Pre-B-cell colony-enhancing factor gene polymorphisms and risk of acute respiratory distress syndrome*

Ednan K. Bajwa, MD; Chu-Ling Yu, DSc; Michelle N. Gong, MD, MPH; B. Taylor Thompson, MD; David C. Christiani, MD, MPH

Objective: **Pre-B-cell colony-enhancing factor (PBEF) levels are elevated in bronchoalveolar lavage fluid and serum of patients with acute lung injury. There are several suspected functional polymorphisms of the corresponding PBEF gene. We hypothesized that variations in PBEF gene polymorphisms alter the risk of developing acute respiratory distress syndrome (ARDS).**

Design: **Nested case-control study.**

Setting: **Tertiary academic medical center.**

Patients: **We studied 375 patients with ARDS and 787 at-risk controls genotyped for the PBEF *T-1001G* and *C-1543T* polymorphisms.**

Interventions: **None.**

Measurements and Main Results: **Patients with the *-1001G* (variant) allele had significantly greater odds of developing ARDS than wild-type homozygotes (odds ratio, 1.35; 95% confidence interval, 1.02–1.78). Patients with the *-1543T* (variant) allele did not have significantly different odds of developing ARDS than wild-type homozygotes (odds ratio, 0.86; 95% confidence interval, 0.65–1.13). When analysis was stratified by ARDS risk factor, *-1543T* was associated with decreased odds of developing ARDS in septic shock patients (odds ratio, 0.66; 95% confidence interval, 0.45–0.97). Also, *-1001G* was associated with increased hazard of intensive care unit mortality, whereas *-1543T* was associated with decreased hazard of 28-day and 60-day ARDS mortality, as well as shorter duration of mechanical ventilation. Similar results were found in analyses of the related *GC* (*-1001G:-1543C*) and *TT* (*-1001T:-1543T*) haplotypes.**

Conclusions: **The PBEF *T-1001G* variant allele and related haplotype are associated with increased odds of developing ARDS and increased hazard of intensive care unit mortality among at-risk patients, whereas the *C-1543T* variant allele and related haplotype are associated with decreased odds of ARDS among patients with septic shock and better outcomes among patients with ARDS. (Crit Care Med 2007; 35:1290–1295)**

KEY WORDS: acute respiratory distress syndrome; genetic predisposition to disease; genetic polymorphism

Acute respiratory distress syndrome (ARDS) is characterized by an inflammatory response to lung injury (1). The American-European Consensus Committee on ARDS (AECC) suggested that the type and severity of injury may affect the likelihood of developing the syndrome, but it remains unclear why up to 50% of individuals are not affected despite experiencing similar exposures (1, 2). Genetic variation between individuals probably accounts for at least some of this observed heterogeneity. Variations in the genes encoding for a variety of proteins have been associated with the risk of developing or dying from ARDS. These proteins include myosin light chain kinase, angiotensin-converting enzyme, surfactant proteins, and some cytokines (3–9).

Pre-B-cell colony-enhancing factor (PBEF) is a cytokine that was originally isolated in 1994 from a human lymphocyte DNA library (10). The protein's function is not completely understood, but it has been implicated in pre-B-cell colony formation, normal and preterm labor, sepsis, colorectal cancer, obesity, and diabetes mellitus (11–17). Recently, Ye and colleagues (18) demonstrated that PBEF gene expression is increased in human and animal models of acute lung injury (ALI) and that PBEF protein levels are increased in *in vitro* models of ALI and in serum and bronchoalveolar lavage fluid from humans and animals with ALI. In addition, those authors detected 11 single-nucleotide polymorphisms (SNPs) of the PBEF gene through resequencing. Genotyping of 271 subjects for two of these SNPs showed that the *T-1001G* variant allele (*-1001G*) was present more frequently in patients with sepsis and sepsis-associated ALI than in healthy controls, whereas the *C-1543T* variant allele (*-1543T*) was present less frequently in patients with sepsis-associated ALI than in healthy controls. However, no association was found between the PBEF polymorphisms and ALI compared with septic patients without ALI (18). Thus, it is not clear whether the association between the PBEF polymorphism and ALI is due to its association with ALI or the underlying risk factor for ALI (sepsis). In addition, this study was not able to evaluate the effect of genotype on mortality, leaving open the question of whether these polymorphisms are also associated with altered severity of illness.

We sought to investigate the association between the *T-1001G* and *C-1543T* polymorphisms in the PBEF gene and the

*See also p. 1424.

From the Pulmonary and Critical Care Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA (EKB, BTT, DCC); Department of Environmental Health, Harvard School of Public Health, Boston, MA (CLY, DCC); and Division of Pulmonary and Critical Care Medicine, Department of Medicine, Mount Sinai School of Medicine, New York, NY (MNG).

Supported, in part, by NHLBI grants HL60710, HL67197, and HL07874.

The authors have not disclosed any potential conflicts of interest.

Address requests for reprints to: David C. Christiani, MD, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115. E-mail: dchristi@hsph.harvard.edu

DOI: 10.1097/01.CCM.0000260243.22758.4F

development of ARDS in a hospital-based, nested case-control study consisting of patients at risk for ARDS from septic and noninfectious insults. We hypothesized that the *-1001G* allele is associated with increased development of ARDS whereas the *-1543T* allele is associated with decreased development of ARDS.

METHODS

Study Enrollment and Design. Study design and exclusion criteria were described previously (5). Adult intensive care unit (ICU) admissions to the Massachusetts General Hospital (Boston, MA) from September 1999 to May 2005 were screened for risk factors for ARDS development (Table 1). Eligible patients were approached and enrolled in the prospective cohort after informed written consent was obtained from subjects or surrogates. Study design is illustrated in Figure 1. Patients were screened daily until death or ICU discharge and defined as having ARDS if they developed respiratory failure requiring intubation and met AECC criteria (1) for ARDS development. A nested case-control study was constructed using ARDS patients as cases and patients without ARDS as controls. The Massachusetts General Hospital Human Subjects Committee approved the study.

Genotyping. A 10-mL blood sample was collected from each patient in an ethylenediaminetetraacetic-acid tube. DNA was extracted using PureGene kits (Gentra Systems, Research Triangle, NC) and genotyped using 5' nuclease (Taqman) assays with custom primers and probes (Applied Biosystems, Foster City, CA). For *T-1001G*, the forward primer sequence was 5'-ACGGGCCAAGCCTTTGA-3', reverse primer sequence was 5'-CCAACTCGTTTCCCAGGATTTA-AAG-3', and reporter sequence was 5'-TCAGTG/TTCGCACCCTG-3'. Corresponding *C-1543T* sequences were 5'-GCAAAGATCATGGAAGTG-GAAGGTA-3', 5'-CCTCGTTGCTGAAAATAATTG-TAAGTGT-3', and 5'-CACCAG/AGCACTCAC-3'.

Genotyping personnel were blinded to case-control status. Two separate readers interpreted results, and 5% of samples were randomly reanalyzed for quality control. No genotyping errors were found.

Statistical Analysis. Deviation from Hardy-Weinberg equilibrium (19) was determined using SAS Genetics software package (version 9.1, SAS Computing) PROC ALLELE test. Linkage disequilibrium was measured using Lewontin's D' in controls (20). Univariate analysis was performed using chi-square tests for categorical variables and *t*-tests for continuous variables as appropriate. Variables with $p \leq .2$ were entered into a logistic-regression model using a backward-selection algorithm and eliminated if $p > .2$. The final model included gene effect; variables from backward elimination; significant interactions; clinically relevant variables such as age, gender, and ARDS risk factor; whether patients had received transfusion of blood products; and Acute Physiology and Chronic Health Evaluation (APACHE) III score. APACHE III scores were revised to remove Pao_2/Fio_2 and age from the score to avoid colinearity in the model (21). Likelihood ratio testing suggested that a dominant model of inheritance fit the data best.

Because existing data (18) focused on septic patients, we decided *a priori* to stratify analysis by patients with infectious and noninfectious risk factors to determine whether gene effects vary by risk factor.

Cox proportional hazards models were used for multivariate analysis of the effect of genotype

Table 1. Predefined risk factors for acute respiratory distress syndrome (ARDS) required for study inclusion

Risk Factor	Definition
Sepsis	Known or suspected source of systemic infection and at least two of the following: a) temperature >38°C or <36°C; b) heart rate >90 beats/min; c) respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg; d) WBC >12,000·mm^3, <4000·mm^3, or >10% bandemia.
Septic shock	Definition of sepsis plus one of the following: a) SBP <90 mm Hg or reduction of ≥40 mm Hg from baseline for ≥30 mins, unresponsive to 500 mL of fluid resuscitation; b) need for vasopressors to maintain SBP ≥ 90 mm Hg or within 40 mm Hg of baseline.
Pneumonia	Two or more of the following: a) new airspace opacity on chest radiograph; b) temperature >38.3°C or <36.0°C, WBC >12,000·mm^3 or <4000·mm^3 or >10% bandemia; c) positive microbiological culture.
Aspiration	Defined as witnessed or documented aspiration event or the retrieval of gastric contents from the oropharynx, endotracheal tube, or bronchial tree.
Trauma	Defined as multiple fractures and/or pulmonary contusions. Multiple fractures are defined as a fracture of two long bones, an unstable pelvic fracture, or one long bone and a pelvic fracture. Pulmonary contusion is defined as airspace opacity on chest radiograph within 8 hrs of admission to the emergency room and evidence of blunt trauma to the chest, for example, fractured ribs or ecchymosis overlying airspace opacity.
Multiple transfusions	Defined as receiving ≥8 units of packed RBCs within 24 hrs.

WBC, white blood cell count; SBP, systolic blood pressure; RBCs, red blood cells.

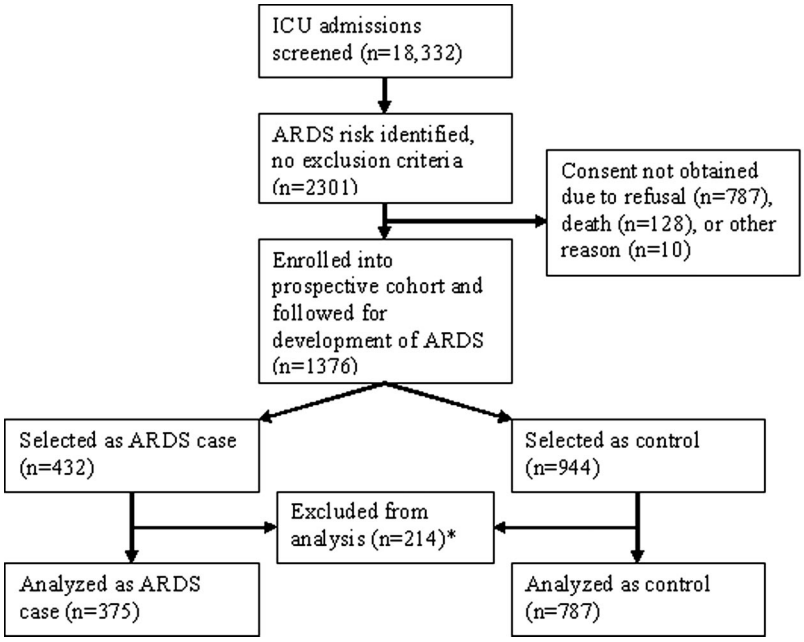

Figure 1. Schematic of patient selection and study design. *Excluded for prior enrollment or history of acute respiratory distress syndrome (*ARDS*) (each subject analyzed once, duplicate enrollment excluded) (n = 97), genetic heritage other than white (n = 99), failure of genotyping (n = 10), or other reasons (n = 8). *ICU,* intensive care unit.

on all-cause ICU mortality as well as 28-day and 60-day mortality. The 28-day and 60-day analyses were restricted to patients with ARDS, because other patients were not followed after ICU discharge. Candidate variables were selected for inclusion into the model using a process similar to that described for the multivariable logistic regression model. The final proportional hazards model included covariates for gene effect, age, gender, APACHE III, severity of illness/organ dysfunction, and the presence of the ARDS risk factors sepsis, septic shock, or direct pulmonary injury. Covariates were tested to ensure they did not violate the proportional hazards assumption. All covariates complied with this assumption.

Haplotypes were generated using SAS software and the Happy macro (http://www.hsph.harvard.edu/faculty/kraft/soft.htm), which infers haplotypes using an "expectation substitution" algorithm (22, 23). This approach gives accurate estimates for common haplotypes (>4%) with moderate relative risks (24). Haplotype scores were entered as covariates in the multivariable logistic regression model, again assuming dominant inheritance.

Assuming allele frequencies of 22% for *-1001G*, 25% for *-1543T*, α-error of .05, and 80% power, our sample (375 patients, 787 controls) could detect minimum odds ratios (ORs) for ARDS development of 1.43 for *-1001G* and 0.68 for *-1543T*. Analyzing sepsis patients (99 patients, 298 controls) would provide minimum detectable ORs of 1.90 for *-1001G* and 0.48 for *-1543T*, septic shock patients (219 patients, 342 controls) would have minimum detectable ORs of 1.63 for *-1001G* and 0.59 for *-1543T*, and patients with other risk factors (57 patients, 147 controls) would have minimum detectable ORs of 2.34 for *-1001G* and 0.35 for *-1543T*, providing sufficient power to detect effects similar to those previously reported.

RESULTS

Cohort Characteristics. From October 1999 to February 2005, 18,332 adult ICU admissions were screened for this study (Fig. 1), and 1,376 patients were enrolled in the cohort. Patients with a genetic heritage other than white (n = 99), patients who had been previously enrolled or had a history of ARDS (n = 97), patients who could not be genotyped (n = 10), and other patients (n = 8) were excluded from analysis, leaving 1,162 patients in the final analysis, including 375 patients with ARDS and 787 controls.

Characteristics of the study population are shown in Table 2. Data relevant to clinical risk factors for ARDS have been described previously and detailed (5).

Genetic Analysis and Risk of ARDS. In all, 418 patients were heterozygous and 74 patients were homozygous for *-1001G*, with an allele frequency of 0.24. Meanwhile, 414 patients were heterozygous and 64 patients were homozygous for *-1543T* allele, with an allele frequency of 0.23. These genotype frequencies did not differ significantly from those predicted by Hardy-Weinberg equilibrium ($p > .25$) and are comparable to the prior report (18). Both polymorphisms were highly linked (Lewontin's $D' = 1.00$).

Genotype and haplotype frequencies are presented in Table 3. There was a significant difference in the proportion of cases and controls with regard to genotype at the *T-1001G* locus ($p = .05$). Patients with ARDS were significantly more likely to have at least one copy of the variant allele compared with controls (178 of 375, or 47%, vs. 314 of 787, or 40%; $p = .02$). ARDS patients were less likely to have one or more copies of the *-1543T* allele compared with controls (144 of 375, or 38%, vs. 334 of 787, or 42%), but this was not statistically significant ($p = .2$). Of the four possible haplotypes, the *GT* haplotype (both variant alleles) was not observed in the study population.

Results of multivariable analysis are presented in Table 4. Patients with the *-1001G* allele had greater odds of developing ARDS than wild-type homozygotes (OR_{adj}, 1.35; 95% confidence interval

Table 2. Characteristics of study population

Characteristic	At-Risk Controls	ARDS	*p*
Total no.	787	375	
Age, mean ± SD	63.43 ± 17.1	60.11 ± 18.7	.004
Female gender, n (%)	218 (32)	157 (32)	.79
APACHE III score, mean ± SD	68.03 ± 23.2	77.33 ± 23.6	<.0001
Sepsis syndrome, n (%)	298 (38)	99 (26)	<.0001
Pulmonary source	153 (19)	79 (21)	<.0001
Extrapulmonary source	145 (18)	20 (5)	
Septic shock, n (%)	342 (43)	219 (58)	<.0001
Pulmonary source	163 (21)	161 (43)	<.0001
Extrapulmonary source	179 (23)	58 (15)	
Trauma, n (%)	64 (8)	29 (8)	.81
Need for blood transfusion, n (%)	404 (51)	234 (62)	.0003
Aspiration, n (%)	58 (7)	34 (9)	.32
>1 risk factor for ARDS, n (%)	73 (9)	49 (13)	.05
Direct pulmonary injury, n (%)	381 (48)	271 (72)	<.0001
Indirect pulmonary injury, n (%)	406 (52)	104 (28)	

ARDS, acute respiratory distress syndrome; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 3. Genotype and haplotype frequencies

	At-Risk Controls	ARDS	*p*
Total no.	787	375	
T-1001G, n (%)			
Wild-type (TT)	473 (60)	197 (53)	.05
Heterozygous variant (TG)	267 (34)	151 (40)	
Homozygous variant (GG)	47 (6)	27 (7)	
C-1543T, n (%)			
Wild-type (CC)	453 (58)	231 (62)	.24
Heterozygous variant (CT)	293 (37)	121 (32)	
Homozygous variant (TT)	41 (5)	23 (6)	
Haplotypes, n (%)			
TC (-1001T:-1543C)	617 (78)	278 (74)	.10
GC (-1001G:-1543C)	314 (40)	178 (47)	.02
TT (-1001T:-1543T)	334 (42)	144 (38)	.19
GT (-1001G:-1543T)	0	0	—

ARDS, acute respiratory distress syndrome.

[CI], 1.02–1.78). The association between the *-1543T* allele and development of ARDS was not statistically significant (OR_{adj}, 0.86; 95% CI, 0.65–1.13). Results of haplotype analysis (Table 5) showed similar effects on ARDS development associated with the *GC* haplotype, with a borderline association for the *TT* haplotype. Covariates found to be significantly associated with ARDS development on multivariable analysis were evaluated for differences in distribution across genotype. There were no significant differences in age, APACHE III score, proportion of patients with direct lung injury, or proportion of patients who received blood transfusions when patients with variant genotypes were compared with wild-type homozygotes ($p > .1$).

Multivariable modeling was also used to evaluate whether genotype was associated with sepsis or septic shock. When we used sepsis or septic shock in the model as the dependent variable, the *-1001G* and *-1543T* alleles were not significantly associated with either sepsis (OR_{adj}, 1.0 for *-1001G*; 95% CI, 0.78–1.37; OR, 1.2 for *-1543T*; 95% CI, 0.9–1.5) or septic shock (OR 1.1_{adj}, for *-1001G*; 95% CI, 0.85–1.48; OR, 0.82 for *-1543T*; 95% CI, 0.62–1.07).

To determine whether gene effect varied by ARDS risk factor, the association between the variant alleles and ARDS was examined further in the multivariable model after stratifying the population into groups of patients with sepsis, septic shock, or noninfectious (all other) risk factors for ARDS. Among the 397 patients with sepsis syndrome, the odds of developing ARDS were significantly increased by presence of *-1001G* (OR_{adj}, 2.09; 95% CI, 1.23–3.56). Among the 561 patients with septic shock, the odds of developing ARDS for patients with *-1543T* (OR_{adj}, 0.66; 95% CI, 0.45–0.97) were significantly decreased. For patients with noninfectious risk factors, the odds of developing ARDS were significantly increased with the presence of *-1001G* (OR_{adj}, 2.3; 95% CI, 1.14–4.72). The association between ARDS and *-1001G* was not statistically significant in the subgroup of patients with septic shock ($p = .55$). Likewise, the association between ARDS and *-1543T* was not statistically significant in the subgroups of patients with either sepsis or noninfectious risk factors ($p = .53$, $p = .74$).

Exploratory analyses were conducted to explain why the association between *-1001G* allele and ARDS differed between sepsis and septic shock. First, the septic and septic shock groups were compared. Septic shock patients were significantly older ($p < .0001$), had higher APACHE III scores ($p < .0001$), were more likely to have received blood transfusions ($p < .0001$) or to have multiple risks for ARDS ($p = .05$), and were more likely overall to develop ARDS ($p < .0001$). We hypothesized that effect modification by age or severity of illness might have resulted in dilution of the gene effect in this group of patients who appeared to be at highest risk of ARDS. To explore this, all patients were analyzed using the multivariable model after stratification by APACHE III score quartile. This revealed that the estimated odds ratios for the effect of *-1001G* on ARDS development decreased with increasing severity of illness (Fig. 2), suggestive of an interaction between APACHE III score, *-1001G* allele, and ARDS. However, the results are not statistically significant, given the reduced sample size in each stratum. Analyzing age independently in this fashion did not reveal a similar interaction.

The results of Cox proportional hazards analyses are shown in Table 6. The *-1001G* allele and the related *GC* haplotype were associated with significantly increased hazard of ICU mortality. When restricted to ARDS patients, the haplotype association remained significant; *-1543T* and the associated *TT* haplotype were significantly associated with 28-day and 60-day mortality in ARDS. The *TT* haplotype, but not the *-1543T* allele, was significantly associated with ARDS ICU mortality, as well.

Days of mechanical ventilation were recorded as a secondary outcome. ARDS patients with the *-1543T* allele had significantly more ventilator-free days

Table 4. Multivariate analysis

Variable	OR_{adj}	95% CI
T-1001G variant allele	1.35	1.02–1.78
C-1543T variant allele	0.86	0.64–1.13
Age	0.98	0.97–0.99
Female gender	1.13	0.86–1.49
APACHE III score	1.01	1.00–1.02
Cirrhosis	1.24	0.65–2.37
Sepsis	0.78	0.47–1.29
Septic shock	1.42	0.88–2.29
Direct pulmonary injury	3.32	2.49–4.44
Diabetic	0.56	0.40–0.78
Received blood transfusion	1.87	1.40–2.49

OR_{adj}, odds ratio for effect on development of acute respiratory distress syndrome; CI, confidence interval; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 5. Haplotype analysis

Haplotype	OR_{adj}	95% CI	*p*
TC (-1001T:-1543C)	0.81	0.60–1.10	.19
GC (-1001G:-1543C)	1.40	1.07–1.83	.01
TT (-1001T:-1543T)	0.79	0.60–1.04	.09
GT (-1001G:-1543T)	NA	NA	—

OR_{adj}, odds ratio for effect on development of acute respiratory distress syndrome; CI, confidence interval; NA, not applicable.

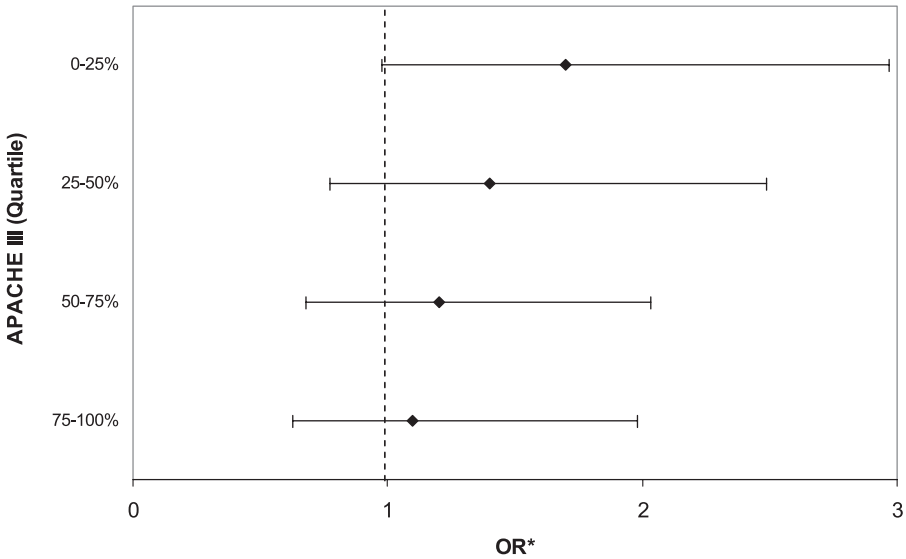

Figure 2. *T-1001G* genotype analysis stratified by Acute Physiology and Chronic Health Evaluation (*APACHE*) III score. *OR*, odds ratio for effect of *T-1001G* variant allele on development of acute respiratory distress syndrome.

Table 6. Gene effects on mortality

	ICU Mortality (All Patients)			ICU Mortality (ARDS Only)			28-Day Mortality (ARDS Only)			60-Day Mortality (ARDS Only)		
	HR_{adj}	95% CI	*p*	HR_{adj}	95% CI	*p*	HR_{adj}	95% CI	*p*	HR_{adj}	95% CI	*p*
T-1001G variant	1.60	1.20–2.12	.001	1.37	0.93–2.02	.11	1.19	0.82–1.71	.36	1.20	0.86–1.67	.28
GC haplotype	1.56	1.18–2.06	.002	1.53	1.07–2.20	.02	1.33	0.94–1.90	.11	1.35	0.98–1.86	.07
C-1543T variant	1.11	0.83–1.48	.5	0.72	0.47–1.08	.11	0.62	0.42–0.92	.02	0.60	0.42–0.86	.005
TT haplotype	1.00	0.75–1.33	.9	0.64	0.43–0.94	.03	0.59	0.40–0.87	.008	0.57	0.41–0.81	.002

ICU, intensive care unit; ARDS, acute respiratory distress syndrome; HR_{adj}, adjusted hazard ratio; CI, confidence interval.

than wild-type homozygotes (8.1 vs. 6.2, $p = .03$).

DISCUSSION

Our results demonstrate an association between the variant *T-1001G* allele and related haplotype and increased risk of poor outcomes, as well as an apparently protective role for the variant *C-1543T* allele and related haplotype, in a case-control study of critically ill patients. This study design has a number of strengths. Using the AECC definition of ARDS (1) to prospectively identify cases helps minimize bias due to misclassification in the absence of a gold standard for diagnosis of this disease. As one of the largest studies reported on development of ARDS, this study has more power than other prior reports and allows us to examine gene-environment interactions via stratified analysis. The selection of patients with risk factors for ARDS as controls is preferable to using healthy controls, as healthy patients without a stimulus for lung injury would not be expected to develop ARDS based on genetic factors alone. Including heterogeneous risk factors for ARDS in both groups also minimizes confounding due to association of genotype with a single risk factor. Using only white patients in the analysis also reduces the possibility of confounding by population stratification, although this can still occur at a potentially significant level (25).

However, only including whites also results in a potential limitation of the study, as the results cannot be extrapolated to other genetic heritages. Another potential limitation of the study is that the functional significance of these PBEF polymorphisms is not confirmed in the patient population. Also, because of the study design, the results cannot be generalized to the community-hospital setting, to immunocompromised patients, to patients without risk factors for ARDS, or to patients with different clinical risks for ARDS than those studied.

In the prior study by Ye and colleagues (18), frequencies of the *-1001G* allele and the associated haplotype were found to be increased in patients with sepsis and sepsis-associated ALI compared with healthy controls. Our study confirms and extends this finding by demonstrating that this allele and haplotype are also associated with increased susceptibility to ARDS among at-risk patients with both septic and nonseptic risk factors for ARDS. We also present the novel finding that the *-1001G* allele and *GC* haplotype are associated with ICU mortality in the entire population of at-risk patients and that the haplotype is also associated with ICU mortality among ARDS patients. In our study, the presence of either variant genotype was not shown to be associated with the presence or absence of sepsis syndrome or septic shock, decreasing the probability that association between the gene and development of a septic phenotype confounds association with ARDS development.

After stratification by risk factor for ARDS, the association between the variant *-1001G* allele and ARDS development was stronger in the group of patients with sepsis syndrome and those with noninfectious risk factors for ARDS. Also, the *-1543T* allele was found to be associated with ARDS development in the group of patients with septic shock but not in the larger patient population with sepsis syndrome and noninfectious risk factors for ARDS. It is unclear why the *-1001G* association was different in patients with septic shock compared with patients with sepsis syndrome. Our exploratory analyses suggest the presence of an interaction between *-1001G* and severity of illness whereby the effect of the variant on the development of ARDS decreases with the increasing severity of illness seen in septic shock. In addition, other factors that increase the risk of ARDS were present more frequently in the septic shock group. It is possible that among the most severely ill patients, the relative protective effect of the homozygous wild-type genotype is diminished by the greater nongenetic clinical risk factors for lung injury seen more frequently in septic shock. However, this interaction was found on *post hoc* exploratory analyses and likely did not have the power to draw meaningful conclusions. These findings will need to be confirmed and further studied in a larger population.

Ye and colleagues (18) found that the *-1543T* allele was present less frequently in patients with sepsis-associated ALI than healthy controls. In contrast, we did not find a significant difference in frequency of this allele or the related haplotype between cases and controls except among patients with septic shock. We attribute the difference in these findings to difference in selection of controls between the two studies. Indeed, Ye et al. (18) did not find any association between *-1543T* and development of ALI when compared with non-ALI sepsis patients. The protective effect of *-1543T* appears to be in opposition to the increased risk conferred by *-1001G* allele, and the two polymorphisms appear to exert influence in different subgroups of patients. This suggests the presence of complex gene-environment interactions that should be studied further.

We also found that the *-1001G* allele is associated with increased ICU mortality, whereas the *-1543T* allele is associated with improved outcomes later in the course of ARDS, including 28-day and 60-day mortality, and fewer days of mechanical ventilation. In terms of biological rationale for the observed effects, it is known that PBEF expression is increased in response to inflammatory stimuli, such as in human fetal membranes *in vitro* and *in vivo* in response to mechanical distention and infection (15, 26). In turn, PBEF increases expression of other inflammatory mediators, such as interleukin-6 and interleukin-8 (11). Additional effects of PBEF include inducing de-

lays in neutrophil apoptosis (17) and mediating pulmonary capillary permeability (14), both of which processes are implicated in the pathogenesis of lung injury (11–14, 17, 27). The *-1543T* allele is associated with decreased PBEF expression *in vitro*, and although the *-1001G* allele was not associated with altered expression levels, it is plausible that increased expression might account for its apparent opposing effect to *-1543T*. Given these data, it is possible to envision a process by which increased PBEF expression in response to inflammatory stimuli results in increased ARDS development and worse outcomes early in disease. It is less clear why the protective effect of *-1543T* emerges more strongly later in the disease process. One possibility is that this genotype enhances down-regulation of expression once the initial inflammatory insult has subsided.

With regard to functional significance, because the *T-1001G* SNP is located in the promoter region of the gene (18), variation in this SNP would not by itself result in the transcription of a different protein product but could alter the amount of protein expressed. In light of the findings by Ye and colleagues (18) that variation in this promoter polymorphism did not alter the level of PBEF expression *in vitro*, it is possible that this SNP serves as a marker for an as-yet unknown polymorphism within the gene with functional significance. Linkage disequilibrium with an external gene is also possible for either of the SNPs studied, but the chromosomal location of PBEF at 7q22 contains mainly genes that are not suspected of involvement in inflammatory processes, such as *SYPL1* and *PIK3CG*.

CONCLUSIONS

In our cohort of critically ill patients with risk factors for ARDS, the variant allele of the *T-1001G* SNP in the PBEF gene and the related haplotype are associated with increased risk of developing ARDS. Stratification of the analysis by risk factor for ARDS points to a potential gene-environment interaction between this gene, sepsis syndrome vs. septic shock as the risk factor for ARDS, and APACHE III score. The variant *C-1543T* allele of the PBEF gene was found to be associated with decreased risk of developing ARDS among the subgroup of patients with septic shock. In addition, the variant *T-1001G* allele and related haplotype are associated with increased ICU mortality, whereas the variant *C-1543T* allele and related haplotype are associated with decreased 28-day and 60-day mortality and fewer days of mechanical ventilation. Further study is warranted to determine the pathophysiologic and genetic bases of these associations.

ACKNOWLEDGMENTS

We thank T. McCabe and J. Shin for patient recruitment, A. Shafer for research support, L. Su and Z. Wei for laboratory expertise, and J. Frelich, M. Chertok, and L. Pothier for data management.

REFERENCES

1. Bernard GR, Artigas A, Brigham KL, et al: The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994; 149(3 Pt 1):818–824
2. Hudson LD, Milberg JA, Anardi D, et al: Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995; 151(2 Pt 1):293–301
3. Gao L, Grant A, Halder I, et al: Novel polymorphisms in the myosin light chain kinase gene confer risk for acute lung injury. *Am J Respir Cell Mol Biol* 2006; 34:487-495
4. Gong MN, Wei Z, Xu LL, et al: Polymorphism in the surfactant protein-B gene, gender, and the risk of direct pulmonary injury and ARDS. *Chest* 2004; 125:203–211
5. Gong MN, Zhou W, Williams PL, et al: -308GA and TNFB polymorphisms in acute respiratory distress syndrome. *Eur Respir J* 2005; 26:382–389
6. Gong MN, Zhou W, Williams PL, et al: Polymorphisms in the mannose binding lectin-2 gene and acute respiratory distress syndrome. *Crit Care Med* 2007; 35:48–56
7. Lin Z, Pearson C, Chinchilli V, et al: Polymorphisms of human SP-A, SP-B, and SP-D genes: Association of SP-B Thr131Ile with ARDS. *Clin Genet* 2000; 58:181–191
8. Marshall RP, Webb S, Bellingan GJ, et al: Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2002; 166:646–650
9. Zhai R, Zhou W, Gong MN, et al: Inhibitor κB-α haplotype GTC is associated with susceptibility to acute respiratory distress syndrome in Caucasians. *Crit Care Med* 2007; [Epub ahead of print]. Accessed March 1, 2006
10. Samal B, Sun Y, Stearns G, et al: Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 1994; 14:1431–1437
11. Ognjanovic S, Bryant-Greenwood GD: Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. *Am J Obstet Gynecol* 2002; 187:1051–1058
12. Rongvaux A, Shea RJ, Mulks MH, et al: Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur J Immunol* 2002; 32:3225–3234
13. Van der Veer E, Nong Z, O'Neil C, et al: Pre-B-cell colony-enhancing factor regulates NAD+-dependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. *Circ Res* 2005; 97:25–34
14. Ye SQ, Zhang LQ, Adyshev D, et al: Pre-B-cell-colony-enhancing factor is critically involved in thrombin-induced lung endothelial cell barrier dysregulation. *Microvasc Res* 2005; 70:142–151
15. Ognjanovic S, Bao S, Yamamoto SY, et al: Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. *J Mol Endocrinol* 2001; 26:107–117
16. McGlothlin JR, Gao L, Lavoie T, et al: Molecular cloning and characterization of canine pre-B-cell colony-enhancing factor. *Biochem Genet* 2005; 43:127–141
17. Jia SH, Li Y, Parodo J, et al: Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J Clin Invest* 2004; 113:1318–1327
18. Ye SQ, Simon BA, Maloney JP, et al: Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. *Am J Respir Crit Care Med* 2005; 171:361–370
19. Czika W, Yu X, Wolfinger RD: An introduction to genetic data analyses using SAS/genetics. SAS Customer Support Center. Available at: http://support.sas.com/rnd/papers/sugi27/genetics.pdf. Accessed December 1, 2005
20. Devlin B, Risch N: A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 1995; 29:311–322
21. Knaus WA, Wagner DP, Draper EA, et al: The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. *Chest* 1991; 100:1619–1636
22. Stram DO, Haiman CA, Hirschhorn JN, et al: Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered* 2003; 55:27–36
23. Zaykin DV, Westfall PH, Young SS, et al: Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum Hered* 2002; 53:79–91
24. Kraft P, Cox DG, Paynter RA, et al: Accounting for haplotype uncertainty in matched association studies: A comparison of simple and flexible techniques. *Genet Epidemiol* 2005; 28:261–272
25. Campbell CD, Ogburn EL, Lunetta KL, et al: Demonstrating stratification in a European American population. *Nat Genet* 2005; 37:868–872
26. Marvin KW, Keelan JA, Eykholt RL, et al: Use of cDNA arrays to generate differential expression profiles for inflammatory genes in human gestational membranes delivered at term and preterm. *Mol Hum Reprod* 2002; 8:399–408
27. Chen MP, Chung FM, Chang DM, et al: Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91:295–299