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Molecular Analysis of HLA Class II-Associated Susceptibility to Neuroinflammatory Diseases in Korean Children

The work was done to study immunogenetic peculiarities of neuroinflammatory diseases among Korean children. A total of 13 children with neuroinflammatory diseases (8 males and 5 females; mean age 4.6±2.6 yr) were consecutively recruited. Genomic typing was performed on their HLA DRB/HLA DQB genes using PCR-SSOP/SSP techniques with gel immunoelectrophoresis. The frequencies of HLA-DR1*15 in children with acute disseminated encephalomyelitis (ADEM) (31%) and DQB1*06 in other neuroinflammatory diseases (38%) were significantly increased compared with control subjects. The frequencies of HLA-DRB3*0202 (100%), HLA-DRB1*1302 (67%), HLA-DRB3*0301 (67%), and HLA-DQB1*0301 (67%) were significantly increased in children with multiple sclerosis and the frequencies of HLA-DRB1*1501 (40%) and HLA-DRB5*0101 (40%) were significantly increased in children with ADEM. HLA-DRB1*1401, HLA- DRB3*0202, and HLA-DQB1*0502 were found in children with acute necrotizing encephalopathy. In conclusion, HLA-DR1*15 and DQB1*06 may be involved in susceptibility to inflammation in Korean children. The frequencies of HLA-DRB1*1501, HLA-DRB5*0101, HLA-DRB3*0301, and HLA-DQB1*0602 were not as high in Korean children with multiple sclerosis as in western children. However, HLA-DRB3*0202 was seen in all children with multiple sclerosis. Our data may provide further evidence that the immunogenetic background of neuroinflammatory diseases in Korean is distinctly different from the ones in western countries. Further studies are necessary to confirm this finding.

Key Words : *Neuroinflammatory Diseases; HLA-DR Antigens; HLA-DQ Antigens; Encephalomyelitis, Acute Disseminated; Multiple Sclerosis; Leucoencephalitis, Acute Hemorrhagic*

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INTRODUCTION

Neuroinflammatory diseases are clinically heterogeneous and one of the major causes of acquired neurological disability. An underlying complex genetic susceptibility may play an important role in their etiologies; however, the role of genetic factors in determining their clinical features is still unclear. Multiple sclerosis (MS), one of the major neuroinflammatory diseases, recently was recognized and subsequently well-characterized in children, but the awareness of pediatricians and pediatric neurologists, especially in Asian countries to the occurrence of MS, is still unsatisfactory. Some data provided evidences that Asian and Western type MS are distinct regarding their immunogenetic background (1-3). Acute disseminated encephalomyelitis (ADEM) is a postinfectious encephalitis that is usually preceded by an infectious illness or vaccination. The clinical manifestation has a wide spectrum and complementary examinations are not specific, except for magnetic resonance imaging (MRI) findings which show multifocal white-matter lesions similar to those seen in MS (4-9). Acute necrotizing encephalopathy (ANE), likely postinfectious encephalomyelitis, is not uncommon in Asian countries but is very rarely seen in Western countries. The etiology is still unknown, but immunogenetic mechanism has been suggested (10-15). Some data suggest that children with postinfectious encephalomyelitis are genetically predisposed to this demyelinating disease (16). These postinfectious encephalomyelitides and multiple sclerosis have clinical, immunologic, and neuroradiographic similarities. Therefore we studied HLA determinants in thirteen children consecutively diagnosed with neuroinflammatory disease to study the immunogenetic peculiarities of neuroinflammatory diseases in Korean children.

MATERIALS AND METHODS

A total of 13 children (8 males and 5 females) with neuroinflammatory diseases were consecutively recruited at the section of Pediatric Neurology, Kyungpook National University Hospital, Daegu, Korea, between January 2000 and April 2003. The subject's ages ranged from 1-10 yr. The studied

subjects included five children with ADEM, three with MS, three with acute necrotizing encephalopathy, and two with transverse myelitis. Three age-matched children with no neurological illnesses were also evaluated as controls.

The children with neuroinflammatory diseases and three age-matched children with no neurological illnesses as controls were molecularly typed for HLA class II genes. Genomic DNA was prepared from peripheral blood leukocytes by using a DNA purification kit (Promega, Madison, WI, U.S.A.) according to standard procedures. Molecular analysis was performed on HLA-DRB/HLA-DQB genes (chromosome 6p21) of 13 unrelated children with neuroinflammatory diseases using PCR-SSOP/SSP kits (INNO-LiPA HLA-DRB, Ghent, Belgium and Pel-Freez SSP UniTray with *Taq* polymerase, Basel, Switzerland) for low and high resolution typing, according to the manufacturer's instructions along with augmentation of gel immunoelectrophoresis for HLA-DQB. Alleles were assigned according to the nomenclature for factors of the HLA system.

Table 1. Demographic features of subjects (n=13)

Sex (male:female)	8:5
Age (yr)	4.6±2.6
Family history of IND	none
Clinical diagnosis	
ADEM	5
CDMS/CIS	3 (2/1)
AEBTT	2
ANEBG	1
Transverse myelitis	2

IND, inflammatory neurological diseases; ADEM, acute disseminated encephalomyelitis; CDMS/CIS, clinically definite multiple sclerosis/clinically isolated syndromes suggestive of MS; AEBTT, acute encephalopathy with bilateral thalamotegmental involvement; ANEBG, acute necrotizing encephalitis with basal ganglia involvement.

RESULTS

Thirteen children with neuroinflammatory diseases were typed for HLA class II genes. Their demographic and clinical characteristics are summarized in Table 1. Eight males and five females were enrolled in the study. Their ages ranged from one to ten years (mean age 4.6±2.6 yr). The three major conditions were ADEM, ANE, and MS. As shown in Table 2, the frequency of HLA-DR1*15 was significantly increased in patients with ADEM (31%) and the frequency of DQB1*06 was also increased in other neuroinflammatory diseases (38%; 4 ADEM, 1 multiple sclerosis, 1 transverse myelitis) compared with control subjects as well as known data of HLA-DRB alleles in Koreans. In Korean children with multiple sclerosis, the frequencies of HLA-DRB1*1501, HLA-DRB5*0101, HLA-DRB3*0301, and HLA-DQB1*0602 were not as high as in western children, but the frequencies of HLA-DRB3*0202 (100%), HLA-DRB1*1302 (67%), HLA-DRB3*0301 (67%), and HLA-DQB1*0301 (67%) were significantly increased instead (Table 3). The frequencies of HLA-DRB1*1501 (40%) and HLA-DRB5*0101 (40%) were significantly increased in children with ADEM compared with control subjects. In addition, three common alleles, HLA-DRB1*1401, HLA-DRB3*0202, and HLA-DQB1*0502, were found in children with acute necrotizing encephalopathy with bilateral thalamotegmental involvement (ANEBTT) (Table 4).

DISCUSSION

In the absence of a biological marker, the distinction between ADEM and MS cannot be made with certainty at the time of initial manifestation and a preceding or concurrent viral illness, high lesion load on MRI, involvement of the deep gray matter, or absence of oligoclonal bands may be more

Table 2. HLA-DRB and DQB alleles in subjects (n=13)

Patient	Sex	Diagnosis	DRB1	DRB3	DRB4	DRB5	DQB1
1	F	ANEBG	0701 1401	0202	0101		0303 0502
2	F	AEBTT	1101 1401	0202 0301		0301	0502
3	M	CDMS	1302 1406	0202 0301		0301	0609
4	M	ADEM	1101 1502	0202		0102	03 0501
5	M	AEBTT	0101 1302	0301			0501 0609
6	M	TM	0405 0701		0101		0202 0401
7	M	ADEM	0403 0701		0101		0202 0302
8	F	CDMS	0101 1101	0202			0301 0501
9	F	ADEM	0101 1501		0101		0501 0602
10	M	CIS	1302 1405	0202 0301		0609	0503
11	M	ADEM	0901 1504		0101?	0101?	0303 0601
12	F	TM	0301 1001	0106			0201 0501
13	M	ADEM	1302 1501	0301		0101	0602 0604

Male (M)/Female (F).
ANEBG, acute necrotizing encephalitis with basal ganglia involvement; AEBTT, acute encephalopathy with bilateral thalamotegmental involvement; CDMS/CIS, clinically definite multiple sclerosis or clinically isolated syndromes suggestive of MS; TM, transverse myelitis; ADEM, acute disseminated encephalomyelitis.

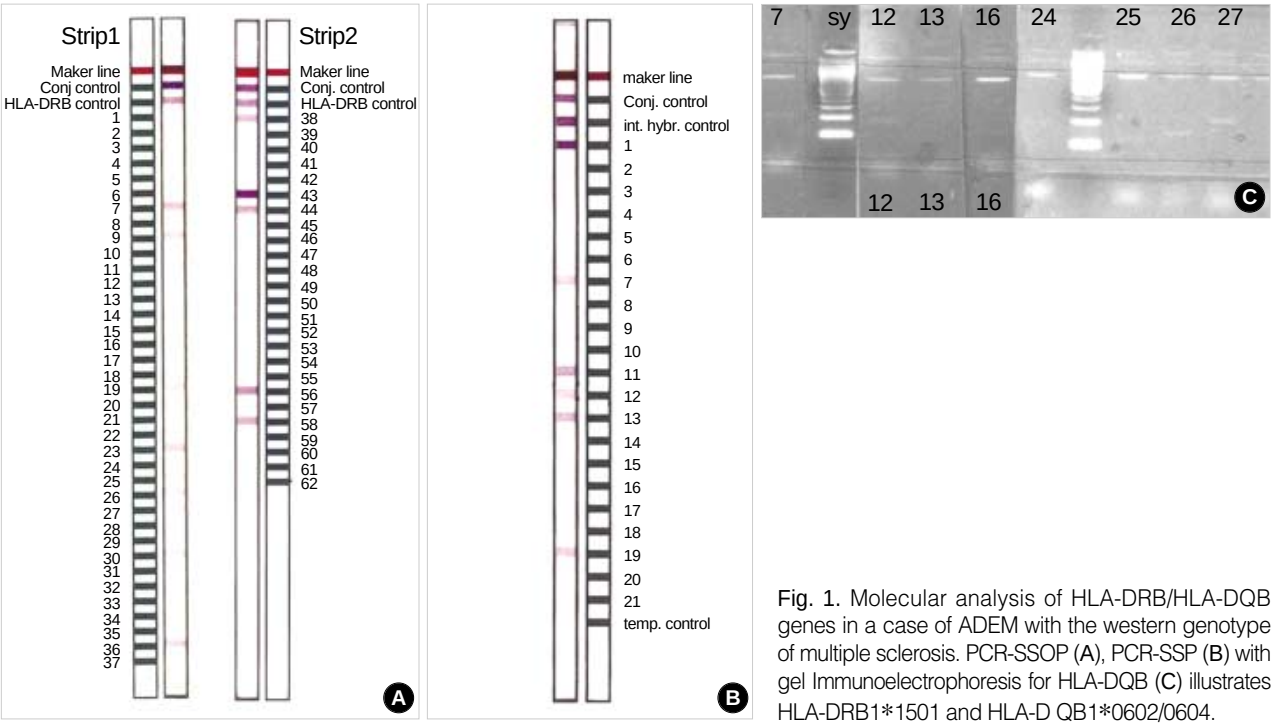

Fig. 1. Molecular analysis of HLA-DRB/HLA-DQB genes in a case of ADEM with the western genotype of multiple sclerosis. PCR-SSOP (A), PCR-SSP (B) with gel Immunoelectrophoresis for HLA-DQB (C) illustrates HLA-DRB1*1501 and HLA-D QB1*0602/0604.

Table 3. Frequent alleles of HLA-DRB and DQB in patients with multiple sclerosis

Korean	Western
DRB3*0202 (100%)	DRB1*1501
DRB1*1302 (67%)	DRB5*0101
DRB3*0301 (67%)	DRB3*0301
DQB1*0301 (67%)	DQB1*0602

Table 4. HLA-DRB and DQB alleles of patients with ANEBTT

	DRB1		DRB3	DQB1	
A	0701	1401	0202	0303	0502
B	1101	1401	0202	0301	0502
C	0101	1302	0301	0501	0609

AEBTT, acute encephalopathy with bilateral thalamotegmental involvement.

indicative of ADEM (5, 17). It will be very interesting if we were able to clarify whether these conditions have their own specific biological markers or share the markers. We sought to assess a possible relationship between HLA class II genes and neuroinflammatory diseases such as MS and ADEM. This is the pilot study to evaluate the immunogenetic background of neuroinflammatory diseases in Asian children.

We analyzed HLA determinants in thirteen Korean children consecutively diagnosed with neuroinflammatory disease. As illustrated in Table 1, ADEM is still the major condition despite the fact that a lot of children with ADEM were not enrolled for many reasons. We had two cases of clinically definite multiple sclerosis (CDMS) who met the diagnostic criteria (18-20). In addition, there was a child with Clinically Isolated Syndromes (CIS) suggestive of MS. Few pediatric cases of CDMS have been reported in Korea. However, recently the number is increasing. This is probably due to rapid westernization of the country, availability of clinically qualified experts, and improvement of diagnostic tools. A few cases of ANEBTT were also included in the study.

The subjects were genotyped using PCR-SSOP/SSP techniques for HLA DRB/HLA DQB genes with augmentation and gel immunoelectrophoresis. As shown in Table 2, the frequencies of HLA-DR1*15 and DQB1*06 were significantly increased in neuroinflammatory diseases compared with control subjects as well as known data of HLA-DRB alleles in Korean (21, 22). The results are similar to previous reports from Western countries and partly supports our hypothesis (23). The five most common alleles in these children were DRB3*0202 (46%), DRB1*0101 (23%), DRB1*1302 (23%), DQB1*0301 (23%), and DQB1*0501 (23%). Like previous Japanese studies (24, 25), the frequencies of HLA-DRB1*1501, HLA-DRB5*0101, HLA-DRB3*0301, and HLA-DQB1*0602 were not as high in Korean children with MS as in western children (1-3, 26-29). A very strong correlation of the condition with HLA-DRB3*0202, HLA-DRB1*1302, HLA-DRB3*0301, and HLA-DQB1* 0301 alleles was verified instead (Table 3). These findings may provide the evidence for correlation of these alleles and susceptibility of Korean children to MS. The frequencies of HLA-DRB1*1501 (40%) and HLA-DRB5*0101 (40%) were significantly increased in children with ADEM compared with control

subjects. Interestingly, there was one child with a clinically strong feature of ADEM, but he had the western genotype of MS, HLA-DRB1*1501 and HLA-DQB1*0602 (Fig. 1). He is still visiting the clinic and has not had any relapse yet. Although the same HLA determinants were found in this patient as in those with MS, further studies on a larger number of patients with postinfectious encephalomyelitis are needed before we can conclude with certainty that the two diseases share a common genetic propensity. Finally, it is noteworthy that three common alleles, HLA-DRB1*1401, HLA-DRB3*0202, and HLA-DQB1*0502 were found in children with ANEBTT (Table 4). With our limited knowledge, ANEBTT is not a common illness worldwide and has never been molecularly evaluated, so this result might be valuable. However, further studies are required to elucidate the condition.

In conclusion, HLA-DR1*15 and DQB1*06 may be involved in susceptibility to inflammation in Korean children. The frequencies of HLA-DRB1*1501, HLA-DRB5*0101, HLA-DRB3*0301, and HLA-DQB1*0602 were not as high in Korean children with MS as in western children. HLA-DRB3*0202 was seen in all children with MS. Our data may provide further evidence that the immunogenetic background of neuroinflammatory diseases in Asians is distinctly different from that in western countries. Further studies are necessary to confirm this finding.

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