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

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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.\textsuperscript{[1,2]} According to previous research, the incidence of ARDS in China is high, with an annual mortality rate of 52%.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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-α, IL-6, and MyD88 were genotyped, and the associations between these SNPs and ARDS occurrence and prognosis were analyzed.
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.\textsuperscript{[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.\textsuperscript{[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-\textalpha 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 TTC TCT TTC TCT TCT CG-3’. All the samples were tested twice, and the results were identical for both trials (Fig. 1).

2.3. Detection of plasma TNF-\textalpha and IL-6 levels

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

| SNP loci information. |
|-----------------------|
| Gene | Polymorphism | Location | Variation | MAF<sup>*</sup> |
|------|--------------|----------|-----------|-----------------|
| TNF-\textalpha | rs1800629 | Promoter | −308G>A | 0.0571 |
| IL-6 | rs1800796 | Intron | −572C>G | 0.2143 |
| MyD88 | rs7744 | 3’UTR | 1244A>G | 0.3048 |

<sup>*</sup>MAF = minor allele frequency in Chinese Han population.

Figure 1. SNP site sequencing results. A is the sequencing result for different genotypes of TNF-\textalpha 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 (Tecnol 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 PrimeScript™ 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 Taq™ II kit (Takara Bio, Inc.). The primer sequences for MyD88 mRNA were Fw: 5'-GCA CAG AGA GAG AAG AGA GG-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^{-ΔΔCq} 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 χ² test. Hardy-Weinberg equilibrium was evaluated using χ² 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, and 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).

#### 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). The population carrying TNF-α rs1800629 locus A allele was more prone to developing ARDS than the population carrying the G allele (adjusted OR = 1.452, 95% CI: 1.373–1.741, P < .001). The population carrying the homoygous 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 ARDS risk and allele frequencies of TNF-α rs1800629, IL-6 rs1800796, and MyD88 rs7744.

| SNP          | 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) | .001     | 1.000 (Reference)   | .001       |
| 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|              |                   |        |                  |          |                     |            |
| G            | 493 (82.17%)   | 561 (93.50%)      | 1.000  | (Reference)      | .059     | 1.000 (Reference)   | .059       |
| 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) | .001     | 1.000 (Reference)   | .001       |
| CG           | 65 (21.67%)    | 85 (28.33%)       | 0.810  | (0.544–1.205)    | .276     | 0.892 (0.710–1.101) | .320       |
| GG           | 48 (16.00%)    | 17 (5.67%)        | 2.990  | (1.604–5.624)    | <.001    | 1.520 (1.229–1.733) | <.001      |
| Dominant model|              |                   |        |                  |          |                     |            |
| C            | 439 (73.17%)   | 481 (80.17%)      | 1.000  | (Reference)      | .004     | 1.205 (1.058–1.358) | 0.005      |
| 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) | .001     | 0.497 (0.373–0.646) | <.001      |
| AG           | 43 (14.33%)    | 107 (35.67%)      | 0.295  | (0.192–0.451)    | <.001    | 0.924 (0.687–1.172) | .621       |
| GG           | 32 (10.67%)    | 28 (9.33%)        | 0.838  | (0.469–1.407)    | .525     | 0.619 (0.501–0.756) | <.001      |
| Dominant model|              |                   |        |                  |          |                     |            |
| A            | 493 (82.17%)   | 437 (72.83%)      | 1.000  | (Reference)      | .001     | 0.748 (0.631–0.876) | <.001      |
| 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 (≤ 60, > 60) and sex (men, women), the increased ARDS risk in TNF-α rs1800629 A allele carriers (GA/AA) was found only in patients ≤ 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 (G/A/A) 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 ≤ 60 years (adjusted OR = 0.504, 95% CI: 0.383–0.633, 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).
**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) | .001    | 1.468 (1.170–1.759) | .001 |
| GA/AA          | 51 (23.94%)  | 26 (11.66%)     | 2.385 (1.383–4.131) | <.001   | 1.404 (0.986–1.809) | .060 |
| >60            |              |                 |                  |         |            |            |
| GG             | 63 (72.41%)  | 66 (85.71%)     | 1.000 (Reference) | .038    | 1.548 (1.230–1.843) | <.001 |
| GA/AA          | 24 (27.59%)  | 11 (14.29%)     | 2.286 (0.971–5.457) | .174    | 1.315 (0.943–1.706) | .107 |
| **Gender [n (%)]** |              |                 |                  |         |            |            |
| Male           |              |                 |                  |         |            |            |
| GG             | 138 (74.59%) | 162 (89.50%)    | 1.000 (Reference) | .038    | 1.548 (1.230–1.843) | <.001 |
| GA/AA          | 47 (25.41%)  | 19 (10.50%)     | 2.904 (1.571–5.406) | <.001   | 1.315 (0.943–1.706) | .107 |
| Female         |              |                 |                  |         |            |            |
| GG             | 87 (75.65%)  | 101 (84.87%)    | 1.000 (Reference) | .076    | 1.315 (0.943–1.706) | .107 |
| GA/AA          | 28 (24.35%)  | 18 (15.13%)     | 1.806 (0.892–3.674) | .004    | 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.

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**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) | .145    | 1.157 (0.941–1.407) | .174 |
| GA/GG          | 86 (40.38%)  | 75 (33.63%)     | 1.336 (0.888–2.012) | .584    | 0.917 (0.638–1.326) | .703 |
| >60            |              |                 |                  |         |            |            |
| CC             | 60 (68.97%)  | 50 (64.94%)     | 1.000 (Reference) | .584    | 0.917 (0.638–1.326) | .703 |
| GA/GG          | 27 (31.03%)  | 27 (35.06%)     | 0.633 (0.412–1.684) | .145    | 1.157 (0.941–1.407) | .174 |
| **Gender [n (%)]** |              |                 |                  |         |            |            |
| Male           |              |                 |                  |         |            |            |
| CC             | 123 (66.49%) | 125 (69.06%)    | 1.000 (Reference) | .038    | 1.059 (0.839–1.313) | .678 |
| GA/GG          | 62 (33.51%)  | 56 (30.94%)     | 1.125 (0.709–1.787) | .377    | 1.125 (0.848–1.479) | .453 |
| Female         |              |                 |                  |         |            |            |
| CC             | 64 (55.65%)  | 73 (61.34%)     | 1.000 (Reference) | .377    | 1.125 (0.848–1.479) | .453 |
| GA/GG          | 51 (44.35%)  | 46 (38.66%)     | 1.265 (0.726–2.203) | .094    | 1.125 (0.848–1.479) | .453 |

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

* logistic regression modeling adjusted age, sex, and risk factor.

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**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) | .001    | 0.504 (0.383–0.653) | <.001 |
| 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) | .015    | 0.598 (0.450–0.778) | <.001 |
| 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) | .004    | 0.651 (0.464–0.892) | .006 |
| AG/GG          | 43 (23.24%)  | 80 (44.20%)     | 0.382 (0.238–0.614) | <.001   | 0.651 (0.464–0.892) | .006 |
| Female         |              |                 |                  |         |                     |            |
| AA             | 83 (72.17%)  | 64 (53.78%)     | 1.000 (Reference) | .004    | 0.651 (0.464–0.892) | .006 |
| 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.
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 GA 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...
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%. 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. 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. 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. 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, 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. 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. 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. The genetic variation for IL-6 is associated with diseases caused by abnormal inflammatory responses. Studies have shown that IL-6 expression levels are abnormally elevated after acute inflammatory response, 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. The TLR4-mediated MyD88 signaling pathway regulates the expression of multiple genes involved in inflammation during the immune response to pathogen invasion. 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. Studies have shown that the single nucleotide polymorphism at the rs7744 locus of MyD88 gene is closely related to the susceptibility of ulcerative colitis. 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

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References

[1] Pan C, Liu L, Xie JP, et al. Acute respiratory distress syndrome: challenge for diagnosis and therapy. Chin Med J (Engl) 2018;131:1220–4.
[2] Belluni G, Laffey JG, Pham T, et al. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA 2016;315:788–800.
[3] Zhang Z, Chen L, Ni H. The effectiveness of Corticosteroids on mortality in patients with acute respiratory distress syndrome or acute lung injury: a secondary analysis. Sci Rep 2015;5:17654.
[4] Avasarala S, Zhang F, Liu G, et al. Curcumin modulates the inflammatory response and inhibits subsequent fibrosis in a mouse model of viral-induced acute respiratory distress syndrome. Plos One 2018;3:e57285.
[5] Malcolm KC, Kret JE, Young RL, et al. Bacteria-specific neutrophil dysfunction associated with interferon-stimulated gene expression in the acute respiratory distress syndrome. Plos One 2011;6:e21958.
[6] O'Mahony DX, Glavan BJ, Holden TD, et al. Inflammation and immune-related candidate gene correlations with acute lung injury susceptibility and severity: a validation study. Plos One 2012;7:e51104.
[7] Ramos I, Fernandez-Sesma A. Modulating the innate immune response to influenza a virus: potential therapeutic use of anti-inflammatory drugs. Front Immunol 2015;6:361.
[8] Medzhitov R, Janeeway C Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev 2000;173:89–97.
[9] Rosato M, Curtale G, Tamassia N, et al. IL-10-induced microRNA-187 negatively regulates TNF-alpha, IL-6, and IL-12p40 production in TL1R4-stimulated monocytes. Proc Natl Acad Sci U S A 2012;109: E3101–3110.
[10] Li W, Liu HD, You WC, et al. Enhanced cortical expression of myeloid differentiation primary response protein 88 (Myd88) in patients with traumatic brain injury. J Surg Res 2013;180:133–9.
[11] Chen L, Chen H, Chen P, et al. Development of 2-amino-4-phenyl-thiazole analogues to disrupt myeloid differentiation factor 88 and prevent inflammatory responses in acute lung injury. Eur J Med Chem 2019;161:22–38.
[12] Zhou L, Liu Z, Wang Z, et al. Astragalus polysaccharides exerts immunomodulatory effects via TLR4-mediated MyD88-dependent signaling pathway in vitro and in vivo. Sci Rep 2017;7:44822.
[13] Mandal RK, Khan MA, Hussam A, et al. A trial sequential meta-analysis of TNF- alpha -308G>A (rs800829) gene polymorphism and susceptibility to colorectal cancer. Biosci Rep 2018;39: doi: 10.1042/BSR20181052.
[14] Zhao B, Li X, Li R. Genetic relationship between IL-6 rs1800796 Polymorphism and Susceptibility to Periodontitis. Immunol Invest 2018;1–5.
[15] Sun D, Sun L, Xu Q, et al. SNP-SNP interaction between TLR4 and MyD88 in susceptibility to coronary artery disease in the chinese han population. Int J Environ Res Public Health 2016;13.
[16] Force ADT, Raman VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012;307:2526–33.
[17] Moore JH, Andrews PC. Epistasis analysis using multifactor dimensionality reduction. Methods Mol Biol 2015;1253:301–14.
[18] Beauchamp CA, Sheean PM, Peterson SJ, et al. Intensive nutrition in acute lung injury: a clinical trial (INTACT). JPEN J Parenter Enteral Nutr 2015;39:13–20.
[19] Silversides JA, Ferguson AJ, McAuley DF, et al. Fluid strategies and outcomes in patients with acute respiratory distress syndrome, systemic inflammatory response syndrome and sepsis: a protocol for a systematic review and meta-analysis. Syst Rev 2015;4:162.
[20] Fujishima S, Morisaki H, Ishizaka A, et al. Neutrophil elastase and systemic inflammatory response syndrome in the initiation and development of acute lung injury among critically ill patients. Biomed Pharmacother 2008;62:333–8.
[21] Butt Y, Kordowska A, Allen TC. Acute lung injury: a clinical and molecular review. Arch Pathol Lab Med 2016;140:343–50.
[22] Yang Z, Zhang XR, Zhao Q, et al. Knockdown of TNFalpha alleviates acute lung injury in rats with intestinal ischemia and reperfusion injury by upregulating IL10 expression. Int J Mol Med 2018;42:926–34.
[23] Wilson RM, Goddard ME, O’Dea KP, et al. Differential roles of p35 and p73 tumor necrosis factor receptors on stretch-induced pulmonary edema in mice. Am J Physiol Lung Cell Mol Physiol 2007;293:L60–68.
[24] Azevedo ZM, Moore DB, Lima FC, et al. Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA) single nucleotide polymorphisms: importance in ARDS in septic pediatric critically ill patients. Hum Immunol 2012;73:661–7.
[25] Yang ML, Wang CT, Yang SJ, et al. IL-6 ameliorates acute lung injury in influenza virus infection. Sci Rep 2017;7:43829.
[26] Zhang H, Neuhofe P, Song L, et al. IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. J Clin Invest 2013;123:1019–31.
[27] Assiku IP, Yamal JM, Doshi P, et al. Plasma cytokines IL-6, IL-8, and IL-10 are associated with the development of acute respiratory distress syndrome in patients with severe traumatic brain injury. Crit Care 2016;20:288.
[28] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014;6:a016295.
[29] Taniguchi K, Karin M. IL-6 and related cytokines as the critical lymphins between inflammation and cancer. Semin Immunol 2014;26:54–74.
[30] Jordan SC, Choi J, Kim I, et al. Interleukin-6, A cytokine critical to inflammation syn- drome and survival among end stage renal disease patients. J Interferon Cytokine Res 2013;33:384–91.
[31] Wang XM, Hamza M, Wu TX, et al. Upregulation of IL-6, IL-8 and TNF-alpha gene polymorphism with malnutrition in surgical intensive care units in 50 countries. JAMA 2016;315:788–800.
[32] Matsunaga K, Tahara T, Shiroeda H, et al. The *1244A>G polymorphism of MyD88 (rs744) is closely associated with susceptibility to ulcerative colitis. Mol Med Rep 2014;9:28–32.

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