

## Original Article

# Correlation of *TNF- $\alpha$* gene polymorphisms with sepsis susceptibility

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**Abstract:** Objective: Current study was aimed to explore the association between single nucleotide polymorphisms (SNPs) of tumor necrosis factor- $\alpha$  (*TNF- $\alpha$* ) gene (rs1800629 and rs361525) and sepsis susceptibility. Methods: 115 sepsis patients and 108 healthy individuals were enrolled in this case-control study. Cases and controls were in accordance with each other in age and gender. *TNF- $\alpha$*  gene rs1800629 and rs361525 polymorphisms were genotyped via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach. Differences of genotype and allele frequencies of the two SNPs between case and control groups were analyzed by chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to indicate relative susceptibility of sepsis. Results: GA genotype and A allele frequencies of rs1800629 polymorphism were significantly different between case and control groups, indicating a significant association with the susceptibility of sepsis ( $P = 0.035$ , OR = 2.501, 95% CI = 1.405-5.988;  $P = 0.030$ , OR = 2.289, 95% CI = 1.063-4.929). However, genotypes and alleles of rs361525 SNP in case and control groups had no significant association with sepsis susceptibility ( $P > 0.05$ ). Conclusion: *TNF- $\alpha$*  gene variant allele of rs1800629 polymorphism might act as a susceptible factor for sepsis occurrence. *TNF- $\alpha$*  gene rs361525 SNP was not related to the incidence of sepsis.

**Keywords:** *TNF- $\alpha$* , polymorphisms, sepsis, PCR-RFLP

## Introduction

On traditional viewpoint, sepsis is a serious systemic symptom because bacteria intrude into blood circulation, continue being inside and breed rapidly to produce plenty of toxins. At present, sepsis is a clinical process of systemic inflammatory response induced by bacterial infections [1]. Sepsis is the common complication and one of the main causes of death in hospitalized patients [2]. Mortality of sepsis is very high [3-5]. Obviously, sepsis has been one of significant diseases posing threat to human health. Consequently, it's necessary to identify sepsis in early-stage. Exploration of the pathogenesis will contribute to the diagnosis of sepsis. Specific pathogenesis of sepsis involves systemic inflammatory network effect, immunity dysfunction, damage of tissue and correlates with pathophysiologic alteration of multisystem and multiple organ in organism [1]. Infection factors may activate diverse inflammatory cells

in organism and release a large amount of inflammatory mediators.

As we all know, tumor necrosis factor- $\alpha$  (*TNF- $\alpha$* ) is a cytokine which is mainly released by mononuclear macrophage. It has potential effects on growth and differentiation of various cells and effectively regulates autocrine mechanism. *TNF* involve in systemic inflammation and could regulate the immune cells. Main biological functions of *TNF- $\alpha$*  are causing inflammatory response and resisting neoplasms [6]. In human, *TNF- $\alpha$*  gene locates in the short arm of sixth chromosome and includes 4 exons and 3 introns. *TNF- $\alpha$*  gene polymorphisms affect transcription and output of this gene and finally impact the susceptibility of diseases including sepsis [7-9].

Therefore, this study explored the correlation of *TNF- $\alpha$*  gene rs1800629 and rs361525 polymorphisms with sepsis susceptibility. This study

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**Table 1.** Primer sequences of *TNF- $\alpha$*  gene rs1800629 and rs361525 polymorphisms

SNP	Primer sequences	
Rs1800629	Upstream	5'-AGGCAATAGGTTTTGAGGGCCAT-3'
	Downstream	5'-GAGCGTCTGCTGGCTGGGTG-3'
Rs361525	Upstream	5'-ATGAGCACTGAAAGCATGATCCGG-3'
	Downstream	5'-GCAATGATCCCAAAGTAGACCTGCC-3'

will contribute to understand the pathogenesis of sepsis.

### Materials and methods

#### Sample features

All the objects of this study were Chinese Han population and there were no genetic connection among them. Written informed consent was obtained from all participants before enrollment. This research was reviewed and consented by Ethics committee of The First Affiliated Hospital of Guangxi Medical University. Sample collection was based on ethics criteria of national human genome research.

115 sepsis patients and 108 healthy controls were enrolled in this study. Sepsis patients were diagnosed in The First Affiliated Hospital of Guangxi Medical University from January 2013 to January 2015, including 55 females and 60 males (age range 22-67). Sepsis patients were with a history of chronic heart, renal, liver or pulmonary failure, including trauma subjects. Healthy controls were recruited from healthy check-up center of the same hospital during the same times, including 51 females and 57 males (age range 25-65). Health controls were individuals without any clinical medical history. Controls were according to the cases in age and gender.

#### Sample collection and DNA extraction

5 ml peripheral venous blood were collected from participants, anticoagulated by 0.5% EDTA (pH 8.0), and then stored at -20°C. Genomic DNA was extracted by TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering CO., LTD, China) and stored at -20°C.

#### Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Primer sequences of *TNF- $\alpha$*  gene polymorphisms were designed by Primer Premier 5.0,

and synthesized by Sangon Biotech (Shanghai, China) (**Table 1**). PCR amplification was adopted a 25  $\mu$ l system, including 2  $\mu$ l template DNA, 1  $\mu$ l upstream primer, 1  $\mu$ l downstream primer, 5  $\mu$ l 10  $\times$  Buffer, 0.5  $\mu$ l Taq DNA polymerase, 2  $\mu$ l dNTP, and 13.5  $\mu$ l deionized sterile water. This process was going in ice bath.

Reaction tube was put on PCR amplification cyclor after uniform agitation of reaction mixture with transient centrifugation. PCR procedure was as follows: 94°C initial denaturation for 5 min, followed by 35 cycles of 94°C denaturation for 45 s, 62°C annealing for 45 s and 72°C extension for 50 s; 72°C final extension for 5 min and reduced to 4°C saved. PCR products of rs1800629 and rs361525 were digested with Nco I and BsaI, respectively. Then polymorphisms were confirmed on 10% polyacrylamide gel electrophoresis according to the fragment length. The results were observed in an ultraviolet transilluminator.

#### Statistical analysis

Statistical analysis was accomplished through PASW statistics 18.0 statistical software. Hardy-Weinberg equilibrium (HWE) was used to test the representativeness of cases and controls. Genotype and allele frequencies of rs1800629 and rs361525 polymorphisms were calculated by direct counting. Differences of the polymorphisms distributions between case and control groups were assessed by  $\chi^2$  test. Association of *TNF- $\alpha$*  gene polymorphisms with sepsis susceptibility was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs). The differences had statistical significance when  $P < 0.05$ .

### Results

#### HWE test

Genotype and allele distributions of *TNF- $\alpha$*  gene rs1800629 and rs361525 SNPs were displayed in **Table 2**. Via chi-square test, allele frequencies distributions of the two *TNF- $\alpha$*  gene loci in patients and healthy controls conformed to HWE test ( $P > 0.05$ ), testifying the representativeness of participants.

#### Distributions of *TNF- $\alpha$* gene polymorphisms

As the **Table 2** shown, GG, GA and AA genotype frequencies of rs1800629 were respectively

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**Table 2.** Genotype and allele distributions of *TNF- $\alpha$*  gene rs1800629 and rs361525 polymorphisms in case and control groups

Genotype/ Allele	Case n = 115 (%)	Control n = 108 (%)	$X^2$	P	OR (95% CI)
<b>Rs1800629</b>					
GG	94 (81.74)	99 (91.67)	-	-	1
GA	19 (16.52)	8 (7.41)	4.451	0.035	2.501 (1.045-5.988)
AA	2 (1.74)	1 (0.92)	0.381	0.537	2.106 (0.188-23.617)
G	207 (90.00)	206 (95.37)	-	-	1
A	23 (10.00)	10 (4.63)	4.689	0.030	2.289 (1.063-4.929)
<b>Rs361525</b>					
GG	102 (88.70)	99 (91.67)	-	-	1
GA	13 (11.30)	9 (8.33)	0.553	0.457	1.402 (0.574-3.427)
G	217 (94.35)	207 (95.83)	-	-	1
A	13 (5.65)	9 (4.17)	0.524	0.469	1.378 (0.577-3.292)

81.74%, 16.52%, 1.74% in cases and 4.63% in controls. GA genotype distribution of rs1800629 polymorphism in two groups had statistical significance, indicating an obvious association with the susceptibility of sepsis ( $P = 0.035$ , OR = 2.501, 95% CI = 1.405-5.988). Besides, A allele was observed high frequencies in cases compared with controls, demonstrating a positive relationship with sepsis susceptibility ( $P = 0.030$ , OR = 2.289, 95% CI = 1.063-4.929). These results demonstrated that *TNF- $\alpha$*  gene rs1800629 polymorphism correlated with sepsis susceptibility.

*TNF- $\alpha$*  gene rs361525 polymorphism was genotyped too. However, AA genotype was not observed both in cases and controls. GG and GA genotype frequencies of rs361525 were respectively 88.70%, 11.30% in case group and 91.67%, 8.33% in control group; G and A allele frequencies were respectively 94.35%, 5.65% in case group and 95.83%, 4.17% in control group. Differences of rs361525 genotype distribution and allele frequency had no statistical significance between those two groups ( $P > 0.05$ ). Therefore, this result suggested that *TNF- $\alpha$*  gene rs361525 polymorphism had no obvious association with sepsis risk.

### Discussion

Sepsis is a common complication in patients undergoing severe traumas, burns or major surgeries. It is the clinical manifestation of systemic inflammatory response syndrome (SIRS)

caused by infections [10]. Severe sepsis is determined with the concurrence of single or more than one organ dysfunction [11]. Further development of sepsis may lead to septic shock and multiple organ dysfunction syndrome (MODS), if persistent SIRS can't be controlled efficiently early. What's more, the further development of MODS may result in multiple organ failure (MOF), which is one of the main causes of death in patients in the intensive care unit (ICU) [12]. Besides, MOF is the most serious

consequence of sepsis in irreversible phase. In spite of the significant developments of modern surgical technique and antibiotic therapy, sepsis continues to be a great obstacle to clinical treatment for severe illness.

Sepsis has been a great challenge in modern critical care medicine. It's reported that the mortality of sepsis is 30%-40% in non-shock patients and reaches 70%-90% in shock patients [13-15]. Sepsis has high morbidity, high death rate and high treatment cost [16, 17]. Meanwhile, the morbidity of sepsis is increased year by year. Consequently, prevention and treatment of sepsis are still the focus of researches in current medical domain. Therefore, it is necessary to understand the pathogenesis of sepsis. Many researches showed that the occurrence and development of sepsis is influenced by multiple genetic and environmental factors [18-20]. Among these factors, gene is the critical factor for sepsis incidence.

TNF is a kind of cytokine, it could regulate the immunologic function and mediated the inflammatory process [21]. TNF includes two types (*TNF- $\alpha$*  and *TNF- $\beta$* ) in accordance with resources. *TNF- $\alpha$*  is produced by multiple cells, and mainly in activated mononuclear macrophage. *TNF- $\alpha$*  is a cell signaling cytokine involving in inflammation. It is closely related to NF- $\kappa$ B pathway [22]. *TNF- $\alpha$*  has extensive biology activities. It could activate and promotes the proliferation and differentiation of immune cells. Besides, *TNF- $\alpha$*  affects migration, activa-

tion, proliferation and differentiation of lymphocytes, and regeneration of lymphoid tissues [23]. TNF- $\alpha$  can remove damaged cells to maintain homeostasis in the pathological state. TNF- $\alpha$  is encoded by *TNF- $\alpha$*  gene. DNA damages may cause functional disorders of the TNF- $\alpha$ , and then lead to disorders in inflammatory response and tissue cancerization. Baghel et al. [8] suggested that *TNF- $\alpha$* -308 G/A polymorphism increased the expression level of TNF- $\alpha$ .

With the continuous progression of human genome researches, it is known that different genetics mechanism is the internal material basis of the occurrence and development of many diseases. More and more evidences show that sepsis is affected by multiple gene polymorphisms [24, 25]. Association between gene polymorphisms and sepsis has been reported in previous studies [7, 8, 25, 26]. Studies about correlation between genetic factors and diseases discover remarkable diversity in different races and regions. Therefore, current study explores the correlation of *TNF- $\alpha$*  gene rs1800629 and rs361525 polymorphisms with sepsis susceptibility in 223 Chinese Han population. It concludes that GA genotype and A allele frequencies of rs1800629 are obvious higher in case group than that in control group and the differences have statistical significance. But genotype and allele frequencies of rs361525 are not significantly different between case and control groups.

In short, we draw a conclusion that *TNF- $\alpha$*  gene rs1800629 polymorphism may correlate with sepsis susceptibility and contribute to the occurrence of it. Although *TNF- $\alpha$*  rs1800629 SNP plays a significant role in sepsis, but its accurate mechanism is still not clearly. Because the small sample size, only one ethnicity and unadjusted results, the conclusion was insufficient to understand the pathogenesis of sepsis. So the well designed further studies about *TNF- $\alpha$*  and sepsis is necessary.

### Disclosure of conflict of interest

None.

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