ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Evaluation of HLA-B*51 Subtypes in Behçet's Patients with Uveitis

*Behçet Üveitli Hastalarda HLA-B*51 Alt Tipinin Değerlendirilmesi*

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ABSTRACT

Aim: *The HLA-B*51 allele has been determined to be the most important genetic factor in the pathogenesis of Behçet's disease (BD). This relationship has been demonstrated in various ethnic groups and many studies have shown sequence alterations in B*51 protein coding regions. To date, 116 different subtypes of HLA-B*51 (HLA-B*51:01-B*51:122) have been identified (IMG/HLA 3.5.0, June 14, 2011). This study investigated the distribution of B*51 subtypes in patients diagnosed with BD according to the 1990 International Study Group criteria and positive for B*51 compared to healthy controls.*

Material and Method: *DNA was isolated from 40 unrelated B*51-positive BD patients and 54 healthy volunteer bone marrow donors. B*51 subtype analysis was done by polymerase chain reaction with sequence specific primers (PCR-SSP) (One Lambda Inc., CA, USA). Chi-square and Fisher's exact tests were used in the statistical analysis (SPSS version 17.0).*

Results: *There were no statistically significant associations between B*51 subtype and BD patients' clinical characteristics or laboratory parameters ($p<0.05$). No significant difference was found between BD patients and controls in the frequency of B*51 subtypes.*

Conclusion: *Although there has been much emphasis on the association between BD and the HLA*5101 subtype, which is a common finding in BD patients in the Turkish population and in other ethnic groups, the presence of this subtype at a comparable frequency in the control group indicates that the development of BD is not attributable to HLA*5101 alone. Our data suggest that in addition to genetic factors, certain environmental factors also play a role in the development of BD.*

Key words: *Behçet's disease; uveitis; allele; HLA-B*51; PCR-SSP*

ÖZET

Amaç: *Behçet hastalığının (BH) bilinen patogenezindeki genetik faktörlerin en önemli bulgusu HLA-B*51 alleli olarak tespit edilmiştir. Bir çok farklı etnik grupta söz konusu ilişki gösterilmiştir. B*51 proteinini kodlayan bölgelerdeki dizi değişimleri birçok çalışmada gösterilmiştir. HLA-B*51'in şimdiye kadar 116 farklı alttipi (HLA-B*51:01-B*51:122) tanımlanmıştır (IMG/HLA 3,5,0, 14 Haziran 2011). Bu çalışmada, 1990 Uluslararası Çalışma Grubu kriterlerine göre BH tanısı almış B*51'i pozitif hasta ve sağlıklı kontroller üzerinde B*51 alttip dağılımı araştırıldı.*

Materyal ve Metot: *Çalışmada, Behçet tanısı almış, akraba olmayan, B*51'i pozitif 40 hasta ve 54 gönüllü kemik iliği vericisinden DNA izolasyonu yapıldı. Sekansa spesifik primerler ile polimeraz zincir reaksiyon (PCR-SSP) yöntemi ile B*51 alttip tiplendirmesi yapıldı (One Lambda Inc CA, USA). İstatistiksel verilerin değerlendirilmesinde SPSS versiyon 17, Ki-kare-Fisher exact istatistiksel analiz yöntemi kullanıldı.*

Bulgular: *Elde edilen bulgulara göre, B*51 alttipleri ile BH hastalarının klinik özellikleri ve laboratuvar parametreleri arasında istatistiksel olarak anlamlı bir ilişki bulunamamıştır ($p<0,05$). Aynı şekilde, hasta ve kontrollerin B*51 alttiplerinin frekansında anlamlı bir farklılık gözlenmemiştir.*

Sonuç: *Toplumumuzda ve diğer etnik gruplardaki Behçet hastalarında sıkça rastlanan HLA*B5101 alttipinin hastalıkla ilişkili olduğu sıklıkla vurgulansa da elde edilen veriler kontrol grubunda aynı alttipin tek başına hastalığın gelişimine katkısı olmadığını göstermektedir. Sonuç olarak, BH'nın gelişiminde genetik faktörler dışında bazı çevresel faktörlerinde rol oynadığını düşündürmektedir.*

Anahtar kelimeler: *Behçet hastalığı; üveit; allel; HLA-B*51; PCR-SSP*

Introduction

Behçet's disease (BD), first described in 1937 by Turkish dermatologist Hulusi Behçet, is a symptomatic triad consisting of recurrent oral and genital ulcers and skin lesions. BD is a chronic inflammatory disease characterized by recurrent attacks, and may involve multiple systems[1].

Although the etiology and pathology of BD has not been fully elucidated, it is believed to be triggered by

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environmental factors in individuals with certain genetic backgrounds. Proinflammatory cytokines released by various cells with genetic predisposition are thought to be responsible for the increased inflammatory reaction seen in BD. The association between BD and HLA-B*51 has been clearly documented[2–4].

The distribution of BD varies worldwide. The countries with highest prevalence are Turkey, Iraq, Greece, Italy, Spain, China, Japan and Korea[5].

The HLA-B*51 allele is the most important genetic factor in the pathogenesis of BD. The link between the disease and HLA-B*51 is thought to possibly be related to either a direct role of the

HLA-B*51 molecule in BD pathogenesis, or a connection with another gene in the HLA-B region which causes disequilibrium and acts as a susceptibility gene[6].

To date, 116 different subtypes of the HLA-B*51 antigen have been identified (HLA-B*51:01-B*51:122) (IMG/HLA Database Release 3.5.0, 14 July 2011).

Investigation of the link between BD and other HLA-B alleles revealed a weak association with the HLA-B*2702 allele. Comparative sequence analysis of the HLA-B*51 and B*2702 alleles revealed a common Bw4 motif between amino acids 77–83. It is notable that this common sequence is known to bind with KIR3DL1 found on natural killer (NK) cells. KIR3DL1 has been described as a specific inhibitor receptor of HLA-Bw4[15,16].

There is evidence of a strong association between BD and MICA (major histocompatibility complex class I-related chain A). The MICA gene is located within a 46-kb centrometric region of HLA-B, and its MICA*009 allele has been shown to increase risk of BD. Furthermore, a significant relationship between BD and MICA*006 and MIC-A6 TM alleles has been reported. It has been emphasized that MIC-A may be a candidate gene for BD[4,17–19].

Therefore, another gene in the region with high disequilibrium linkage with B*51 may be involved in susceptibility to BD. HLA-Cw14 and Cw15 were found significantly more often in BD patients[20,21].

Other studies regarding the relationship between BD and HLA in different populations have shown associations between the disease and HLA A26, HLA B*3901, HLA B52, HLA B56, Cw1, Cw14, Cw15, Cw16, HLA DR*B104, and HLA DR*B107 alleles[14–17].

In this study we performed polymerase chain reaction (PCR) at low-and high-resolution subtype HLA-B loci in order to determine the frequency of HLA-B*51 and its suballeles in Turkish BD patients and healthy controls.

Material and Method

The study was approved by the Ataturk University Faculty of Medicine Ethics Committee.

Twenty-one male and 19 female BD patients from various provinces of Turkey who presented to the Ophthalmology and Skin and Venereal Disease outpatient clinics of the Erzurum Ataturk University Yakutiye Research Hospital were included in the study. All patients were diagnosed and treated in accordance with criteria defined by the 1990 International Behçet's Disease Study Group. The control group consisted of 54 unrelated individuals with no systemic diseases who underwent HLA tissue typing. All patients and control subjects provided written informed consent for their participation in accordance with the Helsinki Declaration.

Automated genomic DNA isolation was performed (MagNA Pure LC DNA Isolation Kit I, Roche) on blood samples obtained from all patients and control subjects. HLA-B tissue typing was done with by high resolution sequence-specific oligonucleotide (PCR-SSO) (Luminex 200, USA). HLA-B*51 gene polymorphisms were identified using low-resolution sequence specific primers (PCR-SSP) (Onelambda, CA, USA).

SPSS version 17.0 statistical software package was used for statistical analyses. The chi-square test was used to determine HLA-B*51 subgroup allele distributions.

Results

In this study, the HLA-B*51 subtypes of 40 BD patients and 54 unrelated healthy individuals were evaluated.

There was a significant difference in B*51 subtype frequencies between the patient and control groups. B*5101 was the most common B*51 subtype seen in this study; in addition, the B*5109 allele was found in one BD patient and HLA-B*5108 subgroup was found in two controls.

In summary, in this study of BD patients, the common HLA-B*5101 subtype was observed, as well as HLA-B*5109 allele in one patient (Table 1).

Table 1. *Distribution of HLA-B*51 subtypes in the patients with Behçet's disease and in controls*

	Patients		Controls	
B*51 allele	n=40	%	n=54	%
Het*5101	37	92.5	52	96.3
Hom*5101	2	5	0	0
*5108	0	0	2	3.7
*5109	1	2.5	0	0

Discussion

Behçet's disease is a systemic vasculitis featuring attacks as well as a long-term disease course, and its etiopathology is not completely understood[22]. The strongest genetic predisposition to BD is the HLA-B*51 antigen. The HLA-B*5101 and 5108 alleles are the most common HLA-B*51 subgroups found in BD patients[9]. It has been reported that BD patients with HLA-B*51 exhibit more severe clinical symptoms and ocular involvement.

The distribution of HLA-B*51 varies by population, with rates of HLA-B*51 positivity ranging from 62 to 98% [23–26]. Studies indicate that HLA-B*51 is more common in male BD patients[23–27]. In the current study, all 21 of the male BD patients were positive for HLA-B*51, compared to 47.5% of the female patients.

To date, 116 subtypes of HLA-B*51 have been identified[9]. HLA-B*5101 and HLA-B*5108 are the most commonly reported suballeles[8,9,14,23] and data from various populations show HLA-B*5101 frequency of 62–98% [14,24,28–30]. The most common subtype of the BD patients in our study was heterozygous HLA-B*5101 (92.5%), while homozygous HLA-B*5101 was identified in 2 patients (5%).

Takemoto et al. found that all of the BD patients carrying HLA-B*5101 alleles in their study had the HLA-B*5101 subtype[31]. Demirseren et al. found that the HLA-B*5101 allele was the most common subtype in their study at a frequency of 97.2% ; they detected HLA-B*5101 in 94.3% and HLA-B*5108 in 57% of their patients[23].

A study of the Turkish and German BD population revealed that HLA-B*5107 subtype had a negative effect on BD[14,17]. The B*5108 and *5109 subtypes have also been detected in BD patients, and their sequence analysis should be investigated and compared with that of *5101 (35). HLA-B*5108 is the second most common suballele associated with BD[14]. Its incidence in the Turkish, Japanese, German, Greek, Spanish and Italian populations ranges from 10–30%[7,8,10,13,23,32]. However, we did not detect HLA-B*5108 in the BD patients in our study. In a study by Kera et al. employing SBT, HLA-B*5101 and HLA-B*5108 were found at rates of 52% and 17.9%, respectively[7]. Demirseren et al. reported that the HLA-B*5109 subtype may be protective against the development of papulopustular lesions[23]. One patient in our study carried the HLA-B*5109 allele.

In conclusion, the results of our study indicate that the HLA-B*51 subgroup is not a causative factor in the pathogenesis of BD.

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