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Association of FCγRIIA (CD32) Polymorphism with Susceptibility to Brucellosis

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Abstract

Background: Brucellosis is the major bacterial zoonoses of global importance caused by *Brucella spps.* FCγRIIA receptor plays a central role in phagocytosis of IgG2-opsonized bacteria. FCγRIIA exhibits allelic polymorphisms with different capacities for binding IgG2 and phagocytosis. Cells expressing FcγRIIa-H131, bind more efficiently to complexes of IgG2 than those expressing the FcγRIIA-R131 variant. The purpose of this study was to evaluate the association of FCγRIIA polymorphisms with susceptibility to or severity of brucellosis.

Materials and Methods: In this study we evaluated FCγRIIA polymorphisms (R/R131, R/H131, H/H131) in 67 patients with brucellosis and 67 age, sex and geographical matched healthy volunteers. FCγRIIA genotyping was performed by using a sequence-specific primer polymerase chain reaction (SSP-PCR).

Results: Comparison of the FCγRIIA genotypes distribution in patients with brucellosis and controls showed a higher frequency in FCγRIIA-R/R131 homozygosity in patients than controls (47.8% vs. 28.4%). Logistic regression analysis showed that there is a significant correlation between R/R131 genotype and brucellosis (OR=2.3, 95%CI=1.3-4.2, P=0.04). Although the frequency of the FCγRIIA-R/R131 was higher in patients with chronic brucellosis compared with acute brucellosis, we did not find any statistically significant differences (53.8% vs. 46.3%, P=0.65).

Conclusion: The result of this study showed that the homozygous genotype of FCγRIIA-R/R131 in patient with brucellosis may be associated with susceptibility to brucellosis as a genetic risk factor.

Keywords: Brucellosis; FCγRIIA; Polymorphism

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Introduction

Brucellosis is a major source of bacterial zoonoses of global importance caused by organisms belonging to the genus Brucella, gram negative, intracellular bacteria (1). Phagocytosis is the main host defense against *Brucella Spp.* via Immunoglobulin G (IgG) receptors. IG2 is the subclass of antibodies produced by the immune system in response to bacterial polysaccharide antigens (2-3).

Human FCγRs are glycoproteins bind the Fc region of IgG and mediate a variety of immune functions like antigen presentation, phagocytosis, ADCC, and cytokine production (4-5). FCγRs have important function in the pathogenesis of inflammatory diseases in human and functional polymorphisms of FCγR have strong effects on susceptibility and severity of the diseases (6). There are 3 leukocyte receptors for human immunoglobulin G: FCγRI (CD64), FCγRII (CD32), and FCγRIII (CD16). Among these receptors, five classical low affinity FCγ receptors such as FCγRIIA, FCγRIIB, FCγRIIC, FCγRIIIA, and FCγRIIIB code by five genes (FCGR2A, FCGR2B, FCGR2C, FCGR3A, and FCGR3B) in the 1q23 chromosome (7-9). Each FCγR has a variety of isoforms with differing IgG affinities, tissue distribution, and expression levels (9). Balancing

between the activating FCγRs (FCγRIIA, FCγRIIC, FCγRIIIA, and FCγRIIIB) and the inhibitory FCγRIIB is crucial in immune responses and outcomes of local and systemic inflammations. FCγRIIA (CD32) is an important member of the FC receptor family that plays a central role in the regulation immunity and autoimmunity (10-11). In addition, it is the only FCγR binds with human IgG2 efficiently (12). A single nucleotide polymorphism (SNP) at position 494 (A to G) is present in exon 4 human FCγRIIA gene resulting in an amino acid substitution from histidine (H) to arginine (R) at position 131 in the second extracellular Ig-like domain of this receptor (13-15). This polymorphism is known to affect receptor affinity and specificity and also essential for the binding of human IgG2 (16-18). Polymorphisms of FCγRIIA critically affect interaction with antibodies that human IgG2 binds effectively to FCγIIA-H/H131, but not to FCγRIIA-R/R131 (19-20). IgG2-opsonized bacteria are efficiently internalized by phagocytes of FCγRIIA-H/H131 homozygous individuals, in contrast to those from FCγRIIA-R/R131 homozygous individuals (8, 18). Whereas the 131-R variant has weaker binding affinity of IgG, less-effective phagocytosis, and a lower capacity for immune activation, the His131 allotype has more-effective phagocytosis and is thought to be associated with hyperactivation of immune cells (17). The clinical importance of FCγRIIA polymorphism has been evaluated for encapsulated bacterial infections, in which IgG2 plays a critical role in host defense. Several recent case-control studies have shown an association between FCγRIIA-H/H131 and protection from encapsulated bacterial infections, whereas the poorly IgG2-binding allotype FCγRIIA-R/R131 is associated with increased susceptibility to these pathogens (12). The FCgRIIA-H/R 131 SNP has been reported to be associated with ulcerative colitis, Kawasaki diseases , systemic lupus erythematosus, and chronic inflammatory disorders such as periodontitis and Guillain-Barre´ syndrome, infections including recurrentbacterial respiratory tract infections, pneumococcal pneumonia, severe acute respiratory syndrome, severe sepsis, HIV, and Epstein Barr virus and Dengue virus (9) infection (8,19). In this study, we determined the distribution of genetic variants of FCγRIIA in brucellosis patients compared with controls.

Materials and Methods

Subjects

The study population contained of 67 (43 male and 24 female) brucellosis patients with a mean age of 43.31±17.84 years, from the university hospitals of Mazandaran, north of Iran. The patients were diagnosed as brucellosis with serological tests and clinical symptoms. From 67 patients, 54 with acute brucellosis were defined as the presence of the disease course (less than one year), clinical symptoms and serological tests (Wright ≥1/160) and 13 patients with chronic brucellosis were diagnosed with low fever, exhausting and local symptoms like arthritis, spondylitis, serological tests (Coombs-Wright) and the course of the disease (more than one year). 67 age, sex, and geographical matched healthy volunteers with mean age; 37.57±17.84 years who negative in standard tube agglutination and C-reactive protein tests, were used as controls. The protocol of study was approved by Ethic Committee of Mazandaran University of Medical Sciences and all subjects gave informed consent to participate to study.

DNA Extraction and FCγRIIA Genotyping

Genomic DNA was extracted from 5-10 milliliter whole blood-treated with 50 mM EDTA by the standard salting out method (21). FCγRIIA R/H 131 genotyping was performed by sequence-specific polymerase chain reaction (SSP-PCR). The primers employed to amplify Arg or His allele of FCγRIIA gene is in table 1.

Table 1. Primer sequences for FCγRIIA R/H 131 genotyping

Primers name	Primer sequences (5′-3′)
Forward (131H)	GGAGAAGGTGGGATCCAAAT
Forward (131R)	GGAGAAGGTGGGATCCAAAC
Reverse (S131) Common primer	CAAGTTCTGTGAGTAACGTAC
Internal control	
HGH-Forward	CAGTGCTTCCCAACCATTCCCTTA
HGH-Reverse	ATCCACTCACGGATTTCTGTTGTGTTT

The PCR was performed by adding 0.2 ng DNA in to 25 µL solution containing PCR buffer (10mM Tris-HCl PH=8.3, 50mM KCl, 1.5 mM MgCl 2), 200µM of each oligonucleotide, 10 pM specific primers, 5 pM internal primers and 0.5 U DNA Taq polymerase. The PCR conditions were; initial denaturation at 94 ˚C for 2 min; followed by 10 cycles of 94 ˚C for 10 sec, 65 ˚C for 1 min; 20 cycles of 94 ˚C for 10 sec, 60.5 ˚C for 50 sec, 72 ˚C for 30 sec; then a final extension was down at 72 ˚C for 4 min. The final amplified products were analyzed on a 1% agarose gel stained by ethidium bromide, and viewed under ultraviolet light.

Statistical analysis

Genotype and allele frequencies of the FCγRIIA-131 H/R polymorphisms were determined by direct counting. Comparison of genotype and allele

frequencies between brucellosis and controls were performed using Chi-square ($\chi 2$).

Chi-square goodness of fit analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg equilibrium. Analysis of continuous variables was performed with student's t test. A forward stepwise conditional logistic regression model was applied by adjusting for age and sex to analyze the impact of associated SNP on the development of brucellosis and the results were expressed as odds ratio (OR) and 95% CI. All P values were evaluated in a two-sided model, and $P< 0.05$ was considered statistically significant.

Results

FCγRIIA genotypes of 67 patients with brucellosis (64.2% males and 35.8% females) and 67 age, sex and geographical matched controls (50.7% males and 49.3% females) were determined. 35 (81.4%) out of 43 males were defined as acute brucellosis and 8 (18.6%) with chronic brucellosis. From 24 women, 19 (79.2%) were diagnosed as acute brucellosis and 5 (20.8%) with chronic brucellosis. The mean age of patients and controls was 43.31±17.84 and 37.57±15.66 years, respectively.

Figure 1 shows electrophoretic pattern of 253 bp fragment of target gene which containing the polymorphic site and a 428 bp control gene.

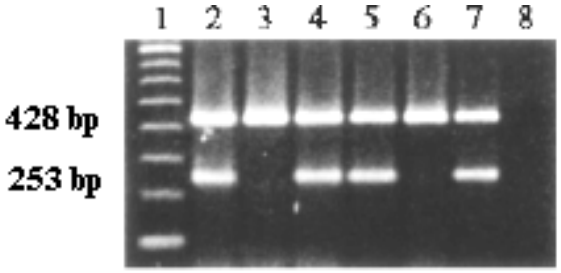

Figure 1. The result of FCγRIIA genotyping by SSCP-PCR. The HGH PCR product (428bp) is present in all reactions. Lane 1 represents the DNA ladder; Lanes 2 and 3 represent an FCγRIIA-H/H131-homozygous person; Lane 4 and 5 shows an FCγRIIA-H/R131–heterozygous subject; Lane 6 and 7 exemplify an FCγRIIA-R/R131-homozygous person.

Distribution of FCγRIIA allele and genotype in patients and controls

Table 2 summarizes the frequencies of FCγRIIA genotypes and alleles in the brucellosis patients and healthy individuals. Genotype frequencies did not deviate from expectations of the Hardy-Weinberg equilibrium in each group (P=0.15 in patients and p=0.06 in controls). As it was showed in Table 2, Allele distribution of FCγRIIA-R131 among patients was at a higher frequency (71.64%) than controls (58.21%). But this difference did not meet statistical significance (P=0.2). The frequency of the R/R131 genotype was significantly higher in patients than control group (47.8% vs. 28.4%, P= 0.04). However, 131R allele frequency was not significantly differed between two study groups.

To examine whether FCγRIIA genotypes are associate with clinical characteristics of brucellosis by fixing covariates sex and age using a logistic regression analysis, we found that the FCγRIIA-R/R131 genotype was significantly associated with susceptibility to brucellosis (P=0.04).

Table 2. Genotypes and alleles frequencies of FCγRIIA-H/R131 polymorphism in brucellosis patients and controls.

FCγRIIA-131 H/R	Brucellosis	Controls	P-value
Allele frequency- n (%)			
H131	38 (28.36)	56 (41.79)	
R131	96 (71.64)	78 (58.21)	0.2
Genotype frequency- n (%)			
H/H	3 (4.4)	8 (11.9)	
H/R	32 (47.8)	40 (59.7)	
R/R	32 (47.8)	19 (28.4)	0.04

As the Table 3 shows a significant differences in the sex distribution of FCγRIIA genotypes in brucellosis patients (P=0.044). On the other hand, frequency of FCγRIIA-H/H131, FCγRIIA-H/R131, and FCγRIIA-R/R131 genotypes were 0%, 35.5%, and 46.5% in male patients compared with 12.5%, 37.5%, and 50% among female patients.

Table 3. Distribution of FCγRIIA genotypes among patients with brucellosis and controls based on gender.

FCγRIIA-131 H/R	Brucellosis		Controls	
	Males	Females	Males	Females
H/H	0	3 (12.5)*	5 (14.7)	3 (9.1)
H/R	23 (35.5)	9 (37.5)	20 (58.8)	20 (60.6)
R/R	20 (46.5)	12 (50)	9 (26.5)	10 (30.3)

* Statistical analysis revealed a significant difference at p=0.044

Effect of FCγRIIA polymorphism on disease development

Table 4 displays the distribution of genotype and allele frequencies among acute and chronic brucellosis. Distribution of FCγRIIA-R/R131 genotype (53.8% vs. 46.3%) and 131R (76.9% vs.

70.4%) allele in chronic brucellosis were higher than acute brucellosis. Meanwhile, statistical analysis in fixed model was not shown significant difference (p=0.65).

Discussion

Study of the role of SNPs in host genes potentially involved in immune responses, help us to discover the pathophysiology of infectious diseases, especially how these polymorphisms influence both the susceptibility to disease and the course of disease development (22). Unlike many other genes where genetic variants have no clear functional contribution to a population disease profile, the FCγRIIA exhibits a clear functional difference between R131 and H131 allotypes and has relevance for some infectious and autoimmune diseases (23).

Table 4. Genotypes and alleles frequencies of FCγRIIA-H/R131 polymorphism in patients with acute and chronic brucellosis.

FCγRIIA	Acute brucellosis	Chronic brucellosis	P-value
Allele frequency- n (%)			
H131	32 (29.6)	6 (23.1)	0.76
R131	76 (70.4)	20 (76.9)	
Genotype frequency –n (%)			
H/H	3 (5.6)	0	
H/R	26 (48.1)	6 (46.2)	
R/R	25 (46.3)	7 (53.8)	0.65

In this study, we have aimed to analyze the frequency of immunogenetic marker, FCγRIIA-H/R 131, a low-affinity FC receptor, and an important protein in the host defense against infection. The results of our study demonstrated that FCγRIIA-R/R131 genotype was in higher frequency in patients with brucellosis compared with controls. Bredus *et al* showed that the R/R131 genotype was more frequent among patients with meningococcal disease than in controls (24). Also Platonov *et al* found that in children older than 5 years of age, the R131 variant was associated with a greater risk of severe meningococcal disease (17). FCγRIIA has been studied directly in relation to malaria susceptibility showing an association between FCγRIIA H/R131 polymorphism and malaria (12). As this receptor is responsive to different IgG subtypes, part of the heterogeneity may be down to which antibody is being produced by individuals and it may also be under control of genetic factors (17). FCγR-IIA has functional effect and has been associated with autoimmune diseases and susceptibility to bacterial infections (25, 17). H/R 131 is located in the IgG-binding site of FCγRIIA and the amino acid substitutions within the extracellular domain of FCγRs that lead to polymorphism in the FCγRIIA-131 H/R, change the ligand–binding capacities for IgG binding and phagocytosis of polymorphonuclear leukocytes. The R131 variant has weaker binding affinity of IgG, less-effective phagocytosis, and a lower capacity for immune activation, while the H-131 variant has more-effective phagocytosis and immune cells hyperactivation (17). Whereas immunoglobulin G2 is the main immunoglobulin isotype induced in response to many bacterial polysaccharide antigens in humans (16, 26), therefore the affinity of FCγRIIA-H131 for IgG2 is higher than the FCγRIIA-R131 and the H/R131 polymorphism may have functional consequences for IgG2- mediated phagocytosis of bacteria (17, 27). Therefore predominance of FCγRIIA-R/R131 genotype in patients with brucellosis in this study, imply the importance of this genotype as a risk factor for susceptibility to brucellosis. In this study, the frequency of the FCγRIIA-H/H131 genotype was higher in controls than patients with brucellosis. There is experimental evidence suggesting that homozygous H/H131 of genotype FCγRIIA may be advantageous for handling IgG2-opsonized bacteria (24). Rodriguez´s group showed that FCγRIIA-H/H131 homozygous polymorphonuclear cells and cells transfected with FCγRIIA-H/H131 gene were better able to phagocytosis the opsonized *S. pneumonia in vitro* than FCγRIIA-R/R131 cells (16).

In vitro, FCγRIIA polymorphism has been shown to be important in defense against other encapsulated bacteria such as group B type III streptococci, and also it has been shown the H/H131 genotype may be protective in infection with severe meningococcal diseases (16, 28). FC□RIIA-131 H/H genotype and the H allele have been shown to be associated with higher IgG1, IgG2, and IgG3 antibodies (12, 17).

In the presence of the H/H131 genotype, IgG1, IgG2, and IgG3 immunoglobulin subtypes are able to

activate the immune system using the FCγRIIA molecule, and the presence of the H131 allele is essential for effective IgG2-mediated cellular activation through this mechanism (29, 25). There is accumulating evidence show that some IgG2 antigen-specific responses correlate with disease protection (29). IgG2, which requires the presence of the H131 allele for efficient activity, is known to be important in infection protection against capsulated bacteria including *Nisseria meningitides*, *Streptococcus pneumonia* and *Haemophilus influenza* and the high affinity of IgG2 to FCγRIIA-H/H131 cells indicate the protective role of this allele (16). In this study, however, the frequency of the FCγRIIA-R131 allele and genotype were higher in chronic brucellosis compared with acute brucellosis, but this difference did not reach statistical significance. In addition of FCγRIIA polymorphism that influence on susceptibility of brucellosis, other factors such as cytokines may contribute to severity of brucellosis. One study demonstrated the role of L-selectin in development of brucellosis. In their study the hypothesis was the abnormal binding function of the L-selectin 206Leu variant on lymphocytes which lead to impaired migration to sites of brucella colonization (30). In addition, host intrinsic factors, such as genetic factors, influence on cytokine production, may contribute to severity of brucellosis. Death from meningococcal disease has been associated with a tumor necrosis factor-α (TNF- α) gene promoter polymorphism, low level of tumor necrosis factor production and a high level of interleukin-10 production in patients with severe meningococcal disease (27). However, previously published data from south of Iran has shown no significant association between FCγRIIA polymorphism and brucellosis showed no difference (31). Also estimation of the association between FCγR polymorphisms and systemic lupus erythematosis (SLE) and renal involvement in Egyptian patients demonstrated lack of association of FCγRIIA polymorphism with SLE in the Egyptian patients (32). As the FCγRIIA, FCγRIIIA and FCγRIIIB genes are most likely derived from a common gene and are clustered in very close proximity on chromosome 1q22, FCγR genotype combinations may represent more relevant risk markers than single FCγR genotypes. Furthermore, genetic linkage may not be confined to the FCγR locus but comprise other genes on chromosome 1. Many potential immunological relevant genes have been mapped near the FCγR locus, including a family of FCR homologs, CRP and SAP (11, 33). Thus this linkage disequilibrium may exist between other adjacent genes and FCγR genotypes, therefore this may complicate the association between FCγR polymorphism and disease and other host genetic factors that may influence the susceptibility and severity of disease should be evaluated in future studies (33).

Taken to gather, our data suggest that the frequency of FCγRIIA-R/R131 genotype was higher in patients with brucellosis compared with controls and FCγRIIA H/R131 polymorphism has significant association with brucellosis. Whereas the R131 variant has weaker binding affinity of IgG, less-effective phagocytosis, and lower capacity for immune activation, the R131 allele of FCγRIIA might have evolved to play as a risk factor for susceptibility of infectious disease such as brucellosis.

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