Genotypes and haplotypes of *VEGF* gene are associated with higher ARDS Mortality and lower VEGF plasma levels

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Background: Endothelial injury is an important prognostic factor in acute respiratory distress syndrome (ARDS). Vascular endothelial growth factor (VEGF) plays a critical role in endothelial destruction and angiogenesis. Genetic variations of *VEGF* have been associated with VEGF production. A study was undertaken to investigate the impact of *VEGF* gene polymorphisms on ARDS development and clinical outcomes.

Methods: Three *VEGF* polymorphisms *(-460C/T, +405C/G, and +936C/T)* were determined in 1253 ICU patients with risk factors for ARDS. Among them 394 patients developed ARDS. Patients were followed for 60-day survival. Plasma VEGF levels were measured in 71 patients with ARDS.

Results: The *+936TT* (OR=4.29; 95%CI, 1.12-16.40; p=0.03) and *+936CT+TT* (OR=1.98; 95%CI, 1.14-3.42; p=0.01) genotypes were significantly associated with increased ARDS mortality. Plasma VEGF levels in ARDS patients with the *+936CT+TT* genotype (median 49pg/ml, IQR 16-98pg/ml) were significantly (p=0.02) lower than that in subjects with the *+936CC* genotype (median 112pg/ml, IQR 47-162pg/ml). At haplotype levels, haplotype *TCT (-460T+405C+936T*) was significantly associated with higher ARDS mortality (OR=2.89; 95%CI, 1.30-6.43; p=0.009). Haplotype *CGT (-460C+405G+936T*) was associated less strongly with increased ARDS mortality (OR=1.90; 95%CI, 0.94-3.84; p=0.07). Lower plasma VEGF levels were correlated to the probability of haplotype *CGT* (coefficient=-0.26, $p<0.05$) but the same trend of correlation was not significant to haplotype *TCT*.

Conclusions: *VEGF* polymorphisms may contribute to ARDS prognosis and inter-individual variations in circulating VEGF levels.

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Acute respiratory distress syndrome (ARDS) is lung injury characterized by alveolar injury and increased pulmonary vascular permeability (1-2). The pathogenetic basis of ARDS is incompletely understood; however, emerging evidence has suggested that severity and outcome of ARDS depend significantly on the balance between alveolar epithelial and/or vascular endothelial injuries and their repair mechanisms (2-6).

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that promotes angiogenesis and mediates vascular permeability (6-7). Over-expression of VEGF in the lungs induces an increased pulmonary vascular permeability, resulting in pulmonary edema (8). However, VEGF expression in human lung epithelial cells can also increase neovascularization, thereby contributing to the repair of endothelium injuries (6, 9). In human lungs, low VEGF levels were associated with the severity of ARDS, while elevated VEGF levels were associated with ARDS recovery, indicating a role of VEGF in the repair process of lung injury (10-13).

The *VEGF* gene is located on chromosome 6p31.3. Several functional single nucleotide polymorphisms (SNPs) in this gene have been described in the literature. The *+936C/T* polymorphisms (rs3025039) have been related to lower levels of plasma VEGF (14-15), whereas the *-460C/T* (rs833061) and *+405C/G* (rs2010963) polymorphisms have been shown to significantly increase VEGF production (16). Recent studies have indicated that these three SNPs are implicated in the risk of several disorders in which vascular injury and acceleration of inflammation are critical in disease development (17-18). However, the combined effect of these polymorphisms on ARDS risk and prognosis has not been evaluated. Based on the biological function of these three *VEGF* polymorphisms on VEGF production, and the pathologic significance of VEGF in ARDS, we hypothesized that *VEGF* polymorphisms may

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contribute to the development of and poor outcomes in ARDS and correlate to circulating VEGF levels in patients with ARDS.

METHODS

Study subjects

The present study was part of an ongoing research investigating the effects of multiple genetic variants on the risks or prognosis of ARDS. Recruitment of subjects was started in September 1999 and will continue until 2010. Details of the study have been described previously (19-20). Briefly, all admissions to the intensive care units (ICU) at Massachusetts General Hospital (MGH, Boston, MA) were screened daily for study-defined clinical risk factors for ARDS such as sepsis, trauma, aspiration or massive transfusions and were followed prospectively during their ICU stay for the development of ARDS (Figure 1.). Subjects were classified as ARDS cases if they were intubated on positive ventilation and met the American European Consensus Committee (AECC) criteria for ARDS: (1) Pao_2/fraction of inspired oxygen ratio of ≤ 200mmHg; (2) bilateral infiltrates seen on chest radiographs; and (3) pulmonary arterial occlusion pressure≤ 18 mm Hg or no clinical evidence of left atrial hypertension. All cases of ARDS were followed for all causes 60-day mortality. This study was approved by The Human Subjects Committees of MGH and the Harvard School of Public Health (Boston, MA), and informed written consent was obtained from all subjects or their appropriate surrogates.

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DNA isolation and genotyping assays

Genome DNA was extracted from peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. The

allelic discrimination of the *VEGF* gene was assessed with the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster city, CA), using the fluorogenic 5' nuclease

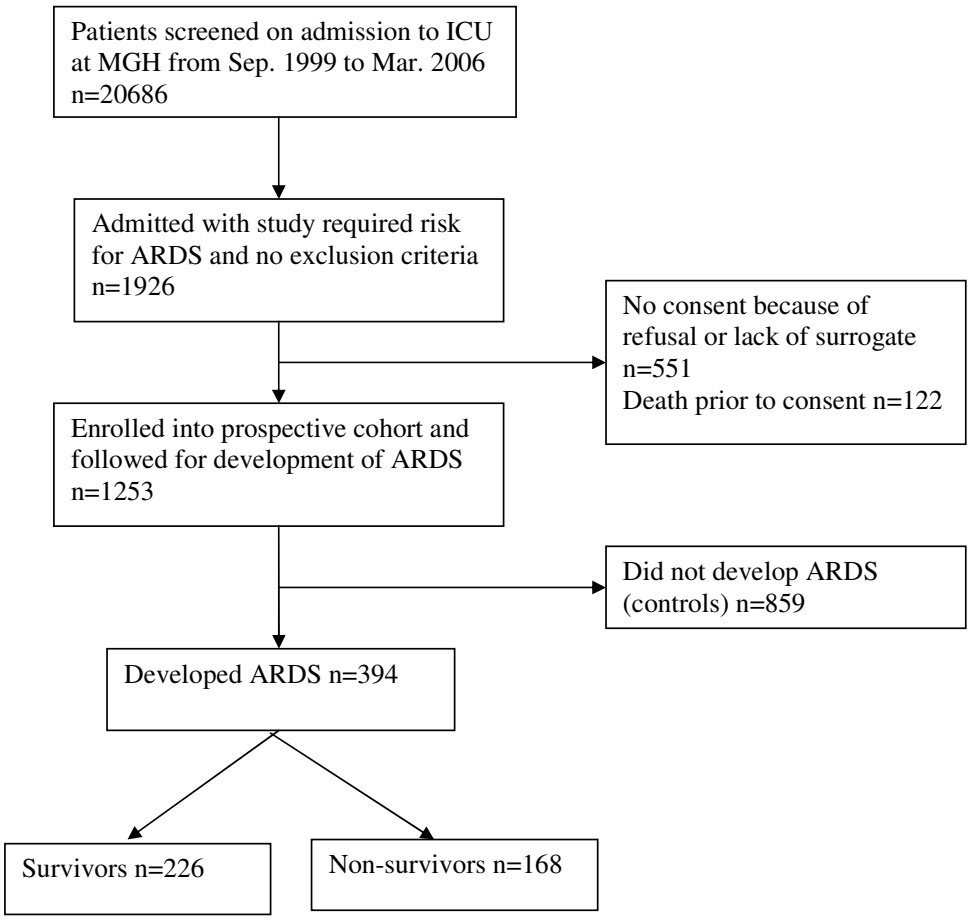

Figure 1. Flow diagram of study design and patient selection for the case-control study. ICU: Intensive care unit. MGH: Massachusetts General Hospital, Boston, MA, USA. Patients who had previous history of ARDS, who were previously admitted to the ICU were excluded from being controls**.** Subjects listed in this Figure were all Caucasians. Non-caucasians were also enrolled but they were excluded from the analysis because they accounted for only 4% of all enrollment.

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assay with Taqman Minor Groove Binder (MGB) probes. The wide-type Taqman MGB probes were FAM labeled and the mutant probes were VIC labeled. The primers and probes

for the *-460C/T*, *+405C/G*, and the *+936C/T* polymorphism assays were ordered from Applied Biosystems. Genotyping was performed by laboratory personnel blinded to subject status, and a random 10% of the samples were repeated to validate genotyping procedures. Two authors reviewed independently all genotyping results.

Analysis of VEGF levels in plasma

Plasma samples from day 2 after development of ARDS were available for 71 ARDS patients for the measurement of circulating VEGF levels. Blood samples were collected in 10ml vacuum tubes and were centrifuged for 10 minutes. Plasma samples were stored at -80^{0}C until analysis. Plasma VEGF levels were quantified in duplicate according to the manufacturer's recommendations with commercially available ELISA kit (R&D Systems, MN, USA). No statistically significant differences were observed between patients with plasma samples and those without plasma samples in terms of age, gender, APACHE III, and risk factors for ARDS.

Statistical analysis

The demographic variables between different groups were compared by chi-square tests for categorical variables and by Student *t* test and or nonparametric test for continuous variables. The Hardy-Weinberg-equilibrium was evaluated using Chi-square test. Logistic regression model was used to assess the effect of *VEGF* polymorphisms on the mortality of ARDS with adjustments for potential confounding factors such as age, gender, APACHE III score, diabetes, history of steroid use, number of units of red cells transfused, and chronic liver disease. Linkage disequilibrium between the SNPs, haplotypes and their frequencies was estimated using the expectation maximization algorithm. Association between haplotypes and

risk of ARDS was assessed using an "expectation substitution" approach to account for unknown phase (21). Haplotypes were coded as an additive fashion. ARDS mortality was regressed on haplotype counts by logistic regression, using the most common haplotype as reference haplotype. The multiple Cox regression model was applied to test the effect of the *VEGF* polymorphisms on overall survival, adjusting for confounding factors such as age, gender, APACHE III score, diabetes, history of steroid use, and chronic liver disease. The correlations between haplotype probabilities and plasma VEGF levels were estimated by Spearman correlation test. All statistical analysis were performed using the SAS statistical software package (version 9.1, SAS Inc., Cary, NC).

RESULTS

Patient population

Between September 1999 and March 2005, 1253 Caucasian patients with risk factors of ARDS were enrolled into the prospective cohort. In this study population, 394 (31.4%) were diagnosed as ARDS cases and 859 did not develop ARDS (Figure 1).

Table 1. Characteristics between survivals and non-survivors in patients with ARDS

Covariables	Non-survivors (n=168)	Survivors (n=226)	p
Age (mean±SD)	67.98 (±14.75)	53.88 (±18.62)	<0.01
Female No (%)	72 (42)	92 (41)	0.67
APACHE III score (mean±SD)	89.60 (±22.28)	68.95 (±21.15)	<0.01
Diabetes N (%)	31 (18)	38 (17)	0.70
Chronic liver disease N (%)	8 (10)	6 (3)	<0.01
History of steroid use N (%)	27 (16)	19 (8)	0.02
Bilirubin>2.0mg/L N (%)	41 (27)	27 (14)	<0.01
Creatinine>2.0mg/L N (%)	63 (38)	54 (24)	0.10
Haematological failure N (%) (platelets $\leq 80000 mm^{-3}$)	48 (29)	35 (16)	<0.01

Clinical risk factors for ARDS and baseline characteristics between subjects with and without ARDS are shown in Table 1 and Table 2 in the online data supplement. Among 394

ARDS cases studied, the 60-day mortality was 42.6% (168/394). Logistic analysis showed that older age, APACHE III, steroid use, hematological failure, and liver disease were the major prognostic factors for survival (Table 1).

***VEGF* genotypes in relation to and ARDS mortality**

Genotyping of the *-460C/T, +405 C/G,* and *+936 C/T* polymorphisms was successfully achieved for all subjects and followed the Hardy-Weinberg equilibrium. The genotype frequencies of these three polymorphisms in the current study were broadly similar to those reported by others in Caucasian populations (22-28). When overall ARDS cases were compared with non-ARDS patients, no significant difference was found in the distribution of genotypes for any polymorphisms studied （P>0.05）.

We tested for association between ARDS mortality and any of the three SNPs among the 394 patients with ARDS (Table 2). No significant association was found between the *-460C/T* or *+405C/G* polymorphisms and the risk of ARDS mortality (P>0.05). However, the *+936TT* genotype was significantly associated with higher ARDS mortality as compared with the *+936CC* genotype (adjusted OR=4.29; 95%CI, 1.12-16.40; p=0.01). Similar association was also observed for the *+936CT* genotype, but this association was not statistically significant (adjusted OR=1.60; 95%CI, 0.90-2.80; p=0.10). When the mutant *+936TT* and *+936CT* were combined to form a united genotype, this combined *+936CT+TT* genotype was significantly associated with increased ARDS mortality compared with *+936CC* genotype (adjusted OR=1.98; 95%CI, 1.14-3.42; p=0.01). No significant interactions between any genotypes and age, gender, or types of injury were detected in the present study.

Table 2. Associations of *VEGF* genotypes and ARDS mortality

Genotypes	Genotype frequency (%)	OR* (95% CI)	p* value
All patients (n=394)			
460TT	29.4	1.00	
460CT	46.7	1.33 (0.83-2.14)	0.24
460CC	23.9	0.77 (0.44-1.35)	0.37
405GG	43.9	1.00	
405CG	43.4	1.37 (0.84-2.24)	0.20
405CC	12.7	0.87 (0.43-1.74)	0.69
936CC	73.9	1.00	
936CT	22.8	1.60 (0.90-2.80)	0.10
936TT	3.3	**4.29** (1.12-16.40)	**0.03**
936CT+TT	26.1	**1.98** (1.14-3.42)	**0.01**
Ptrend#		**1.93** (1.10-1.94)	**0.005**

*Estimated by Logistic regression models, adjusting for age, gender, history of alcohol abuse, diabetes, steroid use, APACHE III score, and multiple (>1) risk factors for ARDS.
The genotypes were coded as continuous variables (1, 2, and 3 for wild type, hyterozygous, and homozygous genotype respectively) and entered together into the logistic regression model.

Association between *VEGF* haplotypes and ARDS mortality

Haplotype analyses were conducted to evaluate the combined effect of the three polymorphisms on ARDS survival. Consistent with observations from early studies (29), *VEGF -460C/T* and *+405C/G* polymorphisms in our study were in strong linkage disequilibrium (correlation coefficient, R=0.68; Lewontin's D`, D`=0.99), whereas their linkage with the *+936C/T* polymorphism was much weaker (R=0.04; D`=0.1 and R=0.1; D`=0.20, respectively). Six haplotypes with frequencies greater than 1% were estimated of the eight possible haplotypes (Table 3). No association was detected between any haplotype and risk of ARDS development (P>0.05). Interestingly, the *-460T+405C+936T (TCT)* haplotype

was significantly associated with increased risk of ARDS mortality (adjusted OR=2.89; 95%CI, 1.30-6.43; p=0.009), and the *-460C/+405G/+936T (CGT)* haplotype was marginal significantly associated with higher risk of ARDS mortality (adjusted OR=1.90; 95%CI, 0.94-3.87; p=0.07). No associations were demonstrated between other haplotypes and ARDS mortality in this study population.

Table 3. Associations of *VEGF* haplotypes and ARDS mortality

Haplotypes[#]	Haplotype frequency (%)	OR* (95% CI)	p value
Global test			0.026
CGC	39.6	1.0	
TCC	28.9	0.85 (0.59-1.24)	0.4
TGC	16.6	0.95 (0.58-1.55)	0.8
CGT	7.4	**1.90 (0**.94-3.83)	**0.07**
TCT	5.3	**2.89** (1.30-6.43)	**0.009**
TGT	1.7	1.09 (0.20-5.84)	0.92

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[#] The order of the polymorphisms is as follows: *-460C/T, +405C/G, +936C/T.*
* Estimated by Logistic regression models, adjusting for age, gender, history of alcohol abuse, diabetes, steroid use, APACHE III, multiple risk of (>1) of ARDS.

Relationship between *VEGF* polymorphisms and plasma VEGF levels

To investigate the relationship between *VEGF* polymorphisms and circulating VEGF levels, plasma VEGF levels were categorized according to *VEGF* polymorphisms. Since the plasma VEGF values were not normally distributed (Kolmogorov-Smirnov test, p=0.01), a nonparametric median two-sample test was used to compare the VEGF levels between different genotype carriers. Median plasma VEGF level in individuals carrying the *+936CT+TT* genotype (median=49pg/ml; IQR, 16-98) was significantly (p=0.02; power>0.99) lower than that in individuals with the *VEGF+936CC* genotype (median=112pg/ml; IQR, 47-162). Based on the sample sizes and median plasma VEGF levels between the *+936CT+TT* and the *VEGF+936CC* carriers, the power to detect a difference at α=0.05 level was

calculated to be 99%. At haplotype levels, the plasma VEGF levels were inversely correlated with haplotype *CGT* probability (Spearman coefficient, -0.26, $p<0.05$). A similar correlation was also observed between plasma VEGF levels and the haplotype *TCT* probability, but this correlation did not reach statistical significance (coefficient, -0.14, $p=0.26$). Thus, haplotypes containing the *936T* allele tended to correlate to lower plasma VEGF levels. There was no significant difference between survivors and non-survivors in plasma VEGF levels ($p>0.05$).

Association between *VEGF* polymorphisms and ARDS Survival

Cox proportional hazard model was used to analyze the associations between *VEGF* polymorphisms and ARDS survival. After adjusting for other predictors of survival (age, gender, APACHE III score, diabetes, history of steroid use, hematological failure, and chronic liver disease), both the *+936CT+TT* genotype (HR=1.71; 95% CI, 1.09-2.72; $p=0.02$) and haplotype *TCT* (HR=2.11; 95%CI, 1.32-3.37; $p<0.01$) were significantly associated with lower ARDS survival, consistent with the results assessed by Logistic regression models.

DISCUSSION

Our data showed that the *+936TT* and *+936CT+TT* genotypes of *VEGF* gene were significantly associated with increased risk of ARDS mortality. At haplotype levels, the *TCT haplotype* was significantly associated with higher ARDS mortality. In addition, we demonstrated that *VEGF* polymorphisms contributing to increased ARDS mortality were correlated with lower plasma VEGF levels.

This study has a number of strengths. Firstly, the prospective determination of ARDS using the AECC definition minimized phenotype misclassification, since there is no diagnostic gold standard for ARDS. Secondly, the *VEGF* polymorphisms and the circulating VEGF levels were determined in a parallel manner. Thus, the functional effect of *VEGF* polymorphisms can be assessed. Thirdly, restricting analyses to patients of Caucasian reduced the possibility of altered genotype frequencies in different ethnicities.

Among these three functionally SNPs, none of the *–460C/T* and *+405C/G* polymorphisms was found to significantly influence ARDS mortality. However, the *+936TT* and the combined*+936CT+TT* genotypes were significantly associated with ARDS mortality, suggesting that *+936* polymorphisms may play a stronger role than either the *–460C/T* or *+405C/G* polymorphisms in *VEGF* gene functions. Recently, a smaller case-control study found that the frequency of the combined*+936CT+TT* genotype in ARDS cases was significantly higher than that in controls. This study also found that the *+936CT* and *TT* genotypes were associated with APACHE III score among the ARDS patients, but association between the *+936 CT /TT* genotype and increased ARDS mortality did not reach statistically significance (OR>1.80, p>0.05) (26). In the present, larger study, although *+936CT+TT* genotype was not associated with ARDS susceptibility, a significant association

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between the *+936CT+TT* genotype and ARDS mortality was found. At haplotype level, haplotype containing the *+936T* allle was also associated with ARDS mortality, , further supporting the effects of *+936C/T* variants on ARDS outcomes.

Our data showed that the *+936 CT/TT* genotype was significantly correlated to lower plasma VEGF levels, consistent with the functions of this polymorphism reported by other studies (14-15). Although the plasma VEGF levels were measured at only one point that may not reflect fully the temporal VEGF response over the course of ARDS, our results suggested that VEGF may be a protective molecule in ARDS severity. Decreased VEGF concentrations in the lungs have been correlated to higher lung injury score and lower ARDS survival rates (10, 11, 31), whareas increased VEGF production in the lungs resulted in less lung injury (33). In animal models, administration of VEGF could prevent lung injury-induced death (30, 32, 33). In alveolar epithelial cells VEGF mRNA expression increased during recovery from oxygen injury (34). In neonatal rats exposed to high oxygen, intraocular injection of VEGF at the onset of experimental hyperoxia prevents apoptotic death of endothelial cells and rescues the retinal vasculature (35). Taken together, these findings indicate that the VEGF may function as a protective factor in ARDS.

Although the haplotypes predicted in the present study were identical to those previously reported, the overall distribution of haplotype frequencies in the present study was different from that in Chinese and Korean populations (29, 36). Different haplotype frequencies in different populations may be due to different ethnic backgrounds or different disease states. Interestingly the frequencies of *TCT* (5.3%) and *CGT* (7.4%) haplotypes in the present study were similar to those reported in the Chinese population (6.0% and 6.4% for *TCT* and *CGT*, respectively) (29). In Asian populations, both the *TCT* and *CGT* haplotypes were significantly

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implicated in reduced risk of lung cancer, and the *CGT* was associated with decreased breast survival (29, 36). These observations suggested that *TCT* and *CGT* haplotypes may play important roles in disease processes. However, since these previous studies did not measure circulating VEGF levels, the mechanisms connecting the *VEGF* haplotypes and disease development or severity were unclear. Our study not only indicated that the *TCT* haplotype was associated with ARDS mortality, but also demonstrated that the *CGT* and *TCT* haplotypes correlated with lower circulating VEGF protein levels, suggesting that these functional haplotypes may contribute to ARDS mortality by affecting VEGF production.

We acknowledge some potential limitations to this study. Firstly, sample size in this study was relatively small. Additional studies with larger populations are required to confirm the associations between the *VEGF* haplotypes and ARDS mortality. Secondly, the *VEGF* gene is a polymorphic gene, and the present study only investigated three SNPs. Further studies on other functional *VEGF SNPs* are needed to define the role of the *VEGF* polymorphisms in ARDS prognosis. Thirdly, plasma VGEF measurement was done at one time point so the role of plasma VEGF level in the natural history of ARDS remained to be defined. Finally, since genetic polymorphisms often vary between ethnic groups, further studies are necessary to clarify the association between the *VEGF* polymorphisms and ARDS outcome in diverse ethnic populations.

In summary, our study suggested that *+936TT*, *+936CT+TT* genotypes and *TCT* haplotype of *VGEF* gene are attributable to increased ARDS mortality and to inter-individual variations in plasma VEGF levels. The associations found in this study are biologically plausible and may have significant implications for ARDS treatment.

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REFRENCES

1 **Matthay MA**, Zimmerman GA, Esmon C, *et al.* Future research directions in acute lung injury: summery of a National Heart, Lung, and Blood Institute working group. *Am J Respir Criti Care Med* 2003; **167:** 1027-35.

2 **Piantadosi CA**, Schwartz DA. The acute respiratory distress syndrome. *Ann Intern Med* 2004; **141:** 460-70.

3 **Berthiaume Y**, Lesur O, Dsgenais A. Treatment of adult respiratory distress syndrome: plea for rescue therapy of the alveolar epithelium. *Thorax* 1999; **54:** 150-60.

4 **Bernard GR**, Artigas A, Brigham KL, *et al*. The American –European Consensus Conference on ARDS. Definition, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994: **149:** 818-24.

5 **Ware LB**, Conner ER, Matthay MA von Willebrand factor antigen is an independent marker of poor outcome in patients with early acute lung injury. *Criti Care Med* 2001; **29:** 2325-31.

6 **Mura M**, dos Santos CC, Stewart D, *et al.* Vascular endothelial growth factor and related molecules in acute lung injury. *J Appl Physiol* 2004; **97:** 1605-17.

7. **Boussat S**, Eddahibi S, Coste A, *et al.* Expression and regulation of vascular endothelial growth factor in human pulmonary epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2000; **279:** L371-L78.

8. **Ferrara N**. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 2004; **25:** 581-11.

9. **Kaner RJ**, Ladetto JV, Singh R, *et al.* Lung overexpression of the vascular endothelial

growth factor gene induces pulmonary edema. *Am J Respir Cell Mol Biol* 2000; **22:** 657-64.

10 **Thickett DR**, Armstrong L, Millar AB. A role for vascular endothelial growth factor in acute and resolving lung injury. *Am J Respir Criti Care Med* 2002; **166:** 1332-37.

11 **Abadie Y**, Bregeon F, Papazian L, *et al*. Decreased VEGF concentration in lung tissues and vascular injury during ARDS. *Eur Respir J* 2005; **25:** 139-146.

12 **Hanaoka M**, Dromam Y, Naramoto A, *et al*. Vascular endothelial growth factor in patients with high-altitude pulmonary edema. *J Appl Physiol* 2003; **94:** 1836-40.

13 **Maniscalco WM**, Watkins RH, Finkelstein JN, *et al.* Vascular endothelial growth factor mRNA increases in alveolar epithelial cells during recovery from oxygen injury. *Am J Respir Cell Mol Biol* 1995; **13:** 377-86.

14 **Renner W**, Kotschan C, Hoffman B, *et al.* A common *936C/T* mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res* 2000; **37:** 443-48.

15 **Sciacca FL**, Ciusani E, Silvani A, *et al*. Genetic and plasma markers of venous thromboembolism in patients with high grade Glioma. *Clin Cancer Res* 2004; **10:** 1312-17.

16 **Watson CJ**, Webb NJA, Bottomley MJ, *et al*. Identification of polymorphisms within the vascular endothelial growth factor (*VEGF*) gene: correlation with variation in VEGF protein production. *Cytokine* 2000; **12:** 1232-35.

17 **Han SW**, Kim GW, Soe JS, *et al*. *VEGF* gene polymorphisms and susceptibility to rheumatoid arthritis. *Rheumatology* 2004; **43:** 1173-77.

18 **Shahbazi M**, Fryer AA, Pravica V, *et al***.** Vascular endothelial growth factor gene

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polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol* 2002; **13:** 260-64.

19 **Gong MN**, Zhou W, Xu LL, Miller D, *et al*. Polymorphism in the surfactant protein-B gene, gender, and the risk of direct pulmonary injury and ARDS. *Chest* 2004; **125:**203-11.

20 **Gong MN**, Zhou W, Williams P, *et al.* *-380GA* and *TNFB* polymorphisms in acute respiratory distress syndrome. *Eur Respir J* 2005; **26:**382-89.

21 **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:** 261-72.

22 **Sakao SK**, Tatsumi T, Hashimato H, *et al.* Vascular endothelial growth factor and the risk of smoking –related COPD. *Chest* 2003; **124:** 323-27.

23 **Marohashi K**, Takada T, Omori K, *et al*. Vascular endothelial growth factor gene polymorphisms in Japanese patients with Sarcoidosis. *Chest* 2003; **123:** 1520-26.

24 **Awata T**, Inoue K, Kurihara S, *et al.* A common polymorphism in the 5'-untranslated region of the *VEGF* gene is associated with diabetic retinopathy in type 2 diabetes. *Diabetes.* 2002; **51:** 1630-35.

25 **Stevens A**, Soden J, Brenchley PE, *et al.* Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. *Cancer Res* 2003; **63:** 812-16.

26 **Medford ARL**, Keen LJ, Bidwell JL, *et al*. Vascular endothelial growth factor gene polymorphism and acute respiratory distress syndrome. *Thorax* 2005; **60:** 244-48.

27 **Krippl P**, Langsenlehner U, Renner W, *et al.* A common *936 C/T* gene olymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk.

Int J Cancer 2003; **106:** 468-71.

28 **Cancer Genome Anatomy Project**: SNP500 cancer Database. http://snp500cancer.nci.nih.gov/home_1.cfm

29 **Lu H,** Shun XO, Cui Y, *et al.* Association of genetic polymorphisms in the *VEGF* gene with breast cancer survival. *Cancer Res* 2005; **65:** 5015-9.

30 **Corne J,** Chupp G, Lee CG, *et al.* IL-13 stimulates vascular endothelial cell growth factor and protects against hyporoxic acute lung injury. *J Clin Invest* 2000; **106:** 783-91.

31 **Maitre B**, Boussat S, Jean D, *et al*. Vascular endothelial growth factor synthesis in the acute phase of experimental and clinical lung injury. *Eur Respir J* 2001; **18:** 100-06.

32 **Compernolle V**, Brusselmans K, Acker T, *et al*. Loss of HIF-2 alpha and inhibition of VEGF impair foetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. *Nat Med* 2002; **8:** 702-10.

33 **Corne J**, Chupp G, Lee CG, *et al.* IL-13 stimulates vascular endothelial cell growth factor and protects against hyporoxic acute lung injury. *J Clin Invest* 2000; **106:** 783-91.

34 **Maniscalco WM**, Watkins RH, Finkelstein JN, *et al*. Vascular endothelial growth factor mRNA increases in alveolar epithelial cells during recovery from oxygen injury. *Am J Respir Cell Mol Biol* 1995; **13:** 377-86.

35 **Alon T**, Hemo I, Itin A, *et al*. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995; **10:** 1024-28.

36 **Lee SJ,** Lee SY, Jeon HS, *et al.* Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. *Cancer Epidemiol Biomarker Preven* 2005; **14:** 571-75.