Original Article Lack of association between FOXO1 polymorphisms and bacteremia

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Abstract: Increasing evidence suggests that FOX01, one critical gene related to the human immune system, probable is closely to the human infection. In the present study we aimed to investigate genetic association of FOX01 with bacteremia in Han Chinese. 188 patients with bacteremia diagnosed with blood culture and 250 healthy blood donors without signs of infection were studied, two tagging SNPs of FOX01 (rs9532571, rs3751436) were selected and genotyped using predesigned TaqMan allelic discrimination assays. The results showed that the allele frequency of rs9532571 and rs3751436 in FOX01 was not associated with an increased risk of bacteremia (P=0.762, OR=1.05, 95% CI 0.77-1.43; P=0.059, OR=1.34, 95% CI 0.99-1.81 respectively), the genotype distribution of these two SNPs was also not significantly different between bacteremia patients and control groups (P=0.9; P=0.16). Haplotypes in this block were not significantly associated with bacteremia risk. Conclusion: the association between FOX01 genetic polymorphism and bacteremia has not been observed in the study, maybe a larger sample population and more SNPs in the FOX01 need to reveal the role in bacteremia in the future.

Keywords: Bacteremia, FOXO1, SNP, association

Introduction

Bloodstream infections can be caused by a wide variety of pathogens and remain a significant cause of morbidity and mortality especially in the immune compromised patients. The immune response is the first-line response to defense microbial infections. Increasing evidence suggests that genetic variation especially single nucleotide polymorphisms (SNPs) in the innate and adaptive immune system may influence the risk of patients for serious infection [1, 2].

FOXO transcription factors are highly conserved among distantly related species, in humans, four isoforms (FOXO1-4) exist [3]. FOXOdependent regulating the antimicrobial peptides gene is known to play an important role in the innate immune defense against bacterial infections [4]. Recent studies showed that FOXO1 regulates inflammation by enhancing TLR4-mediated signaling and IL-1β expression in human macrophages [5, 6]. These studies suggested that FOXO1 transcription factor acts as modulators of innate immune functions. FOXO1 also is indispensable for T cell responses to infection. Previous studies have revealed a critical role for FOXO1 in the control of naive T cell homeostasis, which is in part dependent on the induction of IL-7Ra expression [7, 8]. It has been recently shown the FOXO protein is predominant expressed in mature regulatory T cells and mediate regulatory T cell function in part via the inhibition of the expression of the pro-inflammatory cytokine interferon-g (IFN-g) [9]. All these research suggest that FOXO1, one critical gene related to the human immune system, probable is closely to the human infection.

The genetic susceptibility of FOXO1 has been related with the disease of diabetes, atherosclerosis and longevity [10-12]. Considering the possible role of FOXO1 in the maintenance the immune cells, in the present study we aimed to investigate the intrinsic association of FOXO1 with bacteremia in Han Chinese. Two tagging

Subgroup	Number	Male	Female	Age
Health control	250	162	88	42.3±11.8
Bacteremia	188	122	66	46.5±17.0
Species				
Gram-	72			
Gram+	99			
Fungi	14			
Polymicrobial	3			

Table 1. Demographics and phenotypes of thestudy subjects

SNPs of FOXO1 (rs9532571, rs3751436) were selected in this study and genotyped to investigate the susceptibility to bacteremia.

Materials and methods

Ethics statement

The study conformed to guidelines set forth by the Declaration of Helsinki and was approved by the ethics committee of the Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from each patient.

Study population

In this study, 188 patients with culture-proven bacteremia were enrolled from Sep 2014 to April 2015, Blood cultures positive for following microorganisms generally considered to be contaminants, including staphylococcus and Penicillium spp., were excluded from analysis. All the patients were: age >18 years, fever (>38.0°C) and/or a history of fever and chills within 24 hours before presentation. For the control group, 250 health examination volunteers without infection, with sex and age matched, were selected from our hospital database, and the blood samples were obtained from the clinical laboratory. The exclusion criteria were patients with immune suppression of any etiology, including cancer, current immunosuppressive therapy or chemotherapy, human immunodeficiency virus (HIV) infection, liver insufficiency and severe chronic renal disease with dialysis therapy.

Genotyping

Genomic DNA was extracted from whole blood as previously described [13]. The SNPs in

FOX01 were genotyped using predesigned Taq Man allelic discrimination assays in a Vii A 7 real-time polymerase chain ® reaction (PCR) from Applied Bio systems including universal master mix, amplifying primers, and probes. One allelic probe was labeled with FAM dye and the other with VIC dye. PCR was run in the TagMan universal master mix at a probe concentration of 20×. The reaction was performed in a total reaction volume of 25 mL including 20 ng of genomic DNA. The reaction plates were heated for 2 min at 50°C and for 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 90 s. The fluorescence intensity of each well was subsequently read, and fluorescence data files from each plate were analyzed by automated software.

Statistical analysis

All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). A. ×2-test was used to compare the distribution of genotypes among patients and controls. The association between the FOXO1 SNPs and the disease status was expressed in odds ratio (OR) and their 95% confidence intervals (CI). A P <0.05 was considered to be statistically significant. The distributions of genotype for SNPs were analyzed for deviation from Hardy-Weinberg Equilibrium (HWE) using ×2 analysis. A cut-off *p* value of 0.05 was set for HWE. The haplotype analysis of these SNPs was determined using the SNP Stats analysis platform, as described previously [14].

Results

Characteristics and grouping of the study population

188 patients with bacteremia (122 male and 66 female) and 250 healthy blood donors without signs of infection (162 male and 88 female) were studied. No significant differences in age or gender distribution were detected between the cases and the controls (**Table 1**). The mean age was 42.3 ± 11.8 years for the patients with bacteremia and 46.5 ± 17.0 years for the controls. Gram-negative infection (38.2%), fungi (7.4%) and Gram-positive infection (52.7%), polymicrobial infection (1.6%) were the primary pathogens.

SNP	Control (n=250)	Case (n=188)	OR (95% CI)	Ρ
rs9532571				
Analyzed sample	234	178		
CC	22	17	1.0	
СТ	84	60	0.91 (0.59-1.38)	0.9
TT	128	101	0.98 (0.49-1.94)	
C allele	128	94		
T allele	340	262	1.05 (0.77-1.43)	0.762
rs3751436				
Analyzed sample	227	174		
AA	34	16	1.0	
AG	91	68	0.85 (0.55-1.29)	0.16
GG	102	90	0.53 (0.28-1.03)	
A allele	159	100		
G allele	295	248	1.34 (0.99-1.81)	0.059

Table 2. Prevalence of two SNPs in FOXO1 and bacteremiasubjects in Han Chinese

 Table 3. Association of two SNPs in FOXO1 with bacteremia

 subjects both in dominant and recessive model

SNP	Model	Control	Case	OR (95% CI)	Р
rs9532571	dominant	106	77	1.09 (0.73-1.61)	0.68
	(CC+CT/TT)	128	101		
	recessive	22	17	0.98 (0.51-1.91)	0.96
	(CC/CT+TT)	212	161		
rs3751436	dominant	125	84	1.31 (0.88-1.95)	0.18
	(GG+AG/GG)	102	90		
	recessive	34	16	1.74 (0.93-3.27)	0.08
	(GG/AG+GG)	193	158		

 Table 4. Haplotype analysis of the association of two SNPs in FOXO1 with bacteremia subjects

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Haplotype	Frequency	OR (95% CI)	Р
TA	0.414	1	-
TG	0.316	0.77 (0.56-1.05)	0.096
CA	0.263	0.87 (0.63-1.20)	0.4

Association analyses of FOXO1 polymorphisms with susceptibility to bacteremia

The genotyping success rates ranged from 92.6% to 94.7%, and all of the genotype distributions were consistent with Hardy-Weinberg equilibrium (data not shown). The allele and genotype distributions of these SNPs in healthy controls and patients with bacteremia are listed in **Table 2**. When patients with bacteremia

were compared with healthy controls, two tag SNPs in FOXO1 were observed in association with bacteremia susceptibility. The allele frequency of rs9532571 and rs-3751436 in FOXO1 was not associated with an increased risk of bacteremia (P=0.762, OR=1.05, 95% CI 0.77-1.43; P=0.059, OR=1.34, 95% CI 0.99-1.81 respectively), furthermore, the genotype distribution of these two SNPs was also not significantly different between bacteremia and control groups (P=0.9; P=0.16). We also did not observe there was any significant statistical difference in the dominant and recessive model (Table 3).

Then haplotype analysis was performed to investigate whether the haplotypes in the genes were associated with bacteremia. Three common haplotypes with a frequency of greater than 3%: TA, TG, CA were generated. In the global test, haplotypes in this block were not significantly associated with bacteremia risk (P=0.096, OR=0.77, 95% CI 0.56-1.05; P=0.4, OR=0.87, 95% CI 0.63-1.20) (Table 4).

Discussion

FOX01 is a member of the Forkhead

box protein family of proteins, (the other members being FOXO3a, FOXO4 and FOXO6) that respond to environmental stimuli (growth factors, oxidative stress, nutritional availability) and regulate the expression of many genes involved in cell growth, proliferation, differentiation, and survival [15, 16]. In recent years, several studies provided evidence that FOXO transcription factors play a role in inflammation and infection. Therefore, in this study, FOXO1 genetic polymorphisms were used to investigate the susceptibility to bacteremia.

However, our results showed that these two tag SNPs in FOXO1 were not significantly associated with susceptibility to bacteremia. Consistent with the single SNP analyses, the haplotype analysis also did not to reveal the association with the risk of bacteremia. Which has not been deserved the similar conclusion in another FOXO3 in the role of infection, the research has shown that a genetic variant in FOXO3 (rs12212067: T>G) associates with the increased susceptibility to severe malaria in Kenyan and Vietnamese patients [17]. The different results may suggest that FOXO1 have different role from FOXO3 in the immune system. Another explanation is that FOXO1 may be only susceptibility to some forms infection.

FOX01 is specifically upregulated during T cell maturation and is constitutively active in resting T cells and many other tissues [18-20]. When FOXO1 inactivation in the rest CD4+T cell enhance HIV virus replication [21], also have the similar result in EBV infection [22], suggesting that FOXO1 genetic polymorphsims might susceptibility to prior to viral infection. FOXO1 is also an important node in a dynamic network of transcription factors that orchestrate B-cell differentiation and specialization via PI3K-AKT axis [23]. Recently, an interesting research has shown that lungs of mice were infected with either Pseudomonas aeruginosa or nontypeable Haemophilus influenza (NTHI) resulted in the activation of FOXO transcription factors in respiratory epithelial cells [24]. All these research has taken a surge that FOXO1 play an important role in the development of innate immunity and adaptive immunity in the host.

There are several reasons that we have got the negative results in the study. First, the study has recruited a relative small sample size, the statistical power is low. Second, although we have recruited the bacteremia at randomized, but there is still exist selection bias in this study. Third, the two SNP selected were based on the haploview, there are other tag SNPs that has not been selected and the data does not cover the whole gene. The conclusion is limited.

Although there are above limitations, this is the first report attempt to reveal the genetic FOXO1 susceptibility to bacteremia, a larger sample population and more SNPs in the FOXO1 need to reveal the role in bacteremia.

Disclosure of conflict of interest

None.

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