HLA-B51 and its allelic types in association with Behcet's disease and recurrent aphthous stomatitis in Korea

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Abstract

Objective

This case-control study was undertaken to evaluate the association of HLA-B51 antigen and its allelic types with Behçet's disease (BD) and with recurrent aphthous stomatitis (RAS), to investigate the degree of this association with diagnostic types and clinical variables of BD.

Methods

The DNA typing of HLA-B51 by nested PCR-SSP was performed in 61 patients with BD, 56 patients with RAS, and in 70 healthy controls. Also, blind quality control study was done to assess the accuracy of nested PCR-SSP in HLA-B51-positive and negative BD patients on the microlymphocytotoxicity. In addition, direct DNA sequencing analysis was carried out in HLA-B51-positive individuals.

Results

*The outcome of nested PCR-SSP showed 100% concordance with those of the microlymphocytotoxicity. The prevalence of HLA-B51 in patients with BD was 55.7%, 16.1% in patients with RAS, and 15.7% in healthy controls. According to the diagnostic types of BD, all ten patients with complete BD had HLA-B51 antigen, and 47.1% in patients with incomplete BD ($p = 0.002$). In addition, the prevalence of HLA-B51 was statistically significant in patients with BD who had uveitis ($p = 0.003$) or erythema nodosum ($p = 0.042$). Direct DNA sequencing analysis revealed that the major allelic types in BD, RAS, and in healthy control were mostly HLA-B*51011.*

Conclusions

Compared to patients with RAS or healthy controls, prevalence of HLA-B51 in the Korean patients with BD was much higher. The BD patients with B51 seemed to be susceptible for manifesting uveitis, erythema nodosum, and the full-blown syndrome as complete BD. Therefore the presence of HLA-B51 antigen in BD patients would be a genetic marker for the severe disease. In addition, there was no difference on the major allelic types of HLA-B51 in BD, RAS, and in healthy control.

Key words

Behçet's disease, HLA-B51 antigen, nested PCR-SSP, allelic types of HLA-B51.

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Introduction

BD is a chronic inflammatory disorder characterized by recurrent oral ulcers, genital ulcers, ocular lesions, skin lesions, arthritis, gastrointestinal involvement, and central nervous system (CNS) involvement. Although the etiology and pathogenesis remain unclear, the expression of disease is believed to be triggered by the environmental factors such as infectious agents in individuals having a particular genetic background (1-3).

HLA-B51 antigen has been well-known genetic factor associated with BD. The prevalence of HLA-B51 in BD appears to be higher in such countries adjacent to the ancient Silk Road as Turkey (4, 5), Iraq (6), Greece (7), Italy (8), Spain (9), China (10), Japan (11, 12), and Korea (13). The global distribution of this antigen among healthy populations shows a striking similarity both to the ancient Silk Road and the distribution of BD (14). In a recent study, the increased prevalence of transmembrane MIC-A6 allele and extracellular MIC-A*009 allele is a consequence of linkage disequilibrium with HLA-B51, and the real disease susceptibility gene involved in the development of BD is the HLA-B51 itself (15, 16).

The oral ulcerations frequently occur as a first clinical manifestation of BD, and constitute the keystone for the diagnosis (3). But it may be indistinguishable from those of recurrent aphthous stomatitis (17). Therefore, the HLA-B51 was examined by a two-step polymerase chain reaction with sequence specific primers (PCR-SSP) to clarify the association of this antigen in Korean patients with BD and with RAS, and to investigate the strength of this association with the diagnostic types and certain clinical variables of BD. In addition, direct DNA sequencing analysis was done to elucidate the HLA-B51 allelic types in HLA-B51-positive individuals.

Materials and Methods

Subjects. The study populations included 61 patients with BD (23 males and 38 females) who fulfilled the 1987 revised criteria by the Japan Behçet's Disease Research Committee (18), 56 patients with RAS (19 males and 37 females), as well as 70 healthy controls (24 males and 46 females). They were ethnically homogenous Koreans who were unrelated each other. The mean age of patients with BD and RAS was 40.36 years (± 10.57) and 40.82 years (± 14.10), respectively. The mean age of the controls was 45.63 years (± 12.56).

DNA extraction. Genomic DNA was isolated using the Chelex extraction method (19).

HLA-B51 by nested PCR-SSP. DNA typing was performed by a two-step PCR-SSP method (20). Briefly, the first amplification step was carried out using the sequence-specific primers for HLA-B5 cross-reactive group (CREG) (63N-163L). The internal positive control primers (HGH I-HGH III) were also used to co-amplify a 1078 bp fragment of the human growth hormone gene. The B5-positive PCR products were subjected to the second amplification with the sequence-specific primers for B51 (81A-114N). The final amplification products were applied to gel electrophoresis on standard 2% agarose gel. After ethidium bromide staining, the results were examined under UV transillumination and were documented by photography.

Blind quality control study. 22 patients with BD who constitute 14 HLA-B51 positive patients and 8 HLA-B51 negative patients using the standard microlymphocytotoxicity were selected. The samples from these 22 patients were coded and afterward typed by the two-step PCR-SSP without giving any information.

DNA sequencing analysis. DNA direct sequencing analysis was performed in HLA-B51-positive individuals. Polymorphic regions (exon 2, intron 2, exon 3) of the HLA-B51 and B52 genes were amplified using a HLA-B group specific PCR primer set, Bin1-ta and Bin3-37. The PCR product was used as a template for direct DNA sequencing using Cycle Sequencing Kits containing AmpliTaq DNA polymerase, FS (PE-Applied Biosystems Inc., USA). Two internal sequencing primers derived from intron 2 (int2f and int2r)

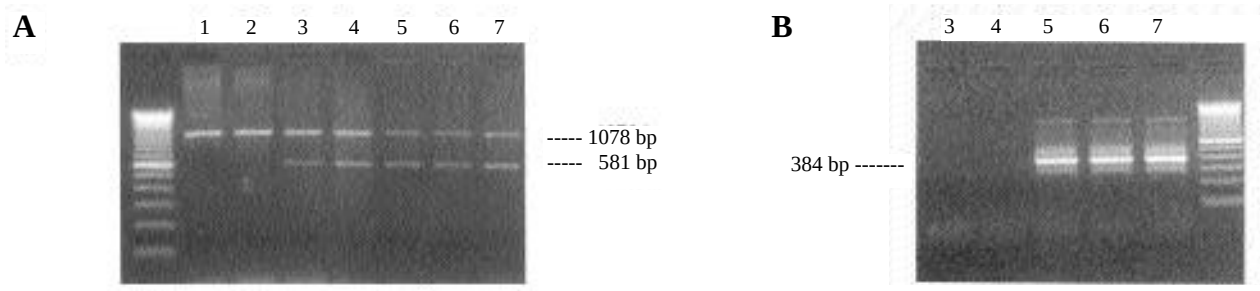

Fig. 1. Illustration of a two-step PCR-SSP
Panel A is a 2 % agarose electrophoresis gel showing first round amplification. A 581 bp bands for HLA-B5 cross-reactive group (CREG) are observed in lane 3 to 6. A 1078 bp fragments shown in each lane are human growth hormone served as an internal control. The resulting amplified DNA with HLA-B5 CREG is subjected to second amplification step. The products of second amplification are shown in panel B, in which lane 5 and 6 at 384 bp carry products for HLA-B51. In the panel A and B, lane 7 represents positive control.

were used in the sequencing reaction to obtain complete sequence information from exons 2 and 3. Procedures and condition for DNA direct sequencing analysis using an automated DNA sequencer (Model 377, PE-Applied Biosystems Inc., USA) have been described (21). Sequences were analyzed using Sequence Navigator and Match-Tool software (PE-Applied Biosystems Inc., USA).

Statistical analysis. The statistical significance was evaluated by Fisher's exact test. P values less than 0.05 were considered to be statistically significant. These results were confirmed by multiple logistic regression analysis. The odds ratio (OR) for each categorical variable was estimated where necessary.

Results

According to Japanese diagnostic criteria, 61 patients with BD were divided into two diagnostic types, 10 patients as complete BD, and 51 patients as incomplete BD. There was female preponderance, as the ratio of male/female was 1:1.65. At the time of the examination and during the follow-up period, 34 patients (55.7%) had erythema nodosum, 16 patients (26.2%) uveitis, 52 patients (85.2%) genital ulcers, 11 patients (18.0%) gastrointestinal involvement, 2 patients (3.3%) CNS involvement, and 16 patients (26.2%) arthritis of peripheral joints.

Blind quality control study

The outcome of nested PCR showed 100% concordance with those of the microlymphocytotoxicity.

Two-step PCR-SSP

The first PCR generated bands of 581 bp for B5 CREG alleles, and the second round of amplification resulted in 384 bp products for B51 allele (Fig. 1). The prevalence of HLA-B51 in patients with BD was 55.7%, 16.1% in patients with RAS, and 15.7% in healthy controls. Compared to healthy controls, the BD patients had much higher prevalence of HLA-B51 ($p < 0.001$). However, there was no difference between patients with RAS and with healthy controls ($p = 1.0$). According to the diagnostic types of BD, all ten patients with complete BD had HLA-B51 antigen,and 47.1 % in patients with incomplete BD. There was a statistical significance between the two diagnostic types ($p = 0.002$) (Table I). In addition, there was no gender difference ($p = 0.791$), as the prevalence of HLA-B51 in male and female patients with BD was 52.2% and 57.9%, respectively.

HLA-B51 antigen was positive in 67.6% of erythema nodosum, 87.5% of uveitis, 57.7% of genital ulcer, 54.5% of gastrointestinal involvement, 43.8% of arthritis, and in all two patients with CNS involvement. There was a statistical significance in uveitis ($p = 0.003$)

Table I. The prevalence of HLA-B51 in patients with Behcet's disease, recurrent aphthous stomatitis, and in healthy controls.

Diagnostic type	Number of patients	Number of HLA-B51-positive patients (%)	p-value
Behcet's disease (BD)	61	34 (55.7)	< 0.001*
Complete BD	10	10 (100)	
Incomplete BD	51	24 (47.1)	0.002**
RAS	56	9 (16.1)	1.0¶
Healthy control	70	11 (15.7)	

*: p-value between BD and healthy control; **: p-value between two diagnostic types of BD; ¶: p-value between RAS and healthy control; RAS: recurrent aphthous stomatitis.

Table II. The relationship between clinical variables and HLA-B51 antigen.

Clinical variables (no. of pts.)	Number of patients with B51 (%)	p-value	OR (95% CI)
Erythema nodosum (34)	23 (67.6)	0.042	3.0 (1.1 ~ 8.7)
Uveitis (16)	14 (87.5)	0.003	8.8 (1.8 ~ 43.1)
Genital ulcer (52)	30 (57.7)	0.492	1.7 (0.4 ~ 7.1)
CNS involvement (2)	2 (100)	0.498	
GI involvement (11)	6 (54.5)	1.0	0.9 (0.3 ~ 3.5)
Arthritis of peripheral joints (16)	7 (43.8)	0.380	0.5 (0.2 ~ 1.6)

OR: odds ratio; CI: confidence interval; CNS: central nervous system; GI: gastrointestinal.

and in erythema nodosum (p = 0.042). The OR for the development of erythema nodosum was 3.0, uveitis 8.8 and genital ulcer 1.7 (Table II). In addition, the OR for the development of BD in Korean persons with HLA-B51 was 6.8 (95% confidence interval; 3.0-15.3).

Direct DNA sequencing analysis

The allelic types in HLA-B51-positive patients with BD were all HLA-B*51011 but one HLA-B*51021. The allelic types in patients with RAS were all HLA-B*51011 except one HLA-B*51021. In healthy controls, all allelic types were HLA-B*51011. There was no difference in allelic types of HLA-B51 between these three groups.

Discussion

BD, also known as the Silk Road disease, is most active between the second and fourth decades of life. Most clinical manifestations of BD take a benign course except for those of the CNS involvement, gastrointestinal lesions, vascular involvement and ocular attacks. Repeated attacks of uveitis can cause blindness (3). The ocular lesions are more prevalent in young males (22, 23). Although males are more frequently affected in Middle Eastern countries, BD is somewhat more common in females of Japan and Korea (3, 24, 25). Current study showed female predominance as the sex ratio was 1:1.65.

The serological tissue typing by the microlymphocytotoxicity for HLA class I is restricted due to its requirement of viable cell and to the limited availability of specific antisera. According to Hein *et al.* who developed a DNA typing system of the HLA-B5 CREG by nested PCR-SSP, this method showed 100% concordance with classical serologic typing for the typing of B5 CREG (20). In our study, this method was applied to the typing of HLA-B51 in BD and RAS. We also acquired the same results to the microlymphocytotoxicity method in blind quality control study.

The HLA-B51 antigen is not only the important contributor to the development of BD in area in which the disease is prevalent, but also relates to the severity of BD, since this antigen is more common among hospital-based patients or among patients with posterior uveitis, erythema nodosum, or CNS involvement (3,7,24,26-28). The current study showed that HLA-B51 was positive in all patients with complete BD who has four major symptoms of recurrent oral ulcerations, skin lesions, ocular lesions and genital ulcerations. The individuals with HLA-B51 appeared to be susceptible for manifesting uveitis and erythema nodosum. However, there was limitation in concluding whether HLA-B51 is a risk factor for CNS involvement, because of only two patients with CNS involvement. The OR of HLA-B51 for BD in the Greek population was 10.48 (95% confidence interval; 4.8-22.8) (7), that in our study was 6.8 (95% confidence interval; 3.0-15.3). As in the Greek population, HLA-B51 in Koreans may be a genetic predisposing factor for BD.

Besides a genetic factor in the pathogenesis of BD, the infectious agents have been suspected. Skin and peripheral blood mononuclear cells of the BD patients showed the intense hypersensitivity to Streptococcal antigens when compared to healthy controls (29). In addition, the mycobacterial 65 kilodalton heat shock protein (hsp) showing significant homology with the human 60 kilodalton hsp has been shown to upregulate the expression of T cell in BD patients (30-32). There have been a few reports about the allelic types in BD, in which major allelic type of B51 was B*5101 (7, 9, 12). In our study, there was no difference of B51 allelic types among BD, RAS and healthy controls, as the major allelic type of these three groups was B*51011. If HLA-B51 would be the most important gene for the development of BD, our results may support the role of environmental factor in persons having specific genetic backgrounds.

RAS is a chronic inflammatory disease characterized by painful, recurring ulceration of the oral mucosa. Except for gingivitis, it is the most common disease affecting the oral mucosa. Approximately 20% of the general population will have this disease at any time of life (33). In the past year, it had been thought that RAS would be the same disease spectrum to BD (34). However, besides ordinary RAS, the recurrent oral ulcerations could be associated with several inflammatory systemic diseases such as BD, Crohn's disease, ulcerative colitis, Reiter's syndrome, and systemic lupus erythematosus (17). The oral aphthous ulcers usually precede the other manifestations of BD. It is very difficult to differentiate RAS and oral ulcer of BD. So far, there have been the conflicting a few reports of the prevalence of HLA-B51 in patients with RAS. The prevalence of HLA-B51 in one study was higher than control subjects (35), that in other studies was not increased (36, 37). In our study, the prevalence of B51 in patients with RAS was similarly low to that of healthy controls.

The conclusions of this case-control study in these Korean patients with BD and with RAS were as follows: 1) HLA-B51 in the Korean population may be a genetic predisposing factor for BD. 2) The BD patients with B51 tended to manifest uveitis, erythema nodosum and the full-blown syndrome as complete BD. Therefore, it was suggested that the presence of HLA-B51 in BD patients would be a genetic marker of the severe disease. 3) The major allelic type of HLA-B51 in BD, RAS, and in healthy control was HLA-B*51011. 4) It seemed that nested PCR-SSP was an accurate method for identification of HLA-B51 antigen. 5) The prevalence of B51 in patients with RAS was similar to that of healthy controls.

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