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-*308GA* and *TNFB* polymorphisms in acute respiratory distress syndrome

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ABSTRACT: The *-308GA* and *TNFB1*/2 polymorphisms of the tumour necrosis factor genes have been associated with increased susceptibility to, and mortality in sepsis, although, prior studies are not consistent. Their role in acute respiratory distress syndrome (ARDS) has not been evaluated. The current authors hypothesised that the *-308A* allele and *TNFB22* genotype would be associated with increased susceptibility to, and mortality in ARDS.

The above hypothesis was investigated in a nested case-control study of 441 Caucasian controls and 212 cases admitted to an intensive care unit with sepsis, trauma, aspiration or hypertransfusions.

The *-308A* and *TNFB1* alleles were in linkage disequilibrium. These polymorphisms were not associated with ARDS susceptibility on crude analysis. On subgroup analyses, they were associated with either increased or decreased odds of developing ARDS depending on whether the clinical risk for ARDS results in direct or indirect pulmonary injury. The *-308A* allele was associated with increased 60-day mortality in ARDS, with the strongest association found among younger patients. There was no association between the *TNFB* polymorphism and ARDS mortality.

The *-308GA*, but not the *TNFB12*, polymorphism was associated with increased mortality in acute respiratory distress syndrome, but their association with acute respiratory distress syndrome susceptibility depended on the site of injury predisposing to acute respiratory distress syndrome.

KEYWORDS: Acute respiratory distress syndrome, genetic susceptibility, tumour necrosis factor

Understanding why some individuals develop and subsequently die from acute respiratory distress syndrome (ARDS), while others do not, is incomplete. Although clinical predictors such as sepsis and trauma are well recognised, only a minority of patients with these risks develop ARDS [1]. It is likely that after the same type and degree of insult, individual differences in susceptibility to developing and dying from ARDS exist. Genetic susceptibility to acute lung injury may explain the observed inter-individual differences in risk and outcomes [2–4].

Studies on the role of tumour necrosis factor (TNF)-α in ARDS have been conflicting. Some found plasma TNF-α to correlate with the development of, and mortality in, ARDS [5–7], while others did not [8, 9]. These discrepancies may be due to temporal and regional variation in TNF-α release [10, 11] and to differences in assay techniques [12]. Clinical heterogeneity and genetic variability in the production of TNF-α may also contribute to such discrepancies [13].

The *-308GA* promoter polymorphism in the TNF-α gene and the *TNFB1/2 Nco*I restriction fragment length polymorphism (RFLP) in the TNF-β gene appear to influence TNF-α level. Carriers of the *-308A* allele (*-308A*) and homozygotes for the *TNFB2* allele (*TNFB22*) have increased TNF-α production [14, 15] and increased susceptibility to, or increased mortality in, septic shock in some studies [15–19], but not in others [20, 21].

In recognition of the clinical heterogeneity in ARDS and the differences between pulmonary and extra-pulmonary ARDS [22], the American European Consensus Conference (AECC) on ARDS suggested dividing aetiologies of lung injury into direct or indirect pulmonary injuries [23]. Recently, the -1580C/T missense mutation in the surfactant protein-B gene was linked to ARDS in patients with direct, but not indirect pulmonary injury [2]. Thus, it is possible that any associations of the *-308GA* and *TNFB1/2* polymorphisms on the development of ARDS may vary with direct *versus* indirect pulmonary injury.

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The present authors describe a nested case-control study of patients at risk for ARDS and hypothesised that the *-308A* of the *-308GA* polymorphism and the *TNFB22* of the *TNF*-β gene would be associated with increased susceptibility to, and increased mortality in, ARDS. In addition, association was explored to determine if it might differ by direct *versus* indirect pulmonary injury.

METHODS

Study subjects

A schematic of the study design is illustrated in figure 1. Admissions to the intensive care units (ICU) of the Massachusetts General Hospital (Boston, MA, USA) were screened daily for study-defined clinical risk factors for ARDS as previously described (table 1) [3]. Exclusion criteria included age <18 yrs, diffuse alveolar haemorrhage or chronic lung diseases and directive to withhold intubation. Patients with immunosuppression or treatment with granulocyte colony-stimulating factor were excluded. After November 2000, patients with immunosuppression secondary to corticosteroid were no longer excluded because of increasing use of steroids in sepsis. Sensitivity analyses revealed that patients enrolled before and after the change in exclusion criteria had identical rates of ARDS (34%), ICU mortality (22%) and ARDS mortality (46%). Adjusting for whether the patient was enrolled before or after dropping steroids as an exclusion criterion, changed the estimates for *-308A* and ARDS mortality <1%.

ICU admissions with at least one defined risk factor for ARDS and no exclusion criteria were eligible for the prospective cohort. The Human Subjects Committees (Boston, MA, USA) approved the study and informed written consent was obtained from all subjects or their appropriate surrogates.

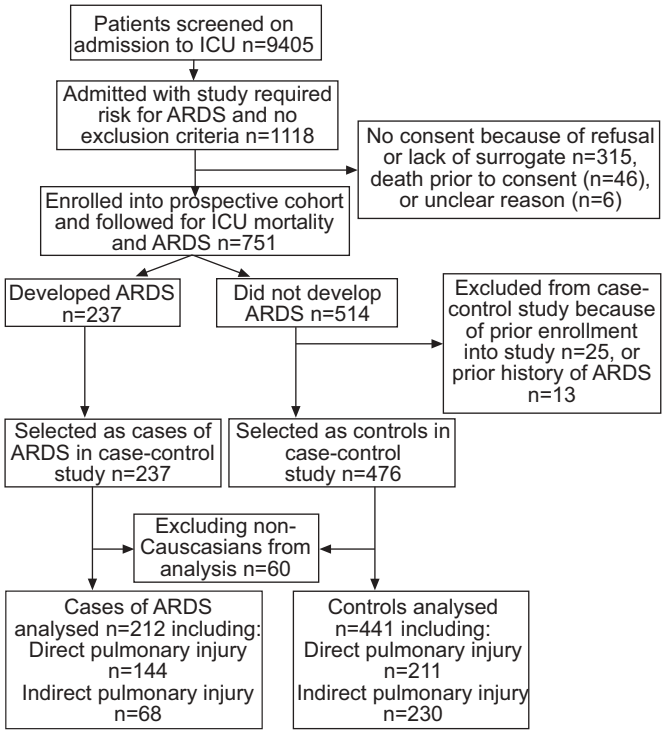

FIGURE 1. Flow diagram of study design and patient selection for the case-control study. ICU: intensive care unit; ARDS: acute respiratory distress syndrome.

TABLE 1 Study-required risk factors for acute respiratory distress syndrome on admission to an intensive care unit

Sepsis	As defined by BONE *et al.* [24] to be a known or suspected source of systemic infection and at least two of the following: 1) temperature >38℃ or <36℃; 2) heart rate >90 bpm; 3) respiratory rate >20 bpm or P_{a,CO_2} <32 mmHg; 4) WBC >12000·mm^{-3}, <4000·mm^{-3} or >10% bands.
Septic shock	Fulfil requirements for sepsis and one of the following: 1) SBP <90 mmHg or reduction of ⩾40 mmHg from baseline for at least 30 min unresponsive to 500 cc fluid resuscitation. 2) The need for vasopressors to maintain SBP⩾90 mmHg or within 40 mmHg of baseline.
Pneumonia	Fulfil two or more of the following: 1) new infiltrate on CXR; 2) temperature >38.3℃ or <36.0℃, WBC >12000 or <4000·mm^{-3} or >10% bandaemia; 3) positive microbiological culture.
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 infiltrates on CXRs within 8 h of admission to the emergency room and evidence of blunt trauma to the chest, *e.g.* fractured ribs or ecchymosis overlying the infiltrate.
Multiple transfusions	Defined as receiving ⩾8 units of packed RBC within 24 h.
Aspiration	Defined as witnessed or documented aspiration event or the retrieval of gastric contents from the oropharynx, endotracheal tube or bronchial tree.

P_{a,CO_2}: carbon dioxide arterial tension; WBC: white blood cells; SBP: systolic blood pressure; CXR: chest radiograph; RBC: red blood cells.

Study design

Baseline clinical information was collected on admission to an ICU. Vital signs and laboratory parameters in the first 24 h after ICU admission were collected for calculation of Acute, Physiology, Age and Chronic Health Evaluation (APACHE) III [25]. Subjects were screened daily for ARDS, defined by respiratory failure requiring intubation and AECC criteria as previously described [3, 23]. Daily chest radiographs were interpreted by two pulmonary and critical care physicians after consensus training on interpretation of infiltrates with disagreements arbitrated by a third physician. All were blinded to the clinical status of the patients. The κ-score for agreement between initial interpretation for bilateral infiltrates was 0.75 (95% confidence interval (CI): 0.62–0.89), comparable to other reports [26].

From the prospective cohort, a case-control study was designed. Cases were those who fulfilled criteria for ARDS during their hospitalisation. All patients who did not develop ARDS during their hospitalisation and had no prior history of ARDS or prior enrolment into the study were selected as controls. All patients were followed for ICU mortality and

ARDS cases were followed for all causes of mortality within a 60-day period.

Methods

Blood (10cc) was collected for DNA extraction and PCR amplification. Genotyping was performed with pyrosequencing for *-308GA* polymorphism (Pyrosequencing AB, Uppsala, Sweden), with the forward primer of 5′-CCAAACACAGGCCTCAGGACTC-3′, biotinylated reverse primer of 5′-TCCTCCCTGCTCCGATTCCG-3′ and sequencing primer of 5′-AGGCAATAGGTTTTGAGGGGCA-3′. The *TNF*-β *Nco I* polymorphism was detected using a modified PCR-RFLP method that involved published primer sequences and Nco I enzyme digestion (New England BioLabs, Beverly, MA, USA) [19]. Results were interpreted by two independent investigators, and genotyping was repeated in a random 5% of samples. Laboratory personnel and research assistants were blinded to the case-control status or genotype of the subjects.

Analysis

Conformity to Hardy Weinberg Equilibrium was determined with a Chi-squared goodness of fit test. Univariate analysis was performed using Fisher's Exact Test, Chi-squared trend test, ANOVA or Wilcoxon Rank Sum tests as appropriate. Variables with p-value $\leqslant 0.2$ on univariate analyses were studied in a backwards selection algorithm and eliminated if they did not meet a p-value of $\leqslant 0.2$. The final multivariate logistic regression model included the gene effect, results from backwards elimination, significant interactions and clinically relevant parameters such as APACHE III scores for the development of ARDS and septic shock for mortality in ARDS. Possible confounders were adjusted for in the final model, rather than matched between cases and controls on analysis. Given the small number of *-308A* homozygotes, homozygotes for the *-308A* allele were grouped with the heterozygotes (*-308A*), and compared with *-308G* homozygotes in the final model, as in prior reports [15, 16]. As the distribution of the *TNFB* genotype among cases and controls did not clearly suggest a dominant or recessive model, the *TNFB* genotype was modelled as in previous reports [17, 18] by comparing *TNFB2* homozygotes (*TNFB22*) with homozygotes and heterozygotes for *TNFB1*. C-statistics (area under the receiver operating characteristic curve) were used to evaluate model fit [27]. An *a priori* decision was made to stratify the analysis by direct *versus* indirect pulmonary injury. Effect modification was tested with an interaction term. A p-value of 0.05 was considered statistically significant.

Detection of linkage disequlibrium between the two polymorphisms was based on Lewontin's D̀ in controls [28]. Haplotypes of the two TNF polymorphisms were generated using the Partition Ligation-Expectation Maximization (PL-EM) version 1.0 [29], as in other association studies [30]. This software uses an efficient variant of the EM algorithm, to reconstruct individual probabilities for individual phasing accuracy based on unphased genotype data, while providing estimates on the overall haplotype frequencies, as well as their standard errors. Odd ratios (OR) were estimated by comparing the individuals with one or more copies of the haplotype of interest with the individuals without the haplotype.

In the initial study design, assuming an α-error of 0.01 (to allow for multiple comparisons), 80% power and allele frequencies of 16% for *-308A* [31] and 65% for *TNFB2* [17], a study with 560 cases and 1,120 controls would have a minimum detectable OR for the development of ARDS of 1.56 for *-308A* and 1.47 for *TNFB22*. Assuming 50% of patients had direct pulmonary injury as a risk for ARDS, the minimum detectable OR was 1.9 for *-308A* and 1.8 for *TNFB22*. Given the actual study size of 653 subjects and α-error of 0.05, the minimum detectable OR would be 1.78 for *-308A*, and 1.68 for *TNFB22*.

RESULTS

Patient population

The case-control study consisted of 237 ARDS cases and 476 controls, recruited between September 9, 1999 and October 15, 2002 (fig. 1). As 92% of the patients were Caucasians, analyses were restricted to the 212 Caucasian cases and 441 Caucasian controls.

Clinical risk factors for ARDS and baseline characteristics on admission to the ICU are shown in tables 2 and 3. ARDS developed a median of 1 day after ICU admission (25–75%; 0–3 days). Results from backwards selection for development of ARDS were; direct pulmonary injury ($p<0.001$), sepsis ($p=0.05$), trauma ($p<0.001$), age ($p=0.002$), female ($p=0.02$), diabetes ($p=0.004$), platelets $\leqslant 80{,}000 \cdot mm^{-1}$ ($p=0.005$), abnormal bilirubin $>2.0\ mg \cdot dL^{-1}$ ($p=0.1$) and blood transfusion ($p<0.001$). A statistically significant interaction was only found between the type of pulmonary injury (direct *versus* indirect) and the *–308A* allele or the *TNFB22* genotype.

Genotype analysis on -308GA and TNFB1/2 polymorphisms and development of ARDS

The allele frequencies were 0.16 for *-308A* and 0.69 for *TNFB2* alleles, similar to prior reports [19, 20, 30] with no departure from Hardy Weinberg Equilibrium among controls ($p>0.2$) and no discrepancy on repeat genotyping. The *-308GA* and *TNFB1/2* genotype did not differ by admission risk factor for ARDS ($p>0.4$). The *-308A* allele was linked to the *TNFB1* allele (D̀=0.90). Applying PL-EM algorithm, the posterior probabilities of individual haplotypes ranged from 0.96–1.0. Therefore, two haplotypes with the highest posterior probability were assigned to each individual.

The genotype frequencies among the cases and controls are detailed in table 4. Genotype and haplotype frequencies did not differ between cases and controls. The C-statistic for the multivariate model for ARDS was 0.73 and 0.74 for *-308A* and *TNFB22*, respectively. On multivariate analyses, an association with ARDS was found for *TNFB22* (adjusted OR: 0.47; 95% CI: 0.25–0.86; $p=0.01$), but not for *-308A* (adjusted OR: 1.7; 95% CI: 0.89–3.1).

After stratifying by direct *versus* indirect pulmonary injury, the association between ARDS and *-308A* or *TNFB22* differed according to the risk factor for ARDS (fig.2). The *-308A* allele was associated with decreased odds of developing ARDS among those with direct pulmonary injury (adjusted OR: 0.52; 95% CI: 0.30–0.91), but a nonsignificant increased odds of ARDS in indirect pulmonary injury (adjusted OR: 1.7; 95% CI: 0.93–3.2). Excluding the 69 patients, with both direct and indirect pulmonary

TABLE 2 Clinical risk factors for acute respiratory distress syndrome (ARDS) between cases, controls, survivors and nonsurvivors[#]

Risk for ARDS	Development of ARDS			Mortality in ARDS		
	Controls	Cases	p-value	Survivors	Nonsurvivors	p-value
Subjects n	441	212		114	98	
Sepsis syndrome	167 (38)	67 (32)	0.1	44 (39)	23 (23)	0.03
Pneumonia source	87 (20)	52 (25)	<0.001	33 (29)	19 (19)	0.6
Extra-pulmonary source	80 (18)	15 (7)		11 (10)	4 (4)	
Septic shock	196 (44)	115 (54)	0.02	52 (46)	63 (64)	0.009
Pneumonia source	89 (20)	79 (37)	<0.001	36 (32)	43 (44)	>0.9
Extra-pulmonary source	107 (24)	36 (17)		16 (14)	20 (20)	
Trauma	40 (9.0)	9 (4)	0.03	8 (7)	1 (1)	0.04
Multiple transfusions	51 (12)	26 (12)	0.8	13 (11)	13 (13)	0.7
Aspiration	34 (8)	24 (11)	0.1	13 (11)	11 (11)	>0.9
>1 Risk for ARDS	48 (11)	29 (14)	0.3	16 (14)	13 (13)	>0.9
Direct pulmonary injury[¶]	211 (48)	144 (68)	<0.001	79 (69)	65 (66)	0.7
Indirect pulmonary injury[+]	230 (52)	68 (32)		35 (31)	33 (34)	

Data are presented as n (%) or n, unless otherwise stated. [#]: the number of controls and cases with each risk adding up to >653 patients because of multiple risks in 77 patients; [¶]: pneumonia, aspiration or pulmonary contusions were categorised as direct pulmonary injury; [+]: sepsis from an extra-pulmonary source, trauma without pulmonary contusions and multiple transfusions were categorised as indirect pulmonary injury. Patients (n=69) with both direct and indirect pulmonary injuries were considered to have direct pulmonary injury.

TABLE 3 Baseline characteristics between cases of acute respiratory distress syndrome (ARDS), controls, survivors and nonsurvivors

	Controls	Cases	p-value	Survivors	Nonsurvivors	p-value
Subjects	441	212		114	98	
Females	179 (41)	101 (48)	0.09	50 (44)	51 (52)	0.3
Age yrs	69 (18–94)	65 (18–97)	0.05	51 (18–89)	73 (22–97)	<0.001
APACHE III[#]	64 (14–130)	68 (8–136)	0.07	69 (8–115)	88 (40–150)	<0.001
Diabetes[¶]	116 (26)	34 (16)	0.004	20 (18)	14 (14)	0.6
History of alcohol abuse	42 (10)	27 (13)	0.2	10 (9)	17 (17)	0.07
Tobacco abuse[+]	217 (49)	105 (50)	0.4	57 (50)	48 (49)	0.7
Chronic liver disease[¶]	18 (4)	12 (6)	0.2	4 (4)	8 (8)	0.2
End stage renal disease	23 (5)	6 (3)	0.7	2 (2)	4 (4)	0.4
History of steroid use	37 (8)	20 (9)	0.7	6 (5)	14 (14)	0.03
Transfusion of PRBC	218 (49)	133 (63)	0.001	64 (56)	69 (70)	0.03
Number of PRBC transfused	0 (0–74)	2 (0–63)	0.005	1 (0–31)	2 (0–63)	0.01
Systolic BP <90 mmHg	304 (69)	163 (77)	0.04	85 (75)	78 (80)	0.4
Creatinine >2.0 mg·L^{-1}	149 (34)	65 (31)	0.5	28 (25)	37 (38)	0.05
Bilirubin >2.0 mg·dL^{-1}	52 (12)	39 (18)	0.03	14 (12)	24 (24)	0.03
Haematological failure platelets ⩽80000·mm^{-3}	60 (14)	47 (22)	0.007	20 (18)	27 (28)	0.1

Data are presented as n, n (%) or median (range), unless otherwise stated. APACHE: Acute Physiology, Age and Chronic Health Evaluation; PRBC: packed red blood cells; BP: blood pressure. [#]:for development of ARDS, APACHE III physiology score for cases and controls were calculated without the arterial oxygen tension/inspiratory oxygen fraction component. For survivors and nonsurvivors in ARDS, the APACHE III physiology score was calculated with all components; [¶]: chronic health information was missing on one case and two controls; [+]: tobacco history was missing in 56 (26%) cases and 97 (22%) controls.

injury from the analysis, did not change the estimate for ARDS in direct pulmonary injury (adjusted OR: 0.52; 95% CI: 0.27–1.0). As might be expected from the linkage disequilibrium, the association between *TNFB22* and ARDS was opposite of that for *-308A*, (adjusted OR: 1.5; 95% CI: 0.91–2.3, and adjusted OR: 0.48; 95% CI: 0.26–0.87, in direct and indirect pulmonary injury, respectively). Results for the *-308A:TNFB1* haplotype was similar to *-308A* (fig. 2). The type of injury significantly modified the association between ARDS and *-308A* (p=0.01), *TNFB22* (p=0.006) and the

TABLE 4 Genotype and haplotype frequencies for the *-308GA* and *TNFB* polymorphism among cases, controls, survivors and nonsurvivors in acute respiratory distress syndrome

	Cases	Controls	p-value	Nonsurvivors	Survivors	p-value
Subjects	212	441		98	114	
***-308GA* polymorphism**[#]						
-308GG	73 (155)	73 (320)		65 (64)	80 (91)	
-308GA	22 (47)	24 (106)	0.6	28 (27)	18 (20)	<0.05
-308AA	5 (10)	3 (15)		7 (7)	3 (3)	
***TNFB* polymorphism**[#]						
TNFB11	8 (18)	8 (37)		6 (6)	11 (12)	
TNFB12	48 (101)	45 (198)	0.8	53 (52)	43 (49)	0.3
TNFB22	44 (93)	47 (206)		41 (40)	46 (53)	
Haplotypes[¶]						
-308G:TNFB1	33 (70)	30 (130)	0.4	27 (26)	39 (44)	0.08
-308G:TNFB2	90 (190)	90 (396)	>0.9	91 (89)	89 (44)	0.7
-308A:TNFB1	26 (56)	27 (121)	0.9	35 (34)	19 (101)	0.01
-308A:TNFB2	2 (5)	2 (8)	0.8	3 (3)	2 (2)	0.7

Data presented as % (n), unless otherwise stated. [#]: for the *-308GA* and *TNFB* polymorphisms, independence was tested between the genotypes with Fischer's exact test; [¶]: for haplotypes, Fischer's exact p-value was obtained by comparing those individuals with one or more copies of the haplotype to individuals with no copies.

-308A:TNFB1 haplotype (p=0.007). If the type of injury and its interaction term was not adjusted for in the final model, *TNFB22* was no longer associated with ARDS on multivariate analysis (adjusted OR: 0.94; 95% CI: 0.67–1.3).

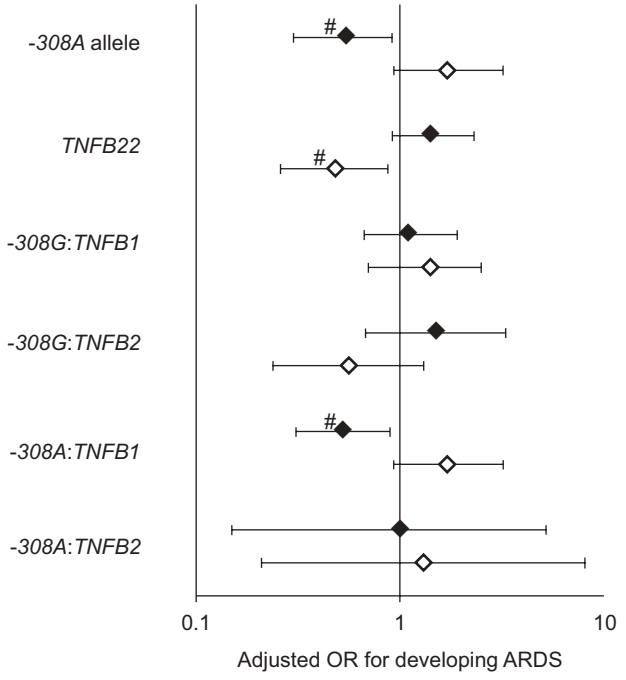

FIGURE 2. Adjusted odds ratio (OR) for development of acute respiratory distress syndrome (ARDS) by individual genotypes and haplotypes of polymorphisms after stratification by direct (◆) *versus* indirect (◇) pulmonary injury. There were 355 subjects with direct pulmonary injury at risk for ARDS and 298 subjects with indirect pulmonary injury. There was significant effect modification between the type of injury and the *-308A* allele (p=0.01), *TNFB22* genotype (p=0.006) and *-308A:TNFB1* haplotype (p=0.007). [#]: p≤0.01.

Mortality in ARDS

The 60-day mortality for the 212 ARDS cases was 46%. Clinical risks for ARDS and baseline characteristics between survivors and nonsurvivors of ARDS are shown in tables 2 and 3. Predictors of increased ARDS mortality from backwards elimination include: age (p<0.001), higher APACHE III score (p=0.001), trauma (p=0.07), steroid treatment before admission (p=0.005), total bilirubin $\geqslant 2.0\ mg \cdot dL^{-1}$ (p=0.05), and blood transfusion (p=0.03). The only significant interaction was between transfusion and *-308A* (p=0.05) and *TNFB22* (p=0.02). The C-statistics for the final model for ARDS mortality was 0.85 for both *-308A* and *TNFB22*.

ARDS mortality differed significantly depending on the *-308GA*, but not the *TNFB1/2* polymorphism (table 4) with the numbers of *-308A* alleles associated with increasing 60-day ARDS mortality (p=0.01; fig. 3). The *-308A* allele was associated with a significantly increased 60-day mortality in ARDS (crude OR: 2.1; 95% CI: 1.1–3.9; adjusted OR: 3.5; 95% CI: 1.4–8.6; p=0.007). This association was strongest among the 117 patients <67 yrs (adjusted OR: 14.9; 95% CI: 3.0–74; p<0.001). No association with mortality was found among the 95 ARDS patients >67 yrs (p=0.3). The *-308A* allele was associated with mortality in both direct (adjusted OR: 4.8; 95% CI: 1.3–18) and indirect pulmonary injury (adjusted OR: 5.5; 95% CI: 0.99–31) with no evidence of effect modification by type of injury (p=0.2). *TNFB22* was not associated with ARDS mortality (adjusted OR: 0.50; 95% CI: 0.21–1.2). As expected from the linkage disequilibrium, the *-308A:TNFB1* haplotype was associated with increased ARDS mortality (adjusted OR: 3.7; 95% CI: 1.5–9.1; table 3).

DISCUSSION

The present study reports the results of a molecular epidemiology study of ICU patients with clearly defined common

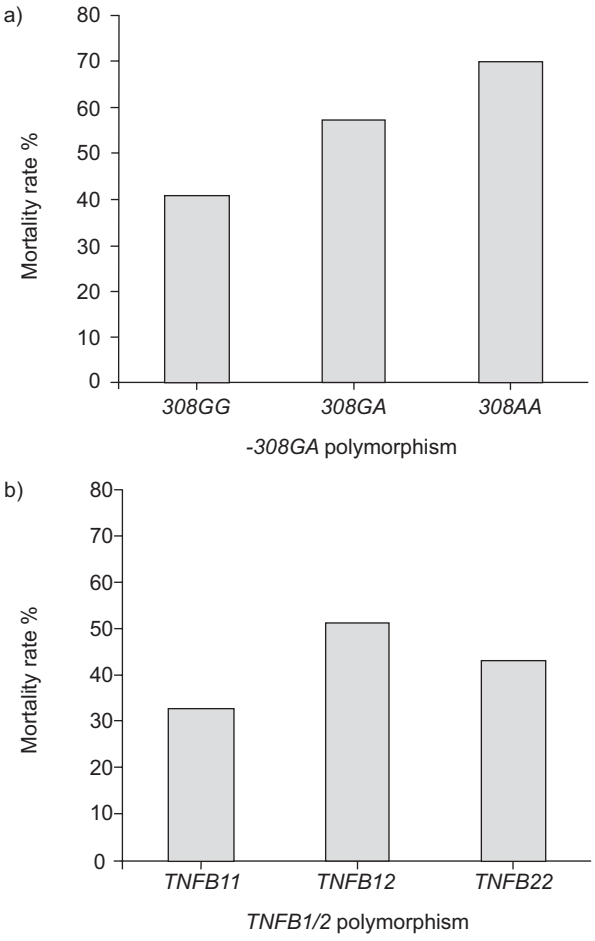

FIGURE 3. Graph showing acute respiratory distress syndrome (ARDS) 60-day mortality for the 212 patients with ARDS by *-308GA* and *TNFB1/2* polymorphisms. *308GG*: n=155; *308GA*: n=47: *308AA*: n=10; *TNFB11*: n=18; *TNFB12*: n=101; *TNFB22*: n=93. The p-value for the Chi-Squared test of trend for *-308A* alleles was 0.01 for ARDS mortality. There was no statistically significant trend between the number of *TNFB2* alleles and ARDS mortality ($p \geq 0.8$).

risk factors for ARDS. Significant associations were found between mortality in ARDS and *-308A*, but not *TNFB22*, especially among the younger patients. The association between these polymorphisms and ARDS susceptibility is heterogeneous and depended on the site of injury that places the patient at risk for ARDS.

The current study has a number of strengths. First, the prospective determination of ARDS using the AECC definition helps minimise phenotype misclassification given there is no diagnostic gold standard for ARDS. Secondly, clearly defined at-risk controls were used in this study. Using critically ill controls that have the opportunity to develop the outcome is more clinically relevant than using healthy individuals. In addition, this reduces the confounding from any possible association between the gene and the risk condition such as sepsis or pneumonia.

In support of the current authors hypothesis, the reportedly high TNF producing *-308A* allele of the *-308GA* polymorphism was associated with increased mortality in ARDS, consistent with other studies in malaria and sepsis [15, 16, 30] This study suggests that genetic heterogeneity among patients may partially explain the differences in prior studies in circulating TNF-α in ARDS and sepsis [5–10].

The association between *-308A* and the *-308A:TNFB1* haplotype and mortality in ARDS was particularly strong among younger patients even though younger ARDS patients tended to have lower mortality (table 3). Genetic contribution to complex diseases is greater in diseases with early age of onset [32].

While an association was found between the *TNFB22* and ARDS susceptibility on multivariate analysis, this was found to be driven by its differing association with ARDS among subgroups of patients with direct and indirect pulmonary injury. A similar heterogeneous effect was seen with the *-308A*. Such effect modification by the type of injury on the association between the TNF polymorphisms and ARDS suggests a possible gene-environment interaction in ARDS. The significance of this finding is not clear. The inflammatory response and the radiological, histological and mechanical properties of the lung differ depending on whether the site of infection or the aetiology of ARDS is pulmonary or extrapulmonary [22, 33, 34]. Alternatively, it is possible that these findings are due to the lower sensitivity and specificity of the AECC criteria for ARDS in patients with direct *versus* indirect pulmonary injury. A recent study found that bilateral pneumonia can often be misdiagnosed as ARDS by AECC criteria [35]. While bilateral pneumonia, bilateral aspiration and pulmonary contusion are indistinguishable from ARDS clinically, they have different histology and pathogenesis. Therefore, it is difficult to know whether the high TNF-α producing *-308A* allele is associated with decreased progression to bilateral pneumonia or a decreased odd of developing the diffuse alveolar damage of ARDS. It is possible that an adequate or high TNF-α response may limit the direct pulmonary injury of pneumonia or aspiration of oral pathogens and decrease the odds of developing bilateral pneumonia. However, even with such misclassification, most patients with direct pulmonary injury who do fulfil AECC criteria do have ARDS histologically, indicating that the ARDS cases may be more homogeneous [24]. However, given the reduced sample size in the subgroups, these findings will need to be confirmed in a larger population of patients with clinically homogenous risks for ARDS.

In the present study, the haplotype analysis closely parallels the results of the *-308A* allele. This suggests that the *-308GA* polymorphism is more important than the *TNFB* polymorphism in ARDS mortality, with little contribution from the *TNFB* polymorphism to the overall findings. However, since the *-308GA* and *TNFB1/2* polymorphisms lie in the highly polymorphic, major histocompatibility region, the possibility that the *-308A* polymorphism is linked to another polymorphism that is the disease locus cannot be excluded. The current study focused on two particular polymorphisms, specifically because of their previous association with variable function and sepsis, but there are certainly many more polymorphisms in the TNF gene. A formal haplotype analysis after genotyping multiple polymorphisms would be preferable for defining the linkage disequilibrium in the genes and their relationship to ARDS.

The authors acknowledge some other limitations to the present study. The functional significance of these TNF polymorphisms is not confirmed in the patient population. Due to the study design, the results may not be generalised to the community setting, to immunocompromised hosts, to patients without risk factors for ARDS or to patients with different clinical risks for ARDS. In addition, the analyses were restricted to Caucasians, which reduces the possibility of confounding from different genetic make-up, but it does not permit extrapolation of the results to other ethnic groups.

In conclusion, the present study demonstrated an association between the *-308A* allele and mortality in acute respiratory distress syndrome, especially among younger patients. The association between the *-308GA* polymorphism and the development of acute respiratory distress syndrome is heterogeneous and may depend upon the type of injury predisposing to acute respiratory distress syndrome. Additional studies are needed to confirm these findings in other populations with other risk factors.

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