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MUC5B Promoter Polymorphism and Development of Acute Respiratory Distress Syndrome

To the Editor:

Although acute respiratory distress syndrome (ARDS) and acute exacerbations of idiopathic pulmonary fibrosis (IPF) can differ markedly in their clinical presentations, they share a cardinal feature: histopathologic evidence of diffuse alveolar damage on lung biopsy. Genetic variation in *MUC5B*, which is highly expressed in bronchial epithelium and produces gel-forming mucin (mucin 5B), has emerged as a key risk factor for IPF yet has not been studied in ARDS.

The gain-of-function *MUC5B* promoter polymorphism rs35705950 is a promising candidate in ARDS for several reasons. First, it is common, with a minor allele frequency of approximately 10% among non-Hispanic whites, meaning that a substantial proportion of the population carries this risk allele. It is associated with not only an unusually high risk of IPF (with an odds ratio >6 per allele) (1) but also interstitial changes on chest computed tomography (CT) scan in undiagnosed carriers (2). These interstitial changes are typically found in adults older than 50 years of age. Patients

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Table 1. Baseline and Clinical Characteristics of Patients with Acute Respiratory Distress Syndrome versus At-Risk ICU Control Subjects

Characteristic	Copies of rs35705950	ARDS (n = 419)	At-Risk Control Subjects (n = 1,115)	P Value
Age, yr		51 ± 18	53 ± 17	0.09
Male sex, %		58	61	0.18
ARDS risk factor, %				<0.0001
Sepsis		27	28	
Pneumonia		19	9	
Trauma		35	33	
Multiple transfusions		2	21	
Aspiration		14	5	
Other		3	5	
White race, %		86	82	0.04
Current cigarette smoking, %		40	35	0.06
Diabetes, %		22	27	0.09
APACHE II score		29 ± 8	25 ± 8	<0.0001
<i>MUC5B</i> variant frequency, n				0.04*
Age ≥ 50 yr (n = 903)	0	179	543	0.01
	1	46	120	
	2	9	6	
Age < 50 yr (n = 631)	0	152	368	0.83
	1	32	74	
	2	1	5	

Definition of abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; ARDS = acute respiratory distress syndrome.

Data shown represent percentage or n (as indicated) for dichotomous variables (P from Fisher's exact test or χ^2 test) and mean ± SD for continuous variables (P from Wilcoxon's test).

**MUC5B* P value for interaction by age.

with interstitial abnormalities have been found to be at increased risk of ARDS (3). Thus, we hypothesized that *MUC5B* risk allele carriers over age 50 would be at increased risk for the development of ARDS.

Methods

To test this hypothesis, we genotyped more than 2,000 subjects enrolled in the VALID (Validating Acute Lung Injury Biomarkers for Diagnosis) study, a prospective, observational cohort of

Table 2. Logistic Regression Modeling for *MUC5B* ARDS Risk Remains Significant in Subjects 50 Years of Age and Older after Adjustment for Race and ARDS Risk Factors

Model	OR	95% CI	<i>MUC5b</i> P Value*
<i>MUC5B</i> alone	1.42	1.04–1.94	0.03 [†]
<i>MUC5B</i> + race + smoking status	1.42	1.03–1.93	0.03
<i>MUC5B</i> + race + smoking status + APACHE II	1.42	1.02–1.96	0.04
<i>MUC5B</i> + race + smoking status + APACHE II + direct lung injury [‡]	1.36	0.97–1.9	0.07

Definition of abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II; ARDS = acute respiratory distress syndrome; CI = confidence interval; OR = odds ratio.

*All genetic analyses in the additive model.

[†]P value using logistic regression differs slightly from that obtained using Fisher's exact test (P = 0.01), as presented elsewhere in this article.

[‡]Direct lung injury: aspiration or pneumonia as ARDS risk factor.

critically ill patients at high risk for ARDS, enrolled at Vanderbilt University Medical Center since 2006 and approved by the Vanderbilt Institutional Review Board (4, 5). At the time of enrollment, a blood sample was collected for DNA extraction. To select a population with the highest confidence of ARDS, we included as ARDS patients those who 1) were both mechanically ventilated and met Berlin criteria on at least two study days (6), and 2) had at least one ARDS risk factor. Subjects with ARDS were compared with those with at least one ARDS risk factor who never met ARDS criteria.

MUC5B rs35705950 genotyping was performed by TaqMan genotyping as described previously (1, 2); 98.4% of VALID samples were successfully genotyped. To assess for the presence of interstitial lung abnormalities, we reviewed official radiologic interpretation of all clinically obtained chest and abdominal CT scans for subjects at least 50 years old and who were homozygous for the *MUC5B* variant.

All genetic analyses were performed in the additive model. Association testing was conducted using *t* test or Wilcoxon's test for continuous variables and Fisher's exact test for categorical variables. Logistic regression was used to adjust for race and known ARDS risk factors. Given that interstitial changes in carriers of the *MUC5B* variant are limited to adults aged 50 years or older, we tested for genotype by age interaction, and present age-stratified results dichotomized at age 50. All statistical analyses were performed with R version 3.0.1.

Results

A total of 419 subjects with ARDS were compared with 1,115 critically ill control subjects. Baseline characteristics of the subjects

are presented in Table 1. Eighty percent of subjects with ARDS had moderate to severe disease ($\text{PaO}_2:\text{FiO}_2$ or $\text{SaO}_2:\text{FiO}_2 \leq 200$ on day of enrollment); ARDS severity did not vary by age. Among all 1,534 subjects, there was no significant association of *MUC5B* promoter genotype and ARDS ($P = 0.09$). However, consistent with prior evidence that *MUC5B* promoter variant carriers are only at increased risk for interstitial changes in older age groups (age, ≥ 50 yr), we noted significant evidence of an interaction between *MUC5B* promoter genotype and age in the risk for ARDS development ($P = 0.04$); thus we present only age-stratified results.

Among the 903 subjects at least 50 years of age, 234 had ARDS. Carriers of the *MUC5B* promoter variant had an odds ratio of 1.47 of developing ARDS (95% confidence interval, 1.02–2.1; $P = 0.01$). The increased risk of ARDS was driven by the homozygous carriers, with 60% of homozygous subjects (9 of 15) developing ARDS, compared with 28% of heterozygotes and 25% of wild-type individuals ($P = 0.01$; Fisher's exact test). This association remained significant when stratified to the 800 white subjects ($P = 0.01$); as expected, the minor allele frequency of rs35705950 was lower in the 103 nonwhite subjects (minor allele frequency, 4%; $P = 1.0$). This increased risk remained significant even after adjustment for race and known ARDS risk factors including Acute Physiology and Chronic Health Evaluation II score and smoking status (all $P < 0.05$; Table 2). Additional adjustment for direct lung injury as ARDS risk factor attenuated the association ($P = 0.07$). *MUC5B* carrier status was not associated with ICU outcomes including ventilator days or hospital mortality among patients with ARDS.

We manually reviewed the medical records of the 15 patients over age 50 years who were homozygous carriers of the *MUC5B* promoter variant. None carried a clinical diagnosis of interstitial lung disease. Fourteen of the subjects had a clinically obtained CT scan either before ($n = 2$) or during ($n = 12$) their index hospitalization. Nine of the 14 subjects (including both subjects who underwent imaging before the hospitalization, and three who never developed ARDS) had evidence of interstitial changes on chest imaging.

Discussion

The major finding of this study is that subjects who are homozygous for the *MUC5B* promoter variant (highest risk category for IPF) appear to be at risk of developing ARDS. Similar to the associations with interstitial lung abnormalities (2), the association between the *MUC5B* promoter polymorphism and ARDS appears to be limited to those over age 50 years. We found evidence of interstitial abnormalities in more than one-half of the homozygous carriers of the *MUC5B* promoter variant. This work suggests that either *MUC5B* is involved in the pathogenesis of ARDS or that individuals with preclinical pulmonary fibrosis are at risk of ARDS (3).

We note that our findings contrast with the apparent protective association of the same *MUC5B* variant in patients with chronic obstructive pulmonary disease with interstitial changes (7). In that study, *MUC5B* promoter variant carriers had a decreased risk of chronic obstructive pulmonary disease exacerbations and hospitalizations, suggesting possible heterogeneity of genetic effects.

This study has several limitations. First, although the VALID study includes more than 2,000 ICU patients, the *MUC5B* genotype association was limited to the 922 subjects over age 50 years, of whom 234 developed ARDS. Thus, although we found no evidence that usual ARDS risk factors, such as smoking, influenced the genotype effect estimate or that genotype status affected ARDS outcomes, our power to appreciate these differences was limited. Second, given that ARDS and interstitial lung disease are both characterized by chest X-rays with infiltrates, our analysis is at risk for identifying "false-positive ARDS." To mitigate against this, 100 ARDS chest X-rays were reviewed by two of the authors (R.M.B. and L.B.W.), who concurred with ARDS phenotype in all cases. Further study, particularly with longitudinal chest imaging, in large critically ill populations is needed. Replication in a large, well-phenotyped, independent population of at-risk critically ill adults, such as the PETAL (Preventional and Early Treatment of Acute Lung Injury) network VIOLET (Vitamin D to Improve Outcomes by Leveraging Early Treatment) population, would add confidence to the importance of this variant in ARDS pathogenesis.

In summary, we found that critically ill carriers of the *MUC5B* promoter variant rs35705950 over age 50 years were at increased risk for ARDS, particularly those with two copies of the minor allele. This increased risk remained significant after adjustment for known ARDS risk factors. These findings suggest that ARDS and undiagnosed interstitial lung disease or pulmonary fibrosis may share a potential pathogenic overlap in some cases. ■

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Therapeutic Engagement of the Histone Deacetylase IIA–Myocyte Enhancer Factor 2 Axis Improves Experimental Pulmonary Hypertension

To the Editor:

Pulmonary arterial hypertension (PAH) is a fatal disease of the pulmonary vasculature, characterized by abnormal remodeling

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of pulmonary vessels and elevated pulmonary arterial pressure, ultimately leading to right ventricular (RV) failure and death. MEF2 (myocyte enhancer factor 2) is a transcription factor that regulates multiple genes that are important during cardiovascular development and for vascular homeostasis (1), with MEF2C known to play a crucial role in regulating endothelial integrity, survival, and angiogenesis (2). A wealth of evidence supports regulation of MEF2 activity by the class IIA subset of HDACs (histone deacetylases), which directly bind to and inhibit its transcriptional activity (3). Our previous study demonstrated the involvement of the HDAC IIA–MEF2 axis in PAH, where increased HDAC IIA activity in pulmonary artery endothelial cells (PAECs) from subjects with PAH was strongly associated with impaired MEF2 function (4). Here, we used experimental rodent pulmonary hypertension (PH) models to determine whether genetic or pharmacologic targeting of the HDAC IIA–MEF2 axis can affect PH pathogenesis. Some of the results of these studies have been previously reported in the form of an abstract (5).

Methods

To investigate the role of endothelial MEF2 function in the pathogenesis of PH, we generated adult mice with inducible, endothelial-specific deletion of the *Mef2c* allele, using a tamoxifen-responsive *Cdh5* promoter-driven Cre recombinase crossed to mice with *Mef2c*^{fl/fl} alleles (henceforth called *Mef2c*^{ECKO} mice) (6, 7). Invasive hemodynamic and morphometric parameters were assessed at baseline and after exposure to 3 weeks of chronic hypoxia (10% F_IO₂). For the SU-5416/hypoxia rat models, male Sprague-Dawley rats (200–250 g) were injected subcutaneously with SU-5416 (Sigma; 20 mg/kg body weight). For drug administration, rats were given injections of either vehicle or TMP269 (50 mg/kg of body weight intraperitoneally; DC Chemical), or Tasquinimod (10 mg/kg body weight by oral gavage; DC Chemical) daily.

Results and Discussion

Immunofluorescent staining confirmed the endothelial-specific deletion of *Mef2c* in the lungs after injection of tamoxifen (Figure 1A). These mice did not display any appreciable pulmonary vascular phenotype up to 6 weeks after *Mef2c* deletion, but on chronic hypoxia exposure, the *Mef2c*^{ECKO} mice developed significantly higher right ventricular systolic pressure (Figure 1B). We also observed increased right ventricle to left ventricle plus septum weight ratios in the *Mef2c*^{ECKO} mice, although there was variability in the extent of right ventricular hypertrophy (Figure 1B). Lung morphometric analyses showed significantly increased percentage of smooth muscle actin–positive muscularized pulmonary arterioles in *Mef2c*^{ECKO} mice compared with controls. Overall, these findings provide the first genetic evidence that endothelial MEF2C is crucial for maintaining pulmonary vascular homeostasis *in vivo*, and that its genetic deletion results in exacerbation of PH.

We sought to further investigate the efficacy of HDAC IIA inhibition in inducing MEF2 activation. Stimulation with TMP269 (8), a highly selective HDAC IIA inhibitor, led to significant increase in *KLF2* and *KLF4* expression and