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ORIGINAL ARTICLE

IL-8 gene locus is associated with risk, severity and 28-day mortality of sepsis in a Chinese population

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Abstract

Interleukin (IL)-8 has been reported to be associated with the progression of sepsis. Recent studies have explored the relationship between the IL-8 − 251 A/T polymorphism and sepsis risk. This study evaluated the association between the IL-8 − 251 A/T polymorphism and sepsis susceptibility in a Chinese Han population. We designed a case–control study with 254 sepsis cases and 322 controls. Genotyping was performed using the polymerase chain reaction–restriction fragment length polymorphism method. SPSS 20.0 software was used for all statistical analysis (SPSS Inc., Chicago, USA). This study showed that the IL-8 − 251 A/T polymorphism was associated with a decreased risk of sepsis. Stratified analyses found that this association held true in females, non-smokers, and older individuals (age > 60 years). The IL-8 − 251 A/T polymorphism was also related to the severity and 28-day mortality of sepsis. The IL-8 − 251 A/T polymorphism is associated with a decreased risk of sepsis.

Keywords IL-8 · Sepsis · Case–control study · Polymorphism

Abbreviations

PCR-RFLR	Polymerase chain reaction–restriction fragment length polymorphism
MODS	Multiple organ dysfunction syndrome
IL-8	Interleukin-8
SNP	Single-nucleotide polymorphism
OD	Optical density
ORs	Odds ratios
Cis	Confidence intervals
BMI	Body mass index
HWE	Hardy–Weinberg equilibrium

Introduction

Sepsis is a life-threatening organ disorder in which the host loses control of a severe infection [1]. In high-income countries, 2–8 million deaths are associated with sepsis annually [2]. The causes of sepsis include the serious infections from the lungs, abdomen, blood, and urinary tract [3–5]. Of these, pulmonary infections account for approximately 64% of all sepsis cases. Multiple organ dysfunction syndrome is the most common and serious complication secondary to sepsis, which makes sepsis a leading cause of mortality worldwide [6]. The sepsis inpatient mortality reaches 25–30% [7]. Early recognition is likely to improve the prognosis of sepsis [8, 9]. Therefore, the management of sepsis patients relies primarily on early recognition, which allows timely therapeutic measures to be initiated.

Inflammation is one of the most important clinical manifestations of sepsis [10–12]. The pro-inflammatory cytokine interleukin (IL)-8 is a founding member of the chemokine family [13] and plays an important role in several illnesses [14, 15]. During inflammation, mononuclear macrophages secrete IL-8 from the blood in tissues [16]. IL-8 and its functions in angiogenesis, tissue remodeling, and tumor progression have been studied extensively. Bacterial and viral products induce IL-8 rapidly [17, 18]. Upregulation of IL-8 in vitro predicts death from sepsis [19, 20]. In addition, IL-8

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has been reported to be associated with the progression of sepsis [21, 22].

A functional single-nucleotide polymorphism (SNP), -251A/T, in the promoter region of IL-8 gene was reported to influence the expression of IL-8 [20]. Several recent studies have explored the correlation between single-nucleotide polymorphisms (SNPs) of IL-8 − 251 A/T (rs4073) polymorphism and sepsis susceptibility [23, 24]. However, the results were conflicting. Thus, this study was aimed to investigate the association between the IL-8 − 251 A/T polymorphism and sepsis risk in a Chinese Han population.

Patient and methods

Subjects

This study enrolled 254 sepsis patients and 322 controls from the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University. All sepsis patients were diagnosed according to criteria established by the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [1]. Sepsis was classified as sepsis and septic shock. Sepsis is a systemic response to infection, and this disorder is characterized as two or more of the following: body temperature > 38.5 °C or < 35.6 °C, tachypnea > 20/min, leukocytosis > 12,000/μL, tachycardia > 90/min, or leucopenia < 4000/μL [25]. Exclusion criteria were as follows: patients < 18 or > 80 years old, patients with uremia or end-stage renal disease, patients with cardiac arrest history, pregnancy, or cancer patients. The controls were selected from the same hospital. Individuals with potential infection, heart disease history, or receiving immunosuppressive therapy were excluded. All of the cases and controls were enrolled consecutively.

Clinical information including age, sex, smoking, alcohol, and body mass index (BMI) was collected using a written questionnaire. Smokers were defined as smoking more than 1 cigarette per day for at least 1 year. Drinkers were classified as consuming alcoholic beverages at least once a week for more than 1 year. This study was approved by the Ethics Committee of the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University and met the standards of the *Declaration of Helsinki*. Written informed consent was obtained from all subjects.

Blood sampling and genotyping

Genomic DNA of cases and controls was extracted from peripheral blood leukocytes using a TIANamp Blood DNA kit (Tiangen Biotech, Beijing, China). Extracted DNA was stored at − 20 °C. The quality and concentration of the extracted DNA were measured at 260 and 280 nm using a NanoDrop (Thermo Scientific, Waltham, MA, USA). The IL-8 − 251 A/T polymorphism was genotyped using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLR) method. The primers used for the nucleotide extension reaction were 5′-TGGCTGGCTTAT CTTCACCATCA-3′ (forward) and 5′-TCAGGGCAAACC TGAGTCATCA-3′ (reverse). Approximately, 10% of the samples were randomly re-examined by genotyping the SNP to validate the accuracy. The results were 100% concordant.

Statistical analysis

The differences in epidemiological variables and clinical data of the cases and controls were evaluated using the Chi-square test (χ^2 test). The differences in genotype and allele frequencies of the IL-8 − 251 A/T polymorphism were evaluated using the χ^2 test. Hardy–Weinberg equilibrium (HWE) among the controls was tested using a goodness-of-fit Chi-square test. Using logistic regression analysis, the crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to assess the relationship between the IL-8 − 251 A/T polymorphism and the sepsis risk. Subgroup analyses were performed by sex, age, alcohol consumption, smoking, and BMI. SPSS 20.0 software was used for all statistical analyses (SPSS Inc., Chicago, USA). $P < 0.05$ was deemed significant.

Results

Characteristics of the study population

In this case–control study, 254 sepsis patients and 322 controls were recruited. Demographic information and clinical characteristics of all individuals are shown in Table 1. HWE analysis revealed no difference in the control group. The case and control groups were matched in age and sex. The percentage of drinkers and smokers was higher in the sepsis patients than in the controls. The mean Acute Physiology and Chronic Health Evaluation (APACHE) II score of the sepsis patients was 21.49 ± 4.90. The sepsis patients included 157 sepsis and 97 septic shock patients.

Relationship between IL-8 − 251 A/T polymorphism and sepsis risk

The genotype and allele frequencies of the IL-8 − 251 A/T polymorphism are presented in Table 2. Data showed that carriers of the TT genotype were associated with a decreased risk of sepsis (TT vs. AA: $P = 0.011$, OR = 0.54, 95%CI = 0.34–0.87). Similarly, the A allele carriers had a lower susceptibility to sepsis. Furthermore, the results were remained significant after adjusting for sex and age.

Table 1 Patient demographics and risk factors in sepsis

Characteristics	Case ($N=254$)	Control ($N=322$)	P
Age	64.9 ± 9.01	64.1 ± 9.76	0.338
Sex			0.449
Male	139 (54.7%)	166 (51.6%)	
Female	115 (45.3%)	156 (48.4%)	
Smoking			**0.026**
YES	82 (32.3%)	77 (23.9%)	
NO	172 (66.7%)	245 (76.1%)	
Alcohol			**0.031**
YES	76 (29.9%)	71 (20.0%)	
NO	178 (70.1%)	251 (78.0%)	
BMI	23.50 ± 2.49	23.16 ± 2.88	0.135
APACHE II score	21.49 ± 4.90		
Sepsis status (n, %)			
Sepsis	157 (61.8%)		
Septic shock	97 (38.2%)		
Pathogens (n, %)			
Gram-positive	46 (18.1%)		
Gram-negative	129 (50.8%)		
Mixed Gram-negative and -positive	58 (22.8%)		
Fungus	21 (8.3%)		
Source of infection, n (%)			
Respiratory tract infection	175 (69.0%)		
Abdominal infection	43 (17.0%)		
Urinary tract infection	12 (4.7%)		
Catheter-associated infection	10 (3.9%)		
Others	14 (5.4%)		
Mortality, 28 days, n (%)	62 (24.4%)		

Statistically significant values are in bold ($P < 0.05$)

Table 2 Genotype frequencies of IL-8 − 251 A/T polymorphism in cases and controls

Models	Genotype	Case[a] (n, %)	Control[a] (n, %)	OR (95% CI)	P value	*OR (95% CI)	*P-value
Co-dominant	AA	72 (28.3%)	67 (20.8%)	1.00 (reference)	–	–	–
Heterozygote	AT	126 (49.6%)	159 (49.4%)	0.74 (0.49–1.11)	0.142	0.72 (0.48–1.09)	0.119
Homozygote	TT	56 (22.0%)	96 (29.8%)	**0.54 (0.34–0.87)**	**0.011**	**0.53 (0.33–0.86)**	**0.009**
Dominant	AA	72 (28.3%)	67 (20.8%)	1.00 (reference)	–	–	–
	TT + AT	182 (71.6%)	255 (79.2%)	**0.66 (0.45–0.97)**	**0.036**	**0.65 (0.44–0.96)**	**0.030**
Recessive	AT + AA	198 (77.9%)	226 (70.2%)	1.00 (reference)	–	–	–
	TT	56 (22.0%)	96 (29.8%)	**0.67 (0.46–0.97)**	**0.036**	**0.66 (0.45–0.98)**	**0.037**
Allele	A	270 (53.1%)	293 (45.5%)	1.00 (reference)	–	–	–
	T	238 (46.9%)	351 (54.5%)	**0.80 (0.70–0.91)**	**0.001**		

[a]The genotyping was successful in 254 cases and 322 controls for IL-8 − 251 A/T polymorphism

Bold values are statistically significant ($P < 0.05$)

*Adjusting for sex and age

Next, we conducted stratified analyses of age, sex, alcohol, smoking, and BMI (Table 3). There was a decreased risk of sepsis shown in non-smokers, females, and older individuals (age ≥ 60 years). Nevertheless, no significant results were observed in the stratified analyses by alcohol and BMI.

Table 3 Stratified analyses between IL-8 − 251 A/T polymorphism and the risk of sepsis

Variable	(Case/control)			AT versus AA	TT versus AA	TT versus AA + AT	TT + AT versus AA
	AA	AT	TT				
Sex							
Male	37/38	73/88	29/40	0.85 (0.49–1.47); 0.567	0.75(0.39–1.44); 0.380	0.83 (0.48–1.43); 0.502	0.82 (0.49–1.38); 0.452
Female	35/29	53/71	27/56	0.62 (0.34–1.14); 0.121	**0.40 (0.20–0.78); 0.008**	**0.54(0.32–0.94); 0.029**	**0.52(0.30–0.92); 0.024**
Smoking							
Yes	19/13	46/39	17/25	0.81 (0.35–1.84); 0.610	0.47 (0.18–1.19); 0.109	0.54 (0.27–1.11); 0.096	0.67 (0.31–1.48); 0.325
No	53/54	80/120	39/71	0.68 (0.42–1.09); 0.109	**0.56(0.33–0.96); 0.037**	0.72 (0.46–1.13); 0.151	**0.64(0.41–0.99); 0.044**
Alcohol							
Yes	25/15	33/33	18/23	0.60 (0.27–1.34); 0.212	0.47 (0.19–1.14); 0.096	0.65 (0.31–1.34); 0.241	0.55 (0.26–1.15); 0.111
No	47/52	93/126	38/73	0.82 (0.51–1.32); 0.405	0.58 (0.33–1.00); 0.052	0.66 (0.42–1.04); 0.072	0.73 (0.46–1.14); 0.169
Age (years)							
< 60	21/22	25/57	13/29	0.48 (0.22–1.04); 0.062	0.53 (0.22–1.28); 0.158	0.85 (0.41–1.77); 0.659	0.50 (0.24–1.02); 0.056
≥ 60	51/45	101/102	43/67	0.86 (0.53–1.39); 0.532	**0.54(0.31–0.96); 0.031**	**0.60(0.39–0.94); 0.026**	0.73 (0.46–1.16); 0.181
BMI	48/46	93/122	45/74	0.73 (0.50–1.12); 0.205	0.58 (0.34–1.01); 0.053	0.73 (0.47–1.11);0.144	0.68 (0.43–1.07); 0.092
< 25	24/21	33/37	11/22	0.78 (0.37–1.65); 0.517	0.44 (0.17–1.11); 0.079	0.51 (0.23–1.15); 0.099	0.65 (0.32–1.32); 0.223
≥ 25							

Bold values are statistically significant ($P < 0.05$)

Last, we explored the association between the IL-8 − 251 A/T polymorphism and the clinicopathological characteristics of the sepsis patients (Table 4). We found that the IL-8 − 251 A/T polymorphism was associated with the severity and 28-day mortality of sepsis.

Discussion

In this study, the IL-8 − 251 A/T polymorphism was related to the susceptibility to sepsis. Subgroup analysis showed that the IL-8 − 251 A/T polymorphism was associated with decreased risk of sepsis in females, non-smokers, and older individuals (age > 60 years). In addition, we found that this SNP was correlated with the severity and 28-day mortality of sepsis.

IL-8, as a pro-inflammatory cytokine, has been widely studied in sepsis. Most studies focused on the relationship between IL-8 serum level and sepsis incidence and mortality [23, 26–28]. Recently, two studies investigated the relationship between the IL-8 − 251 A/T polymorphism and sepsis risk [23, 24], but obtained different results. In 2017, Hu et al. indicated that the homozygote TT genotype and T allele of the − 251 A/T polymorphism showed protective effects for males in a Chinese population [24]; however, the IL-8 − 251 A/T polymorphism was not associated with levels of IL-8 in sepsis patients. Yousef et al. found a positive correlation between survival and the IL-8 − 251 A/T polymorphism mutant allele in patients from Egypt [26], which was inconsistent with Hu et al. [24]. Interestingly, Georgitsi et al. revealed that AA genotype carriers were protected from developing severe sepsis/septic shock [23]. In this study, we observed that the TT genotype or T allele carriers showed a decreased risk of sepsis, but unlike the findings of Georgitsi et al. Several aspects may explain the conflicting findings. One, race differences were nonnegligible. Two,

Table 4 Associations between IL-8 − 251 A/T polymorphism and clinical characteristics of sepsis

Characteristics	Genotype distributions			
	AA	AT	TT	AT + TT
Sepsis status				
Sepsis/Septic shock	49/23	81/45	27/29	108/74
OR (95%CI); *P*-value	1.0 (reference)	0.85 (0.46–1.56); 0.591	**0.44 (0.21–0.90); 0.023**	0.69 (0.39–1.22); 0.198
Death				
NOT/YES	62/10	97/29	33/23	82/95
OR (95%CI); *P*-value	1.0 (reference)	0.54 (0.25–1.18); 0.120	**0.23 (0.10–0.54); 0.001**	**0.40 (0.19–0.85); 0.014**

Bold values are statistically significant ($P < 0.05$)

exposure factors and the severity of sepsis differed. Three, clinical heterogeneity of sepsis varied. Four, the sample sizes were also differed. In addition, we found that the IL-8 − 251 A/T polymorphism was associated with a decreased risk of sepsis in females, non-smokers, and older individuals (age > 60 years), indicating that exposing to these risk factors may not be prone to sepsis. Furthermore, we observed that the − 251 A/T polymorphism was related to the severity and 28-day mortality of sepsis. Data revealed that individuals with TT genotype showed a decreased incidence of sepsis, but had no effects on sepsis shock incidence. The reasons why − 251 A/T polymorphism exerted effect on sepsis incidence but not sepsis shock were unclear actually. We assumed that this SNP was only involved in the early stage of sepsis, but not the severe stage of sepsis. Maybe, this SNP interacts with some environmental or genetic factors at the early stage of sepsis, thereby decreasing the susceptibility to sepsis. However, further studies are urgently needed to verify these assumptions. Besides, TT or AT + TT genotype carriers showed decreased mortality for sepsis patients.

Several study limitations should be noted. One, the study sample size was not large. Two, this study did not validate whether the IL-8 − 251 A/T polymorphism was associated with the levels of IL-8. Three, the follow-up data on the sepsis patients were limited. Four, studying one SNP of IL-8 was not sufficient.

Conclusions

In conclusion, IL-8 − 251 A/T polymorphism is associated with decreased risk of sepsis in this Chinese population. Further studies with larger sample sizes are urgently needed in other populations to verify the findings of this study.

Author contributions Peng Fu conceived and designed the experiments. Shouxiang Xie and Xiangcheng Zhang performed the experiments. Xiangcheng Zhang analyzed the data. Peng Fu contributed reagents/materials/analysis tools. Xiangcheng Zhang and Shouxiang Xie wrote the paper.

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Availability of data and materials The data can be available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there are no competing interests associated with the manuscript.

Ethical approval This study was approved by the Ethics Committees of the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University and met the standards of Declaration of Helsinki.

Informed consent Informed consent was obtained from all individual participants included in the study.

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