

Macrophage Migration Inhibitory Factor –173GC Variant Might Increase the Risk of Behçet’s Disease

Ayşe Feyda Nursal^a Serbulent Yigit^b Ercan Tural^c Goknur Kalkan^d
Mehmet Kemal Tumer^e Akin Tekcan^f

^aDepartment of Medical Genetics, Faculty of Medicine, Hitit University, Corum, Turkey; ^bDepartment of Medical Biology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, Turkey; ^cHavza Vocational School, Department of Physiotherapy Programme, Ondokuz Mayıs University, Samsun, Turkey; ^dDepartment of Dermatology, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey; ^eDepartment of Maxillofacial Surgery, Faculty of Dentistry, Gaziosmanpaşa University, Tokat, Turkey; ^fDepartment of Medical Biology, Faculty of Medicine, Ahi Evran University, Kirsehir, Turkey

Significance of the Study

- In this study, individuals with the MIF gene –173GC variant CC genotype were more likely to develop Behçet’s disease in a Turkish group. Therefore, the MIF –173GC variant could be a potential biological marker for the occurrence of Behçet’s disease.

Keywords

Behçet’s disease · Macrophage migration inhibitory factor · Variant

Abstract

Objective: The aim of the present study was to investigate any possible association between the macrophage migration inhibitory factor (MIF) –173GC variant and Behçet’s disease (BD) in a group of Turkish patients. **Subjects and Methods:** A total of 111 patients with BD and 100 healthy controls were enrolled in this study. Genomic DNA was extracted from peripheral lymphocytes. The MIF –173GC variant was genotyped using polymerase chain reaction restriction fragment length polymorphism. The allele and genotype frequencies of patients and controls were compared using the

χ^2 test. **Results:** A statistically significant difference in the distribution of the genotype was observed between BD patients and healthy controls. The homo-genotype CC was more prevalent in the patient group compared to the control group ($p = 0.008$, OR: 0.24, 95% CI: 0.05–0.78). A significant association was observed when the patients were compared with the controls according to GG + GC versus CC genotypes ($p = 0.003$, OR: 1.21, 95% CI: 0.06–0.063). Allele frequencies of the MIF –173GC variant did not show any statistically significant difference between patients and controls. **Conclusion:** In this study, we conclude that the CC genotype of the MIF –173GC variant may be a risk factor in the pathogenesis of BD in the Turkish population. However, further studies with larger samples are needed to address the exact role of this variant in BD.

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Introduction

Behçet's disease (BD) is a chronic relapsing systemic inflammatory illness; it manifests as oral and genital ulcers, skin lesions, and uveitis [1]. Arthritis, vasculitis, central nervous system disease and gastrointestinal ulceration are among the other manifestations [1]. BD is more common along the Mediterranean coast and in Eastern Asia [1]. The etiology remains still unknown; however, it has been suggested that BD could be a result of various environmental triggering factors, ranging from infectious agents to pollutants. This disease is also subject to background genetic susceptibility [1]. Four factors imply a genetic impact on BD susceptibility: geographical distribution, familial aggregation, association with HLA-B51 class I antigen, and polymorphisms in genes that modulate immune responses [2]. Some studies in different ethnic groups have shown that there is a correlation between HLA-B51 and BD, and this correlation with HLA-B51 is believed to be the strongest genetic susceptibility factor for BD, and it is assumed to be involved in neutrophil activation [1]. Cytokines play crucial roles in the pathogenesis of BD, due to their impact and regulatory functions in immune and inflammatory reactions [3]. Overexpression of proinflammatory cytokines, mainly Th1 and Th17 from several cellular sources, seems to account for the increased inflammatory response in BD and may be linked with genetic susceptibility [2].

Macrophage migration inhibitory factor (MIF) is a crucial proinflammatory cytokine with a strong modulatory impact on immune and inflammatory responses [4]. MIF is produced in response to various stimuli such as lipopolysaccharide, proinflammatory cytokines including tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ), and hypoxia [4]. The MIF gene (Genebank GeneID:307284) is located in chromosome 22q11.2 in humans [5]. One of them is a single-nucleotide polymorphism at position -173 (rs755622) [6]. It has been suggested that the functional impact of MIF -173GC accounts for the formation of a binding site for the transcription factor AP4 [7]. This variant is related to high plasma concentrations of MIF, enhanced risk and severity of inflammatory disease, and decreased response to glucocorticoid medication [8].

The -173 C allele has been associated with an increased production of MIF protein [8]. So far, the association between the MIF -173GC functional variant and BD has not been fully studied. In this study, we investigated the relationship between the MIF -173GC variant and BD for the first time in a group of Turkish patients.

Subjects and Methods

Study Population

One hundred eleven patients with BD were studied in the Dermatology Department of Gaziosmanpaşa University Research Hospital, Tokat, Turkey. Forty-three of the patients were male and 68 were female. Medical assessments were performed by dermatologists using the International Study Group criteria for BD [9]. Detailed clinical characteristics including disease duration, treatment duration, aphthous stomatitis, genital involvement, skin lesions (papulopustules, erythema nodosum), ocular involvement, deep vein thrombosis, skin involvement, dosage of colchicine, and response of colchicine were reported by dermatologists.

The control group included 100 healthy controls, 41 males and 59 females. Controls were selected from healthy individuals who had no history of any chronic and autoimmune disease. The mean age of the patients and controls was 36.0 ± 9.4 and 38.5 ± 9.2 years, respectively. All participants were informed of the study protocol, and written informed consent was obtained from them. The Institutional Ethics Committee approved this study which was conducted in accordance with the Helsinki Declaration.

Genotyping

Blood samples were drawn from all subjects. Deoxyribonucleic acid (DNA) was extracted from whole-blood samples using a DNA extraction kit, according to the manufacturer's instructions (Sigma-Aldrich, Taufkirchen, Germany). The MIF -173GC variant was genotyped in all the subjects by the polymerase chain reaction (PCR) restriction fragment length polymorphism analysis, as described previously [10, 11]. A set of primers, 5'-ACT-AAG-AAA-GAC CCG-AGG-C-3' (forward) and 5'-GGG-GCA-CGT-TGG-TGT-TTA-C-3' (reverse), was used to amplify the MIF -173GC site. Genomic DNA was amplified in a 25- μ L final reaction. PCR cycles were 95°C (5 min) for 1 cycle, followed by 95°C (45 s), 60°C (45 s), and 72°C (45 s) for 30 cycles. A final cycle of 72°C for 7 min completed the reaction. The amplified PCR product was digested in a 30- μ L final reaction volume using 2 μ L of reaction buffer and 1 μ L of *AluI* restriction enzyme at 37°C for 12 h. Controls of known genotypes were included for every set of digestions carried out. The digested products were resolved on 2% agarose gel stained with ethidium bromide and visualized using ultraviolet transillumination. Two fragments (268 and 97 bp) were seen when the G allele was present at position -173, while 3 fragments (206, 97, and 62 p) were observed when the C allele was present.

Statistical Analysis

All statistical analyses were performed with the Statistical Package for the Social Science for Windows (version 18.0; SPSS Inc., Chicago, IL, USA) Statistical Program Version 16.0 and the OpenEpi info 2.2 software package program. Continuous data were given as means \pm SD and minimum/maximum. The χ^2 test was used to measure significance of differences in the allele frequency and genotype distribution between the two study groups. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. A *p* value <0.05 was considered statistically significant.

Table 1. Clinical and demographic features of controls and patients with BD

Characteristic	Control group	Patient group
Gender (male/female), <i>n</i> (%)	41/59 (40.60/59.40)	43/68 (38.7/61.3)
Age (mean ± SD), years	38.48±9.24	36.00±9.39
Disease duration (mean ± SD), years		7.45±6.82
Treatment duration (mean ± SD), years		5.93±6.70
Aphthous stomatitis		
No/yes, %	–	0/100
Genital involvement		
No/yes, %	–	12.6/87.4
Papulopustule		
No/yes, %	–	27/73
Erythema nodosum		
No/yes, %	–	45.9/54.1
Ocular involvement		
No/yes, %	–	8.1/91.9
Deep vein thrombosis		
No/yes, %	–	25.2/74.8
Skin involvement		
No/yes, %	–	5.4/94.6
Dosage of colchicine, %	–	14.9
500 mg/kg	–	4.8
1,000 mg/kg	–	21
1,000–1500 mg/kg	–	17.1
1,500 mg/kg	–	57.1
Response to colchicine		
No/yes, %	–	33/67

SD, standard deviation.

Table 2. Genotype and allele frequencies of the MIF –173GC variant in patients and controls

MIF	Patients (<i>n</i> = 111), <i>n</i> (%)	Controls (<i>n</i> = 100), <i>n</i> (%)	<i>p</i>	OR (95% CI)
Genotypes				
GG	49 (44.14)	58 (58)	<i>0.008</i>	1.31 (0.82–2.1)
GC	44 (39.63)	38 (38)		0.95 (0.57–1.60)
CC	18 (16.21)	4 (4)		0.24 (0.05–0.78)
HWE	0.139	0.466		
GG:GC + CC	49 (44.14):62 (55.85)	58 (58):42 (42)	>0.05	0.57 (0.31–1.02)
GG + GC:CC	93 (83.78):18 (16.21)	96 (96):4 (4)	<i>0.003</i>	0.21 (0.06–0.63)
Alleles				
G	142 (69.98)	154 (77)	>0.05	1.10 (0.74–.63)
C	80 (36.03)	46 (23)		

Data were analyzed by χ^2 test. If $p < 0.05$ – not consistent with Hardy-Weinberg equilibrium (HWE). The results that are statistically significant are shown in italics.

Results

Baseline characteristics of the subjects according to the distributions of the MIF –173GC variant are shown in Table 1. The results of the genotype and allele frequencies of the MIF –173 variant in patient and control groups are pre-

sented in Table 2. Evaluation of variant within the –173GC region of MIF showed the presence of the GG genotype in 49 (44.14%) and 58 (58%), the GC genotype in 44 (39.63%) and 38 (38%), and the CC genotype in 18 (16.21%) and 4 (4%) patients and controls, respectively. The genotype dis-

tribution of the MIF -173GC variant was statistically different between the patients and controls ($p = 0.008$). The MIF -173GC variant CC genotype was more prevalent in the patient group (OR: 0.24, 95% CI: 0.05–0.078). A significant association was observed when the patients were compared with the controls according to GG + GC versus CC genotypes ($p = 0.003$, OR: 0.21, 95% CI: 0.06–0.063) (Table 2).

In this study, we detected the following: MIF -173GC alleles G ($n = 142$, 69.98%) and C ($n = 80$, 36.03%) in the BD patients, and G ($n = 154$, 77%) and C ($n = 46$, 23%) in the controls. Allele frequencies of the MIF -173GC variant did not show any statistically significant difference between patients and the controls ($p > 0.05$).

Discussion

In the present study, we investigated for the first time the association between the human MIF -173GC functional variant and BD in the Turkish population. The current study identified a significant association of the MIF -173GC with BD susceptibility. Our results show that in the Turkish population the MIF -173GC CC genotype was associated with an increased risk of BD (OR = 0.24) (Table 2). In addition, a significant association was observed when patients were compared with controls in terms of GG + GC versus CC genotypes ($p = 0.003$).

Overexpression of proinflammatory cytokines (mainly T helper 1 type) from various cellular sources appears to account for the increased inflammatory reaction in BD, and it may be related with a genetic tendency [11]. Besides cellular immune activation, the occurrence of autoantibodies against particular kinds of tissues (e.g., oral mucosa), endothelial antigens, and retinal proteins all suggest an abnormal humoral immune response. The activation of the inflammatory mechanism in BD is thought to be driven by proinflammatory cytokines including interleukin (IL)-1, IL-6, TNF- α , IL-8, and IL-10 which are increased in the sera of BD patients [11–14].

MIF, a crucial proinflammatory cytokine released by innate immune cells including macrophages and monocytes, could maintain proinflammatory function of macrophages by hindering p53-dependent apoptosis [15]. MIF is a largely expressed component of the immune system that is produced in response to various stimuli such as lipopolysaccharide (via toll-like receptor-4), proinflammatory cytokines including TNF- α and IFN- γ , and hypoxia [16]. MIF then upregulates the expression of toll-like receptor-4 and proinflammatory cytokines, and plays a role as a cofactor for the activation of T cells.

Research has shown that MIF levels are increased in patients with rheumatoid arthritis [17], systemic lupus erythematosus [18], insulin resistance [19], and type 2 diabetes mellitus [20]. As all these disorders are associated with persistent inflammation, it is important to elucidate the role of MIF in the pathogenesis of the disease. Furthermore, elevated MIF levels were found in the serum and cerebrospinal fluid of patients with multiple sclerosis and neuro-Behçet [21]. Also, the mean MIF levels in the sera of patients with Behçet's uveitis and iridocyclitis were found to be higher than those of healthy control subjects [22, 23]. Also, it was reported that the mean serum level of MIF in patients with BD was higher in patients with BD than in healthy controls [24, 25].

The -173GC variant was the first MIF gene variant described in 2001 by Donn et al. [26]. Polymorphisms in promoters have an impact on the basal and/or induced transcription activity of MIF.

Coban et al. [27] reported that MIF -173GC variant C allele carriage tends to predict new-onset diabetes. Also, Ozsoy et al. [28] found that subjects carrying the variant C allele in the MIF -173 position were at a significantly higher risk of osteoporosis than subjects carrying the wild-type G allele in a Turkish population. In a previous study, it was found that the MIF -173GC variant was not associated with susceptibility to BD in Korean patients [29]. Also, Bahgat et al. [30] found that MIF gene -173GC and +254TC single-nucleotide polymorphisms were not implicated in the genetic susceptibility to BD among Egyptian patients. On the contrary, Zheng et al. [31] reported that frequencies of the MIF -173GC variant genotype GG and G allele were significantly decreased in BD patients compared to controls in the Han Chinese population. In addition, they showed that the expression of MIF mRNA in individuals carrying the CC genotype of the -173GC variant was 1.78- to 1.92-fold higher than in those carrying the GC or GG genotype.

Conclusion

We have demonstrated an association between the functional MIF -173GC variant and BD in a Turkish cohort. Our results reflect the small sample size of patients but we think it will help to enrich the data on the relation between the MIF -173GC variant and BD.

Disclosure Statement

There are no conflicts of interest.

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