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Relationship between β-defensin-1 gene polymorphism and susceptibility and prognosis of acute respiratory distress syndrome

Qijia Feng, PhD[a], Nan Liu, PhD[b], Shuping Song, PhD[b], Yufei Ma, PhD[a,*]

Abstract

Objective: The 1st exon 5′ noncoding region rs1799946 (-52A/G), rs1800972 (-44C/G), rs11362 (-20A/G) 3 single-nucleotide polymorphisms (SNPs) on human β-defensin-1 (HBD-1) gene affect its transcription and posttranscriptional mRNA stability then affect the activity of HBD-1. This study was to investigate the effects of HBD-1 gene rs1799946, rs1800972, and rs11362 locus SNPs on genetic susceptibility and prognosis of acute respiratory distress syndrome (ARDS).

Methods: A total of 300 patients with ARDS (ARDS group) and 240 patients who were admitted to the intensive care unit and had a high risk of ARDS but did not progress to ARDS (control group) were included in this study. The genotypes of HBD-1 gene rs1799946, rs1800972, and rs11362 locus and serum HBD-1 were detected. Patients were followed for 60 days with development of ARDS as a primary outcome, ARDS-related mortality and organ dysfunction were secondary outcomes.

Results: HBD-1 gene rs1799946 and rs11362 gene mutations were not risk factors for ARDS ($P > .05$). Mutation allele G of rs1800972 locus in HBD-1 gene was a risk factor for ARDS. There was no significant difference in serum HBD-1 levels between patients with different genotypes of rs1799946 and rs11362 locus in the HBD-1 gene ($P > .05$). HBD-1 gene rs1800972 locus wild type, heterozygous, and mutant homozygous serum levels of HBD-1 gradually decreased, the difference was statistically significant ($P < .001$). The 60-day survival rate of subjects with wild type, heterozygous, and mutant homozygote at the rs1800972 locus of HBD-1 gene decreased sequentially (81.7%, 48.9%, and 39.7%), and the difference was statistically significant ($P < .05$).

Conclusion: The SNP of rs1800972 (-44C/G) in HBD-1 gene is associated with the risk of ARDS. The rs1800972 locus G allele carriers are more likely to develop ARDS and have a poor prognosis.

Abbreviations: AECC = American-European Consensus Conference, APACHE = Acute Physiology and Chronic Health Evaluation, ARDS = acute respiratory distress syndrome, BMI = body mass index, CI = confidence interval, ELISA = enzyme-linked immunosorbent assay, HBD-1 = human β-defensin-1, IL = interleukin, NF-κB = nuclear factor kappa B, OR = odds ratio, PCR = polymerase chain reaction, SD = standard deviation, SNP = single-nucleotide polymorphism.

Keywords: acute respiratory distress, human β-defensin-1, prognosis, single-nucleotide polymorphism

1. Introduction

Acute respiratory distress syndrome (ARDS) is one of the main causes of death in critically illness patients. In recent years, it has been one of the hotspots in the research of respiratory and critical medicine.[1] Despite the continuous improvement in the understanding and treatment of ARDS in recent years, the mortality rate of patients is still as high as 30% to 50%.[2,3] The common risk factors of ARDS include sepsis (without shock), trauma, septic shock, aspiration, pneumonia, but most of these people do not develop into ARDS.[4] In addition, genetic factors play an important role in the development of ARDS, and the genetic variation of proinflammatory cytokines and anti-inflammatory cytokines affects the incidence, severity, and mortality of ARDS.[5–9]

Human β-defensin-1 (HDB-1) is a class of cationic peptides with antibacterial activity, which play an important role in the antimicrobial process of respiratory mucosa.[10] Studies have shown that HBD-1 gene polymorphism is related to the susceptibility and prognosis of pulmonary infectious diseases such as chronic obstructive pulmonary disease and asthma.[11–13] However, there are few relevant studies about whether HBD-1 gene polymorphism affects the susceptibility to ARDS and the survival time of prognosis. The HBD-1 gene is located on the 8p23 chromosome. The 3 single-nucleotide polymorphisms (SNPs) of the 1st exon's 5′ noncoding region, including rs1799946 (-52A/G), rs1800972 (-44C/G), and rs11362 (-20A/G), influence the transcription of the HBD-1 gene and influence the stability of posttranscriptional mRNA, which subsequently affect the activity of HBD-1.[14] Studies have shown that this region may be the binding site of nuclear factor kappa B (NF-κB), regulating the expression of β-defensin in human epithelial cells.[15] Therefore, it was speculated that the

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polymorphism may have some correlation with the occurrence and development of ARDS.

This study aims to use case–control study to explore the effects of SNPs of rs1799946, rs1800972, and rs11362 on the susceptibility of ARDS and prognosis of survival, as well as to analyze the roles of genetic factors in the occurrence and development process of ARDS.

2. Material and methods

2.1. Subjects

A total of 300 patients diagnosed with ARDS and 240 patients who had a high risk but did not progress to ARDS (control group) in emergency intensive care unit (ICU) were enrolled in this study from August 2015 to October 2017 at Hangzhou Lin'an District People's Hospital and Sir Run Run Shaw Hospital. The diagnostic criteria for ARDS refer to the American-European consensus conference on ARDS set in 1994.[16] The symptoms of patients in control group mainly includes sepsis (no shock), septic shock, aspiration, pneumonia, and trauma. Inclusion criteria: age ≥20 years; consistent with the diagnostic criteria for ARDS or have risk factors of ARDS; the clinical data of patients was complete. Exclusion criteria: have autoimmune disease or acquired immunodeficiency syndrome; receive chemotherapy or radiotherapy recently; have a serious respiratory illness; have serious chronic liver disease (Child–Pugh score > 10). This study was approved by the Medical Ethics Committee of our hospital and signed informed consent was obtained from all patients. Collect the clinical data of all patients within 24 hours after being diagnosed with ARDS, including age, gender, body mass index (BMI), morbidity of diabetes, smoking, drinking, and risk factors of ARDS, such as sepsis (without shock), septic shock, aspiration, pneumonia, trauma, etc, and evaluate the severity of patients with Acute Physiology and Chronic Health Evaluation (APACHE) II scoring system.[17]

2.2. Genotyping and single-nucleotide polymorphism

About 10 mL fasting blood of ulnar vein was collected. Leukocyte genomic DNA was isolated using QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) after plasma separation. The genotypes of HBD-1 gene rs1799946 (-52A/G), rs1800972 (-44C/G), and rs11362 (-20A/G) loci were analyzed by polymerase chain reaction (PCR)/Sanger sequencing. The forward and reverse primers are shown as Table 1. The PCR system: 100 ng gDNA, 2.5 μL 10× buffer, 1.5 μL 25 mol/L $MgCl_2$, 0.25 μL 5 U/μL Taq DNA polymerase, 0.2 μL dNTP, 1 μL 10 mmol/L forward primer, 1 μL 10 mmol/L reverse primer, add sterile water to 25 μL. The PCR condition: 95°C initial denaturation 5 minutes, 95°C denaturation 30 seconds, annealing temperature 60°C 30 seconds, 72°C extension 45 seconds, 25 cycles, 72°C extension 10 minutes. After PCR, the nucleotide sequence of the target fragment was detected by Sanger sequencing, as shown in Figure 1.

Table 1

The primer sequence for HBD gene amplification.

SNPs	Sequence from 5′ to 3′
rs1799946 (-52A/G)	
Forward primer	TCTGGAAGCCTCTGTCAGCTC
Reverse primer	AGGCAACACCCAGGATTTCAG
rs1800972 (-44C/G)	
Forward primer	AGGCAACACCCAGGATTTCA
Reverse primer	GGAAGCCTCTGTCAGCTCAG
rs11362 (-20A/G)	
Forward primer	CTCCAAAGGAGCCAGCGTCT
Reverse primer	GGAAGTTCTCATGGCGACTGG

HBD = human β-defensin, SNPs = single-nucleotide polymorphisms.

2.3. Serum HBD-1 level detection

Enzyme-linked immunosorbent assay (ELISA) was used to detect the serum HBD-1 level of the subjects. The ELISA detection kit for serum HBD-1 level was purchased from Shanghai Enzyme-linked Biotechnology Co, Ltd (Shanghai City, China; No: ml038480) and all the operations were carried out in strict accordance with the kit instructions.

2.4. Statistical analysis

Quantitative data were analyzed using the Statistical Package for the Social Sciences, version 20.0 (SPSS 20.0) and were presented as mean ± standard deviation (SD). Mann–Whitney test was used for compare among groups. The difference of genotype frequency and allele frequency between ARDS group and control group was tested by Chi-squared test or Fisher exact probability test, and the odds ratio (OR) value and 95% confidence interval (CI) of allele frequency were calculated. Hardy–Weinberg equilibrium of alleles was tested by Chi-squared test. Logistic regression analysis was used to correct the confounding factors that affect ARDS susceptibility and Bonferroni method was used for multiple test correction. Haplotypes were reconstructed from population genotype data using Phase software (version 2.1).[18,19] We conducted a study of grade 2 outcomes for patients progressed to ARDS, including mortality in 60 days and organ dysfunction determined by the daily multiple organ dysfunction syndrome score. The subjects with different genotypes were compared, and the mortality was analyzed in the time-event analysis of the Cox proportional hazard model. $P < .05$ was statistically significant.

3. Results

3.1. General clinical characteristics

A total of 300 patients diagnosed with ARDS and 240 patients who had a high risk but did not progress to ARDS (control group) in emergency ICU were enrolled in this study. The general information of patients in ARDS group and control group are shown as Table 2. The common risk factors of ARDS include sepsis (without shock), trauma, septic shock, aspiration, pneumonia. There is no significant difference between ARDS group and control group in age, gender, BMI, morbidity of diabetes, smoking, drinking, and risk factors of ARDS ($P < .05$). The APACHE II score of ARDS group patients was significantly higher than that of control group patients ($P < .05$).

3.2. Association of HBD-1 gene polymorphisms with occurrence risk of ARDS

The genotype distributions and allele frequencies in rs1799946, rs1800972, and rs11362 loci of HBD-1 gene in ARDS group and control group are shown in Table 3. Chi-squared test demonstrated that the genotype frequencies of HBD-1 gene SNPs were all in Hardy–Weinberg equilibrium ($P > .05$), and

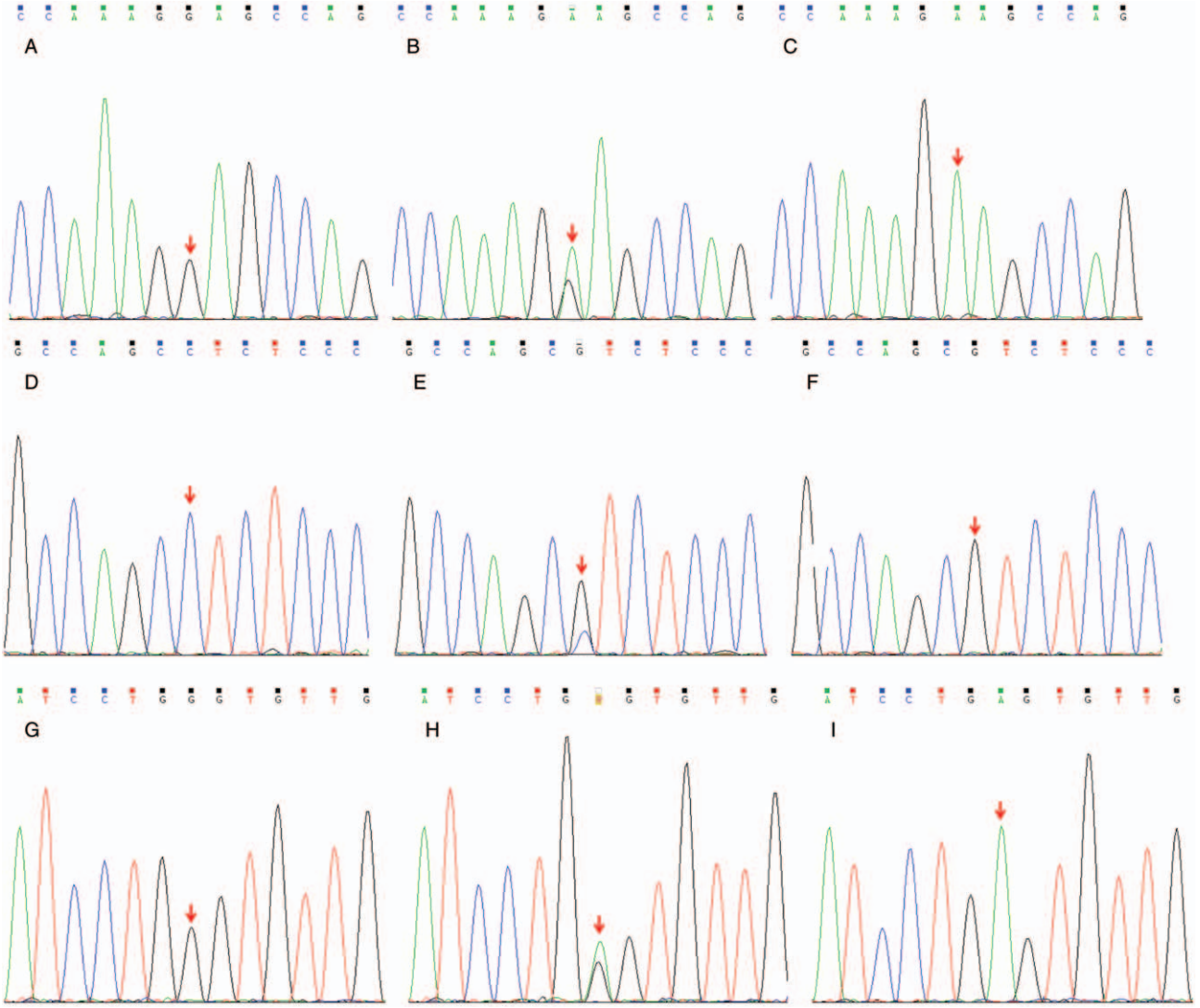

Figure 1. Sanger sequencing results. (A–C) rs1799946 GG, GA, AA genotype. (D–F) rs1800972 CC, CG, GG genotype. (G, H) rs11362 GG, GA, AA genotype.

Table 2

General clinical characteristics of subjects in ARDS group and control group.

Characteristic	ARDS group (n = 300)	Control (n = 240)	*P*-value
Age (mean ± SD)	60.2 ± 12.7	59.6 ± 13.5	.596
Female, n (%)	115 (38.3)	102 (42.5)	.326
BMI (kg/m^2,mean ± SD)	22.9 ± 3.3	23.2 ± 2.7	.246
APACHE II score (mean ± SD)	19.9 ± 3.4	15.6 ± 2.2	<.001
Diabetes, n (%)	51 (17.0)	39 (16.3)	.816
Smoking history, n (%)	134 (44.7)	97 (40.4)	.321
Alcohol abuse history, n (%)	27 (9.0)	29 (12.1)	.243
Risk factors, n (%)			
Sepsis (without shock)	136 (45.3)	93 (37.8)	.124
Septic shock	180 (60.0)	135 (56.3)	.380
Aspiration	9 (3.0)	10 (4.2)	.465
Pneumonia	204 (68.0)	149 (62.1)	.151
Trauma	21 (7.0)	20 (8.3)	.561

ARDS = acute respiratory distress syndrome, APACHE = Acute Physiology and Chronic Health Evaluation, BMI = body mass index, SD = standard deviation.

there was linkage disequilibrium among the 3 gene loci (Fig. 2). The mutation of rs1799946 loci and rs11362 loci of HBD-1 gene were not the risk factors of ARDS ($P > .05$), while the mutant allele G in rs1800972 loci of HBD-1 gene was a risk factor of ARDS (OR = 1.297, 95% CI = 1.136–1.449, $P < .001$). Haploid detection showed that there were 3 haplotypes in the 3 SNP loci of rs1799946, rs1800972, and rs11362, with GGG, GCA, and ACG respectively. Refer to the GGG haplotype of rs1799946, rs1800972, and rs11362 loci, ACG haplotype (OR = 1.292, 95% CI = 1.032–1.644, $P = .023$), but not GCA haplotype ($P > .05$), was the risk factor of ARDS.

3.3. Serum HBD-1 expression level

The ELISA method was used to detect the serum HBD-1 level of all subjects, and the results showed that the serum HBD-1 level of ARDS group patients was significantly lower than control group ([94.5 ± 23.1] μg/L vs [548.2 ± 55.3] μg/L, $P < .001$). The comparison of serum HBD-1 level among different genotypes in rs1799946, rs1800972, and rs11362 loci of HBD-1 gene are shown in Fig. 3. There was

Table 3

Comparison of genotype distributions and allele frequencies of human β-defensin-1 gene SNPs between ARDS group and control group.

SNP	ARDS group (n = 300)	Control group (n = 240)	OR (95% CI)*	P*
rs1799946				
Genotype				
GG	195 (65.0%)	150 (62.5%)	1.0	
GA	87 (29.0%)	73 (30.4%)	0.962 (0.800–1.141)	.722
AA	18 (6.0%)	17 (7.1%)	0.910 (0.600–1.234)	.689
Allele				
G	477 (79.5%)	373 (77.7%)	1.0	
A	123 (20.5%)	107 (22.3%)	0.953 (0.824–1.088)	.522
rs1800972				
Genotype				
CC	186 (62.0%)	192 (80.0%)	1.0	
CG	93 (31.0%)	43 (17.9%)	1.390 (1.175–1.608)	<.001
GG	21 (7.0%)	5 (2.1%)	1.641 (1.199–1.914)	.004
Allele				
C	483 (77.5%)	427 (89.0%)	1.0	
G	117 (22.5%)	53 (11.0%)	1.297 (1.136–1.449)	<.001
rs11362				
Genotype				
GG	87 (29.0%)	80 (33.3%)	1.0	
GA	141 (47.0%)	110 (45.8%)	1.078 (0.895–1.310)	.471
AA	72 (24.0%)	50 (20.8%)	1.133 (0.906–1.403)	.294
Allele				
G	315 (52.5%)	270 (56.3%)	1.0	
A	285 (47.5%)	210 (43.8%)	1.069 (0.958–1.192)	.243
rs1799946/rs1800972/rs11362				
Haplotype				
GGG	53 (17.7%)	57 (23.8%)	1.0	
GCA	125 (41.7%)	109 (45.4%)	1.109 (0.881–1.425)	.429
ACG	122 (40.7%)	74 (30.8%)	1.292 (1.032–1.644)	.023

ARDS = acute respiratory distress syndrome, CI = confidence interval, OR = odds ratio, SNP = single-nucleotide polymorphism.
* Correction according to age, gender, body mass index, diabetes, smoking, drinking, and other factors.

no significant difference of serum HBD-1 level among different genotype subjects in rs1799946 and rs11362 loci of HBD-1 gene ($P > .05$). However, the level of serum HBD-1 was gradually decreased in the wild type, heterozygous genotype and homozygous mutant of HBD-1 rs1800972 loci, and the difference was statistically significant ($P < .001$).

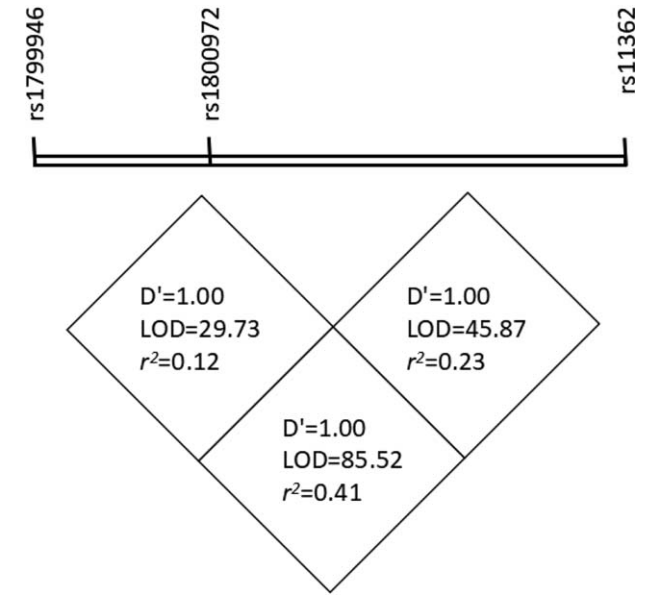

Figure 2. Linkage disequilibrium between position rs1799946, rs1800972, and rs11362 of the human β-defensin-1 gene. D′ = D prime, LOD = logarithmic odds score, r^2 = correlation coefficient.

3.4. Association of HBD-1 gene polymorphisms with prognosis survival period of ARDS

The prognosis survival period of subjects with different genotypes in rs1799946, rs1800972, and rs11362 loci of HBD-1 gene is shown in Figure 4. The prognosis 60-day survival rate of subjects gradually decreased from GG genotype (78.5%), GA genotype (75.7%) to AA genotype (48.3%) in rs1799946 loci of HBD-1 gene and 1-way analysis of variance (ANOVA) showed that there was no significant difference in the 60-day mortality among different genotypes of this locus ($P = .566$, Fig. 4A). The prognosis 60-day survival rate of subjects gradually decreased from CC genotype (81.7%), CG genotype (48.9%) to GG genotype (39.7%) in rs1800972 loci of HBD-1 gene and 1-way ANOVA analysis showed that there was significant difference in the 60-day mortality among different genotypes of this locus ($P = .029$, Fig. 4B). The prognosis 60-day survival rate of subjects gradually decreased from GG genotype (64.5%), GA genotype (60.7%) to AA genotype (52.4%) in rs11362 loci of HBD-1 gene and 1-way ANOVA analysis showed that there was no significant difference in the 60-day mortality among different genotypes of this locus ($P = .586$, Fig. 4C).

4. Discussion

This study provides evidence of the relationship between the susceptibility and prognosis of ARDS and 3 SNPs in rs1799946

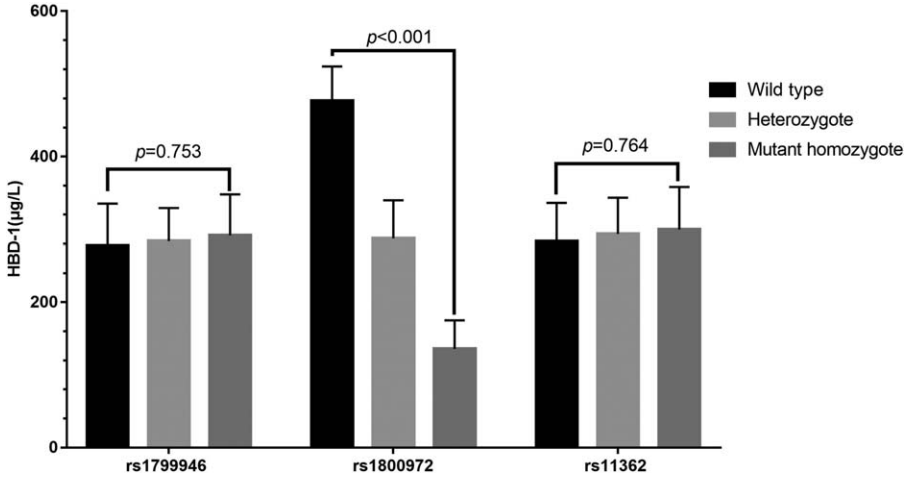

Figure 3. Serum human β-defensin-1 (HBD-1) levels ELISA test results. rs1799946 locus wild type: GG genotype, heterozygote: GA, mutant homozygote: AA. rs1800972 locus wild type: CC genotype, heterozygote: CG, mutant homozygote: GG. rs11362 locus wild type: GG genotype, heterozygote: GA, mutant homozygote: AA.

(-52A/G), rs1800972 (-44C/G), and rs11362 (-20A/G) loci of HBD-1 gene. Currently, the research on genetic susceptibility to ARDS is mainly focused on inflammatory mediator-related encoding genes. For example, Gong et al[20] reported that subjects with 54BB genotype of mannose binding lectin-2 gene are more susceptible to ARDS. And Tejera et al[21] have found the polymorphism in rs2664581 loci of PI3 gene was associated with increased risk of ARDS. Moreover, the results of Zhang et al[22] showed that the gene mutation of protein C was related to the genetic susceptibility of ARDS in Chinese Han population, and the variation of this gene structure affects the plasma APC concentration in patients with ARDS. However, few studies have reported the effects of HBD-1 gene on ARDS genetic susceptibility so far.

The HBD-1 is widely distributed in respiratory epithelial tissue. Studies have shown that HBD-1 present inherent persist expression in the proximal and distal bronchoalveolar epithelial cells of human airway,[23] and play an important role in the process of anti-microbial of respiratory mucosa.[10] β-Defensin family has several members, with genes locating on p23 region of chromosome 8. HBD-1 has been one of the earliest discovered β-defensins. However, the role and mechanism of β-defensins in occurrence and development of disease is not clear, therefore this study focused on the correlation between HBD-1 gene with ARDS using genetic association.

Kalus et al[24] found that the polymorphisms in the 5′UTR region of the HBD-1 gene regulate the expression level of the HBD-1 and HBD-3. Further Nurjadi et al[14] found that the expression level and the probability of continuous delivery of HBD-3 and HBD-1 in the injured skin were negatively correlated with the genetic polymorphisms of -52 (G > A), -44 (C > G) and -52/-44/-20 ACG haploid. Therefore, HBD-1 gene promoter polymorphism is the genetic basis of altered HBD expression. These study found that there was no significant difference between the ARDS group and control group in the genotype and allele frequencies of the HBD-1 gene rs1799946 and rs11362 loci ($P > .05$), but the frequency of mutant G allele in the rs1800972 loci of HBD-1 gene in the ADRS group was significantly higher than that in the control group. We analyzed the expression level of HBD-1 in subjects with different genotypes at these three SNP loci and found that there was no statistical difference in the serum HBD-1 level between subjects with different genotypes at rs1799946 and rs11362 loci of HBD-1 gene ($P > .05$), while the serum HBD-1 level gradually decreased from subjects with wide type, heterozygous to mutant homozygous at the rs1800972 loci ($P < .001$). Therefore, we consider that the SNPs of

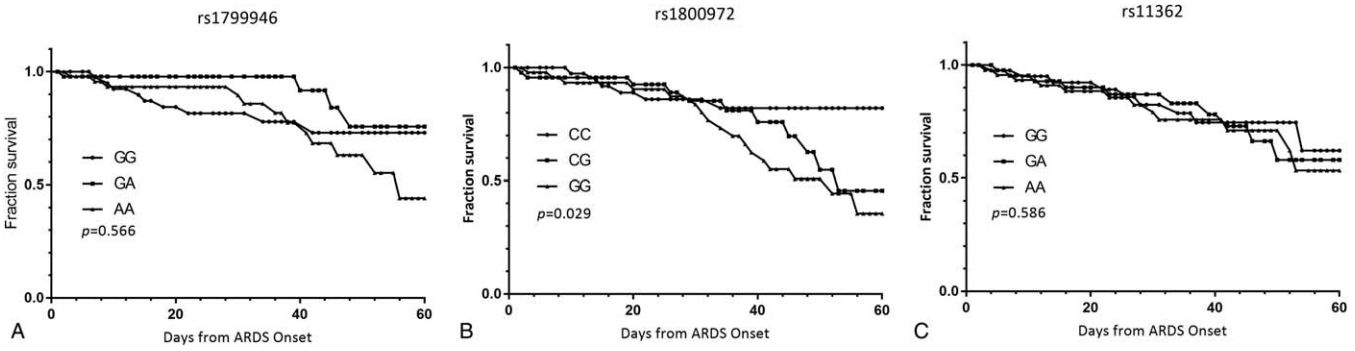

Figure 4. Kaplan–Meier analysis of 60-day survival by genotype of human β-defensin-1 gene single-nucleotide polymorphisms. (A) 60-day survival for rs1799946 locus different genotype. (B) rs1800972. (C) rs11362. *P*-value stated is for Cox proportional hazards model of adjusted hazard of death within 60 days. ARDS = acute respiratory distress syndrome.

rs1799946 (-52A/G) loci and rs11362 (-29A/G) loci in the HBD-1 gene do not affect the expression of HBD-1 protein, and these 2 loci are not the core region regulating HBD-1. While the SNP of rs1800972 (-44C/G) loci is the key region regulating HBD-1 and affect the expression level of HBD-1, with low expression level of HBD-1 in the subjects with mutant genotypes. The upper and lower gene sequence of rs1800972 loci is adjacent to the motif of CCAAT (cytosine-cytosine-adenosine-adenosine-thymidine)-enhancer-binding protein B homogeneity and NF-κB-binding site.[25] CCAAT is the functional regulation area of interleukin 6 (IL-6), IL-8, IL-12, and nitric oxide synthase genes, while NF-κB is an inducible factor binding zone of inducible defension[26]; therefore, the polymorphism of rs1800972 loci may affect the transcription of HBD-1 gene. Polesello et al[27] found that the expression level of HBD-1 in the -52G/G carriers was higher than that in the G/A and A/A carriers and the expression level of HBD-1 in the -44C/G carriers is lower than that in the wild type subjects, while there was no significant difference in the HBD-1 expression level between subjects with different genotypes in the -20G > A site. However, in this study, we only found that the HBD-1 expression level gradually increased from the mutant homozygote, heterozygote to wild-type subjects in the -44C/G loci, which were not consistent with the results of Polesello et al. However, there was no change in the HBD-1 expression level of subject with different genotypes in the other loci. We consider that this could be related to the sample size, and whether it is related to different races is lack of support by large clinical data at present.

To further verify the relationship between the 3 SNPs of rs1799946 (-52A/G), rs1800972 (-44C/G), and rs11362 (-20A/G) loci and the prognosis of patients with ARDS, we followed up for 60 days. The results showed that the 60-day survival rate in patients with wild genotypes of -52A/G and -20A/G loci was higher than those with mutant genotypes, but the difference was not statistically significant which was believed that the polymorphism of -52A/G and -2A/G loci is not related to the prognosis of patients with ARDS. This can be attributed to the fact that the SNPs of these 2 loci dose not significantly alter the expression level of HBD-1. However, the expression level of serum HBD-1 in patients with mutant genotype of rs1800972 (-44C/G) loci was significantly reduced, and the 60-day survival rate was therefore significantly reduced.

From the results of this study, it can be found that genetic factors play an important role in the occurrence and prognosis of ARDS. The results suggest that the occurrence and development of ARDS may be significantly correlated with the expression of genes related to inflammatory mediators. Through this discovery, new ideas can be provided for the prevention and treatment of ARDS, and more active preventive and therapeutic measures can be taken for specific populations, especially high-risk groups.

There are several advantages in this study. Firstly, we determine ARDS according to the standard of AECC and minimize the mistake of phenotypic classification. Secondly, we use the critical patients with the risk factors of ARDS, instead of the healthy subjects or the other population, as the control. However, there are some shortcomings in this study. We did not restrict patients to be in same area and to be the same ethnic group. Therefore, it is not well known whether geographical and racial factors have an impact on the results of this study.

5. Conclusion

The SNP of rs1800972 (-44C/G) in HBD-1 gene is associated with the risk of ARDS. The rs1800972 locus G allele carriers are more likely to develop ARDS and have a poor prognosis. Moreover, the ACG haploid carrier of HBD-1 gene rs1799946/rs1800972/rs11362 loci is a high incidence group of ARDS.

Author contributions

QJF conducted, designed, and analyzed the experiments and wrote the paper. NL analyzed some of the experiments. SPS performed a few of the experiments. YFM designed the experiments, analyzed some data, and wrote the study.
Conceptualization: Qijia Feng, Yufei Ma.
Data curation: Qijia Feng.
Formal analysis: Qijia Feng, Yufei Ma.
Investigation: Qijia Feng, Nan Liu, Shuping Song.
Methodology: Nan Liu.
Resources: Shuping Song.
Software: Nan Liu.
Writing – original draft: Qijia Feng.
Writing – review & editing: Yufei Ma.

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