Original Article

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Relation between interleukin-13 gene polymorphisms and susceptibility to brucellosis in Iranian population

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Abstract

Brucella is an intracellular Gram-negative bacterium. Previous reports showed that gene polymorphisms of cytokines can affect resistance or susceptibility to *Brucella* infection. Interleukin-13, a cytokine secreted by Th2 lymphocytes, has an important role in immune responses against established infections. In this study, we investigated the association of three polymorphic sites of IL-13 with susceptibility to brucellosis in Iranian population. In this study 169 patients with brucellosis and 71 healthy controls were included. DNA was extracted and genotyped for three bi-allelic polymorphisms of IL-13 gene at positions -1512A/C, -1055C/T, and +2044G/A by polymerase chain reaction-restriction fragment length polymorphism method. None of the studied alleles and genotypes of IL-13 gene (-1512A/C, -1055C/T, and +2044G/A) showed significant relationship with susceptibility to brucellosis. However, among eight haplotypes, the distribution of TCG and CAA haplotypes were significantly higher in the patients compared with those in the controls (P=0.002 and P=0.034, respectively). Although, the later did not tolerate Bonferroni correction. On the contrary, the distribution of TCA haplotype was higher in the controls compared to that in the patients (P=0.01). Furthermore, TAG/TCA haplogenotypes were significantly higher among controls compared to the brucellosis patients (P=0.025). P value resulted from TCA and TAG/TCA did not tolerate Bonferroni correction. There is no association between the inheritance of different alleles and genotypes of interleukin-13 gene and susceptibility to brucellosis. However, it seems that the inheritance of some haplotypes and haplogenotypes of IL-13 can impact the susceptibility to brucellosis.

Keywords: Brucella, gene variant, Interleukin-13.

1. Introduction

Brucellosis is an infectious disease caused by *Brucella* species. Brucella is an obligatory intracellular pathogen, which can replicate within monocytes and macrophages (1). These bacteria could be transmitted to humans through consumption of contaminated milk and dairy products, or sometimes through ingestion of meat from the infected animals. The disease has a worldwide distribution, while its most important endemic regions are the Middle East, the Mediterranean countries, the South America, and India (1). The mechanism of host protection to *Brucella* infection is incompletely understood. But, similar to the other intracellular bacteria, cell mediated immunity seems to play the major role in immune responses against this organism (1).

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Some studies have shown that the cytokines produced by Th-1 cells such as interferon-γ (IFN-γ), IL-12, and IL-18 promote cellular immunity. These cytokines can modulate activation of macrophages; enhance expression of their Major Histocompatibility Complex (MHC) molecules and increase their antigen presentation capability, and promotes lymphocyte differentiation to Th1 cells (2). They are key mediators in the protection against *Brucella*. An increased level of these cytokines in acute human brucellosis has been reported (2-3). On the contrary, it is shown that increase of Th2 related cytokines, such as IL-4 and IL-13, which can suppress cell mediated immunity, is related to susceptibility to *Brucella* infection. These cytokines promote Th0 to Th2 differentiation and inhibit Th0 to Th1 differentiation and macrophage activation. In general, secretion of these cytokines downregulates cell mediated immunity, which is the main immunity against *Brucella* infection (4-6).

Previous studies showed that cytokine levels vary among different people facing the same organism, which correlates with polymorphisms in the cytokine genes (7-8). Moreover, it is shown that Single-nucleotide polymorphisms (SNPs) in cytokine genes could play important roles in resistance or susceptibility of different individuals to the same infectious diseases (9-18). According to the importance of IL-13 in the defense against *Brucella* infection and the effect of IL-13 gene polymorphisms on its production, in this study, we decided to investigate probable relation between IL-13 gene polymorphisms and susceptibility to brucellosis.

2. Materials and Methods

2.1. Study groups

169 brucellosis patients (age range 7–82 years and mean±SD; 31.4±18.2) and 71 controls (age range 5–76 years, mean±SD; 29.3±32) were included in this study. The control group subjects were selected randomly in healthy animal husbandmen, who had close contacts with animals infected with Brucella and consumed their milk and dairy products, but did not show any clinical manifestations after a 6-month follow up. Brucellosis in their animals was confirmed by serological tests carried out in the laboratory of Fars Province Veterinary Administration.

Brucellosis was diagnosed in patients according to clinical findings such as fever, night sweats, weight loss, weakness, splenomegaly, microbial cultures, and serological tests. Positive serological tests were defined as high titers of standard agglutination test (SAT≥1/160) and confirmed by a titer≥1/160 in 2-mercaptoethanol test (2ME) at the time of infection.

The ethnicity and place of residency of the controls were the same as those of the patients. This study conforms to the tenets of the Helsinki declaration and was approved by the Ethics Committee of Shiraz University of medical sciences. All subjects were briefed about the study and written informed consents were obtained from all cases before sampling.

2.2. DNA extraction and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

DNA was isolated from peripheral blood mononuclear cells by salting out method. Amplification of the three polymorphic sites of IL-13 gene (-1055C/T, -1512A/C, and +2044G/A) was carried out using specific primers. The sequences of the forward and reverse primers are shown in table 1. PCR reactions were carried out in 10 µl volume. The reaction conditions and reagent concentrations were: 500 picomole of each primer (Primm-Italy), 1 unit of *Taq* DNA polymerase, 1X PCR buffer, 2.5 mM of $MgCl_2$, 200 µM of each dNTPs (all from Cinna Gen- Iran) and 250 ng of genomic DNA. After an initial denaturation at 94 °C for 10 minutes, 35 cycles were run in a Thermal Cycler set (Eppendorf, Germany) each comprising 60 seconds at 94 °C, 60 seconds at a specific temperature (Table 1), 60 seconds at 72 °C followed by a final extension for 10 min. at 72 °C. Then, 10 µl of each amplified DNA was digested with 1 unit of specific enzyme (Fermentas, Lithuania). The digested DNA was resolved by electrophoresis in 3% Agarose NA gel (Amersham Bioscience, Sweden) in 1X TAE buffer. After staining the gel with ethidium bromide, fragments were visualized by UV transilluminator and photographed. Specific restriction enzymes and DNA product sizes after

digestion are shown in table 1.

2.3. Statistical analysis

The differences between the experimental and control groups in genotype distribution were analyzed by χ^2 test using EPI info 2000 and SPSS software. To estimate the Hardy–Weinberg equilibrium and haplotype and haplogenotype frequencies, Arlequin software version 3.1 was used. 95% confidence interval (95% CI) and odds ratio were calculated by EPI info 2000. Linkage disequilibrium measures (LD), D' and P-value, were predicted by LD2SNPing program V 2.0 (http://www.bio.kuas.edu.tw/LD2SNPing).

For allele analysis, *P* values less than 0.05 were considered to indicate a statistically significant difference. The Bonferroni correction was done to adjust *P* values for genotype, haplotype, and haplogenotypes analysis. In this study, 3 genotypes, 8 haplotypes, and 24 haplogenotypes were observed. So, the new critical P values for genotypes, haplotypes, and haplogenotypes analysis were 0.016, 0.006, and 0.002, respectively.

3. Results

3.1. Allele and genotype frequencies

In this study, three single nucleotide polymorphisms (SNPs) of interleukin 13 gene at positions -1512A/C, -1055C/T, and +2044G/A were analyzed. Their electrophoretic patterns are shown in figure 1. The allele and genotype frequencies of interleukin 13 gene polymorphisms in the control and patient groups are shown in table 2. The results showed that there was not any significant relationship between allele and genotype frequencies in patients compared to controls in all the polymorphic sites.

3.2. Haplotype frequencies

Among eight haplotypes, CAG, TAA, CCG, TAG, and CCA haplotypes (positions -1055C/T, -1512A/C, and +2044G/A, respectively) did not showed any significant difference between the patients and the controls. However, the distribution of TCG and CAA haplotypes were significantly higher in the patients compared with those in the controls (6.8% vs. 0.0%, P=0.002 and 10.05% vs. 4.22%%, P=0.034, respectively), though the later did not tolerate Bonferroni correction. On the contrary, the distribution of TCA haplotype was higher in the controls compared with that in the patients (11.97% vs. 5.32%, P=0.01) (Table 3), but it did not tolerate Bonferroni correction.

3.3. Haplogenotype frequencies

Among 24 observed haplogenotypes, only one of them (TAG/TCA) showed significant difference between the patients and controls (P=0.025). The frequency of this haplogenotype in the control

Table 1. Primer sequences, annealing temperatures and restriction enzymes.

Polymorphic sites	Sequences (5' to 3')	Annealing Temp.(°C)	Restriction enzymes	Fragment sizes (bp)
-1512A/C	F: 5'- CAACCGCCGCGCCAGCGCCTTCTC -3'	65.5	Bsh1236	C: 192 and 22
	R: 5'- CCGCTACTTGGCCGTGTGAC-CGC--3'			A: 214
-1055C/T	F: 5'- ACTTCTGGGAGTCAGAGCCA -3'	63.5	HPY991	C: 339 and 33
	R: 5'- TACAGCCATGTCGCCTTTTCCT-GCTCTTCCGTC -3'			T: 372
+2044G/A	F: 5'- CTTCCGTGAGGACTGAATGAGAC-GGTC -3'	62.5	NlaIV	G: 187, 32 and 26
	R: 5'- CAAATAATGATGCTTTC-GAAGTTTCAGTGGA -3'			A: 216 and 26

group was 5.6% versus 0% in the patient group (Table 4). It should be mentioned that this difference did not tolerate Bonferroni correction.

3.4. Linkage disequilibrium measures

Strong LDs (P<0.001 for all comparisons) were detected between positions -1055C/T, -1512A/C, and +2044G/A of IL-13. The LD measures, D′, and P-value are shown in figure 2.

4. Discussion

Several factors such as immunity, environment, and heredity affect the susceptibility to infectious diseases. Studies have shown that single nucleotide polymorphism in different genes can affect the regulation of the immune reaction (8-11). Such polymorphism patterns may have predictive value for Brucella infection. Considering the role of IL-13 in the immunity against brucellosis and the known effect of gene polymorphisms on cytokine production and function, the aim of this study was to investigate the probable relationship between IL-13 gene polymorphisms and susceptibility to Brucella infection. IL-13 gene is

Table 2. Genotype and allele frequencies of IL-13 SNPs at positions -1055C/T, -1512A/C, +2044A/G.

IL-13	Patients N (%)	Controls N (%)	X^2	*P* value	OR (95% CI)
	Total Number=169	Total Number=71			
Position -1055					
Genotype					
TT	34 (20.1)	13 (18.3)	0.1	0.747	1.2 (0.53-2.43)
CC	69 (40.8)	29 (40.8)	0.0	0.998	1.00 (0.55-1.83)
CT	66 (39.1)	29 (40.8)	0.07	0.795	0.93(0.51-1.70)
Allele					
T	134 (39.6)	55 (38.7)	0.03	0.851	1.04 (0.68-1.58)
C	204 (60.4)	87(61.3)			
Position -1512					
Genotype					
AA	114 (67.5)	47 (66.2)	0.04	0.849	1.06 (0.56-1.98)
CC	9 (5.3)	2(2.8)	0.72	0.396*	1.94 (0.38-13.37)
AC	46 (27.2)	22 (31)	0.35	0.55	0.83 (0.44-1.60)
Allele					
A	274 (81.1)	116 (81.7)	0.03	0.872	0.96 (0.56-1.63)
C	64(18.9)	26 (18.3)			
Position +2044					
Genotype					
AA	15 (8.9)	2 (2.8)	2.79	0.094	3.36 (0.71-21.89)
GG	81 (47.9)	34 (47.9)	0.0	0.995	1.00 (0.55-1.81)
GA	73 (43.2)	35 (49.3)	0.75	0.385	0.78 (0.43-1.42)
Allele					
A	103 (30.5)	39 (27.5)	0.43	0.509	1.16 (0.73-1.83)
G	235 (69.5)	103 (72.5)			

Each *P* value is the result of comparing corresponding row with the sum of other related rows.

* *P* value is measured by Fisher exact test.

N: Absolute number, CI: Confidence interval, OR: odds ratio.

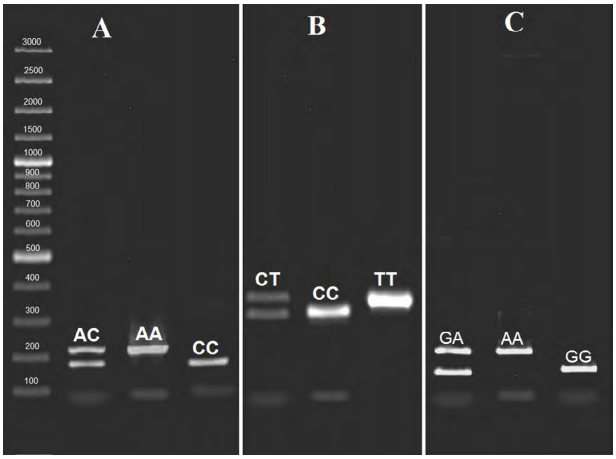

Figure 1. The electrophoretic patterns of Il13 gene polymorphisms. A: position -1512, B: position -1055, C: position +2044

located on chromosome 5q3133 and several SNPs are determined in its gene. Among these polymorphisms, two SNPs that are located in the promoter region (C-1055T and A-1512C) and one SNP in the coding region of exon 4(G +2044 A) are the most popular ones (12-14). It was suggested that -1055 region of IL-3 gene contains a binding site for nuclear factor of activated T-cells (NFAT). It is shown that the change of C to T nucleotide at this position could increase the affinity of NFAT to the IL-13 promoter, and consequently improve IL-13 expression (15-21). Moreover, it has been revealed that +2044 position in IL-13 is directly involved in the interaction of this cytokine with its receptor and A variant in this region might result in a protein with enhanced binding affinity to its receptor (15-21). So, we predicted that +2044A and -1055T alleles, which are related to higher production and more functional form of IL-13, might be more frequent in patients compared to the controls. However, our results showed that there was no significant difference between the distribution of alleles and genotypes in the three loci (-1055C/T, -1512A/C, and +2044G/A) among patient and control groups, indicating that inheritance of these SNPs cannot be considered as a predisposing factor for brucellosis in Iranian population. Additionally, according to our results, there is a significant linkage disequilibrium between positions -1055C/T, -1512A/C, and +2044G/A in IL-13 gene. So, we proposed that

Table 3. Distributions of the most common IL-13 haplotypes (positions -1055C/T, -1512A/C and +2044G/A, respectively).

Haplotype	Patients N (%) Total Number=338	Controls N (%) Total Number=142	X2	P value	OR (95% CI)
CAG	146 (43.1%)	72 (50.70%)	2.27	0.131	0.74(0.49-1.12)
TAA	47 (13.9%)	11 (7.74%)	3.57	0.058	1.92 (0.93-4.07)
TCG	23 (6.8%)	0 (0%)	10.15	0.001**	----------
TCA	18 (5.32%)	17 (11.97%)	6.53	0.010	0.41 (0.20-0.87)
CCG	19 (5.62%)	4 (2.81%)	1.72	0.189*	2.05(0.64-7.28)
TAG	47 (13.9%)	27 (19.01%)	2.0	0.157	0.69(.40-1.20)
CAA	34 (10.05%)	6 (4.22%)	4.45	0.034	2.54(0.99-6.89)
CCA	4 (1.18%)	5 (3.52%)	3.04	0.095*	0.32(0.07-6.89)

**P* value is measured by Fisher exact test.

***Considered significant after the Bonferroni correction (*P* value threshold of 0.006).

N: Absolute number, CI: Confidence interval, OR: odds ratio

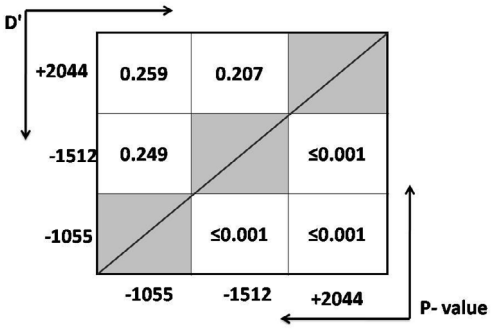

Figure 2. Linkage disequilibrium plot of IL-13 polymorphisms in *D'* and P-value.

haplotype and haplogenotypes reconstructed from +2044A and -1055T alleles, which are related to higher production of IL-13, are frequent in patients versus controls. However, the distribution of TCG and CAA haplotypes were significantly higher in the patients compared with those in the controls. Although the later did not tolerate Bonferroni correction. In contrast, the distribution of TCA haplotype was higher in the controls compared to patients. But this difference did not tolerate Bonferroni correction. Furthermore, TAG/TCA haplogenotype were significantly higher among brucellosis patients versus controls. It did not tolerate Bonferroni correction, too. Based on the good linkage disequilibrium observed between the mentioned loci at IL13 gene, it could be concluded that individuals who inherit TCG and CAA haplotypes and TAG/TCA haplogenotype may express higher level of IL-13 or produce more functional IL-13. So, these haplotypes and haplogenotype could be considered as factors that predispose individuals to brucellosis. On the contrary, TCA haplotype with higher frequency in controls can confer protection against brucellosis in individuals carrying this haplotype. To the best of our knowledge, there has been no study investigating the association of these mentioned haplotypes and haplogenotypes with the level of IL-13; therefore, the expression vectors cloned with these haplotypes can be helpful to clarify the effect of these variants on the IL-13 production.

According to our knowledge, there is no

Table 4. Distributions of the most common IL-13 haplogenotypes (positions -1055C/T, -1512A/C and +2044G/A, respectively).

Haplogenotypes	Patients N (%) Total Number=169	Control N (%) Total Number=71	(95%CI)	OR	*P* value	X^2
CAA/CAG	13 (7.7%)	3 (4.2)	3.5	0.061	3.26	0.82-15.14
CAG/ CAG	31 (18.3%)	18 (25.4%)	0.42	0.51	1.26	0.59-2.67
CAG/ TAG	10 (5.9%)	2 (2.8%)	3.06	0.08	3.69	0.72-25.24
TAA/CAG	11 (6.5%)	5 (7%)	0.63	0.42	1.56	0.47-5.49
TAA/TAG	6 (3.6%)	1 (1.4%)	2.09	0.148	4.03	0.49-97.03
TAG/CAG	7 (4.1%)	6 (8.5%)	0.19	0.66	0.78	0.22-2.78
TAG/TAG	6 (3.6%)	6 (8.5%)	0.49	0.48	0.66	0.18-2.45
TAG/TCA	0 (0%)	4 (5.6%)	6.03	0.025*	0.0	0.0-1.02
TCA/CAG	4 (2.4%)	6 (8.5%)	0.42	0.51	0.65	0.15-2.73

**P* value is measured by Fisher exact test.

N: Absolute number, CI: Confidence interval, OR: odds ratio

published study concerning IL-13 gene variants and brucellosis in other countries. Therefore, it was impossible to compare the results of this study with others. Nevertheless, there are several studies on IL-13 gene variants and susceptibility to other infectious and non infectious diseases. In this regard, association between IL-13 gene SNPs (rs1800925 and rs20541) and the increased risk of chronic obstructive pulmonary disease (COPD) was found by Gong Y *et al* (22). Liu Q and colleagues showed that two loci (IL-13 C-1112T and IL-13 C1923T) have little contribution to development of asthma in Chinese, while IL-13 A2044G is significantly associated with asthma (23). In a study carried out by Okeyo WA and colleagues, the relation between the IL-13 gene polymorphisms at positions -7402 T/G and -4729G/A and susceptibility to severe malarial anemia (SMA) was determined in patients presenting clinical symptoms of falciparum malaria. Their results showed no difference in the proportions of genotypes among children presenting non-SMA and SMA. Furthermore, they found no association between the genotypes -4729G/A and -7402 T/G and SMA. But, they revealed that the proportion of -7402 T/-4729A (TA) haplotype was significantly higher in children with SMA compared to non-SMA (24).

In conclusion, our results revealed that these alleles and genotypes in the mentioned regions of interleukin-13 gene cannot be considered as an influencing factor for brucellosis among Iranian population. However, it seems that inheritance of some haplotypes and haplogenotypes of IL13 gene can affect susceptibility or resistance to brucellosis. As we found no reports on association between IL-13 SNPs and brucellosis, further studies with larger sample sizes on the other IL-13 SNPs and their association with brucellosis are recommended.

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Conflict of Interest

None declared.

5. References

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