Associations of Moyamoya Patients with HLA Class I and Class II Alleles in the Korean Population

Moyamoya disease is characterized by progressive cerebrovascular occlusion at the peripheral internal carotid artery and development of abnormal collateral circulation at the cerebral basal region. Although abnormal thrombogenesis, inflammation and autoimmune process might be involved in the etiology, the genetic pathogenesis of Moyamoya disease is still unknown. To evaluate the association of Moyamoya disease with HLA alleles in the Korean population, we investigated HLA class I and class II alleles in 28 Moyamoya patients and 198 unrelated healthy controls. The frequency of HLA-B35 allele was significantly increased in the patients compared to the controls (32.1% vs. 10.1%, RR=4.2, p<0.008). Further analysis of HLA-B35 on onset age and sex showed that this allele was significantly increased compared to the controls in both late-onset and female group. Especially, HLA-B35 was the most significantly increased in female of late-onset group compared to the controls. These results suggest that HLA-B35 may be an useful genetic marker for Moyamoya disease, and particularly in females of late onset group in the Korean population.

Key Words : Moyamoya Disease; HLA-B Antigens; Female; Age of Onset; Korean

INTRODUCTION

Spontaneous occlusion of the circle of Willis, first described as Moyamoya disease in Japan, is characterized by bilateral stenosis and/or occlusion of the terminal portion of the internal carotid artery and by the development of abnormal net-like vessels at the base of the brain (1). There are remarkable regional differences in the incidence of Moyamoya disease. Moyamoya disease is mostly found in Asians and rare in Caucasian (2). Moyamoya disease is known to be prevalent in children under 10 yr old and young adults in the third decade and occurs more frequently in females (male to female ratio of 2:3) (3). In an epidemiological survey of Moyamoya disease in Korea, the overall clinical background was similar to that of Japanese patients, although Korean Moyamoya disease showed a relatively higher incidence of hemorrhage and adult onset (4-7).

Previous investigations suggested some hypotheses to explain the etiology of Moyamoya disease, such as infection in the upper respiratory tract (8), influence of immune system (9), thrombogenesis on the intima (10, 11), and disorder of the vascular smooth muscle cell (12-14). An epidemiological study showed that Moyamoya disease might be caused by genetic factors rather than environmental factors (15). However, the role of genetic factors in the pathogenesis had not been completely clarified yet.

Human leukocyte antigen (HLA) molecules play an important role in the immune system, which present peptides derived from self and foreign antigens to appropriate T lymphocytes. The alleles of HLA molecules have been reported to be frequently strongly associated with various diseases, such as autoimmune disease, infectious disease, and virus-associated tumor. Therefore, the identification of the association between HLA and disease susceptibility has become important to diagnose and understands the pathogenesis of the disease. Previous studies reported that Moyamoya disease was associated with some HLA alleles (16, 17). But the studies of HLA association of Moyamoya disease have been very limited to only Japanese. Recently, some reports have suggested possible linkage with Moyamoya disease on chromosome 3p24.2-p26 and 17q25 (18, 19). And the marker located on chromosome 6 was also suggested to be in linkage with Moyamoya disease (20).

In this study, we investigated the distributions of HLA alleles in 28 Korean patients with Moyamoya disease to identify HLA markers that may contribute to the genetic susceptibility to Moyamoya disease in the Korean population.
Association of Moyamoya Disease with HLA Alleles in Korean

MATERIALS AND METHODS

Subjects

Twenty-eight Korean patients with Moyamoya disease (11 men and 17 women) who were diagnosed as definite Moyamoya disease according to the criteria for Moyamoya disease were involved in this study. The subjects were collected from 1992 to 2001 at the Neurosurgery Service of Uijongbu St. Mary’s Hospital, Uijongbu, Korea. The ages of the patients ranged from 2 to 68 yr. Their results were compared with those of the control group, which was composed of 198 Korean drawn from the healthy normal population.

HLA-A, B serology typing

Peripheral blood (10 mL) was collected using anticoagulant treated Vacutainer (Becton Dickinson Vacutainer systems, Franklin Lakes, NJ, U.S.A.) and mononuclear cells were isolated by density gradient centrifugation on a Ficoll-Hypaque (Pharmacia Biotech, Wikstoms, Sweden). HLA-A and -B typing was done according to a standard microlymphocytotoxicity technique (21). Cells and serum were incubated together for 30 min at 25°C and incubated at 25°C for 1 hr by the addition of complement. Cell death was assessed by the addition of Eosin-Y and read on a microscope. The standard nomenclature system was followed throughout to describe the HLA alleles.

HLA-B genotyping

To confirm the serological results of HLA-B, we applied molecular approach (ARMS-PCR; Amplification refractory modification system-polymerase chain reaction) for genotyping of HLA-B in this study. DNA was extracted from heparinized blood by the salting out method and HLA-B typing was performed by the ARMS-PCR method described by Bunce et al. and Tonks et al. (22, 23). Each tube contained a primer mix consisting of the allele- or group-specific primer pairs as well as a positive control primer, which matched the non-allelic sequences. PCR reactions were performed in a volume of 7 μL, as modified in the class I ARMS-PCR reference manual of the 12th International Histocompatibility Workshop. PCR product size was defined on 1.5% agarose gel pretained with ethidium bromide.

HLA class II Genotyping

HLA class II typing was performed by the PCR-SSOP (sequence specific oligonucleotide probe) method, which was essentially the same as that described at the 12th International Workshop, with minor modifications (24). For each locus, specific primers were used to amplify products, which were then denatured and immobilized on a nylon membrane and probed with a series of digoxigenin labeled oligonucleotides specific for the known hyper-variable sequences. Stringent washing was performed in the presence of tetramethyl ammonium chloride (TMAC, Sigma Chemical Co. St. Louis, MO, U.S.A.). The hybridized probe was detected according to the manufacturer’s instructions with the anti-digoxigenin antibody conjugated with alkaline phosphatase and followed by the addition of chemiluminescent substrate CSPD (Boehringer Mannheim, GmbH, Germany). Chemiluminescence was detected by exposure to radiography film.

Statistical Analysis

The odds ratio was calculated with Woolf’s formula and, by convention, expressed as a relative risk (RR). Haldane’s modification of the formula was used when one element of the equation was zero (25). The statistical significance of the difference was tested by chi-square test with 1 degree of freedom or by the 2-tailed Fisher’s exact test when the criteria for the chi-square test were not fulfilled. Generally, p values less than 0.05 were considered statistically significant.

RESULTS

Association of HLA class I and class II alleles in Moyamoya Disease

The frequencies of HLA-A and B alleles in patients with Moyamoya disease and normal controls are shown in Table 1. The frequency of HLA-B35 was significantly increased in the patients compared with controls (32.1% vs. 10.1%: Moyamoya patients vs. controls, RR=4.2, p<0.008). However, there were no significant differences between the patients and controls in the frequencies of HLA-A and other B alleles. Table 2 shows the frequencies of HLA-DRB1, -DQA1, and -DQB1 alleles in patients with Moyamoya diseases and normal controls. However, there were no significant differences between the patients and controls in the Korean population.

Distribution of HLA phenotype frequency according to onset age

The average age at onset of the subject in this study is 34 yr. Previous study showed that Moyamoya disease in the Korean population might be classified into two groups, below and above 10-yr at onset (4). Therefore, we divided the patients into two groups according to the age at onset 10 yr, early and late onset group. The frequency of HLA-B35 in late-onset group was significantly increased compared with controls (40.9% vs. 10.1%: Moyamoya patients vs. controls, RR=6.2, p<0.002) (Table 3). Interestingly, HLA-B35 was not appeared...
in early onset group, although the significant difference of the frequency between early and late onset group was not shown, probably due to small number of patients.

Distribution of HLA phenotype frequencies according to gender

When the distributions of HLA alleles were compared between female and/or male and controls, the frequency of HLA-B35 was also found to be higher in female patients than controls (47.1% vs. 10.1%: Moyamoya patients vs. controls, *RR*=7.9, *p* <0.0007) (Table 3). Although the significant differences between female and male were not shown, the frequency of HLA-B35 was increased in female than male. Especially, HLA-B35 was the most significantly increased in female of late onset group compared with controls (53.3% vs. 10.1%: Moyamoya patients vs. controls, *RR*=10.2, *p* <0.0003) (Table 3).

**Discussion**

Moyamoya disease is characterized by progressive cerebrovascular occlusion at the peripheral internal carotid artery and development of abnormal collateral circulation at the cerebral basal region (8, 26). The pathogenesis of Moyamoya disease is still unknown, although several reports suggested that abnormal thrombogenesis, inflammatory process and autoimmune process might be involved in the etiology.

Human leukocyte antigen, presenting peptides derived from self and foreign antigens to T lymphocytes, plays an important role in the immune system. HLA disease associations are of diagnostic and prognostic importance because the presence of certain HLA alleles can raise the risk of developing certain disorders and in specialized situations this...
information can be helpful. Previous studies reported that Moyamoya disease was associated with some HLA alleles. Aoyagi et al. found a significant association of HLA-B51 in their investigation of 32 unrelated Japanese patients with Moyamoya disease (16). Takuya et al. showed that HLA-DRB1*0405, DQB1*0502, and *0401 had significant association with Moyamoya disease in Japanese (17). In this study, we found that the frequency of HLA-B35 was significantly increased in the patients compared with controls (Table 1). We performed molecular approach (ARMS-PCR) for genotyping of HLA-B and confirmed the serological results of HLA-B. Several studies showed that HLA-B35 might be associated with autoimmune and infectious diseases (27, 28). Previous reports suggested that virus and bacterial infection might be involved in the pathogenesis of Moyamoya disease (31). Thus, HLA-B35 may have direct influences on the pathogenesis of Moyamoya disease in some aspects of inflammatory or/and autoimmune process. We did not find a significant association of HLA-B51, DRB1*0405, DQB1*0502, and *0401 although these alleles were reported to be significantly associated with Moyamoya patients in Japanese (16, 17) which are genetically and clinically similar to Koreans. Therefore, these results suggest that the association between HLA antigens and Moyamoya disease may differ between ethnic groups and these different HLA alleles may not be significant susceptibility factors associated with Moyamoya disease, but only genetic marker. Further studies are required to evaluate the more detailed genetic aspects of Moyamoya disease.

Moyamoya disease was reported to occur more frequently in females and be prevalent among patients <10 yr of age (3, 4). In this study, we analyzed the distributions of HLA alleles according to age at onset and sex. HLA-B35 in both late onset and female group was significantly increased compared with controls. Especially, HLA-B35 was the most significantly increased in female of late onset group compared with controls (Table 3). Therefore, these results suggest that HLA-B35 may be a more useful genetic marker for Moyamoya in females, and particularly in females of late onset group in the Korean population. In conclusions, we demonstrated the associations of Moyamoya disease with specific HLA allele in the Korean population. Further studies including family will be required to confirm these data and to investigate new genes associated with Moyamoya disease.

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