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TLR4/NF-κB signaling pathway gene single nucleotide polymorphisms alter gene expression levels and affect ARDS occurrence and prognosis outcomes

Yueping Ding, MD[a], Qijia Feng, MD[b], Jianshi Chen, MD[c], Jia Song, MD[d,*]

Abstract

Background: To study the occurrence and prognosis of acute respiratory distress syndrome (ARDS) using single nucleotide polymorphisms (SNPs) of *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci in the TLR4/NF-κB pathway.

Methods: Genotypes were analyzed for *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci. Plasma TNF-α and IL-6 levels and *MyD88* mRNA expression in peripheral blood mononuclear cells (PBMCs) of 300 ARDS patients and 300 non-ARDS patients (control group) were examined. The patients were followed up for 60 days, and the prognosis outcome was recorded.

Results: The *TNF-α* rs1800629 locus A allele and the *IL-6* rs1800796 locus G allele were found to be risk factors for ARDS (adjusted OR = 1.452, 95% CI: 1.211–1.689, $P < .001$ and adjusted OR = 1.205, 95% CI: 1.058–1.358, $P = .005$, respectively). The G allele at *MyD88* rs7744 locus was a protective factor against ARDS (adjusted OR = 0.748, 95% CI: 0.631–0.876, $P < .001$). Compared with the other groups, homozygotes for *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci had higher expression levels, of which homozygotes for *TNF-α* rs1800629 and *IL-6* rs1800796 loci had lower 60-day survival rates, while *MyD88* rs7744 locus homozygotes had a higher 60-day survival rate.

Conclusion: The effect of *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 SNPs on gene expression level is a likely cause of ARDS occurrence and poor prognosis.

Abbreviations: ALI = acute lung injury, APACHE = Acute Physiology and Chronic Health Evaluation, ARDS = acute respiratory distress syndrome, CI = confidence interval, ICU = intensive care unit, IL-6 = interleukin 6, MAF = minor allele frequency, MDR = multifactor dimensionality reduction, mRNA = messenger RNA, MyD88 = myeloid differentiation factor 88, NF-κB = nuclear factor-kappa B, OR = odds ratio, PBMCs = peripheral blood mononuclear cells, PFS = progression free survival, SD = standard deviation, SIRS = systemic inflammatory response syndrome, SNPs = single nucleotide polymorphisms, TLR4 = Toll-like receptor 4, TNF-α = tumor necrosis factor-α.

Keywords: ARDS, IL-6, MyD88, single nucleotide polymorphism, TNF-α

1. Introduction

Acute respiratory distress syndrome (ARDS) is a respiratory failure characterized by progressive dyspnea and refractory hypoxemia. It is also the final stage of acute lung injury, with a high mortality rate.[1,2] According to previous research, the incidence of ARDS in China is high, with an annual mortality rate of 52%.[3]

From the perspective of pathophysiology, the occurrence of ARDS is associated with abnormal inflammatory reactions, interstitial and alveolar edema, cell infiltration into the alveolar space, and endothelial damage.[4–6] The Toll-like receptor 4 (TLR4)/nuclear factor (NF)-κB pathway may be a key target for inflammatory damage. TLR4 is a pattern recognition receptor from the TLR protein family expressed in lung cells, which activates NF-κB and induces the production of inflammatory cytokines and chemokines such as TNF-α and IL-6.[7–9] Myeloid differentiation factor 88 (MyD88) is a key adaptor of TLR4, which leads to activation of downstream NF-κB and subsequent production of pro-inflammatory cytokines associated with neurotoxicity.[10–12]

This study investigated the effects of genetic variation in *TNF-α*, *IL-6*, and *MyD88* genes on the occurrence and prognosis of ARDS in the TLR4/NF-κB pathway. The three SNP loci for *TNF-*

Editor: Undurti N. Das.

This work was supported by grants from Traditional Chinese Medical science and technology plan of Zhejiang Province (2019ZA061) and (2019ZB055).

The authors have no conflicts of interest to disclose.

[a] Department of Intensive Care Unit, The Second Affiliated Hospital of Zhejiang Chinese Medical University, 318 Chaowang Road, [b] Department of Intensive Care Unit, Hangzhou Lin'an District People's Hospital, 548# Yijin Road, Jincheng Town, Lin'an, Hangzhou, [c] Department of Intensive Care Unit, the Sencond Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 Xueyuan Western Road, Wenzhou, [d] Department of Emergency, the Second Affiliated Hospital of Zhejiang Chinese Medical University, 318 Chaowang Road, Hangzhou, Zhejiang, PR China.

[*] Correspondence: Jia Song, Department of Emergency, The Second Affiliated Hospital of Zhejiang Chinese Medical University, 318 Chaowang Road, Hangzhou, Zhejiang, China (e-mail: ccjia112@163.com).

Medicine (2019) 98:26(e16029)

Received: 11 January 2019 / Received in final form: 27 April 2019 / Accepted: 21 May 2019

http://dx.doi.org/10.1097/MD.0000000000016029

Table 1

SNP loci information.

Gene	Polymorphism	Location	Variation	MAF*
TNF-α	rs1800629	Promoter	−308G>A	0.0571
IL-6	rs1800796	Intron	−572C>G	0.2143
MyD88	rs7744	3'UTR	*1244 A>G	0.3048

* MAF = minor allele frequency in Chinese Han population.

α rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 are located in the non-coding region of the respective genes (Table 1). These non-coding regions may be involved in the regulation of gene expression and have been shown to be sensitive to disease sites.[13–15] This study explored the correlation between the occurrence and prognosis of these three SNPs and ARDS and analyzed the possible underlying mechanisms at the level of gene expression.

2. Data and methods

2.1. General data

From May 2015 to May 2017, 300 patients with ARDS who were admitted to the ICU of the Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou Lin'an District People's Hospital, the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, and Department of Emergency, the Second Affiliated Hospital of Zhejiang Chinese Medical University were enrolled in this study, including 185 men and 115 women, aged 34 to 84 years. During the same period, based on the age and sex of the patients in the ARDS group, 300 non-ARDS patients admitted to the ICU were enrolled as a control group. The inclusion criteria were

1. one or more risk factors for ARDS, such as sepsis (without shock), Septic shock, Pneumonia, Aspiration, Multiple transfusion, and Trauma, and
2. consistent diagnosis of ARDS patients with Berlin's mild, moderate, or severe ARDS criteria.[16]

The criteria for exclusion of patients were

1. age < 18 years,
2. other chronic lung diseases except asthma and chronic obstructive pulmonary disease (COPD),
3. diffuse alveolar hemorrhage,
4. directive to withhold intubation,
5. prior treatment with granulocyte colony-stimulating factor, and
6. immunosuppression not secondary to corticosteroid.

The study protocol was reviewed by an ethics committee, and all the subjects signed informed consent.

2.2. Genotyping

A 5-mL sample of peripheral venous blood was collected from all subjects, and within 30 minutes of the collection, the samples were centrifuged at 1500 × g for 25 minutes. The plasma was separated, and the samples were stored at −80°C in a refrigerator for testing. Peripheral blood mononuclear cells (PBMCs) were separated after centrifugation, Genomic DNA was extracted from PBMC using Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). The experiment was carried out according to the supplier's instructions. The extracted genomic DNA samples were sequenced by Shanghai Shenggong Bioengineering Co. Ltd. The primer sequences for *TNF-α* rs1800629 locus were Fw: 5′-CGG GTC AGA ATG AAA GAA GA-3′ and Rv: 5′-CTC ATC TGG AGG AAG CGG TAG-3′. The primer sequences for *IL-6* rs1800796 locus were Fw: 5′-CTG CAC GAA ATT TGA GGA TGG C-3′ and Rv: 5′-CTT CTG TGT TCT GGC TCT CCC-3′. The primer sequences for *MyD88* rs7744 locus were Fw: 5′-CCA AAC TCT GGA AAG GAC CCA-3′ and Rv: 5′-GCT CTC TTC CTC TCT CTG TGC-3′. All the samples were tested twice, and the results were identical for both trials (Fig. 1).

2.3. Detection of plasma TNF-α and IL-6 levels

Plasma TNF-α and IL-6 levels were measured using an ELISA kit (E-EL-H0109 for TNF-α and E-EL-H0102 for IL-6, Wuhan Elabscience Biotechnology, Wuhan, China). A semi-automated chemiluminescence enzyme-linked immunosorbent assay system

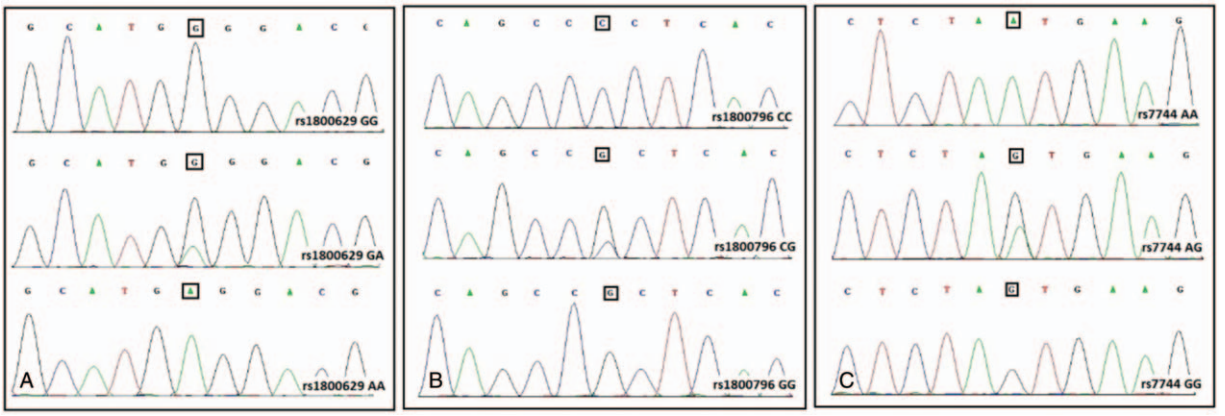

Figure 1. SNP site sequencing results. A is the sequencing result for different genotypes of *TNF-α* rs1800629 locus, B is the sequencing result for different genotypes of *IL-6* rs1800796 locus, and C is the sequencing result for different genotypes of *MyD88* rs7744 locus.

was used for detection (Tecan Group Ltd, Salzburg, Austria). Each sample was tested three times, and the averages are reported.

2.4. MyD88 mRNA detection in PBMCs

Total RNA was extracted from PBMC using TRIzol (Thermo Fisher Scientific, Inc., Waltham, MA) and reverse transcribed into cDNA using the PrimeScriptTM RT reagent kit. The relative expression of *MyD88* mRNA was detected using ABI 7900HT Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Premix Ex TaqTM II kit (Takara Bio, Inc.). The primer sequences for *MyD88* mRNA were Fw: 5′-GCA CAG AGA GAG GAA GAG AGC-3′ and Rv: 5′-TGC TCT GGG AAG GAG AGA GG-3′. The primer sequences for GAPDH were Fw: 5′-CAC CCA CTC CTC CAC CTT TG-3′ and Rv: 5′-CCA CCA CCC TGT TGC TGT AG-3′. The expression level of *MyD88* mRNA relative to GAPDH was analyzed using the $2^{-\Delta\Delta Cq}$ method.

2.5. Follow-up

All the patients were followed up for 60 days and their survival data were recorded.

2.6. Statistics

SPSS20.0 software (IBM, Chicago, IL) was used for statistical analysis. The continuous variables are reported as mean ± SD. Independent sample t-test was used for statistical analysis. The categorical variables are expressed as percentage [n (%)], and the differences between groups were assessed by χ^2 test. Hardy-Weinberg equilibrium was evaluated using χ^2 test. The correlation between SNPs and ARDS risk was determined based on the distribution of allele frequencies and genetic models (dominant and recessive models). Odds ratio (OR) and 95% confidence interval (CI) were used in unconditional logistic regression analysis to adjust factors such as age and sex. Multifactor Dimensionality Reduction (MDR) was used to analyze gene-gene interactions.[17] The correlations between TNF-alpha rs1800629, IL-6 rs1800796, MyD88 rs7744 SNP and 60-day progression-free survival (PFS) were evaluated by Kaplan-Meier curve and logarithmic rank test. All the tests were double-tailed, Kappa > 0.8, and $P < .05$ indicated significant difference.

3. Results

3.1. Demographic characteristics of ARDS and control groups

In this study, 300 patients with ARDS were selected based on the screening conditions. Based on the age and sex distribution within the ARDS group, 300 non-ARDS patients were selected as the control group. The demographic characteristics of ARDS patients and healthy subjects are presented in Table 2, in which ARDS patients had a higher APACHE III score, and the incidence of septic shock and pneumonia was significantly higher in this group than in the control group ($P < .05$). The proportion of patients with sepsis who developed shock was significantly lower than that of the control group ($P < .05$).

Table 2

Demographic characteristics.

Variable	ARDS (n = 300)	Control (n = 300)	P
Age [year, (mean ± SD)]	57.4 ± 8.6	57.7 ± 9.1	.678
Gender [n (%)]			
Male	185 (61.67%)	181 (60.33%)	.738
Female	115 (38.33%)	119 (39.67%)	
APACHE III score (mean ± SD)	72.8 ± 9.7	63.0 ± 10.3	<.001
Risk factors [n (%)]			
Sepsis (without shock)	80 (26.67%)	110 (36.67%)	.009
Septic shock	181 (60.33%)	130 (43.33%)	<.001
Pneumonia	201 (67.00%)	125 (41.67%)	<.001
Aspiration	28 (9.33%)	25 (8.33%)	.666
Multiple transfusion	30 (10.00%)	28 (9.33%)	.782
Trauma	20 (6.67%)	19 (6.33%)	.869

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

3.2. Correlation analysis between genetic polymorphism and ARDS risk

The genotypic distributions for *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci were consistent with the Hardy-Weinberg equilibrium ($P > .05$) (Table 3). The risk of ARDS in *TNF-α* rs1800629 locus homozygotes (AA) was significantly higher than that in the wild type (GG) (adjusted OR = 2.041, 95% CI: 1.683–2.162, $P < .001$). The risk of ARDS in the dominant model and implicit model was significantly increased (adjusted OR = 1.452, 95% CI: 1.211–1.689, $P < .001$/ adjusted OR = 1.988, 95% CI: 1.644–2.103, $P < .001$). The population carrying *TNF-α* rs1800629 locus A allele was more prone to developing ARDS than the population carrying the G allele (adjusted OR = 1.452567, 95%CI: 1.373–1.741, $P < .001$). The population carrying the homozygous *IL-6* rs1800796 locus (GG) was more prone to ARDS than the wild type (CC) (adjusted OR = 1.520, 95% CI: 1.229–1.773, $P < .001$), and the risk of ARDS with a dominance model was significantly high (adjusted OR = 1.568, 95% CI: 1.275–1.815, $P < .001$). Heterozygotes (CG) and recessive models did not have a significant risk of ARDS ($P > .05$). The *IL-6* rs1800796 G allele was a risk factor for ARDS (adjusted OR = 1.205, 95% CI: 1.058–1.358, $P = .005$). *MyD88* rs7744 locus heterozygotes and dominant model carriers were at a lower risk of developing ARDS than the wild-type (adjusted OR = 0.497, 95% CI: 0.373–0.646, $P < .001$/adjusted OR = 0.619, 95% CI: 0.501–0.756, $P < .001$), and the G allele at the *MyD88* rs7744 locus was a protective factor against ARDS (adjusted OR = 0.748, 95% CI: 0.631–0.876, $P < .001$).

3.3. Interaction of SNPs at TNF-α rs1800629, IL-6 rs1800796, and MyD88 rs7744 with respect to ARDS risk

MDR was used to analyze the correlation of SNPs at *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 with respect to ARDS risk (Fig. 2). The results showed that an SNP at the *MyD88* rs7744 locus had the strongest effect on ARDS risk, (vertex effect: 4.54%), followed by SNP at *TNF-α* rs1800629 (vertex effect: 4.29%) and finally *IL-6* rs1800796 (vertex effect: 2.21%) (Table 4). Gene-gene interaction analysis showed that there was a redundancy between *TNF-α* rs1800629 and *IL-6* rs1800796 (edge effect: −0.48%), whereas a synergistic interaction was observed between *TNF-α* rs1800629 and *MyD88* rs7744, as well

Table 3

Correlation between ADRS risk and allele frequencies of *TNF-α*rs1800629, *IL-6* rs1800796, and *MyD88* rs7744.

	ARDS (n = 300)	Control (n = 300)	HWE *P*	crude OR (95%CI)	crude *P*	adjusted* OR (95%CI)	adjusted *P*
rs1800629							
GG	225 (75.00%)	263 (87.67%)	.487	1.000 (Reference)			
GA	43 (14.33%)	35 (11.67%)		1.436 (0.865–2.387)	.138	1.196 (0.925–1.477)	.174
AA	32 (10.67%)	2 (0.67%)		18.702 (4.307–114.160)	**<.001**	2.041 (1.683–2.162)	**<.001**
Dominant model				2.369 (1.506–3.737)	**<.001**	1.452 (1.211–1.689)	**<.001**
Recessive model				17.791 (4.107–108.417)	**<.001**	1.988 (1.644–2.103)	**<0.001**
G	493 (82.17%)	561 (93.50%)		1.000 (Reference)			
A	107 (17.83%)	39 (6.50%)		3.122 (2.088–4.681)	**<.001**	1.567 (1.373–1.741)	**<.001**
rs1800796							
CC	187 (62.33%)	198 (66.00%)	.059	1.000 (Reference)			
CG	65 (21.67%)	85 (28.33%)		0.810 (0.544–1.205)	.276	0.892 (0.710–1.101)	0.320
GG	48 (16.00%)	17 (5.67%)		2.990 (1.604–5.624)	**<.001**	1.520 (1.229–1.773)	**<.001**
Dominant model				1.173 (0.828–1.662)	.349	1.082 (0.909–1.277)	.395
Recessive model				3.171 (1.721–5.900)	**<.001**	1.568 (1.275–1.815)	**<.001**
C	439 (73.17%)	481 (80.17%)		1.000 (Reference)			
G	161 (26.83%)	119 (19.83%)		1.482 (1.122–1.960)	**.004**	1.205 (1.058–1.358)	**0.005**
rs7744							
AA	225 (75.00%)	165 (55.00%)	.087	1.000 (Reference)			
AG	43 (14.33%)	107 (35.67%)		0.295 (0.192–0.451)	**<.001**	0.497 (0.373–0.646)	**<.001**
GG	32 (10.67%)	28 (9.33%)		0.838 (0.469–1.497)	.525	0.924 (0.687–1.172)	.621
Dominant model				0.407 (0.284–0.585)	**<.001**	0.619 (0.501–0.756)	**<.001**
Recessive model				1.160 (0.658–2.046)	.586	1.075 (0.797–1.360)	.683
A	493 (82.17%)	437 (72.83%)		1.000 (Reference)			
G	107 (17.83%)	163 (27.17%)		0.582 (0.437–0.774)	**<.001**	0.748 (0.631–0.876)	**<.001**

ARDS = acute respiratory distress syndrome, CI = confidence interval, OR = odds ratio.

* logistic regression adjusted age, sex, and risk factor. AA is a wild genotype, AG is a heterozygous mutant genotype, and GG is a homozygous mutant genotype. Recessive model: AA vs (AG + GG); dominant model: (AG + AA) vs GG.

as between *IL-6* rs1800796 and *MyD88* rs7744 (Edge effect: 0.59% and 0.62% respectively) (Fig. 2A). The Dendogram results also showed that the interaction between rs1800796 and MyD88 rs7744 was the strongest (Fig. 2B).

3.4. Stratified analysis of the relationship between genetic polymorphism and ARDS risk

According to stratified analysis of age ($\leq 60, > 60$) and sex (men, women), the increased ARDS risk in TNF-α rs1800629 A allele carriers (GA/AA) was found only in patients $\leq$ 60 years old (adjusted OR = 1.468, 95% CI: 1.170–1.759, $P = .001$). *TNF-α* rs1800629 SNP was not associated with ARDS risk in patients > 60 years of age (adjusted OR = 1.404, 95% CI: 0.986–1.809, $P = .060$). Similarly, an elevated ARDS risk in A allele carriers (GA/AA) was observed only in male patients (adjusted OR = 1.548, 95% CI: 1.230–1.843, $P < .001$), and not in female patients (adjusted OR = 1.315, 95% CI: 0.943–1.706, $P = .107$) (Table 4). The correlation between *IL-6* rs1800796 SNP and ARDS risk was not affected by age or sex (Table 5). *MyD88* rs7744 locus G allele carriers (AG/GG) had a reduced risk of ARDS only for patients aged $\leq$ 60 years (adjusted OR = 0.504, 95% CI: 0.383–0.653, $P < .001$). The *MyD88* rs7744 locus G allele carriers (AG/GG) aged > 60 did not have a significant ARDS risk ($P > .05$), while *MyD88* rs7744 locus G allele was a protective factor against ARDS in both male and female patients (adjusted OR = 0.586, 95% CI: 0.450–0.778, $P < .001$ / adjusted OR = 0.651, 95% CI: 0.464–0.892, $P = .006$) (Table 6).

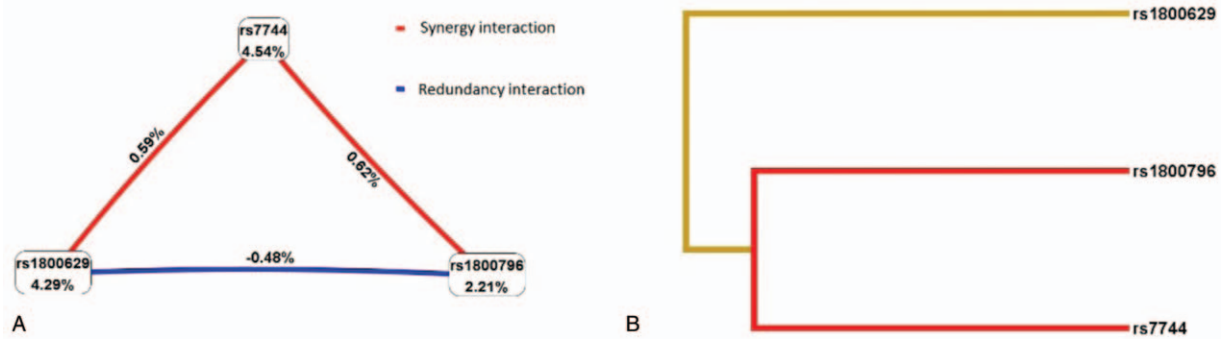

Figure 2. MDR analysis of interaction of SNPs at *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 with respect to ARDS risk. A: Circle graph, red line represents synergistic interaction, blue line represents redundancy, values represent the interaction strength, and the values at the vertices represent the impact on ARDS risk. B: Dendogram. The closer the distance, the stronger the interaction between SNP sites and the farther the distance, the weaker the interaction between SNP sites.

Table 4

Stratified analysis of the relationship between *TNF-α* rs1800629 SNP and ARDS risk.

	ARDS (n = 300)	Control (n = 300)	crude OR (95%CI)	crude *P*	OR (95%CI)*	adjusted *P*
Age [year,n (%)]						
≤60						
GG	162 (76.06%)	197 (88.34%)	1.000 (Reference)			
GA/AA	51 (23.94%)	26 (11.66%)	2.385 (1.383–4.131)	**<.001**	1.468 (1.170–1.759)	**.001**
>60						
GG	63 (72.41%)	66 (85.71%)	1.000 (Reference)			
GA/AA	24 (27.59%)	11 (14.29%)	2.286 (0.971–5.457)	**.038**	1.404 (0.986–1.809)	.060
Gender [n (%)]						
Male						
GG	138 (74.59%)	162 (89.50%)	1.000 (Reference)			
GA/AA	47 (25.41%)	19 (10.50%)	2.904 (1.571–5.406)	**<.001**	1.548 (1.230–1.843)	**<.001**
Female						
GG	87 (75.65%)	101 (84.87%)	1.000 (Reference)			
GA/AA	28 (24.35%)	18 (15.13%)	1.806 (0.892–3.674)	.076	1.315 (0.943–1.706)	.107

ARDS = acute respiratory distress syndrome, CI = confidence interval, OR = odds ratio.
* logistic regression modeling adjusted age, sex, and risk factor.

Table 5

Stratified analysis of the relationship between *IL-6* rs1800796 SNP and ARDS risk.

	ARDS (n = 300)	Control (n = 300)	crude OR (95%CI)	crude *P*	OR (95%CI)*	adjusted *P*
Age [year,n (%)]						
≤60						
CC	127 (59.62%)	148 (66.37%)	1.000 (Reference)			
CG/GG	86 (40.38%)	75 (33.63%)	1.336 (0.888–2.012)	.145	1.157 (0.941–1.407)	.174
>60						
CC	60 (68.97%)	50 (64.94%)	1.000 (Reference)			
CG/GG	27 (31.03%)	27 (35.06%)	0.833 (0.412–1.684)	.584	0.917 (0.638–1.262)	.703
Gender [n (%)]						
Male						
CC	123 (66.49%)	125 (69.06%)	1.000 (Reference)			
CG/GG	62 (33.51%)	56 (30.94%)	1.125 (0.709–1.787)	.598	1.059 (0.839–1.313)	.678
Female						
CC	64 (55.65%)	73 (61.34%)	1.000 (Reference)			
CG/GG	51 (44.35%)	46 (38.66%)	1.265 (0.726–2.203)	.377	1.125 (0.848–1.475)	.453

ARDS = acute respiratory distress syndrome, CI = confidence interval, OR = odds ratio.
* logistic regression modeling adjusted age, sex, and risk factor.

Table 6

Stratified analysis of the relationship between *MyD88* rs7744 SNP and ARDS risk.

	ARDS (n = 300)	Control (n = 300)	crude OR (95%CI)	crude *P*	adjusted OR (95%CI)	adjusted *P*
Age [year, n (%)]						
≤60						
AA	167 (78.40%)	115 (51.57%)	1.000 (Reference)			
AG/GG	46 (21.60%)	108 (48.43%)	0.293 (0.189–0.455)	**<.001**	0.504 (0.383–0.653)	**<.001**
>60						
AA	58 (66.67%)	50 (64.94%)	1.000 (Reference)			
AG/GG	29 (33.33%)	27 (35.06%)	0.926 (0.461–1.859)	.815	0.964 (0.679–1.317)	.945
Gender [n (%)]						
Male						
AA	142 (76.76%)	101 (55.80%)	1.000 (Reference)			
AG/GG	43 (23.24%)	80 (44.20%)	0.382 (0.238–0.614)	**<.001**	0.598 (0.450–0.778)	**<.001**
Female						
AA	83 (72.17%)	64 (53.78%)	1.000 (Reference)			
AG/GG	32 (27.83%)	55 (46.22%)	0.449 (0.251–0.801)	**.004**	0.651 (0.464–0.892)	**.006**

ARDS = acute respiratory distress syndrome, CI = confidence interval, OR = odds ratio.
*logistic regression modeling adjusted age, sex, and risk factor.

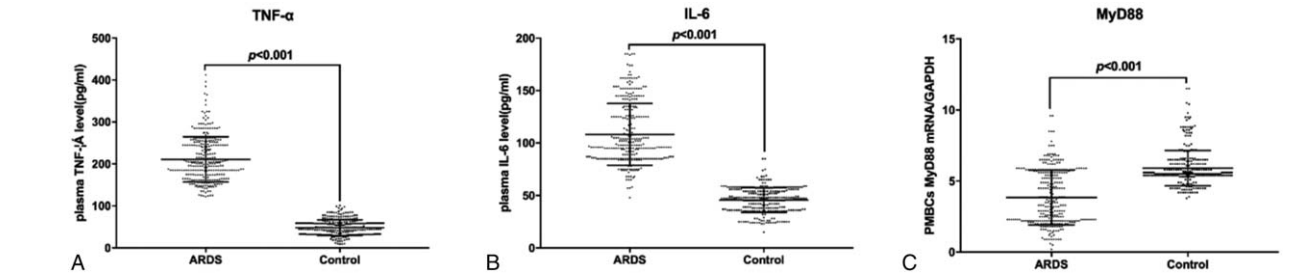

Figure 3. Comparison of TNF-α and IL-6 levels in plasma and *MyD88* mRNA levels in PBMCs between ARDS and control groups. A is plasma TNF-α level, B is plasma IL-6 level, and C is *MyD88* mRNA level in PBMCs. The difference between the ARDS group and the control group was analyzed by t test. The data were expressed as standard deviation and maximum and minimum values.

3.5. Correlation between SNPs at TNF-α rs1800629, IL-6 rs1800796, and MyD88 rs7744 loci and gene expression levels

Analysis of *TNF-α* and *IL-6* mRNA levels in plasma and *MyD88* mRNA levels in PBMCs showed that the *TNF-α* and *IL-6* mRNA levels in the plasma of ARDS patients were higher than those in the control group, whereas the *MyD88* mRNA level in PBMCs was significantly lower than that in the control group ($P < .001$) (Fig. 3). One-way ANOVA showed that the plasma TNF-α level of TNF-α rs1800629 GG locus genotype was significantly lower than that of GA genotype, and the AA genotype was the highest ($P < .05$). The plasma TNF-α level of subjects with IL-6 rs1800796 site CC genotype was significantly lower than that of CG genotype subjects, and the GG genotype was the highest ($P < .05$). The MyD88 mRNA level of subjects with MyD88 rs7744 locus AA genotype was significantly lower than that of AG genotype, and subjects with the GG genotype was the highest. ($P < .05$) (Fig. 4).

3.6. SNPs at TNF-α rs1800629, IL-6 rs1800796, and MyD88 rs7744 loci affect survival prognosis

The 60-day mortality (11.39%) of ARDS patients with TNF-α GG genotype at rs180062 was significantly lower than that of TNF-α GA genotype and AA genotype (22.86%, 39.13%) ($P = .038$) (Fig. 5A). The 60-day mortality of ARDS patients with IL-6 rs1800796 site CC genotype (10.00%) was lower than that of IL-6 rs1800796 CG genotype and GG genotype (25.00%, 26.32%) ($P = .015$) (Fig. 5B). The 60-day mortality of ARDS

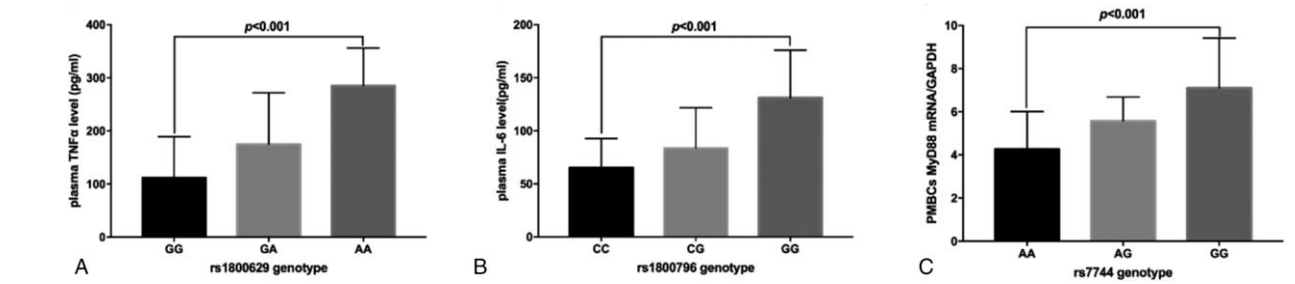

Figure 4. Correlation of SNPs at *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci with the gene expression levels. A is the correlation between *TNF-α rs1800629* SNP and plasma TNF-α levels. B is the correlation between *IL-6 rs1800796* SNP and plasma IL-6 levels. C is the correlation between *MyD88* rs7744 locus SNP and *MyD88* mRNA level in PBMCs.

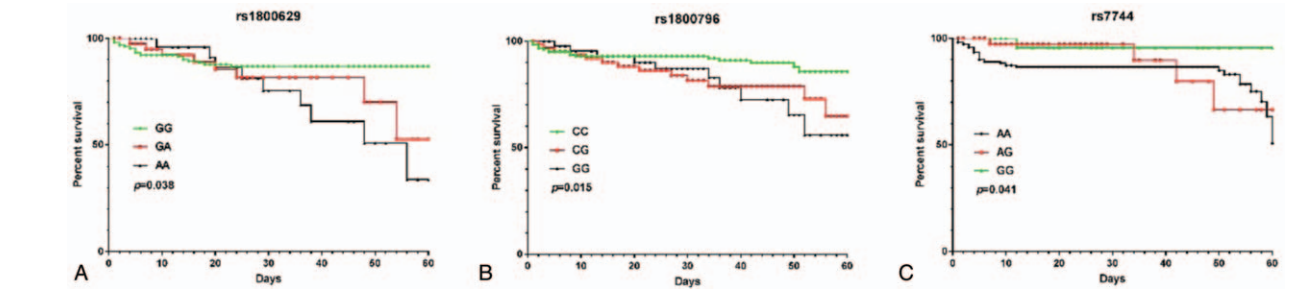

Figure 5. Correlation between SNPs at *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci and survival prognosis of ARDS patients. A is the survival curve of patients with different genotypes for *TNF-α* rs1800629. B is the survival curve of patients with different genotypes for *IL-6* rs1800796. C is the survival curve of patients with different genotypes for *MyD88* rs7744. The Log-rank test was used to assess the correlation between TNF-α rs1800629, IL-6 rs1800796, MyD88 rs7744 SNP and 60-day progression-free survival (PFS).

patients with MyD88 rs7744 locus AA genotype (18.42%) was higher than that of AG and GG genotypes (10.26%, 3.23%) ($P = .041$) (Fig. 5C).

4. Discussion

Acute respiratory distress (ARDS) is the development of acute lung injury (ALI), a common critical illness in the intensive care unit (ICU), with a mortality rate of 30% to 50%[2]. ARDS is a transitional, uncontrolled inflammatory response in the lung caused by a variety of factors. The primary clinical feature is acute and hypoxic respiratory failure.[18] In recent years, studies have found that systemic inflammatory response syndrome (SIRS), caused by infection, trauma, and other factors, is the fundamental cause of ARDS.[19,20] Therefore, studies on pathways associated with inflammatory responses may be of great importance for the prevention and treatment of ARDS.

Among the many cytokines involved in ALI, TNF-α is an early-reactive cytokine that promotes further inflammatory responses, so anti-TNF-α preparations constitute the first form of medication in ALI/ARDS clinical trials.[21,22] TNF-α initiates a downstream signaling pathway by binding with two receptors, p55 and p57, which play different roles in the formation of ARDS pulmonary edema. Studies have shown that the deletion of *p55* has a protective effect against the acute phase of ARDS induced by mechanical ventilation, whereas the effect of *p57* deletion is opposite,[23] demonstrating that TNF-α plays an important role in the development of ARDS. Very few studies have investigated the effect of TNF-α genetic variation on the occurrence of ARDS. In contrast with the results of this study, Azevedo et al[24] had reported that *TNF-α* rs1800629 locus A allele has protective effects against ARDS in the Brazilian population. The researchers reported that the A allele frequency was 13% among the Brazilian population unaffected by ARDS, significantly higher than that in the Chinese Han population (5.71%), which may be one of the reasons for the difference in the results. In addition, the impact of environmental factors cannot be ignored.

IL-6 is a cytokine with multiple immunoregulatory functions and exerts a pro-inflammatory effect in the development and progression of ALI, in addition to having an anti-inflammatory effect.[25,26] Recent studies indicate that IL-6 can enhance the damaging effect of neutrophils on tissues, in addition to participating in the body's immunosuppression response and delaying the apoptosis of neutrophils, which is a key factor in the transformation of innate immunity to acquired immunity. It also plays an important role in the occurrence and development of chronic inflammation.[27–30] The genetic variation for *IL-6* is associated with diseases caused by abnormal inflammatory responses.[14,31] Studies have shown that *IL-6* expression levels are abnormally elevated after acute inflammatory response,[32] so we believe that the abnormal expression of *IL-6*, caused by *IL-6* gene polymorphism, may be related to the occurrence and development of ARDS. It can be seen from the results of this study that the G allele at *IL-6* rs1800796 is a risk factor for ARDS, and the plasma IL-6 level in the population carrying the G allele is significantly higher than in the C allele carrier population, which may be one of the main causes of ARDS.

MyD88 is a key linker in the TLR4 signaling pathway and plays an important role in upstream signaling and the development and progression of disease.[33] The TLR4-mediated MyD88 signaling pathway regulates the expression of multiple genes involved in inflammation during the immune response to pathogen invasion.[34] The *MyD88* gene encodes a cytosolic adaptor protein that plays an important role in both innate and adaptive immune responses, as an essential signal transducer in IL-1 and TLR signaling pathways. Activation of MyD88/NF-κB signaling accelerates the inflammatory response.[35] Studies have shown that the single nucleotide polymorphism at the rs7744 locus of *MyD88* gene is closely related to the susceptibility of ulcerative colitis.[36] This SNP locus is located in the 3'-UTR of the gene and may be involved in the regulation of gene expression. The results of this study showed that the G allele at *MyD88* rs7744 locus is a protective factor against ARDS, and the MyD88 level in PBMCs in G allele carriers is higher than that in A allele carriers, which may be the reason for the decreased risk of ARDS in the presence of G allele.

In addition, we analyzed the correlation between *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 SNPs and survival prognosis in patients with ARDS. The results showed that the 60-day survival rate of wild-type ARDS patients with *TNF-α* rs1800629 and *IL-6* rs1800796 was significantly higher than that of mutants, while that of wild-type ARDS patients with *MyD88* rs7744 was significantly lower than that of mutants, indicating that *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 locus SNPs are significantly associated with survival prognosis in patients with ARDS. We believe that the cascade of reactions may be caused by inconsistent expression levels of inflammatory factors in subjects with different genotypes at SNP sites.

This study had some limitations. First, the occurrence and development of ARDS is likely the result of interactions between genes and environmental factors. Thus, the influence of single SNPs may be limited. Therefore, this study aimed to analyze the effects of SNPs on ARDS in a population with individuals of different ages and sex. The results showed that the correlation between SNP at *TNF-α* rs1800629 and ARDS was influenced by age and sex, whereas the effect of SNP at IL-7 rs1800796 was not dependent on age and sex. The effect of *MyD88* rs7744 locus SNP on ARDS risk was age-related. There may be other factors involved, and further research is needed to uncover their effects. Second, there may be multiple SNP loci involved in the occurrence and development of ARDS. Thus, it may be more fruitful to study the combined effects of SNPs at different genetic loci. The results of this study indicate that *TNF-α* rs1800629 and *IL-6* rs1800796 share redundant effects, whereas interactions between *TNF-α* rs1800629 and *MyD88* rs7744, as well as *IL-6* rs1800796 and *MyD88* rs7744 are synergistic in nature. This suggests that there may be redundancy across some sites, and synergistic interaction across others.

5. Conclusion

The single nucleotide polymorphisms of *TNF-α* rs1800629, *IL-6* rs1800796, and *MyD88* rs7744 loci are associated with poor occurrence and prognosis of ARDS, likely due to the effect of gene expression levels. The combined effects of multiple genes and environmental factors may require further research to reveal the intrinsic and extrinsic mechanisms of ARDS development and progression, for better prevention and treatment of ARDS.

Author contributions

Data curation: Yueping Ding, Qijia Feng, Jianshi Chen.
Formal analysis: Yueping Ding, Qijia Feng, Jia Song.

Investigation: Yueping Ding.
Methodology: Yueping Ding, Jia Song.
Project administration: Yueping Ding, Jia Song.
Software: Qijia Feng, Jianshi Chen.
Supervision: Jia Song.
Visualization: Qijia Feng, Jianshi Chen.
Writing – original draft: Yueping Ding.
Writing – review & editing: Jia Song.

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