Association of chemokine receptor 5 (CCR5) Δ32 mutation with Behçet's disease is dependent on gender in Iranian patients

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ABSTRACT

Objective. *Behçet's disease (BD) is a recurrent multi-system inflammatory disorder caused by the combinations of multiple genetic and environmental factors. CCR5 is a Th1-dominant chemokine receptor whose levels are increased in patients with active BD. It is believed that a 32 bp deletion in the CCR5 gene reduces the expression of this receptor on the cell surface. The aim of the present study was to investigate the association of CCR5 Δ32 allele with BD in Iranian patients.*

Methods. *The study included 100 patients with BD and 380 healthy controls. Polymerase chain reaction (PCR) amplification was used for analysis of CCR5 Δ32 allele.*

Results. *The frequency of CCR5 Δ32 allele was not statistically different between 100 patients with BD and 380 healthy individuals. However, categorizing patients according to gender revealed a significant difference in distribution of the CCR5 Δ32 allele in female patients compared with female control individuals ($p = 0.047$, fisher's exact test, OR = 2.66).*

Conclusion. *The results suggest that the CCR5 Δ32 allele may be a genetic risk factor for BD in Iranian women. These results warrant further investigation to clarify the underlying mechanism of CCR5 deficiency in the initiation of BD.*

Introduction

Behçet's disease (BD) was originally described as a triad of recurrent oral aphthous ulcers, genital ulcers and uveitis (1), but is now recognized as a multi-systemic disease, also involving skin, central nervous system (CNS), joints, lungs, intestine and vessels. The disease has a worldwide distribution, but is most common in countries along the ancient Silk Road (2-4). Besides the different distribution of the disease, its clinical manifestations also differ throughout the world (5). Moreover, it has been found that BD is more frequent and severe in men than women, although recently the numbers of female patients are increasing (4). Iran is among the countries with the high prevalence of the disease and approximate male to female ratio of 1.14/1 (5). The pathogenesis of BD is not fully understood. Streptococcal and viral infections, directly or by cross-reacting with heat shock proteins have been suggested as the most probable environmental factors triggering BD. Genetic factors are also considered to play important roles in the development of the disease (2-4). Class ≤ HLA-B51 allele is the most widely reported risk factor for BD (6) and identification of new susceptibility genes is under investigation (7).

Chemokines are a large family of structurally homogenous cytokines that regulate leukocyte activation and recruitment to sites of inflammation via interaction with a family of chemokine receptors. It has been found that the expression pattern of these receptors determines which cell types respond to which chemokines. CCR5 is a chemokine receptor which binds to proinflammatory chemokines of RANTES, MIP-1α and MIP-1β and its preferential expression is on Th1 cells (8,9). Overproduction of CCR5 has been observed in peripheral blood lymphocytes (PBL) and tissue samples from patients with BD, pointing toward a role for CCR5 in the pathogenesis of the disease (10).

A 32-bp deletion in the open reading frame of the CCR5 gene induces a premature stop codon and results in a truncated protein product that is not transported to the cell surface (8,9). Monocytes from CCR5 Δ2 carriers show a reduced chemotactic response, reflecting that CCR5 Δ32 allele could attenuate

Table I. Frequencies of wild type and mutant genotypes in BD patients and control groups.

Genotypes	Total		Female		Male	
	Patients	Controls	Patients	Controls	Patients	Controls
CCR5 +/+	91(91)	362(95.3)	57 (87.7)	228(95)	34 (97.1)	134 (95.7)
CCR5 +/ Δ32	9 (9)	18 (4.7)	8(12.3)*	12 (5)	1 (2.9)	6 (4.3)
CCR5 Δ32/ Δ32	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	100	380	65	240	35	140

*p = 0.047, calculated by fisher's exact test; P values compare the patient groups with control groups; data are *n* (%).

inflammatory responses at least *in vitro* (11). However, although the *in vivo* blockage of CCR5 in animal models has been associated with reduced leukocyte accumulation in several conditions, i.e., in experimental glomerulonephritis models (8), but in some instances the numbers of immune cells in inflamed area and also the levels of proinflammatory cytokines are increased in CCR5 deficient mice treated with certain pathogens (12-15). Similarly, in humans, the CCR5 Δ32 allele has attributed to the modulation of immune response in some instances such as allograft transplantation and IgA nephropathy (8), while in other conditions such as hepatitis C virus (HCV) infection (16) and multiple sclerosis (MS) (11) has contributed to the disease susceptibility.

To better understanding the ethiopathogenesis of BD and the role of CCR5 in this process, the present study was conducted to investigate the frequency of CCR5 Δ32 allele in southern Iranian patients with BD.

Materials and methods

Subjects

A total of 100 Iranian non-related patients with BD (65 women and 35 men) aged 36.4 years (15-65) were investigated for CCR5 gene polymorphism. The mean age at onset of the disease was 30.7. Patients were recruited from the outpatient Behçet's Disease Clinic of Shiraz University of Medical Sciences. The diagnosis of BD was based on the criteria of International Study Group (17). A total of 380 ethnically matched healthy subjects (240 women and 140 men) were also included in the study. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All the participants were informed of the purpose of the study, and their consents were obtained.

Methods

Peripheral blood samples were collected in 10 ml volumes and genomic DNA was extracted by salting out technique, using the method described by Miller *et al.* (18). Polymerase chain reaction (PCR) was used to amplify the desired fragments of CCR5 gene as described previously (19). Amplification products were 169 bp and 137 bp in the case of the wild type allele and the deleted allele, respectively.

Statistical Analysis

The frequencies of alleles and genotypes between patients and controls were analyzed by Chi-square test or fisher's exact test (two-sided) where appropriate. *p* values of < 0.05 were considered to be significant. All analyses were performed using SPSS statistical software (version 11.5).

Results

Table I shows the frequency and percentage of wild type (+/+), wild type/ mutant (+/-) and mutant/mutant (-/-) genotypes of the CCR5 gene in the studied groups. Nine out of the 100 patients (9%) and 18 out of 380 healthy controls (4.7%) were heterozygous for CCR 5 Δ32 allele. As observed, no individual in studied populations was found homozygous for CCR 5 Δ32. The frequency of CCR5 genotypes was not significantly different between 100 Iranian patients with BD and 380 healthy controls (p = 0.1).

However, subdivision of patients according to gender revealed a significant difference in distributions of the CCR 5 Δ32 allele between female patients and female controls (odds ratio = 2.66, 95% confidence interval = 1.04-6.83, p = 0.047).

Table II. Clinical characteristics of patients with BD in relation to the occurrence of CCR5 Δ32 allele.

Clinical characteristics	CCR5 Δ32 allele	
	n	(%)
Oral ulcers	9/97	9.3
Genital ulcers	9/98	9.2
Skin lesions	8/77	10.4
Pathergy test	7/79	8.1
Ocular lesions	4/51	7.8
Arthritis	5/18	27
Pulmonary lesions	1/4	25
Other lesions	0/15	0
Male	1/35	2.9
Female	8/65	12.3

The observed genotype frequencies of these two SNPs were statistically consistent with the expected distributions according to Hardy–Weinberg equilibrium in the studied groups.

Table II demonstrates the distribution of CCR5 Δ32 allele according to clinical manifestations of patients with BD. As observed, frequency of CCR5 Δ32 allele was the highest in patients with arthritis.

Discussion

CCR5 is a major co-receptor for the entry of human immunodeficiency virus (HIV)-1 into cells and individuals with low CCR5, caused by Δ32 deletion, are more resistant to HIV infection. Despite the well-known effects of CCR5 deficiency on HIV infection, the impact of low levels of this chemokine receptor on other infectious and inflammatory processes is not clear (8, 9). CCR5 gene polymorphism has recently been studied in several inflammatory conditions including HCV infection (16) and MS (11) in which the 32-bp deletion in the CCR5 gene was associated with increased susceptibility to these disorders. In the present study we demonstrated that the CCR5 Δ32 allele conferred susceptibility to BD in Iranian female patients.

CCR5 gene polymorphism has previously been investigated in BD by Yang

et al. As reported, no significant association between CCR5 polymorphism and British, Turkish and Palestinian patients with BD was observed, while no data about the male/female ratio of the studied groups were presented (20). So any interpretation and comparison of their data with ours must be with caution. Association of a chemokine system member, CCR5, with BD by gender is consistent with the results of Chen et al. demonstrating that the contribution of the two other members of chemokine system (CCL2 and CCL5) genes to BD depends on gender (7). There is a possibility that the dissimilarities of men and women in the endogenous factors such as hormones, and/or in the exogenous factors such as different rate or severity of infections (important etiologic factors reported for BD (2-4)) affects CCR5 gene. In this regard, it has been shown that males and females differ in their susceptibility to a variety of infections (21-23). For instance, more severe and prevalent disease has been reported in women than men during Herpes simplex virus (HSV) exposure (21, 22), a virus which is not only important in the occurrence of BD (4), but also its clearance may require CCR5 molecule (12). Thus, HSV infection may be one of the factors exert a pathologic role through CCR5 gene predominantly in women. Whatever the underlying mechanisms might be, the polymorphisms in the chemokine system can be regarded as one of the factors accounting for the gender bias of clinical manifestations in BD.

As a general consideration, the CCR5 Δ32 allele has been found with a high frequency in populations (8,9) with low prevalence of BD (2-4), and also low frequency in populations (8,9) with high prevalence of the disease (2-4). In contrast to CCR5 gene, HLA-B51 has a higher frequency in areas in which BD is more prevalent than areas in which the disease is rare, indicating the importance of HLA-B51 as a contributor to the risk of BD (2-4). These epidemiologic observations may be in favor of the assumption that the increased frequency of CCR5 Δ32 allele in our BD patients is influenced by a particular ethnic background.

As mentioned, in the present study, CCR5 Δ32 heterozygous female patients have shown increased susceptibility to BD. In this respect the impact of CCR5 genotype with lower receptor expression should be addressed in relation to the pathology of the disease. CCR5 and its ligands are up regulated during inflammatory conditions on Th1 cells and in non-lymphoid tissues, respectively, and known to be involved in positioning of Th1 cells in inflamed area. CCR5 ligands, in addition to CCR5, can bind to the other inflammatory-related chemokine receptors (8,9). It has been shown that in the absence of CCR5 the levels of CCR5 ligands are increased which in turn exacerbate the immune response, probably through interaction with other chemokine receptors (24). CCR5 deficiency has also been associated with the overexpression of another inflammatory cytokine, IL-6 (25). Additionally, regulatory T cells express CCR5 and are recruited to inflamed areas through CCR5 ligands (26), pointing toward a role for CCR5 in the modulation of post- inflammatory reactions. More importantly, it has been suggested that CCR5 deficiency may impair the proper function of the immune system to T-cell-dependent infections. CCR5 deficient mice challenged with certain pathogens, e.g., HSV, influenza virus and *mycobacterium tuberculosis*, demonstrate symptoms ranging from increased inflammatory cytokine production and leukocyte infiltration to increased T cell clonal expansion (12-14). These findings have frequently been reported in patients with BD (2-4, 10), a circumstance whose underlying pathology is thought to be the enhanced inflammatory reactions triggered most likely by the microbial infections (2-4).

In conclusion, the results of this study show that the 32-bp deletion in the CCR5 gene is associated with BD in female patients. The data from large numbers of patients are needed to clarify the consequence of CCR5 polymorphism on clinical manifestation of the disease.

References:

1. BEHÇET H: Dermatol Wochenschr 1937; 105: 1152-7.
2. GÜL A: Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2000; 19: S6-12.
3. GUL A: Behçet's disease as an autoinflammatory disorder. *Curr Drug Targets Inflamm Allergy* 2005; 4: 81-3.
4. SUZUKI KUROKAWA M, SUZUKI N: Behçet's disease. *Clin Exp Med* 2004; 3: 10-20.
5. SHAHRAM F, DAVATCHI F, NADJI A *et al.*: Recent epidemiologic data on Behçet's disease in Iran. The 2001 Survey. *Adv Exp Med Biol* 2003; 528: 31-6.
6. MIZUKI N, YABUKI K, OTA M *et al.*: Analysis of microsatellite polymorphism around the HLA-B locus in Iranian patients with Behçet's disease. *Tissue antigens* 2002; 60: 396-9.
7. CHEN Y, VAUGHAN RW, KONDEATIS E *et al.*: Chemokine gene polymorphisms associate with gender in pateints with uveitis. *Tissue antigens* 2004; 63: 41-45.
8. RIBEIRO S, HORUK R: The clinical potential of chemokine receptor antagonists. *Pharmacol Ther* 2005; 107: 44-58.
9. MUELLER A, STANGE PG: The chemokine receptor, CCR5. *Int J Biochem Cell Biol* 2004; 36: 35-8.
10. HOUMAN H, HAMZAOUI A, GHORBAL IM, KHANFIR M, FEKI M, HAMZAOUI K: Abnormal expression of chemokine receptors in Behçet's disease: relationship to intracellular Th1/Th2 cytokines and to clinical manifestations. *J Autoimmun* 2004; 23: 267-73.
11. PULKKINEN K, LUOMALA M, KUUSISTO H *et al.*: Increase in CCR5 Delta32/Delta32 genotype in multiple sclerosis. *Acta Neurol Scand* 2004; 109: 342-7.
12. SORENSEN LN, PALUDAN SR: Blocking CC chemokine receptor (CCR) 1 and CCR5 during herpes simplex virus type 2 infection in vivo impairs host defence and perturbs the cytokine response. *Scand J Immunol* 2004; 59: 321-33.
13. NANSEN A, CHRISTENSEN JP, ANDREASEN SO, BARTHOLDY C, CHRISTENSEN JE, THAMSEN AR: The role of CC chemokine receptor 5 in antiviral immunity. *Blood* 2002; 99: 1237-45.
14. ALGOOD HM, FLYNN JL: CCR5-deficient mice control *Mycobacterium tuberculosis* infection despite increased pulmonary lymphocytic infiltration. *J Immunol* 2004; 173: 3287-96.
15. MORENO C, GUSTOT T, NICAISE C *et al.*: CCR5 deficiency exacerbates T-cell-mediated hepatitis in mice. *Hepatology* 2005; 42: 854-62.
16. WOITAS RP, AHLENSTIEL G, IWAN A *et al.*: Frequency of the HIV-protective CC chemokine receptor 5- Delta32/Delta32 genotype is increased in hepatitis C. *Gastroenterology* 2002; 122: 1721-8.
17. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-1080.
18. MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for extracting DNA

from human nucleated cells. *Nucleic Acids Res* 1998; 16: 1215.

19. GHARAGOZLOU M, DOROUDCHI M, FARJADIAN SH, PEZESHKI AM, GHADERI A: The frequency of CCR5Delta32 and CCR2-64I in southern Iranian normal population. *Immunol Lett* 2005; 96: 277-81.
20. YANG X, AHMAD T, GOGUS F *et al.*: Analysis of the CC chemokine receptor 5 (CCR5) Delta32 polymorphism in Behçet's disease. *Eur J Immunogenet* 2004; 31: 11-4.
21. SULIGOI B, TORRI A, TANZI E, PALU G: The Italian Herpes Management Forum. Seroprevalence and seroincidence of herpes simplex virus type 1 and herpes simplex virus type 2 infections in a cohorot of adolescents in Italy. *Sex Transm Dis* 2004; 31: 608-10.
22. BURGOS JS, RAMIREZ C, SASTRE I, ALFARO JM, VALDIVIESO F: Herpes simplex virus type 1 infection via the bloodstream with apolipoprotein E dependence in the gonads is influenced by gender. *J Virol* 2005; 79: 1605-12.
23. BARNA M, KOMATSU T, BI Z, REISS CS: Sex differences in susceptibility to viral infection of the central nervous system. *J Neuroimmunol* 1996; 67: 31-9.
24. BAO L, ZHU Y, ZHU J, LINDGREN JU: Decreased IgG production but increased MIP-1beta expression in collagen-induced arthritis in C-C chemokine receptor 5-deficient mice. *Cytokine* 2005; 31: 64-71.
25. TAKEUCHI A, USUI Y, TAKEUCHI M *et al.*: CCR5-deficient mice develop experimental autoimmune uveoretinitis in the context of a deviant effector response. *Invest Ophthalmol Vis Sci* 2005; 46: 3753-60.
26. WYSOCKI CA, JANG Q, PANOSKALTSIS-MORTARI A *et al.*: Critical role for CCR5 in the function of donor CD4+CD25+ regulatory T cells during acute graft-versus-host disease. *Blood* 2005; 106: 3300-7.