Different Distribution of HLA Class II Alleles According to Response to Corticosteroid Therapy in Sudden Sensorineural Hearing Loss

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Objective: To investigate the association of HLA class II alleles with the susceptibility to sudden sensorineural hearing loss and with the results of corticosteroid treatment in the Korean population.

Design: HLA-DRB1, -DQA1, -DQB1, and -DPB1 genotyping by the sequence-specific oligonucleotide probes method in 41 patients with sudden sensorineural hearing loss and in 206 healthy control subjects. Initial hearing levels at the onset of hearing loss and final hearing levels after treatment were evaluated for the association with HLA class II alleles.

Setting: Tertiary care referral center, ambulatory and hospitalized care.

Subjects: Forty-one patients (24 men and 17 women; mean age, 49.2 years) were compared with 206 controls. Patients were divided into 2 groups according to their response to corticosteroid therapy (good response vs nonresponse).

Results: The frequencies of HLA-DRB1, -DQA1, -DQB1, and -DPB1 alleles were not significantly different between patients and controls (P>.05). When an association between the results of corticosteroid treatment and the frequency of HLA alleles was evaluated, the frequencies of HLA-DRB1*14 (relative risk [RR]=3.5, P=.02), -DQA1*03 (RR=4.2, P=.02), and -DQA1*05 (RR=3.1, P=.03) were significantly increased, but HLA-DQA1*01 (RR=0.2, P<.004) and -DQB1*06 (RR=0.2, P=.09) were decreased in the group nonresponsive to corticosteroid therapy, compared with the controls. The distribution of HLA-DQA1*01 (P<.04), -DQB1*06 (P<.02), and -DQA1*03 (P<.003) was significantly different between the responsive and the nonresponsive groups. HLA-DQA1 allelic combination analysis showed that the frequencies of DQA1*03 and *05 had a high RR value in patients with sudden sensorineural hearing loss (RR=4.1, P<.001) and in patients in the nonresponsive group (RR=8.9, P<.001), compared with the controls.

Conclusion: The presence of HLA class II alleles may be a useful genetic marker in forecasting a prognosis in Korean patients with sudden sensorineural hearing loss.


Sudden sensorineural hearing loss (SNHL) is a partial or complete, typically unilateral hearing loss characterized by a rapidity of onset or a progression that may occur within a few moments or for a few days. Sudden SNHL has numerous possible causes, including viral infection, vascular occlusion, cochlear membrane breaks, ototoxic drugs, and bacterial infection. Autoimmunity or immunologic disorders have been suggested as possible causes of idiopathic progressive SNHL; however, the role of genetic factors in the pathogenesis has not been studied. Many patients with sudden and rapidly progressive SNHL have circulating cross-reacting antibodies and benefit from glucocorticoid therapy. Treatment of sudden SNHL should be focused on its cause, although when a cause cannot be found, the treatment regimen is much more controversial. The specific mechanism of corticosteroid therapy in the treatment of sudden SNHL is unknown; however, it may improve infectious, inflammatory, and immune-mediated conditions.

Since autoimmune SNHL was first reported by McCabe in 1979, some of the previously ill-defined inner ear diseases, such as Meniere disease, otosclerosis, and idiopathic progressive SNHL, have been believed to be associated with autoimmune. Some autoimmune diseases can be affected by inherited HLA alleles. Identification of the exact locus within the HLA responsible for suspected autoimmune disease susceptibilities has become important in diagnosing and understanding the pathogenesis of the disease. Several genes lo-
PATIENTS AND METHODS

PATIENTS

Forty-one patients with sudden SNHL were included in this study. Sudden SNHL was defined as hearing loss that is greater than 30 dB in 3 contiguous frequencies, occurring within 3 days, and in most cases within several hours. Patients underwent general physical and otologic examinations; audiological testing, including pure tone audiometry, speech audiometry, and auditory brainstem response; routine laboratory tests; and magnetic resonance imaging studies of the auditory canal and temporal bone. No cause for the hearing loss was identified.

These patients had been admitted to the otolaryngology department at St Mary’s Hospital in Seoul, Korea, between March 1995 and December 1998. They consisted of 24 men and 17 women (mean age, 49.2 years; range, 21-74 years). Following the diagnosis of sudden SNHL, treatment was started with oral corticosteroid therapy, low molecular weight dextran infusion, and oral vasodilator. For corticosteroid therapy, 1 to 2 mg/kg of body weight per day (usual dose, 60 mg) of prednisone was given for 6 days and tapered for another 4 to 6 days. Additional corticosteroid therapy in a similar manner was recommended to patients who did not display beneficial results to initial dosages. Administration of oral nicotinic acid was started in a daily dose of 150 to 200 mg and increased until facial flushing appeared.

Audiometric examinations were performed every other day during hospitalization and at subsequent visits. Two hundred six randomly selected Korean subjects with no history of hearing loss, ear disease, or immune-mediated systemic disorders and without a family history of hereditary hearing impairment were used as controls. The control subjects consisted of 160 men and 46 women (mean age, 25 years; range, 23-27 years).

HLA GENOTYPING

DNA was extracted from heparinized blood samples by the salting out method, and class II typing was performed by the polymerase chain reaction sequence-specific oligonucleotide probes method. The method was essentially the same as that described at the 12th International Histocompatibility Conference (D. Charron and R. Frauchet, written communication, June 1996), with minor modifications. 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 hypervariable sequences. Stringent washing was performed in the presence of tetramethyl ammonium chloride (Sigma-Aldrich Corp, St Louis, Mo). The hybridized probe was detected according to the manufacturer’s instructions with the antidigoxigenin antibody conjugated with alkaline phosphatase, followed by the addition of chemiluminescent substrate disodium 3-(4-methoxyspiro[1,2-dioxetane-3,2’(5’-chloro)tricyclo[3.3.1.1(3,7)]decan]-4-yl) phenyl phosphate (CSPD) (Boehringer Mannheim GmbH, Mannheim, Germany). Chemiluminescence was detected by exposure to x-ray film. HLA-DRB1, -DQA1, and -DQB1 genotyping was performed in 41 patients, with HLA-DPB1 genotyping performed in 40 patients because of the absence of 1 patient’s DNA.

HEARING LEVEL

The hearing level of patients was tested from 125 to 8000 Hz in pure tone audiograms, and the result was recorded as the mean of the hearing thresholds at three speech frequencies (500, 1000, and 2000 Hz). The improvement in hearing was assessed at 4 weeks after the start of treatment according to the criteria proposed by Siegel. Patients with no improvement showed less than a 15 dB gain in hearing. Those with slight improvement had more than a 15 dB gain and a final hearing level poorer than 45 dB. Patients with partial recovery gained more than 15 dB in hearing and had a final hearing level between 25 and 45 dB. Patients with complete recovery had a final hearing level better than 25 dB, regardless of the amount of gain.

STATISTICAL ANALYSIS

Comparisons between patients and controls were analyzed by means of 2-tailed Fisher exact tests. Relative risk (RR) was calculated using the method of Woolf (Haldane’s modification was used in sets containing zero). An uncorrected P value for the number of comparisons was used, and P<.05 was considered statistically significant. Three-locus haplotypes (DRB1, DQA1, and DQB1) were assigned to all subjects on the basis of known associations. The frequency of DRB1, DQA1, and DQB1 haplotypes was compared in the patient and control groups using χ² tests.

RESULTS

INITIAL HEARING LOSS AND RECOVERY FROM HEARING LOSS

A mild hearing loss was considered to be in the range of 25 to 40 dB; moderate loss, 41 to 55 dB; moderately severe loss, 56 to 70 dB; severe loss, 71 to 90 dB; and a pro-
found loss was indicated by a threshold greater than 90 dB. Of 41 patients, 7 (17%) were classified in the mild hearing loss category, 6 (15%) in the moderate group, 16 (39%) in the moderately severe group, and 12 (29%) in the severe hearing loss group.

Evaluated according to the criteria proposed by Siegel, the overall rate of recovery among our 41 patients with sudden SNHL was 17 (41%) with no improvement, 13 (32%) with complete recovery from hearing loss, 6 (15%) with partial recovery, and 5 (12%) with slight improvement. Overall, 24 patients (59%) achieved slight improvement of their hearing loss or better. However, we did not find a significant correlation between the level of initial hearing loss and recovery from hearing loss in patients with sudden SNHL.

ASSOCIATION BETWEEN HLA CLASS II ALLELES AND RESULTS OF TREATMENT

The results of HLA class II genotyping in the patients with sudden SNHL and in the controls are shown in Table 1. The frequencies of HLA-DRB1, -DQA1, -DQB1, and -DPB1 alleles were not significantly different between the patients and the controls (P > .05). The frequencies of HLA-DRB1*14 (RR = 2.6), -DQA1*04 (RR = 2.1), -DQA1*05 (RR = 1.8), -DQB1*04 (RR = 2.2), -DPB1*0501 (RR = 2.0), and -DPB1*1701 (RR = 2.2) were higher in the patients, but these differences were not statistically significant (P > .05). Although the frequencies of HLA class II alleles were not significantly different according to severity of initial hearing loss, they were significantly different according to the degree of recovery from hearing loss (P < .05 for both).

Among the 41 patients with sudden SNHL, 24 responded well to corticosteroid treatment (RS group) and 17 did not respond to corticosteroid treatment (NRS group). When an association between the results of treatment and the presence of HLA alleles was evaluated, the frequencies of HLA-DRB1*14 (RR = 3.5, P < .02), -DQA1*03 (RR = 4.2, P < .004), and -DQA1*05 (RR = 3.1, P < .03) were significantly increased and the frequencies of HLA-DRB1*14-DQA1*05 haplotype was significantly increased only in the NRS group, compared with the controls. The frequencies of HLA-DQA1*01 (P < .04) and -DQB1*06 (P < .02) were significantly increased, and the frequency of HLA-DQA1*03 (P < .003) was significantly decreased in the RS group, compared with the NRS group.

The frequency of the HLA-DRB1*04 allele was higher in the NRS group compared with the RS group, but it was not statistically significant (P > .05). A significant association between HLA-DPB1 alleles and the results of corticosteroid therapy in patients with sudden SNHL was not found (P > .05). The frequency of HLA-DPB1*1701 was higher in the NRS group (RR = 4.6) compared with the controls, although it was not statistically significant (P > .05) (Table 1). HLA-DQA1 allelic combination analysis revealed that the genotype frequencies of -DQA1*03 and *05 had a high RR value in patients with sudden SNHL (RR = 4.1, P < .003) and in the NRS group (RR = 8.9, P < .001), compared with the controls. In addition, the genotype frequencies of -DQA1*03 and *05 were significantly different between the NRS group and the RS group (P < .03) (Table 2).

HAPLOTYPE

The frequency of the HLA-DRB1*14-DQA1*05-DQB1*03 haplotype was significantly increased in patients with sudden SNHL (RR = 1.6, P < .04) and in the NRS group (RR = 4.6, P < .03) compared with the controls, but it was not increased in the RS group (P > .05). The frequency of the DRB1*04-DQA1*03-DQB1*03 haplotype was significantly increased only in the NRS group (RR = 5.8, P < .002); however, the frequency of the DRB1*02-DQA1*01-DQB1*06 haplotype was signifi-
cantly decreased only in the NRS group (RR=0.2, P<.04) compared with the controls. In a comparison of the RS group and the NRS group, the frequency of the DRB1*02-DQA1*01-DQB1*06 haplotype was significantly increased in the RS group compared with the NRS group (29.2% vs 0%, P<.02), and the frequency of the DRB1*14-DQA1*05-DQB1*03 haplotype was significantly decreased in the RS group compared with the NRS group (0% vs 23.5%, P<.03) (Table 3).

**COMMENT**

Wilkins and associates treated patients with idiopathic sudden SNHL with a "shotgun" regimen consisting of corticosteroids, vasodilators, carbogen inhalation, dextran, histamine, and diuretics. Despite the inconsistent results of corticosteroid treatment seen in early studies, corticosteroids in moderate doses have become the most widely accepted treatment for idiopathic sudden SNHL. In the present study, patients received oral corticosteroids and vasodilators for a 10- to 12-day course, and none showed serious harmful effects from the drugs. Wilson et al reported that 78% of patients treated with corticosteroid experienced complete or partial recovery, and a similar study also showed a significantly improved recovery rate in a group treated with corticosteroids compared with a nontreated group. However, with the shotgun regimen, Wilkins et al did not achieve better results than the spontaneous recovery that is reported in the literature. Fifty-nine percent of our patients showed slight improvement or better, and this is low compared with the results described in earlier reports. Possible explanations why treatment in this study was not as effective may be the older age of our patients (mean age, 49.2 years) and the more severe levels of initial hearing loss (68% with hearing levels poorer than 56 dB).

In the present study, low molecular weight dextran infusion and oral vasodilators were given to patients to try to improve cochlear blood flow. Redleaf et al noted that 74% of patients with sudden SNHL showed improvement with dextran treatment, but hearing improvement by oral vasodilator treatment alone was not reported. In this study, treatment with oral corticosteroids, low-molecular-weight dextran, and vasodilators did not show significant improvement of hearing, compared with corticosteroid treatment alone or no treatment (P>.05).

Among the possible mechanisms of HLA class II alleles and disease association, inner ear autoimmunity may be related to the phenomenon of molecular mimicry. The shared structure between an individual pathogen and particular class II molecules may serve as a cross-reactive autoantigen and lead to an immune reaction in the inner ear. In this study, there was no significant alteration in HLA class II alleles in the patients with sudden SNHL compared with the controls. The association of HLA with the response to corticosteroid therapy has been reported in some autoimmune diseases, including idiopathic thrombocytopenic purpura, nephrotic syndrome, and giant cell arteritis. In Japanese pediatric patients with corticosteroid-sensitive nephrotic syndrome, DQA1*0103 was significantly lower than in the controls (RR=0.19, P<.04), whereas DQB1*0302 was increased. Our results suggest that the presence of HLA-DRB1*14, -DQA1*03, and -DQB1*05 alleles is associated with a poor recovery from sudden SNHL, and that the presence of HLA-DQA1*01 and -DQB1*06 alleles forecasts a good prognosis in Korean patients with sudden SNHL. HLA-DQA1*03 is the most prominent allele associated with poor recovery from hearing loss in Koreans with sudden SNHL. Significantly higher frequencies of HLA-DQA1*01 and -DQB1*06 and a significantly lower frequency of -DQA1*03 were observed in the RS group, compared with the NRS group. However, no significant association between HLA-DPB1 alleles and the results of corticosteroid therapy in the patients was found. No as-

**Table 2. Frequencies of DQA1*03 and *05 Alleles in Patients With Sudden SNHL and in Healthy Controls**

<table>
<thead>
<tr>
<th>HLA-DQA1</th>
<th>Sudden SNHL (n = 41)</th>
<th>RS (n = 24)</th>
<th>NRS (n = 17)</th>
<th>Controls (n = 206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*03 or *05</td>
<td>10 (24.4)†</td>
<td>3 (12.5)‡</td>
<td>7 (41.2)§</td>
<td>15 (7.3)</td>
</tr>
<tr>
<td>*03 or *05</td>
<td>37 (90.2)</td>
<td>22 (91.7)</td>
<td>15 (88.2)</td>
<td>178 (23.8)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage). Sudden SNHL indicates all patients with sudden sensorineural hearing loss; RS, patients with sudden SNHL with good response to corticosteroid therapy; NRS, patients with sudden SNHL nonresponsive to corticosteroid therapy; and RR, relative risk.
†P<.003, RR = 4.1 vs controls.
‡P<.03 vs NRS group.
§P<.001, RR = 8.9 vs controls.

**Table 3. HLA Class II Haplotypes (HLA-DRB1, -DQA1, and -DQB1) Showing Significant Association With Sudden SNHL**

<table>
<thead>
<tr>
<th>HLA Class II Haplotypes</th>
<th>Sudden SNHL (n = 41)</th>
<th>RS (n = 24)</th>
<th>NRS (n = 17)</th>
<th>Controls (n = 206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1<em>02-DQA1</em>01-DQB1*06</td>
<td>7 (17.1)</td>
<td>7 (29.2)†</td>
<td>0‡</td>
<td>40 (19.4)</td>
</tr>
<tr>
<td>DRB1<em>04-DQA1</em>03-DQB1*03</td>
<td>12 (29.3)</td>
<td>5 (20.8)</td>
<td>7 (41.2)§</td>
<td>49 (23.8)</td>
</tr>
<tr>
<td>DRB1<em>14-DQA1</em>05-DQB1*03</td>
<td>4 (9.8)</td>
<td>0‡</td>
<td>4 (23.5)§</td>
<td>13 (6.3)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage). Sudden SNHL indicates all patients with sudden sensorineural hearing loss; RS, patients with sudden SNHL with good response to corticosteroid therapy; NRS, patients with sudden SNHL nonresponsive to corticosteroid therapy; and RR, relative risk.
†P<.02 vs NRS group.
‡P<.04, RR = .22 vs controls.
§P<.002, RR = 5.8 vs controls.
¶P<.04, RR = 1.6 vs controls.
#P<.03, RR = 4.6 vs controls.
association with HLA-DP alleles was observed, which narrows the disease susceptibility region that is genetically involved to the DR-DQ region. Interestingly, the presence of DQA1*03 and DQA1*05 alleles in the NRS group showed the highest RR values. In type 1 diabetes mellitus, which has been extensively studied for HLA association, susceptibility or protection is known to be primarily associated with given DQ heterodimers. In this study, the HLA-DRB1*04-DQA1*03-DQB1*03 and DRB1*14-DQA1*05-DQB1*03 haplotypes were associated with nonresponsiveness to corticosteroid therapy in the patients with sudden SNHL. In particular, DRB1*04-DQA1*03-DQB1*03 showed more significant and higher RR values than did DQA1*03 in the NRS group, whereas the DRB1*02-DQA1*01-DQB1*06 haplotype was not present in the NRS group. It is interesting that the distributions of HLA alleles are different relative to the response to corticosteroid therapy in patients with sudden SNHL. Rauzy et al studied 41 patients with giant cell arteritis and found it to be associated with HLA-DRB1*04. Also, they reported that the association between giant cell arteritis and HLA-DRB1*04 appears to be accompanied by corticosteroid resistance. Therefore, our results suggest the existence of an immune-mediated response in the inner ear as a possible etiopathogenic factor. In addition, these results suggest that genetic studies of HLA can predict the response of some diseases to corticosteroid treatment.

This study showed that genetically determined factors may affect the course of sudden SNHL. However, our findings should be considered preliminary because of possible differences in HLA status between Koreans and other ethnicities with sudden SNHL. Further studies with a larger number of patients in different populations may better reveal the immunogenetic background of sudden SNHL. In addition to the high resolution typing of the HLA class II alleles, an extensive molecular approach incorporating the gene scan method will provide more useful information for defining the genetic factors associated with responsiveness to corticosteroid therapy or the progression of sudden SNHL.

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