Original Article

Association of genetic variants in the IRAK-4 gene with susceptibility to severe sepsis

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BACKGROUND: The association of genetic variation in the IRAK-1 gene with sepsis outcome has been proved. However, few studies have addressed the impact of the IRAK-4 gene variants on sepsis risk. This study aimed to determine whether the polymorphisms in the IRAK-4 gene are associated with susceptibility to and prognosis of severe sepsis in the Chinese Han ethnic population.

METHODS: In this case-control study, 192 patients with severe sepsis hospitalized in the emergency department of Zhongshan Hospital from February 2006 to December 2009 and 192 healthy volunteers were enrolled. Exclusion criteria included metastatic tumors, autoimmune diseases, AIDS or treatment with immunosuppressive drugs. This study was approved by the ethical committee of Zhongshan Hospital, Fudan University. Sepsis patients were divided into a survival group ($n=124$) and a non-survival group ($n=68$) according to the 30-day mortality. Primer 3 software was used to design PCR and sequencing primers. Genomic DNA was extracted from peripheral blood mononuclear cells. Seven tagSNPs in IRAK-4 were selected according to the data of the Chinese Han population in Beijing from the Hapmap project and genotyped by direct sequencing. The chi-square test was used to evaluate the differences in genotype and allele frequencies between the two groups.

RESULTS: The distributions of all tagSNPs were consistent with Hardy-Weinberg equilibrium. The allele and genotype frequencies of rs4251545 (G/A) were significantly different between the severe sepsis and healthy control groups ($P=0.015$, $P=0.035$, respectively). Carriers of the rs4251545A had a higher risk for severe sepsis compared with carriers of the rs4251545G ($OR=1.69$, 95% CI: 1.10-2.58). The allele and genotype frequencies of all SNPs were not significantly different between the survival group and non-survival group.

CONCLUSION: These findings indicate that the variants in IRAK-4 are significantly associated with susceptibility to severe sepsis in the Chinese Han ethnic population.

KEY WORDS: Severe sepsis; IRAK-4; Single nucleotide polymorphism; Susceptibility; Prognosis; Chinese Han population; Allele; Genotype

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INTRODUCTION

Sepsis is an inflammatory state as a response of the immune system to infection, which is frequently the complication of trauma, burn, shock, infection, ischemia-reperfusion injury and surgery.^[1] In the USA, about 200 000 people died of severe sepsis every year, and the medical

cost for sepsis is up to 16 billion US dollars.^[2,3] It has been proved by numerous studies that besides invading microorganisms, the innate and adaptive immune systems of the host play a very important role in the development and outcome of sepsis. More and more evidences show the complex interactions between genes and the

environment during the process of sepsis, suggesting a more strong genetic influence on the outcome from sepsis than environment.^[4-6] Results from the human genome project (HGP) indicate that single nucleotide polymorphisms (SNPs) determine the individual variability.^[7] Clinical studies have shown that the SNPs of some genes are associated with susceptibility to and prognosis of severe sepsis,^[8-14] for example, the SNPs of Toll/interleukin-1 receptor domain-containing adaptor (TIRAP) gene^[15] and Toll-interacting protein (TOLLIP) gene, encoding the adaptor protein Mal and inhibiting Toll-like receptors (TLRs) signaling pathway-mediated inflammatory responses, as demonstrated in our previous studies.^[16]

Interleukin-1 receptor-associated kinase (IRAK) is implicated in signal transduction of the TLR/IL-1 family. There are four different IRAK-like molecules, of which IRAK-4 is essential for NF- κ B activation in TLR. Knockout mice of IRAK-4 were completely impaired in all TLR/IL-1R responses and strongly diminished IL-1-induced NF- κ B activation, indicating an essential role for its kinase domain.^[17-20] The association of genetic variation in the IRAK-1 gene with sepsis outcome has been proved.^[21-24] However, few studies have addressed the impact of the IRAK-4 gene variants on sepsis risk. Therefore, we performed a case-control study in a Chinese Han ethnic population with sepsis by using a tagSNP approach to examine the association of the IRAK-4 gene with susceptibility to sepsis.

METHODS

Study design and enrollment

From February 2006 to December 2009, 192 individuals with sepsis or severe sepsis were prospectively recruited from the ward of Emergency Department of Zhongshan Hospital, Fudan University. Moreover 192 age- and sex-matched healthy volunteers were recruited from an ethnically similar local population. All subjects were of Chinese Han ethnic origin.

The diagnosis of sepsis and severe sepsis met the criteria recommended by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.^[25] Exclusion criteria included metastatic tumors, autoimmune diseases, AIDS or treatment with immunosuppressive drugs. All the patients were divided into the survival and non-survival groups according to the 30-day mortality. Baseline characteristics, source of infection, and the Acute Physiology and Chronic Health

Evaluation (APACHE) II scores of all patients were obtained after admission. Peripheral blood samples of all patients collected in EDTA-treated tubes were stored under the temperature of -20 °C.

Single nucleotide polymorphism genotyping

Selection of TagSNPs was based on the data from the Hapmap database and the selection strategy was as follows: First, the International Haplotype Mapping (HapMap) (www.hapmap.org) SNP databases were used to select tagSNPs in the IRAK-4 gene region. From this database, genotyping data were obtained and loaded in the Haploview software version 4.1. Second, tagSNPs were selected using a pair-wise tagging algorithm setting the minor allele frequency (MAF) >0.05. Consequently, we choose 7 tagSNPs including rs4251533, rs4251481, rs4251545, rs4238087, rs4251431, rs4251513 and rs1461567 for eventual genotyping in our patients and controls. Primer 3 software was used to design primers.

Genomic DNA was extracted from whole blood using FlexiGene DNA Kit (Qiagen Hilden, Germany) following the manufacturer's protocol. The PCR system was 15 μ L, consisting of 1.5 μ L of 10 $\times$ PCR buffer, 0.45 μ L of four different dNTPs (10 mmol/L), 2.5 μ L of magnesium chloride (25 mmol/L), 0.12 μ L of primers of upstream and downstream each, 1.0 μ L of template DNA (10-20 ng), and sterile double-distilled water to make up for 15 μ L. Real-time PCR was performed on an ABI 7900 HT system (Applied Biosystems). The PCR cycling conditions for both fragments involved a denaturation step at 95 °C for 2 minutes and then 12 cycles of 94 °C for 30 seconds, 63 °C for 60 seconds, 72 °C for 50 seconds and then 30 cycles of 94 °C for 30 seconds, 57 °C for 40 seconds and 72 °C for 45 seconds with a final elongation cycle of 72 °C for 10 minutes. The sequences were resolved using an ABI 3730 Genetic Analyzer (Applied Biosystems).

Statistical analyses

Quality tests were processed using Haploview version 4.1 software for the Hardy-Weinberg equilibrium (HWE) of each SNP. The Chi-square test was performed to determine whether an association existed for alleles and genotypes between the individuals and controls. SPSS software version 17.0 was used to determine the association. The association with susceptibility to severe sepsis was tested by calculating odds ratio (OR) with 95% confidence intervals, and *P* values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of the study population

The characteristics of the study population are shown in Table 1. Age and gender were all well-balanced between the patients and controls. The patients with severe sepsis with a mean age of 64.8 ± 12.4 years comprised more men (59.4%) than women (40.6%), while the controls with a mean age of 66.2 ± 12.1 years had a similar proportion of men (58.3%) and women (41.7%). The mortality of the patients with severe sepsis was 35.4%. There was no significant difference in age and gender between survivors and non-survivors. However, non-survivors showed higher APACHE II scores and longer hospital stay ($P < 0.05$).

Association of IRAK-4 gene SNPs with susceptibility to severe sepsis

The genotyping success rates of tagSNPs ranged from 98.5% to 100%. All the tagSNPs were consistent with HWE. The genotype and allele frequencies of the 7 polymorphisms are summarized in Table 2. The allele and genotype frequencies of rs4251545 (G/A) were significantly different between the severe sepsis and healthy groups ($P = 0.015$, $P = 0.035$, respectively). Carriers of rs4251545A had a higher risk for severe sepsis compared with those of rs4251545G ($OR = 1.69$, 95% CI: 1.10-2.58), whereas other 6 SNPs showed no association at all. The difference of genotype and allele frequencies between the severe sepsis and control groups suggested that polymorphisms in the IRAK-4 gene are associated with susceptibility to severe sepsis.

Association of IRAK-4 gene SNPs with prognosis of severe sepsis

The genotype and allele frequencies of the 7 polymorphisms in survivors and non-survivors are showed in Table 3. We found that there was no significant difference in allele frequencies of tagSNPs between the survival group and non-survival group ($P > 0.05$), even the influence of APACH II score was excluded by logistic correction.

Table 1. Demographic and clinical characteristics of patients with severe sepsis

Variables	Survivors (n=124)	Non-survivors (n=68)	P value
Age (years)	63.5±11.4	67.16±13.02	> 0.05
Males/Females	75 / 49	39 / 29	> 0.05
APACHE II	12.75±1.03	18.55±1.42	< 0.05
Length of hospital stay (days)	11.6	19.60	< 0.05

Table 2. The allele and genotype frequencies of IRAK-4 SNPs regard to susceptibility to severe sepsis in different groups

Variables	Healthy controls	Patients with sepsis	Allelic		Genotypic P
			P	OR	
rs4251533			0.460	1.14 (0.80-1.64)	0.587
AA	127	123			
AG	58	58			
GG	6	10			
rs4251481			0.303	1.31 (0.79-2.17)	0.487
AA	165	160			
AG	25	27			
GG	2	5			
rs4251545			0.015	1.69 (1.10-2.58)	0.035
GG	154	139			
AG	36	43			
AA	2	10			
rs4238087			0.190	0.75 (0.49-1.15)	0.287
AA	140	149			
AG	46	40			
GG	4	1			
rs4251431			0.182	0.74 (0.48-1.15)	0.283
GG	140	151			
GT	50	40			
TT	1	0			
rs1461567			0.162	1.23 (0.92-1.64)	0.286
CC	60	50			
CT	108	109			
TT	21	30			
rs4251513			0.823	0.97 (0.77-1.30)	0.872
CC	58	62			
CG	114	109			
GG	17	18			

Table 3. The allele and genotype frequencies of IRAK-4 SNPs regard to prognosis of severe sepsis in different groups

Variables	Survivors	Non-survivors	Allelic		Genotypic P
			P	OR	
rs4251533			0.217	1.38 (0.83-2.30)	0.055
AA	81	42			
AG	40	18			
GG	3	7			
rs4251481			0.295	1.44 (0.73-2.87)	0.130
AA	107	53			
AG	13	14			
GG	4	1			
rs4251545			0.928	0.97 (0.55-1.72)	0.059
GG	87	52			
AG	33	10			
AA	4	6			
rs4238087			0.346	0.71 (0.35-1.45)	0.145
AA	94	55			
AG	30	10			
GG	0	1			
rs4251431			0.444	1.30 (0.66-2.54)	0.457
GG	101	50			
GT	24	16			
TT	0	0			
rs1461567			0.053	1.52 (0.99-2.33)	0.052
CC	36	14			
CT	74	35			
TT	14	16			
rs4251513			0.724	1.08 (0.70-1.67)	0.765
CC	20	42			
CG	41	68			
GG	6	12			

DISCUSSION

Innate immunity is the first-line defense against microbial infections. The TLR signaling system that recognizes a variety of structures derived from microorganisms modulates the onset and development of the innate immunity. However, prolonged and excessive activation of TLR signaling pathways, which means over production of pro- and anti-inflammatory cytokine by inflammatory cells, breaks the balance of the immune system and contributes to the pathogenesis of organ injury. The increasing levels of serum proinflammatory cytokines such as IL-1, IL-6 and TNF- α were proved to be associated with the onset and outcome of severe sepsis.^[26]

IRAK-4 has an essential role in TLR-mediated signaling. The adaptor molecule MyD88, which is recruited by all of the TLRs, except TLR3, recruits IRAK-4 and IRAK-1. After being phosphorylated by IRAK-4, IRAK1 recruits TNFR-associated factor 6 to the receptor complex. The IRAK-TNFR-associated factor 6 complex dissociates from the receptor to activate TAK1, TAB1 and TAB2, which eventually leads to the activation of NF- κ B. Suzuki et al^[27] have found that IRAK-4-deficient animals are completely resistant to a lethal dose of lipopolysaccharide because of impaired TLR-mediated induction of proinflammatory cytokines and chemokines. Patients with deficient IRAK-4 are vulnerable to infections with gram positive organisms while the level of inflammatory response is lower.^[28]

There are several reports on the association of SNPs in the TLR family with susceptibility and prognosis to severe sepsis.^[5,8-9,15-16,29] SNPs in the TLR1(rs5743551), TLR2 (rs5743708), TLR4 (rs4986790 and rs4986791), TLR9 (rs5743836), IRAK-1 (rs1059703), and TIRAP (rs8177374 and rs7932766) genes have been proved to increase the risk of sepsis. However, no literatures have addressed the impact of genetic variants in IRAK-4 which plays such a critical role in TLR signaling pathways. We evaluated the contribution of SNPs in the IRAK-4 gene to susceptibility to severe sepsis and prognosis in a Chinese population-based case-control study. Rs4251545, located in the first exon region of IRAK-4, is a non-synonymous mutation. The substitution of A for G leads to the change of amino acids (Ala/Thr) coding the proteins, while causing the alteration of the proteins in structure and function. We confirmed that IRAK-4 SNPs (rs4251545) are associated with higher risk to sepsis. Even though we still have no idea whether the change of protein causes the susceptibility. Thus further study should be conducted.

To our knowledge, there is only one study focusing on the association of genetic variants in the IRAK-4 gene with susceptibility to the disease. Tewfik and colleagues^[30] found that three SNPs in IRAK-4 (rs1461567, rs4251513 and rs4251559) were associated with total serum IgE levels in patients with chronic rhinosinusitis and asthma in a Caucasus population, while there was no difference in genotype and allele frequencies in SNP rs4251545 between healthy controls and patients. We also had studied the two SNPs in IRAK-4 (rs1461567 and rs425151). Unlike Tewfik's results, the two tagSNPs did not represent major risk factors for the development of sepsis. SNP (rs4251559) was not genotyped in our study because it was not included in the HapMap CHB data. We believe it is the racial and disease difference that determine the different outcomes between our study and Tewfik's.

In conclusion, we reported for the first time that one tagSNP in the IRAK-4 gene contributes to increased risk of severe sepsis in the Chinese Han ethnic population. But whether the genetic variation is associated with susceptibility to sepsis in other populations needs further exploration.

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Ethical approval: The study was approved by the Ethical Committee of Zhongshan Hospital, Fudan University, Shanghai, China.

Conflicts of interest: The authors have no financial or other conflicts of interest regarding this article.

Contributors: Yin J proposed and wrote the study. All authors read and approved the final manuscript.

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