Int J Clin Exp Med 2018;11(12):13501-13510
www.ijcem.com /ISSN:1940-5901/IJCEM0086491

Original Article

Association of polymorphisms in IL-10 and the TLR4 signaling pathway with the development of postoperative sepsis

Liu Yong[1], Duan Peng[1], Zhipeng Chen[1], Jiandong Lin[2]

[1]*Surgical Intensive Care Unit, Zhangzhou Affiliated Hospital of Fujian Medical University, Zhangzhou City, Fujian Province, China;* [2]*Intensive Care Unit, The First Affiliated Hospital of Fujian Medical University, Fuzhou City, Fujian Province, China*

Received October 9, 2018; Accepted November 9, 2018; Epub December 15, 2018; Published December 30, 2018

Abstract: Objective: To investigate the association of polymorphisms in interleukin-10 (*IL-10*) and the Toll-like receptor 4 (TLR4) signal pathway genes *TLR4* and myeloid differentiation factor 88 (*MyD88*) with the occurrence of postoperative sepsis. Methods: Data from 203 patients who underwent surgery were retrospectively analyzed. The analysis included 104 patients who developed sepsis after surgery as the sepsis group and 99 patients who did not develop sepsis after surgery as the control group. Polymorphisms in *IL-10*, *TLR4*, and *MyD88* were detected by PCR and sequencing. Logistic regression analysis was used to evaluate the relationship between genetic polymorphism and septic shock, organ dysfunction as well as the survival rate in sepsis. Results: There was statistical significance in the distribution frequencies of the *IL-10* rs1800896 AA, GG, and AG polymorphisms, *TLR4* rs10759932 TT, TC, and CC polymorphisms, and the *MyD88* rs6853 AA, GG, and AG polymorphisms between the sepsis and control groups ($P < 0.05$, for all three). The *IL-10* rs1800896 locus was associated with the occurrence of sepsis. Compared with the AA genotype, the GG+AG genotype was associated with an increased risk of sepsis ($P < 0.05$). The *TLR4* rs10759932 locus was also associated with the occurrence of sepsis. Compared with the TT genotype, the TC+CC genotype was also associated with an increased risk of sepsis ($P < 0.05$). The *MyD88* rs6853 locus was associated with the occurrence of sepsis. Compared with the AA genotype, the GG+AG genotype was also associated with an increased risk of sepsis ($P < 0.05$). The IL-10 rs1800896 AA, GG, and AG genotypes, TLR4 rs10759932 TT, TC, and CC genotypes, and MyD88 rs6853 AA, GG, and AG genotypes were not significantly correlated with septic shock, organ dysfunction, or survival ($P > 0.05$). Conclusion: The rs1800896 polymorphic locus of *IL-10*, the rs10759932 polymorphic locus of *TLR4*, and the rs6853 polymorphic locus of *MyD88* were closely related to the risk of postoperative sepsis.

Keywords: Sepsis, IL-10, TLR4 signaling pathway, gene polymorphism, occurrence

Introduction

Sepsis is an inflammatory response syndrome caused by microbial invasion of the body after severe trauma, shock, burn, or major surgery. It is a systemic multiple organ dysfunction syndrome and a major cause of death in critically ill patients [1]. Although great progress has been made in the treatment of critically ill patients with the development of various medical technologies, the rates of sepsis-induced shock and death due to organ dysfunction remain high [2]. Based on in-depth studies of physiology and pathogenesis of sepsis, most researchers believe that, in addition to the virulence factors of invading pathogenic bacteria, genetic variation factors also play an important role in the occurrence and development of sepsis [3, 4]. The balance between cytokine-induced anti-inflammatory and pro-inflammatory responses plays an important role in the occurrence and development of sepsis [5]. Interleukin-10 (IL-10), which is secreted by TH2 cells, is an anti-inflammatory cytokine that inhibits the function of TH1 cells [6]. IL-10 is part of a major cytokine network, and IL-10 levels are closely related to the occurrence, development, and prognosis of sepsis. Studies have shown that the levels of secreted IL-10 are affected by genetic factors, and *IL-10* polymorphisms are closely related to

Table 1. Sequences of 8 SNPs of IL-10, TLR4 and MyD88 Genes

Gene	SNPs	Forward	Reverse	Amplicon size (bp)
IL-10	rs1800871	5'-TCATTCTATGTGCTGGAGATGG-3'	5'-TGGGGGAAGTGGCTAAGAGT-3'	377
	rs1800872	5'-CCTAGGTCACAGCGTGG-3'	5'-GGTGAGCACTACCTGACTAGC-3'	412
	rs1800896	5'-CCAAGACAACACTACTAAGGCTCCTTT-3'	5'-GCTTCTTATATGCTAGTCAGGTA-3'	209
TLR4	rs10759932	5'-ACGTTGGATGTTACAGACCAGAAAGTAAT-3'	5'-ACGTTGGATGTCCCACAAATGGTGTACACG-3'	125
	rs11536889	5'-ACGTTGGATGGAACCCCATTAATTCCAGAC-3'	5'-ACGTTGGATGTTTCCTGTTGGGCAATGCTC-3'	109
	rs27371903	5'-ACGTGGATGAGTGATGATTAGGGCTG-3'	5'-ACGTTGGATGCTCTGAACCACCTCCTCTAC-3'	108
MyD88	rs7744	5'-ACGTTGGATGACTCTGGAAAGGACCCAATG-3'	5'-ACGTTGGATGTGTGTGAGTTTAAGCAGCTC-3'	102
	rs6853	5'-ACGTTGGATGGCGTACAAAACATGTAGAAG-3'	5'-ACGTTGGATGCACCTGTCCCCCTTTAATAC-3'	90

Table 2. Participation in logistic regression analysis of individual variable assignments

Independent variable	Assignment	
Gender	Male = 1	Female = 2
Age	≥ 45 = 1	< 45 = 2
Smoking status	Yes = 1	No = 2
Drinking status	Yes = 1	No = 2
Whether there is chronic disease	Yes = 1	No = 2

its expression, which can affect the occurrence and development of sepsis [7].

Infections after major surgery are mainly caused by gram-negative pathogens. Toll-like receptor 4 (TLR4) specifically recognizes lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria that activates the immune system. It plays an important role in cell maturation, apoptosis, and promotion of inflammatory cytokine release [8]. It is reported that TLR4 gene polymorphisms may alter the extracellular structure of proteins and affect the binding of pathogenic ligands, especially LPS, to alter progression of sepsis [9]. Myeloid differentiation factor 88 (MyD88) is an important molecule in the TLR4 signaling pathway. In the immune response to pathogen invasion, the TLR4-mediated MyD88 signaling pathway can regulate the expression of a variety of related genes [10]. Mutations in the TLR4/MyD88 signaling pathway affect the activation, conduction, and function of the signaling pathway, thereby affecting the body's inherent immunity, making the body's immune system unable to detect pathogens and induce the body's immune response in a timely and effective manner, which have an impact on the occurrence of sepsis [11]. Therefore, we hypothesized that single nucleotide polymorphisms (SNPs) in the loci of *IL-10* and *TLR4* and their mediated signaling pathways may affect the expression and function of some genes, thereby affecting the immune status of the body and reducing the body's response to susceptible factors, which in turn affects the occurrence of sepsis.

Previous studies found that IL-10 and the TLR4 signaling pathway, which includes TLR4 and MyD88, are associated with a variety of diseases, such as pneumonia, diabetes, and asthma [12, 13]. However, there are few studies on the relationships between genetic polymorphisms and postoperative sepsis. By exploring the relationship between polymorphisms in *IL-10*, *TLR4* and *MyD88* and the susceptibility to and prognosis of postoperative sepsis in Chinese Han patients, this study will serve as a reference for future screening of sepsis-susceptible populations and studies on the mechanism of sepsis.

Materials and methods

General information

We retrospectively analyzed the data from 203 patients who underwent surgery at The First Affiliated Hospital of Fujian Medical University from April 2015 to February 2018. The patients included 104 patients who developed sepsis after surgery as the sepsis group and 99 patients who did not develop sepsis as the control group. The sepsis group included 60 males and 44 females, aged 42-65 years, with an average age of 54.75 ± 7.08 years. The control group included 69 males and 30 females, aged 40-66 years, with an average age of 52.15 ± 8.15 years. This study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University.

Table 3. Baseline data for study and control groups [n (%)]/ (x ± sd)

Category	Sepsis group (n = 104)	Control group (n = 99)	χ^2	*P* value
Gender			3.155	0.082
Male	60 (57.69)	69 (69.70)		
Female	44 (42.31)	30 (30.30)		
Age			0.754	0.426
≥ 45	74 (71.15)	75 (75.76)		
< 45	30 (28.85)	24 (24.24)		
Smoking status			0.287	0.647
Yes	33 (31.73)	28 (28.28)		
No	71 (68.27)	71 (71.72)		
Drinking status			0.327	0.587
Yes	20 (19.23)	16 (16.16)		
No	84 (80.77)	83 (83.84)		
Chronic diseases			0.219	0.663
Yes	39 (37.50)	34 (34.34)		
No	65 (62.50)	65 (65.66)		
Septic shock				
Yes	40 (38.46)	-		
No	64 (61.54)	-		
Organ dysfunction				
Yes	85 (81.73)	-		
No	19 (18.27)	-		
Survival				
No	26 (25.00)	-		
Yes	78 (75.00)	-		

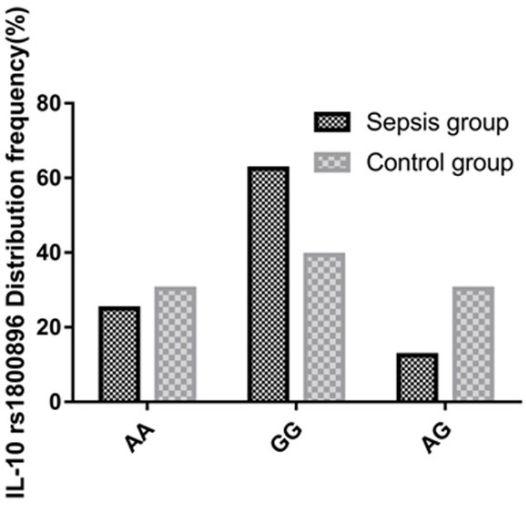

Figure 1. Distribution frequencies of the CC, CT, and TT genotypes at the *IL-10* rs1800871 locus in the study and control groups. Compared with AA, *P<0.05.

The subjects and their families provided full informed consent.

Inclusion and exclusion criteria

Diagnostic criteria for sepsis, septic shock, and organ dysfunction met the standards of the American Society of Critical Care Medicine (SCCM) and the American College of Chest Physicians (ACCP) [14]. Sepsis was defined as a positive bacterial culture at the site of infection or systemic inflammatory response syndrome caused by infection. Septic shock was defined as acute circulatory failure, with a systolic blood pressure < 90 mmHg and basal blood pressure < 40 mmHg [15]. Sequential organ failure was defined as an estimated score of organ dysfunction ≥ 2 points [16]. Age ≥ 40 years old; patients without previous treatments by antibiotics and vasoactive drugs; patients with infection combined with inadequate organ perfusion; high blood lactate levels; oliguria; peripheral circulation disorders; and conscious mind. Exclusion criteria included patients underwent emergency surgery; close relatives, genetic disorders, autoimmune diseases, previous psychosis, and a family history of mental illness, not or unwilling to accept central venous catheterization, or have a catheterization contraindication.

Methods

DNA extraction: Peripheral venous blood (5 ml) was taken in the morning before surgery with an empty stomach and placed in an EDTA anticoagulant tube. DNA was extracted using the DNA extraction kit (Beijing Suolaibao Technology Co., Ltd.), and stored at -20°C until use.

PCR amplification: The single nucleotide polymorphisms (SNPs) of *IL-10*, *TLR4* and *MyD88* that are associated with the development of immune diseases were retrieved from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP), which limits the frequency of minor alleles of candidate SNPs to > 5%. Three SNP loci of *IL-10* (rs1800871, rs1800872 and rs1800896) as well as three SNP loci of *TLR4* (rsl075-9932, rs11536889 and rs27371903) and two SNP loci of *MyD88* (rs7744 and rs6853) were selected. SNP loci primers were designed using

Table 4. Frequency distribution and comparison of genotype frequencies of 3 SNPs in IL-10 gene [frequency (%)]

Gene	SNPs	Genotype	Sepsis group (n = 104)	Control group (n = 99)	χ^2	*P* value
IL-10	rs1800871				0.568	0.753
		CC	62 (59.62)	64 (64.65)		
		CT	33 (31.73)	27 (27.27)		
		TT	9 (8.65)	8 (8.08)		
	rs1800872				1.109	0.537
		AA	6 (5.77)	9 (9.09)		
		AC	33 (31.73)	27 (27.27)		
		CC	65 (62.50)	63 (63.64)		
	rs1800896				13.392	0.001
		AA	26 (25.00)	30 (30.30)		
		GG	65 (62.50)	39 (39.39)		
		AG	13 (12.50)	30 (30.30)		

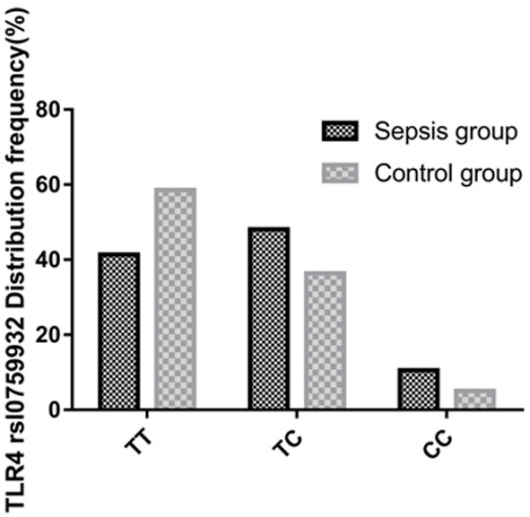

Figure 2. Distribution frequencies of the TT, TC, and CC genotypes at the *TLR4* rs10759932 locus in the study and control groups. Compared with TT, *P<0.05.

Assay Design 3.1 software (Sequenom, USA) and synthesized by Guangzhou Aikey Biotechnology Co., Ltd. The primer sequences are shown in **Table 1**. Each PCR mixture contained the following: 2.5 μl of 10 × buffer, 1 μl of dNTP mix, 0.5 μl of each 10 μmol/L upstream and downstream primer, 0.5 μl of Taq DNase (2.0 U), 1 μl of template, and deionized water to 50 μl. The amplification was performed using a Bio-Rad PCR amplification instrument (Zhejiang Tuopuyunnong Technology Co., Ltd.). The PCR cycling conditions were as follows: pre-denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. Then, 10 μl of the PCR product was incubated with restriction enzymes at 37°C overnight before electrophoresis in a 3.0% agarose gel. The restriction enzyme-digested PCR products were placed in 1 × TBE buffer solution and then electrophoresed on a 3% agarose gel at constant voltage of 90 V for 30 min. Then, the amplification strip was imaged on an automated gel-imaging system.

Statistical methods

SPSS 19.0 (Boyizhixun [Beijing] Information Technology Co., Ltd.) was used for statistical analysis. The data are expressed as mean ± standard deviation (x ± sd). Data were compared between groups using the chi-square test. Logistic regression analysis was used to adjust for gender, age, smoking status, drinking status, and the effects of chronic disease, and analyze the association of SNP genotypes with septic shock, organ dysfunction, and survival. The patients' ages were collapsed into two ranks (< 45 and ≥ 45, **Table 2**). *P* values less than 0.05 were considered statistically significant.

Results

Baseline data of the two groups

General clinical baseline data, such as gender, age, smoking status, drinking status, and chronic diseases, did not differ significantly between the study and control groups (P > 0.05). In the sepsis group, there were 40 cases (38.46%) of septic shock, 85 cases (81.73%) of organ dysfunction, and 26 cases of (25.00%) death (**Table 3**).

Genotype frequency distribution of three SNP loci in IL-10

The distribution frequencies of CC, CT and TT at the rs1800871 locus of the *IL-10* gene were 59.62%, 31.73% and 8.65%, respectively, in the sepsis group; and 64.65%, 27.27% and 8.08%, respectively, in the control group.

Table 5. Frequency distribution and comparison of genotype frequencies of three SNPs in TLR4 gene [frequency (%)]

Gene	SNPs	Genotype	Sepsis group (n = 104)	Control group (n = 99)	X^2	*P* value
TLR4	rs10759932				6.638	0.035
		TT	43 (41.35)	58 (58.59)		
		TC	50 (48.08)	36 (36.36)		
		CC	11 (10.58)	5 (5.05)		
	rs11536889				3.215	0.192
		GG	66 (63.46)	64 (65.64)		
		GC	35 (33.65)	27 (27.27)		
		CC	3 (2.88)	8 (8.08)		
	rs27371903				0.255	0.880
		GG	16 (15.38)	14 (14.14)		
		CA	52 (50.00)	53 (53.54)		
		AA	36 (34.62)	32 (32.32)		

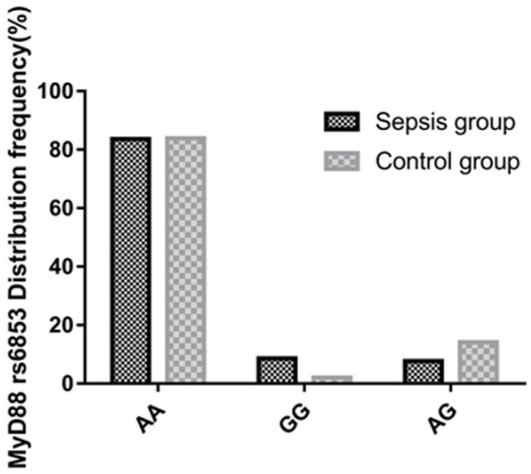

Figure 3. Distribution frequencies of the AA, GG, and AG genotypes at the *MyD88* rs6853 locus in the study and control groups. Compared with AA, *P<0.05.

The difference was not statistically significant (X^2 = 0.568, P = 0.753). The distribution frequencies of AA, AC and CC at the rs1800872 locus of *IL-10* were 5.77%, 31.73% and 62.50%, respectively, in the sepsis group; and 9.09%, 27.27% and 63.64%, respectively, in the control group. The difference was not statistically significant (X^2 = 1.109, P = 0.537). The distribution frequencies of AA, GG and AG at the rs1800896 locus of *IL-10* were 25.00%, 62.50% and 12.50%, respectively, in the sepsis group; and 30.30%, 39.39% and 30.30%, respectively, in the control group. This difference was statistically significant (X^2 = 13.392, P = 0.001; **Figure 1**). After adjustment for gender, age, smoking status, drinking status, and chronic disease by logistic regression, the rs1800896 locus of *IL-10* was associated with the occurrence of sepsis; compared to the AA genotype, the GG+AG genotype showed an increased risk of sepsis (OR = 1.86, 95% CI, 1.16-2.96, P = 0.036). However, there was no significant correlation between rs1800871 and rs1800896 polymorphisms and sepsis risk (P > 0.05; **Table 4**).

Genotype frequency distributions of three SNP loci in TLR4

The distribution frequencies of TT, TC and CC at the rs10759932 locus of *TLR4* were 41.35%, 48.08% and 10.58%, respectively, in the sepsis group; and 58.59%, 36.36% and 5.05%, respectively, in the control group. The difference was statistically significant (X^2 = 6.638, P = 0.035; **Figure 2**). The distribution frequencies of GS, GC and CC at the rs11536889 locus of *TLR4* were 63.46%, 33.65%, and 2.88%, respectively, in the sepsis group; and 65.64%, 27.27% and 8.08%, respectively, in the control group. The difference was not statistically significant (X^2 = 3.215, P = 0.192). The distribution frequencies of GG, CA and AA at the rs27371903 locus of *TLR4* were 15.38%, 50.00% and 34.62%, respectively, in the sepsis group; and 14.14%, 53.56% and 32.32% respectively, in the control group. The difference was not statistically significant (X^2 = 0.255, P = 0.880). After adjustment for gender, age, smoking status, drinking status, and the effects of chronic disease by logistic regression, the *TLR4* rs10759932 locus was associated with the occurrence of sepsis. Compared with the TT genotype, the TC+CC genotype showed an increased risk of sepsis (OR = 1.55, 95% CI, 1.00-2.38, P = 0.048). However, there was no significant correlation between the rs11536889 and rs27371903 allele frequencies and sepsis risk (P > 0.05; **Table 5**).

Table 6. Frequency distribution and comparison of genotypes of two SNPs in the MyD88 gene [frequency (%)]

Gene	SNPs	Genotype	Sepsis group (n = 104)	Control group (n = 99)	X^2	*P* value
MyD88	rs7744				1.504	0.471
		AA	39 (37.50)	40 (40.40)		
		GG	12 (11.54)	16 (16.16)		
		AG	53 (50.96)	43 (43.43)		
	rs6853				6.066	0.040
		AA	87 (83.65)	83 (83.84)		
		GG	9 (8.65)	2 (2.02)		
		AG	8 (7.69)	14 (14.14)		

Table 7. Association of IL-10 (rs1800896), TLR4 (rs10759932), and MyD88 (rs6853) gene polymorphisms with septic shock in patients with sepsis [frequency (%)]

Gene	Septic shock		OR (95% CI)	*P* value
	Have (n = 40)	No (n = 64)		
IL-10 (rs1800896)				
AA	9 (22.50)	17 (26.56)	1 (Ref)	
GG	26 (65.00)	39 (60.94)	2.25 (0.42~12.01)	0.344
AG	5 (12.50)	8 (12.50)	0.94 (0.61~1.43)	0.762
TLR4 (rs10759932)				
TT	17 (42.50)	26 (40.63)	1 (Ref)	
TC	20 (50.00)	30 (46.88)	1.22 (0.79~1.87)	0.374
CC	3 (7.50)	8 (12.50)	0.97 (0.51~1.85)	0.926
MyD88 (rs6853)				
AA	31 (77.50)	56 (87.50)	1 (Ref)	
GG	4 (10.00)	5 (7.81)	3.48 (0.26~27.27)	0.350
AG	5 (12.50)	3 (4.69)	1.00 (0.55~1.83)	0.992

Genotype frequency distributions of two SNP loci in MyD88

The distribution frequencies of AA, GG and AG at the rs7744 locus of *MyD88* were 37.50%, 11.54% and 50.96%, respectively, in the sepsis group; and 40.40%, 16.16% and 43.43%, respectively, in the control group. The difference was not statistically significant (X^2 = 1.504, P = 0.471). The distribution frequencies of AA, GG, and AG in the rs6853 locus of *MyD88* were 83.65%, 8.65% and 7.69%, respectively, in the sepsis group; and 83.94%, 2.02% and 14.14%, respectively, in the control group. The difference was statistically significant (X^2 = 6.066, P = 0.040; **Figure 3**). After adjustment for gender, age, smoking status, drinking status, and the effects of chronic disease by logistic regression, the MyD88 rs6853 locus was associated with the occurrence of sepsis. Compared with the AA genotype, the GG+AG genotype showed an increased risk of sepsis (OR = 1.66, 95% CI, 1.06-2.61, P = 0.027). However, there was no significant correlation between polymorphism at the rs7744 locus and the risk of sepsis (P > 0.05; **Table 6**).

Association of IL-10, TLR4 and MyD88 polymorphisms with septic shock, organ dysfunction, and survival in patients with sepsis

The IL-10 rs1800896 AA, GG and AG genotypes, *TLR4* rs10759932 TT, TC and CC genotypes, and *MyD88* rs6853 AA, GG and AG genotypes were not significantly correlated with septic shock, organ dysfunction, or survival (P > 0.05; **Tables 7**-**9**).

Discussion

With the use of immunosuppressors and invasive arterial pressure monitoring, the misuse and abuse of antibiotics, and the aging of the population, the incidence of the severe infection syndrome, or sepsis, after severe trauma surgery has significantly increased [17]. Sepsis is a complex manifestation of the interaction between invading pathogens and the host's coagulation and immune systems. The invasiveness and drug resistance of pathogens can impact the occurrence and development of sepsis, and the host immune response to the pathogens is an important cause of sepsis. Imbalance in the inflammatory response is the main manifestation of sepsis, as dysregulated immune activation can cause a series of adverse reactions, such as septic shock, organ dysfunction, and even death [18]. Previous studies have shown that the occurrence, development, and prognosis of sepsis have a genetic

Table 8. Association of IL-10 (rs1800896), TLR4 (rs10759932), and MyD88 (rs6853) gene polymorphisms with organ dysfunction in patients with sepsis [frequency (%)]

Gene	Organ dysfunction		OR (95% CI)	*P* value
	Have (n = 85)	No (n = 19)		
IL-10 (rs1800896)				
AA	21 (24.71)	5 (26.32)	1 (Ref)	
GG	55 (64.71)	10 (52.63)	2.13 (0.34~13.26)	0.416
AG	9 (10.59)	4 (21.05)	0.80 (0.49~1.31)	0.381
TLR4 (rs10759932)				
TT	35 (41.18)	8 (42.11)	1 (Ref)	
TC	43 (50.59)	7 (36.84)	0.96 (0.37~2.49)	0.934
CC	7 (8.24)	4 (21.05)	0.86 (0.57~1.31)	0.479
MyD88 (rs6853)				
AA	73 (85.88)	14 (73.68)	1 (Ref)	
GG	7 (8.24)	2 (10.53)	0.59 (0.33~1.04)	0.069
AG	5 (5.88)	3 (15.79)	0.85 (0.53~1.37)	0.512

Table 9. Association of IL-10 (rs1800896), TLR4 (rs10759932), and MyD88 (rs6853) gene polymorphisms with survival in patients with sepsis [frequency (%)]

Gene	Survival		OR (95% CI)	*P* value
	Death (n = 26)	Survive (n = 78)		
IL-10 (rs1800896)				
AA	4 (15.38)	22 (28.21)	1 (Ref)	
GG	18 (69.23)	47 (60.26)	0.87 (0.51~1.51)	0.630
AG	4 (15.38)	9 (11.54)	0.84 (0.42~1.69)	0.629
TLR4 (rs10759932)				
TT	9 (34.62)	34 (43.59)	1 (Ref)	
TC	12 (46.15)	38 (48.72)	0.96 (0.50~1.83)	0.898
CC	5 (19.23)	6 (7.69)	1.02 (0.55~1.92)	0.942
MyD88 (rs6853)				
AA	22 (84.62)	65 (83.33)	1 (Ref)	
GG	3 (11.54)	6 (7.69)	1.14 (0.60~2.16)	0.693
AG	1 (3.85)	7 (8.97)	1.03 (0.68~1.56)	0.908

basis, and genes in the immune pathways are involved. SNPs have been shown to affect the immune response to pathogens [19]. Therefore, genetic factors are involved in regulation of the immune inflammatory response [20].

IL-10 is an important anti-inflammatory factor that inhibits the production of tumor necrosis factor α (TNF-α) by monocyte-macrophages, promotes the secretion of IL-1 receptor antagonists, and has immunomodulatory functions in sepsis. An imbalance in serum IL-10 and TNF-α levels is closely related to the occurrence, development, deterioration, and prognosis of sepsis [21]. Studies by Helminen et al. and Westendorp et al. showed that patients with hereditary high IL-10 and families who commonly suffer from respiratory infections, meningitis, and other diseases are more susceptible to disease progression and poor prognosis [22, 23]. The results of the present study showed that the *IL-10* rs1800896 locus polymorphism was associated with the occurrence of sepsis. Compared with the AA genotype, the GG+AG genotype showed an increased risk of sepsis, suggesting that at the *IL-10* rs1800896 locus, GG+AG genotype was closely associated with susceptibility to postoperative sepsis. It is possible that the rs1800896 locus is located in a positive regulatory region for the *IL-10* gene. Individuals carrying the risk-associated rs1800896 allele showed strong secretion of IL-10, resulting in a low IL-10/TNF-α ratio and immune dysfunction. Thus, when a pathogen invades, causing a local infection, sepsis can develop.

TLR4 is a biomolecule that links free fatty acids, the innate immune system, and inflammation. MyD88 is a key adaptor protein in the TLR4-mediated immune inflammatory signaling pathway [24]. TLR4 specifically recognizes LPS, a cell wall component of gram-negative bacteria, and bacterially-expressed LPS plays an important role in sepsis [27]. Moreover, LPS can activate platelet secretion and enhance platelet

aggregation through the TLR4/MyD88 signaling pathway [25]. Penders et al. showed that the interaction between genes and the environment can affect the incidence of allergic diseases, as the risk of allergic diseases induced by *Escherichia coli* colonization is reduced in children with the TT genotype at the rs10759932 locus and in children without a C allele [26]. The results of this study showed that the *TLR4* rs10759932 locus was associated with the occurrence of sepsis. Compared with the TT genotype, the TC+CC genotype showed an increased risk of sepsis, suggesting that at the *TLR4* rs10759932 locus, the TC+CC genotype was closely related to the susceptibility of postoperative sepsis. Duan et al. showed that non-coding region SNPs can alter gene expression, which in turn affects the release of inflammatory factors [27]. Chen et al. showed that SNPs in the promoter and coding region of *TLR4* can change gene expression levels and the mRNA structure, thus affecting protein function [28]. The *TLR4* polymorphic locus rs10759932 is located in an upstream regulatory region for the *TLR4* gene, and differences in this region may change the secondary structure of the mRNA and TLR4 expression levels, thereby changing the response of TLR4 to LPS [29]. The results of this study showed that the *MyD88* rs6853 locus was associated with the occurrence of sepsis. Compared with the AA genotype, the GG+AG genotype showed an increased risk of sepsis, suggesting that the *MyD88* rs6853 locus genotype GG+AG was closely related to the susceptibility of postoperative sepsis. The rs6853 locus is located in the 3'UTR, and mutations in the 3'UTR can affect gene expression and cause disease. Thus, mutations may affect the biological function of MyD88. Through further research, we showed that patients carrying the *IL-10* rs1800896 AA, GG and AG genotypes, the *TLR4* rs10759932 TT, TC and CC genotypes, and the *MyD88* AA, GG and AG genotypes were not significantly correlated with septic shock, organ dysfunction, or survival. Septic shock, organ dysfunction, and survival are complex processes likely involving more factors and likely influenced by additional genes. Environmental factors had a greater impact on susceptibility to sepsis, and locus changes in *IL-10*, *TLR4* and *MyD88* may primarily affect pathogen identification by the immune system and the inflammatory processes during the initial innate immune response.

Subjects were strictly selected according to inclusion and exclusion criteria to ensure the rigor and reliability of the study results. This study did not detect the expression levels of IL-10 and TLR4 signal pathway genes, and therefore, it has certain limitations. Further prospective, controlled studies may show that IL-10 and genes in the TLR4 signaling pathway influence the occurrence and development of sepsis.

In summary, polymorphisms at the *IL-10* rs1800896 locus, *TLR4* rs10759932 locus, and *MyD88* rs6853 locus are closely related to the risk of postoperative sepsis.

Disclosure of conflict of interest

None.

Address correspondences to: Jiandong Lin, Intensive Care Unit, The First Affiliated Hospital of Fujian Medical University, NO. 20, Tea Middle Road, Fuzhou City 350005, Fujian Province, China. Tel: +86-13605044939; E-mail: linjiandongyx@163.com

References

[1] Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL and Dellinger RP. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Intensive Care Med 2017; 43: 304-377.

[2] Kell DB, Pretorius E. To what extent are the terminal stages of sepsis, septic shock, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome actually driven by a prion/amyloid form of fibrin? Semin Thromb Hemost 2018; 44: 224-238.

[3] Chantratita N, Tandhavanant S, Seal S, Wikraiphat C, Wongsuvan G, Ariyaprasert P, Suntornsut P, Teerawattanasook N, Jutrakul Y, Srisurat N, Chaimanee P, Mahavanakul W,

Srisamang P, Phiphitaporn S, Mokchai M, Anukunananchai J, Wongratanacheewin S, Chetchotisakd P, Emond MJ, Peacock SJ, West TE. TLR4 genetic variation is associated with inflammatory responses in gram-positive sepsis. Clin Microbiol Infect 2017; 23: 47.e1-47.e10.

[4] Asselta R, Paraboschi EM, Rimoldi V, Menegatti M, Peyvandi F, Salomon O, Duga S. Exploring the global landscape of genetic variation in coagulation factor XI deficiency. Blood 2017; 130: e1-e6.

[5] Thamphiwatana S, Angsantikul P, Escajadillo T, Zhang Q, Olson J, Luk BT, Zhang S, Fang RH, Gao W, Nizet V, Zhang L. Macrophage-like nanoparticles concurrently absorbing endotoxins and proinflammatory cytokines for sepsis management. Proc Natl Acad Sci U S A 2017; 114: 11488-11493.

[6] Coomes SM, Kannan Y, Pelly VS, Entwistle LJ, Guidi R, Perez-Lloret J, Nikolov N, Müller W, Wilson MS. CD4(+) Th2 cells are directly regulated by IL-10 during allergic airway inflammation. Mucosal Immunol 2017; 10: 150-161.

[7] Mao ZR, Zhang SL, Feng B. Association of IL-10 (-819T/C, -592A/C and -1082A/G) and IL-6 -174G/C gene polymorphism and the risk of pneumonia-induced sepsis. Biomarkers 2017; 22: 106-112.

[8] Jang JC, Li J, Gambini L, Batugedara HM, Sati S, Lazar MA, Fan L, Pellecchia M, Nair MG. Human resistin protects against endotoxic shock by blocking LPS-TLR4 interaction. Proc Natl Acad Sci U S A 2017; 114: E10399-E10408.

[9] Núñez Miguel R, Wong J, Westoll JF, Brooks HJ, O'Neill LA, Gay NJ, Bryant CE, Monie TP. A dimer of the toll-like receptor 4 cytoplasmic domain provides a specific scaffold for the recruitment of signalling adaptor proteins. PLoS One 2007; 2: e788.

[10] Cheng X, Yang YL, Yang H, Wang YH, Du GH. Kaempferol alleviates LPS-induced neuroinflammation and BBB dysfunction in mice via inhibiting HMGB1 release and down-regulating TLR4/MyD88 pathway. Int Immunopharmacol 2018; 56: 29-35.

[11] Roger T, Froidevaux C, Le Roy D, Reymond MK, Chanson AL, Mauri D, Burns K, Riederer BM, Akira S, Calandra T. Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. Proc Natl Acad Sci U S A 2009; 106: 2348-2352.

[12] Zaffaroni L, Peri F. Recent advances on Toll-like receptor 4 modulation: new therapeutic perspectives. Future Med Chem 2018; 10: 461-476.

[13] Wei W, Wan H, Peng X, Zhou H, Lu Y, He Y. Antiviral effects of Ma Huang Tang against H1N1 influenza virus infection in vitro and in an ICR pneumonia mouse model. Biomed Pharmacother 2018; 102: 1161-1175.

[14] Fernando SM, Barnaby DP, Herry CL, Gallagher EJ, Shapiro NI, Seely AJE. Helpful only when elevated: initial serum lactate in stable emergency department patients with sepsis is specific, but not sensitive for future deterioration. J Emerg Med 2018; 54: 766-773.

[15] Preau S, Bortolotti P, Colling D, Dewavrin F, Colas V, Voisin B, Onimus T, Drumez E, Durocher A, Redheuil A, Saulnier F. Diagnostic accuracy of the inferior vena cava collapsibility to predict fluid responsiveness in spontaneously breathing patients with sepsis and acute circulatory failure. Crit Care Med 2017; 45: e290-e297.

[16] Song JU, Sin CK, Park HK, Shim SR, Lee J. Performance of the quick sequential (sepsis-related) organ failure assessment score as a prognostic tool in infected patients outside the intensive care unit: a systematic review and meta-analysis. Critical Care 2018; 22: 28.

[17] Gaudilliere B, Angst MS, Hotchkiss RS. Deep immune profiling in trauma and sepsis: flow is the way to go! Crit Care Med 2017; 45: 1577-1578.

[18] Straub J, Paula H, Mayr M, Kasper D, Assadian O, Berger A, Rittenschober-Böhm J. Diagnostic accuracy of the ROCHE septifast PCR system for the rapid detection of blood pathogens in neonatal sepsis-a prospective clinical trial. PLoS One 2017; 12: e0187688.

[19] Goh C, Knight JC. Enhanced understanding of the host-pathogen interaction in sepsis: new opportunities for omic approaches. Lancet Respir Med 2017; 5: 212-223.

[20] Kallaur AP, Reiche EM, Oliveira SR, Simão AN, Pereira WL, Alfieri DF, Flauzino T, Proença CM, Lozovoy MA, Kaimen-Maciel DR, Maes M. Genetic, immune-inflammatory, and oxidative stress biomarkers as predictors for disability and disease progression in multiple sclerosis. Mol Neurobiol 2017; 54: 31-44.

[21] Akinosoglou K, Theodoraki S, Gkavogianni T, Pistiki A, Giamarellos-Bourboulis E, Gogos CA. How well does qSOFA correspond to underlying systemic inflammatory response? Cytokine 2018; 110: 288-290.

[22] Helminen M, Nuolivirta K, Virta M, Halkosalo A, Korppi M, Vesikari T, Hurme M. IL-10 gene polymorphism at -1082 A/G is associated with severe rhinovirus bronchiolitis in infants. Pediatr Pulmonol 2008; 43: 391-395.

[23] Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, Vandenbroucke JP. Genetic influence on cytokine production and fatal meningococcal disease. Lancet 1997; 349: 170-173.

[24] Ve T, Vajjhala PR, Hedger A, Croll T, DiMaio F, Horsefield S, Yu X, Lavrencic P, Hassan Z, Morgan GP, Mansell A, Mobli M, O'Carroll A, Chauvin B, Gambin Y, Sierecki E, Landsberg MJ, Stacey KJ, Egelman EH, Kobe B. Structural

basis of TIR-domain-assembly formation in MAL- and MyD88-dependent TLR4 signaling. Nat Struct Mol Biol 2017; 24: 743-751.

[25] Zhang G, Han J, Welch EJ, Ye RD, Voyno-Yasenetskaya TA, Malik AB, Du X, Li Z. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. J Immunol 2009; 182: 7997-8004.

[26] Penders J, Thijs C, Mommers M, Stobberingh EE, Dompeling E, Reijmerink NE, van den Brandt PA, Kerkhof M, Koppelman GH, Postma DS. Host-microbial interactions in childhood atopy: toll-like receptor 4 (TLR4), CD14, and fecal escherichia coli. J Allergy Clin Immunol 2010; 125: 231-236, e1-5.

[27] Duan ZX, Zhu PF, Dong H, Gu W, Yang C, Liu Q, Wang ZG, Jiang JX. Functional significance of the TLR4/11367 polymorphism identified in Chinese Han population. Shock 2007; 28: 160-164.

[28] Chen K, Wang YT, Gu W, Zeng L, Jiang DP, Du DY, Hu P, Duan ZX, Liu Q, Huang SN, Jiang JX. Functional significance of the toll-like receptor 4 promoter gene polymorphisms in the Chinese Han population. Crit Care Med 2010; 38: 1292-1299.

[29] Barber RC, Aragaki CC, Rivera-Chavez FA, Purdue GF, Hunt JL, Horton JW. TLR4 and TNF-α polymorphisms are associated with an increased risk for severe sepsis following burn injury. J Med Genet 2004; 41: 808-813.