
FVC	0.52 (0.32-0.83)	.006	0.53 (0.30-0.93)	.03
DLCO	0.89 (0.85-0.94)	<.001	0.90 (0.83-0.98)	.02
Sex				
Male	1 [Reference]	<.001	1 [Reference]	.06
Female	0.29 (0.14-0.59)		0.47 (0.22-1.02)	

Abbreviations: DLCO, diffusing capacity of carbon monoxide; FVC, forced vital capacity; HR, hazard ratio.

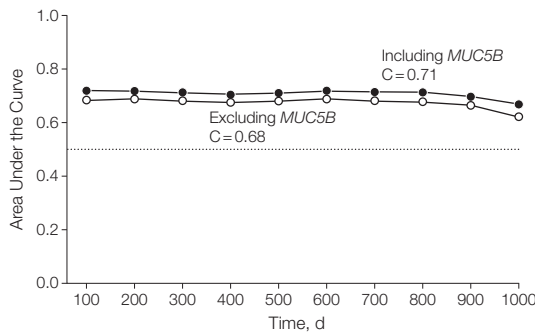
**Figure 1.** Kaplan-Meier Survival Curves by *MUC5B* Genotypes, INSPIRE Cohort



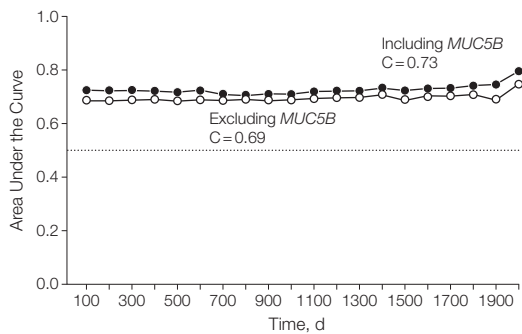
**Figure 2.** Kaplan-Meier Survival Curves by *MUC5B* Genotypes, Chicago Cohort



**Figure 3.** Time-Dependent Area Under the Curve for Adjusted Models Including and Excluding the *MUC5B* Genotype Predictor, INSPIRE Cohort



**Figure 4.** Time-Dependent Area Under the Curve for Adjusted Models Including and Excluding the *MUC5B* Genotype Predictor, Chicago Cohort



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

Variables	Hazard Ratio (95% CI)	P Value
MMP-7, ng/mL		
<5.7	1 [Reference]	.04
≥5.7	2.06 (1.05-4.07)	
<i>MUC5B</i> genotype		
GG	1 [Reference]	<.001
GT	0.46 (0.30-0.70)	
TT	0.21 (0.09-0.49)	
FVC	0.55 (0.34-0.89)	.01
DLCO	0.89 (0.85-0.95)	<.001
Sex		
Male	1 [Reference]	<.001
Female	0.25 (0.12-0.53)	

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

pneumonia and idiopathic pulmonary fibrosis,<sup>12,13</sup> is significantly associated with improved survival in IPF. These findings are consistent with a previous report of an association between the

*MUC5B* variant and less severe pathological changes in familial interstitial pneumonia,<sup>16</sup> as well as another report of slower decline in FVC for patients with IPF.<sup>17</sup> 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.<sup>12</sup>

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

**Table 4.** Patient Characteristics by *MUC5B* Genotype

Characteristics	INSPIRE			Chicago		
	GG (n = 148)	GT (n = 259)	TT (n = 31)	GG (n = 41)	GT (n = 98)	TT (n = 9)
Deaths, No. (%)	36 (24)	35 (14)	2 (6)	26 (63)	36 (37)	2 (22)
Transplant events, No. (%)	2 (1)	5 (2)	0 (0)	3 (7)	7 (7)	0 (0)
Female, No. (%)	39 (26)	68 (26)	8 (26)	6 (15)	27 (28)	2 (22)
Age, mean (SD), y	65.7 (8.22)	67.0 (7.34)	68.5 (4.80)	68.7 (10.22)	69.1 (8.25)	68.4 (7.70)
Ever smokers, No. (%)	114 (77)	174 (67)	24 (77)	25 (66)	66 (76)	5 (56)
FVC, mean (SD), % predicted	71.9 (12.15)	71.7 (12.07)	77.7 (14.74)	64.0 (19.39)	64.1 (18.62)	72.3 (8.48)
FVC, mean (SD), L	2.9 (0.72)	2.8 (0.77)	3.0 (0.82)	2.5 (0.75)	2.5 (0.85)	2.8 (0.73)
DLCO, mean (SD), % predicted	47.1 (9.05)	47.0 (8.36)	50.7 (11.65)	45.3 (18.70)	45.5 (17.67)	60.0 (12.52)
DLCO, mean (SD), mL/min/mm Hg	12.7 (4.22)	12.4 (4.49)	12.6 (4.59)	11.0 (5.27)	10.4 (4.21)	13.6 (3.18)
MMP-7, median (IQR), ng/mL <sup>a</sup>	7.5 (5.1-10.6)	7.9 (5.8-11.6)	8.1 (5.2-10.2)			
Interferon- $\gamma$ 1b treatment, No. (%) <sup>a</sup>	98 (66)	176 (68)	21 (68)			

Abbreviations: DLCO, diffusing capacity of carbon monoxide; FVC, forced vital capacity; IQR, interquartile range; MMP-7, matrix metalloproteinase 7.  
<sup>a</sup>MMP-7 and treatment data unavailable for Chicago cohort.

incorporate *MUC5B* and other molecular features have the potential to affect clinical care of patients. The clinical-radiographic-physiologic scoring system<sup>5</sup> as well as a simpler index<sup>18</sup> including pulmonary function and, most recently, a model that incorporates sex, age, FVC, and DLCO<sup>19</sup> 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.<sup>20-23</sup> 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.<sup>24</sup>

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 pa-

tients 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,<sup>25</sup> 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.<sup>26</sup> 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.<sup>12,13</sup> In the study by Seibold et al,<sup>12</sup> 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.<sup>12,27</sup> 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- $\gamma$ 1b 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- $\gamma$ 1b 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,<sup>2,28</sup> and serum biomarkers, including chemokine ligand 18, KL6, SFTPA, SFTPD, MMP-7, intercellular adhesion molecule 1, and interleukin 8, are potential predictors of disease activity and outcome in patients with IPF.<sup>6-8,29</sup> Further study is necessary to refine the risk estimates and to determine the research and clinical implications of these findings.

**Published Online:** May 21, 2013. doi:10.1001/jama.2013.5827

**Author Affiliations:** Departments of Epidemiology (Drs

Peljto and Fingerlin) and Biostatistics (Drs Zhang and Fingerlin), School of Public Health, Department of Medicine, School of Medicine (Ms Murphy and Drs M. I. Schwarz and D. A. Schwartz), University of Colorado Denver, and Departments of Biostatistics (Dr Silveira), Pediatrics (Dr Seibold), and Medicine (Drs Brown and D. A. Schwartz and Ms Talbert), National Jewish Health, Denver; Department of Medicine, University of Chicago (Drs Ma and Noth), and Institute for Personalized Respiratory Medicine, Department of Medicine, University of Illinois at Chicago (Dr Garcia), Chicago; Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania (Drs Richards, Lindell, Gibson, and Kaminski); and Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee (Drs Steele and Loyd and Ms Markin); InterMune, Brisbane, California (Drs Kossen and Seiwert).

**Author Contributions:** Dr Peljto had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Peljto and Zhang contributed equally as first authors and Drs Noth, M. I. Schwarz, Kaminski, and D. A. Schwartz contributed equally as last authors.

**Study concept and design:** Fingerlin, Garcia, Lindell, Loyd, Noth, Schwarz, Kaminski, Schwartz.

**Acquisition of data:** Zhang, Ma, Lindell, Steele, Gibson, Brown, Talbert, Markin, Kossen, Murphy, Noth, Schwarz, Kaminski, Schwartz.

**Analysis and interpretation of data:** Peljto, Fingerlin, Ma, Richards, Silveira, Loyd, Seibold, Brown, Talbert, Kossen, Seiwert, Noth, Schwarz, Kaminski, Schwartz.

**Drafting of the manuscript:** Peljto, Fingerlin, Richards, Silveira, Gibson, Markin, Noth, Schwarz, Kaminski, Schwartz.

**Critical revision of the manuscript for important intellectual content:** Peljto, Zhang, Fingerlin, Ma, Garcia, Richards, Lindell, Steele, Loyd, Seibold, Brown, Talbert, Kossen, Seiwert, Murphy, Kaminski, Schwartz.

**Statistical analysis:** Peljto, Fingerlin, Ma, Richards, Silveira, Noth, Schwartz.

**Obtained funding:** Seiwert, Noth, Schwarz, Kaminski, Schwartz.

**Administrative, technical, or material support:** Zhang, Lindell, Steele, Loyd, Gibson, Brown, Talbert, Markin, Seiwert, Murphy, Schwartz.

**Study supervision:** Fingerlin, Loyd, Schwarz, Kaminski, Schwartz.

**Conflict of Interest Disclosures:** All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Drs Seibold and D. A. Schwartz have filed a patent for *MUC5B* genetic variants, methods, and compositions for risk prediction, diagnosis, prognosis, and treatment of pulmonary disorders. Drs Kossen and Seiwert are employee shareholders of InterMune Inc, which is pursuing a license to the aforementioned patent, and have filed patents on use of peripheral blood biomarkers in IPF. Dr Noth has contracts for clinical trials with Boehringer Ingelheim, Sanofi, and Stromedix; has served on the speakers' bureau of GlaxoSmithKline; is a consultant to Boehringer Ingelheim and Immuneworks; and has patents related to gene expression profiling and plasma proteins. Dr Kaminski is a consultant to Sanofi, InterMune, Stromedix, Celgene, Promedior, and Vertex; is a recipient of investigator-initiated research grants from Gilead, Celgene, and Centocor; and has filed a patent on use of peripheral blood biomarkers in IPF. No other disclosures were reported.

**Funding/Support:** This research was supported by the National Heart, Lung, and Blood Institute (grants R01-HL095393, R01-HL097163, R01-HL095397, P50-HL0894932, P01-HL092870, RC1-HL09961, and RC2-HL101715) and the Dorothy P. and Richard P. Simmons Endowed Chair for Pulmonary Research (University of Pittsburgh School of Medicine).

**Role of the Sponsor:** The funders had no role in the design and conduct of the study; collection, man-

agement, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Online-Only Material:** The eFigure and 2 eTables are available at <http://www.jama.com>.

**Additional Contributions:** We gratefully appreciate all participating IPF patients for their contributions to IPF research.

## REFERENCES

- Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. *N Engl J Med*. 2001;345(7):517-525.
- Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One*. 2007; 2(5):e482.
- Martinez FJ, Safrin S, Weycker D, et al; IPF Study Group. The clinical course of patients with idiopathic pulmonary fibrosis. *Ann Intern Med*. 2005;142 (12 pt 1):963-967.
- Collard HR, Moore BB, Flaherty KR, et al; Idiopathic Pulmonary Fibrosis Clinical Research Network Investigators. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2007; 176(7):636-643.
- King TE Jr, Tooez JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med*. 2001;164(7):1171-1181.
- Prasse A, Probst C, Bargagli E, et al. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2009;179(8):717-723.
- Takahashi H, Fujishima T, Koba H, et al. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med*. 2000; 162(3 pt 1):1109-1114.
- Richards TJ, Kaminski N, Baribaud F, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2012; 185(1):67-76.
- Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356(13):1317-1326.
- Nogee LM, Dunbar AE III, Wert SE, Askin F, Hamvas A, Whittsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med*. 2001;344(8):573-579.
- Wang Y, Kuan PJ, King C, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet*. 2009; 84(1):52-59.
- 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.
- Zhang Y, Noth I, Garcia JG, Kaminski N. A variant in the promoter of *MUC5B* and idiopathic pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1576-1577.
- King TE Jr, Albera C, Bradford WZ, et al; INSPIRE Study Group. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. *Lancet*. 2009;374(9685):222-228.
- Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics*. 2005;61 (1):92-105.
- Cosgrove GP, Groshong SD, Peljto AL, et al. The *MUC5B* promoter polymorphism is associated with a less severe pathological form of familial interstitial pneu-

monia (FIP) [abstract]. *Am J Respir Crit Care Med.* 2012;185:A6865.

17. Stock CJ, Sato H, Fonseca C, et al. Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax.* 2013;68(5):436-441. doi:10.1136/thoraxjnl-2012-201786.
18. Wells AU, Desai SR, Rubens MB, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. *Am J Respir Crit Care Med.* 2003;167(7):962-969.
19. Ley B, Ryerson CJ, Vittinghoff E, et al. A multi-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 fibrosis: test properties and minimal clinically important difference. *Am J Respir Crit Care Med.* 2011;184(12):1382-1389.
21. Zappala CJ, Latsi PI, Nicholson AG, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. *Eur Respir J.* 2010;35(4):830-836.
22. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2003;168(5):538-542.
23. Flaherty KR, Mumford JA, Murray S, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. *Am J Respir Crit Care Med.* 2003;168(5):543-548.
24. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet.* 2011;378(9807):1949-1961.
25. Raghu G. Idiopathic pulmonary fibrosis: new evidence and an improved standard of care in 2012. *Lancet.* 2012;380(9842):699-701.
26. Fingerlin TE, Murphy E, Zhang W, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis [published online April 14, 2013]. *Nat Genet.* doi: 10.1038/ng.2609.
27. Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet.* 2005;76(6):967-986.
28. Boon K, Bailey NW, Yang J, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). *PLoS One.* 2009;4(4):e5134.
29. Rosas IO, Richards TJ, Konishi K, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. *PLoS Med.* 2008;5(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 know how or why.

—Albert Einstein (1879-1955)