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Genetic association between cluster of differentiation 86 variations and sepsis risk

A case-control study

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Abstract

The aim of this study was to investigate the correlation between cluster of differentiation 86 (*CD86*) gene rs1129055 and rs2715267 single nucleotide polymorphisms and sepsis susceptibility.

One hundred twenty-five sepsis patients and 120 healthy controls were enrolled in this case-control study. *CD86* polymorphisms rs1129055 and rs2715267 were genotyped through polymerase chain reaction-restriction fragment length polymorphism approach. Chi-square test was used to analyze differences in genotype and allele frequencies of the 2 polymorphisms between case and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to present the association strength of the polymorphisms with sepsis susceptibility.

AA genotype and A allele frequencies of *CD86* rs1129055 were significantly lower in sepsis patients than in healthy controls ($P < .05$), revealing their significant associations with decreased disease susceptibility (OR = 0.351, 95% CI = 0.169–0.728; OR = 0.593, 95% CI = 0.415–0.847). Nevertheless, rs2715267 had no significant association with sepsis susceptibility ($P > .05$).

AA genotype and A allele of *CD86* polymorphism rs1129055 might be correlated with decreased sepsis susceptibility in Chinese Han population, but not rs2715267. Further study should be performed to verify our findings.

Abbreviations: *CD86* = cluster of differentiation 86, CIs = confidence intervals, HWE = Hardy–Weinberg equilibrium, IL = interleukin, ORs = odds ratios, PCR = polymerase chain reaction, SNPs = single nucleotide polymorphisms.

Keywords: *CD86*, PCR-RFLP, polymorphism, sepsis

1. Introduction

Sepsis is a clinical process of systemic inflammatory response caused by bacterial infections. This systemic inflammatory response syndrome always makes the body enter into an anti-inflammatory and immunosuppressive state, which leads to the death of patients with severe traumas or burns. In sepsis patients, pro-coagulant and antifibrinolytic pathways may bring about microvascular fibrin deposition, finally leading to multi-organ failure and even death.[1] Foreign epidemiological investigations have shown that the fatality rate of sepsis has exceeded that for myocardial infarction.[2] New diagnosed cases in the United States are over 750,000 per year.[3] And its incidence still shows a rising tendency.[4] Obviously, sepsis is one of the most serious diseases threatening human lives. According to existing documents, sepsis is a complex polyfactorial disease involving genetic and environment factors. However, only a small part of people exposing to risk environmental factors would eventually develop this disease. Moreover, evidences have indicated that genetic polymorphism may influence inflammatory response and sepsis pathology.[5]

Cluster of differentiation 86 (*CD86*, also known as B7-2), a co-stimulatory molecule, resides on antigen-presenting cells and is expressed constitutively in resting monocytes, trigger B cells, and T cells.[6] It plays a crucial role in autoimmunity, transplantation, and tumor immunity.[7,8] CD86 is a type I membrane glycoprotein and contains 2 extracellular Ig-like domains, a transmembrane domain and a cytoplasmic tail. Its encoding gene, *CD86*, is located on chromosome 3q21, and comprised of 8 exons. Genetic variations in the *CD86* gene may lead to immune cell dysfunction and result in subsequent systemic inflammatory responses.[9]*CD86* is a key mediator in activating T cell in immune response.[10] Furthermore, *CD86* gene mutants may result in T cell inactivation and non-response to tumor cells, and finally allows malignant progression of cancer.[11]

Many previous studies have shown that *CD86* participates in the pathogenesis of sepsis.[12–14] A major study has displayed that CD86 level is decreased in monocytes in a cohort of septic patients. Numbers of single nucleotide polymorphisms (SNPs) in *CD86* have been identified to be associated with various human diseases.[15] However, few researches have discussed the correlation between *CD86* polymorphisms and sepsis susceptibility.

Therefore, in the present study, 2 common SNPs (rs1129055 and rs2715267) in the *CD86* gene were selected to explore their

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association with sepsis susceptibility in the Chinese Han population

2. Methods and materials

2.1. Study subjects

This case-control study was reviewed and consented by the Ethics committee of Air Force General Hospital of People's Liberation Army of China (PLA). Sample collection was in line with the ethics criteria of national human genome research. All subjects or their guardians had signed written informed consent and filled out questionnaire (including basic information on the subjects, exposure history, clinical symptoms, and immune history) before enrollment. All of them were the Chinese Han people without any blood connections.

A cohort of 245 subjects including 125 sepsis patients and 120 healthy controls were enrolled in this study. The diagnosis criteria for sepsis, severe sepsis, and septic shock were formulated following the guidance form the American College of Chest Physicians/Society of Critical Care Medicine.[16] Sepsis patients were diagnosed in Air Force General Hospital of PLA, including 59 females and 66 males (aged between 22 and 67). Sepsis patients with a history of diabetes, autoimmune diseases, tumors, and viral infections would be excluded. One hundred twenty healthy volunteers frequency-matched with the cases in age and gender (55 females and 65 males) were recruited from healthy check-up center as controls. Their ages ranged from 20 to 68 years old.

2.2. Sample collection and DNA extraction

Two-milliliter peripheral venous blood was collected from each of the participants, anticoagulated by 0.5% Ethylene Diamine Tetraacetic Acid (pH 8.0), separated into serum and hemocyte, and then stored at −80°C. Genomic DNA was extracted adopting Biospin Whole Blood Genomic DNA Extraction Kit (Bioer Technology Co, Ltd, China) according to the producer's instruction, and then stored at −20°C for standby application.

2.3. Polymerase chain reaction amplifications and genetic typing assay

Genotyping for *CD86* rs1129055 and rs2715267 polymorphisms was conducted through polymerase chain reaction (PCR)-restriction fragment length polymorphism. Primer sequences for *CD86* polymorphisms rs1129055 and rs2715267 were designed by Primer Premier 5.0 software, and synthesized by Sangon Biotech (Shanghai, China) (Table 1). PCR amplification was performed in a mixture of 25 μL, containing 2 μL template DNA, 1 μL upstream primer, 1 μL downstream primer, 5 μL 10× buffer in 0.2 mL reaction tube, 0.5 μL Taq DNA polymerase, 2 μL deoxy-ribonucleoside triphosphate, and 13.5 μL deionized sterile water. PCR procedures were as follows: 94°C initial denaturation for 5 minutes; followed by 35 cycles of denaturing at 95°C for 30 seconds, annealing at 65°C for 30 seconds and extension for 30 seconds at 72°C; a final extension at 72°C for 10 minutes. PCR products were examined via 1% agarose gel electrophoresis.

PCR products were digested with 2 U BbvI and Mly I restriction enzymes (New England BioLabs, Ipswich, MA) in a 10 μL reaction solution. Enzyme digestion reaction was performed through 37°C water bath for 4 hours. Digested fragments were separated via 2.5% agarose gel electrophoresis and the results were observed through ethidium bromide staining under ultraviolet light. Random PCR products were selected for sequencing to check genotyping results.

Table 1

Primer sequences of *CD86* gene rs1129055 and rs2715267 polymorphisms.

SNP		Primer sequences
Rs 1129055	Upstream	5′-TCCATATACCTGAAAGATCTGATGCA-3′
	Downstream	5′-GAGCTGGAGTTACAGGGAGGCT-3′
Rs2715267	Upstream	5′-TACCAGCCGGCTTTGTACTG-3′
	Downstream	5′-ATACAGCTCAGTGCCCTCTTC-3′

CD86 = cluster of differentiation 86, SNP = single nucleotide polymorphism.

2.4. Statistical analysis

Predictive analytics software statistics 18.0 statistical software was employed for statistical analysis. The representativeness of the controls was tested through examining the deviation of their genotype distribution from Hardy–Weinberg equilibrium (HWE). Genotype and allele frequencies of rs1129055 and rs2715267 polymorphisms were determined through direct counting. Differences in genotype and allele frequencies of the polymorphisms between case and control groups were compared via Chi-square test. Association of *CD86* polymorphisms with sepsis susceptibility was evaluated by odds ratios (ORs) and 95% confidence intervals (CIs). Two-sided *P*-values less than .05 were considered as statistically significant.

3. Results

3.1. HWE test

Genotype distributions of rs1129055 and rs2715267 polymorphisms in the control group were consistent with HWE ($P > .05$, Table 2), indicating their representativeness for the general population.

3.2. Genotype and allele distributions of CD86 polymorphisms

Genotype and allele distributions of *CD86* polymorphisms rs1129055 and rs2715267 were shown in Table 2. The frequencies of the GG, AG, and AA genotypes of rs1129055 were 34.40%, 48.00%, 17.60% in the case group and 20.00%, 50.83%, 29.17% in the control group. The frequency of AA genotype in cases was statistically different from controls, showing its association with decreased risk of sepsis ($P = .007$, OR = 0.351, 95% CI = 0.169–0.728). Meanwhile, G and A allele frequencies were 58.40%, 41.60% in cases and 45.42%, 54.58% in controls. Obviously, A allele was less frequent in the case group than in the control group, indicating its repressing effect against sepsis ($P = .005$, OR = 0.593, 95% CI = 0.415–0.847).

The frequencies for the AA, AC, and CC genotypes of rs2715267 were 41.60%, 44.80%, 13.60% in sepsis patients and 54.17%, 35.83%, 10.00% in healthy controls; A and C allele frequencies were 64.00%, 36.00% in cases and 72.08%, 27.92% in controls. Although C allele carriers appeared more frequently in cases than in controls, the difference had no statistical

Table 2

Genotype and allele distributions of *CD86* gene rs1129055 and rs2715267 polymorphisms in case and control groups.

Genotype/allele	Case n = 125 (%)	Control n = 120 (%)	P_{HWE}	*P*	OR (95% CI)
Rs1129055			.782		
GG	43 (34.40)	24 (20.00)		–	1
AG	60 (48.00)	61 (50.83)		.067	0.549 (0.297–1.0140
AA	22 (17.60)	35 (29.17)		.007	0.351 (0.169–0.728)
G	146 (58.40)	109 (45.42)		–	1
A	104 (41.60)	131 (54.58)		.005	0.593 (0.415–0.847)
Rs2715267			.230		
AA	52 (41.60)	65 (54.17)		–	1
AC	56 (44.80)	43 (35.83)		.101	1.628 (0.949–2.792)
CC	17 (13.60)	12 (10.00)		.214	1.771 (0.777–4.037)
A	160 (64.00)	173 (72.08)		–	1
C	90 (36.00)	67 (27.92)		.066	1.452 (0.991–2.129)

95% CI = 95% confidence interval, CD86 = cluster of differentiation 86, HWE = Hardy–Weinberg equilibrium, OR = odds ratio.

significance between 2 groups ($P > .05$), showing that rs2715267 might have no independent association with sepsis susceptibility.

4. Discussion

Sepsis is a common complication in patients undergoing severe traumas, shocks, infections, and major surgeries. Currently, sepsis is a leading cause of death in intensive care unit and the 10th primary cause of death overall.[17] Risk factors for severe sepsis involve not only individuals' predisposition for infection, but also the chance of acute organ dysfunction when infection occurs. Although pathological mechanism of sepsis is still unclear, certain components participating in immune response, such as interleukin (IL)-6 and IL-1β, have been linked to the disease.[18,19] Until now, potential correlation for sepsis onset has been explored with polymorphisms in some genes, such as *1L-6*, tumor necrosis factor-α, and other inflammatory-related ones.[20–23]

CD86 functions as a necessary costimulatory signal for T-cell activation and survival.[24] There are 2 different proteins on T-cell surface, namely CD28 which works for autoregulation and intercellular association and cytotoxic T lymphocyte-associated antigen-4 for attenuating regulation and cellular disassociation. CD86 is the ligand for these 2 proteins and functions in tandem with CD80 to prime T cells. Earlier study has found that CD86 level is reduced in monocytes among patients with sepsis progression.[25] Previous studies have validated that genetic variants may influence gene expression, especially those in promoter regions. A previous study has shown that *CD86* polymorphism rs2715267, in the promoter region of the gene, could regulate the gene transcription and is involved in systemic sclerosis pathogenesis.[26] Liu et al claimed that *CD86* polymorphism rs1129055 might be an important risk factor for chronic obstructive pulmonary disease in Chinese people.[10]*CD86* polymorphism rs17281995 has been reportedly correlated with the risk of cancer particularly colorectal cancer in Caucasians.[27] Hence, genetic factors play an important role in sepsis occurrence.

Various SNPs have been identified in the *CD86* gene. Among them, rs1129055 in exon 8 and rs2715267 in the promoter region have been reported in the Chinese population.[26,28] In the present study, we investigated genotype distributions of *CD86* rs1129055 and rs2715267 in 2 groups and measured their correlation with sepsis risk in the Chinese Han population. We observed significant differences in genotype and allele frequencies of *CD86* polymorphism rs1129055 between sepsis patients and healthy controls. The frequencies for AA genotype and A allele of this polymorphism were significantly decreased in cases, compared with controls. The results indicated AA genotype and A allele might be protective factors against sepsis. In a previous study, Song et al investigated the relationship between *CD86* polymorphism rs1129055 and pneumonia-induced sepsis and reported that this polymorphism significantly decreased the disease risk through regulating *CD86* expression in monocytes.[29] And our conclusion was in accordance with previous evidences However, Fu et al reported that GA genotype of *CD86* polymorphism rs1129055 was significantly associated with the increased risk of sepsis.[30] The discrepancy may be attributed to small sample size and different study populations. Unfortunately, another polymorphism rs2715267 exhibited no independent correlation with sepsis susceptibility in our cohort, through its C allele appeared more frequently in patients than in the control group.

Certainly, some limitations in the present study should be noted. The relatively small sample size might reduce the statistical power of our findings. And only 1 nationality was included, which limited the clinical application of our findings. Moreover, the interaction of our interested polymorphisms with other relevant factors was not considered. Additionally, PCR-sequencing may provide more accurate results in genotyping *CD-86* polymorphisms. Therefore, in further study, we will verify the above results through experiments based on well design, large sample size, and multiple populations, as well as sequencing method for genotyping.

In conclusion, *CD86* polymorphism rs1129055 was significantly correlated with sepsis susceptibility in our research. The results further confirmed that genetic polymorphisms act as a risk or protective factor in sepsis and influence the disease progression. Furthermore, *CD86* gene might participant in the pathogenic mechanism of sepsis.

Author contributions

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