Use of PCR with Sequence-specific Primers for High-Resolution Human Leukocyte Antigen Typing of Patients with Narcolepsy

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Background: Narcolepsy is a neurologic disorder characterized by excessive daytime sleepiness, symptoms of abnormal rapid eye movement (REM) sleep, and a strong association with HLA-DRB1*1501, -DQA1*0102, and -DQB1*0602. Here, we investigated the clinico-physical characteristics of Korean patients with narcolepsy, their HLA types, and the clinical utility of high-resolution PCR with sequence-specific primers (PCR-SSP) as a simple typing method for identifying DRB1*15/16, DQA1, and DQB1 alleles.

Methods: The study population consisted of 67 consecutively enrolled patients having unexplained daytime sleepiness and diagnosed narcolepsy based on clinical and neurologic findings. Clinical data and the results of the multiple sleep latency test and polysomnography were reviewed, and HLA typing was performed using both high-resolution PCR-SSP and sequence-based typing (SBT).

Results: The 44 narcolepsy patients with cataplexy displayed significantly higher frequencies of DRB1*1501 (Pc=0.003), DQA1*0102 (Pc=0.001), and DQB1*0602 (Pc=0.014) than the patients without cataplexy. Among patients carrying DRB1*1501-DQB1*0602 or DQA1*0102, the frequencies of a mean REM sleep latency of less than 20 min in nocturnal polysomnography and clinical findings, including sleep paralysis and hypnagogic hallucination were significantly higher. SBT and PCR-SSP showed 100% concordance for high-resolution typing of DRB1*15/16 alleles and DQA1 and DQB1 loci.

Conclusions: The clinical characteristics and somnographic findings of narcolepsy patients were associated with specific HLA alleles, including DRB1*1501, DQA1*0102, and DQB1*0602. Application of high-resolution PCR-SSP, a reliable and simple method, for both allele- and locus-specific HLA typing of DRB1*15/16, DQA1, and DQB1 would be useful for characterizing clinical status among subjects with narcolepsy.

Key Words: HLA, Genotype, Narcolepsy, Cataplexy
to be primarily associated with alleles of the linked DQ loci (i.e., DQB1*0602 and DQA1*0102), while DR2 association is a secondary effect [4, 5]. A higher prevalence of DQB1*0602 homozygotes and a stronger correlation of this genotype with narcolepsy were observed in the Japanese compared to those in African-Americans and white Americans [1]. DQB1*0602 has been shown to be closely associated with the presence of cataplexy. Previous studies have demonstrated that the presence of DQB1*0602 is reduced among patients in whom cataplexy is absent or ill defined [6, 7]. Differences in the frequency of certain HLA alleles have been observed in previous reports, and these differences have been suggested to be associated with ethnic origin and other factors, including the diagnostic criteria used to identify narcolepsy [2, 8, 9]. More specifically, the frequency and influence of DR2 has been reported to differ according to ethnic origin and the resolution of the HLA typing method. DRB1*1501 has been shown to be a risk factor, while DRB1*1502 has been shown to be a protective factor against the development of narcolepsy [7].

Based on these reports, it is becoming increasingly important to utilize high-resolution molecular techniques, such as sequence-based typing (SBT), to determine HLA types at the allelic level in narcolepsy patients. SBT is a method that can identify all sequence variations as well as new polymorphisms. This method has been regarded as the standard method for HLA typing at the allelic level. However, SBT has several disadvantages, including high equipment costs, time and labor intensiveness, the need for experts, perceived complexity, and occasional inaccuracy [10]. Low to intermediate resolution PCR-reverse sequence-specific oligonucleotide hybridization (PCR-SSO) and PCR with sequence-specific primers (PCR-SSP) have been used for HLA typing at the serological level in clinical settings. These methods have replaced conventional cytotoxicity-based HLA typing methods; however, these methods have been applied to low to intermediate resolution typing. PCR-SSP that includes more probes and primers for the targeted genes of interest, so called high-resolution PCR-SSP, provides high resolution at the allelic level, a relatively short turn-around time, less complexity in both process and interpretation, and requires fewer resources. Therefore, high-resolution PCR-SSP could be a useful and practical method for the detection of a limited number of target alleles related to specific diseases, including narcolepsy.

In this study, we aimed to make use of high-resolution PCR-SSP to analyze the frequencies of DRB1, DQA1, and DQB1 polymorphisms among patients with narcolepsy in Korea and to investigate the association between HLA allele types and clinical features of the disorder, and thus, demonstrate the usefulness of this simple PCR-SSP method for the clinical assessment of narcolepsy patients.

**METHODS**

1. **Patients and controls**
The 67 consecutive Korean patients diagnosed with narcolepsy according to the results of clinical and neurological examinations at the sleep disorder clinic of Samsung Medical Center between July 2009 and January 2010 were enrolled in this study. Written informed consent was obtained from every patient or legal guardian, and the study was approved by the Institutional Ethics Committee. Clinical data, including onset age, the presence or absence of cataplexy, associated features (e.g., sleep paralysis and hypnagogic hallucinations), and the results of the multiple sleep latency test (MSLT) and nocturnal polysomnography were reviewed. The results of polysomnography were available for 64 patients, and the results of the MSLT were available for all 67 subjects. The patients were categorized according to the International Classification of Sleep Disorders (ICSD)-2 [11] into 2 groups: (1) narcolepsy with cataplexy and (2) narcolepsy without cataplexy but with a mean sleep latency of less than or equal to 8 min and the presence of 2 or more sleep-onset REM periods (SOREMPs) during the MSLT. High-resolution PCR-SSP was used to type the HLA-DRB1*1501 and DQB1 alleles in all 67 patients. Simultaneously, SBT was used to type the DRB1 and DQB1 alleles in all 67 patients. For DQA1, 34 patients were typed using only SBT, and 33 were typed using both PCR-SSP and SBT.

In order to compare the results in patients with narcolepsy with the results in the general population, the prevalence of HLA-DRB1, DQA1, and DQB1 was assessed among 467 randomly selected control participants from previous studies performed in Korea [12, 13].

2. **DNA extraction**
Genomic DNA was extracted from 200 μL of an EDTA whole blood sample using a QuickGene DNA whole blood kit S and QuickGene-Mini80 (Fujifilm Corp., Tokyo, Japan) according to the manufacturer’s instructions. This extracted DNA was used for both high-resolution PCR-SSP and SBT. The concentration and purity of the DNA samples were measured as the optical density at 260 nm and the ratio of the optical densities at 260/280 nm, respectively, using a NanoDrop (Thermo Fisher Scientific, Wilmington, DE, USA). The required concentration and purity were 20-50 ng/μL and 1.7-1.9, respectively.
3. SBT
To type DRB1 and DQB1, genomic DNA was amplified using SeCore® Sequencing Kits (Invitrogen Corp., Madison, WI, USA). Master mix (20 μL) containing either DRB1- or DQB1-locus specific primers and FastStart™ Taq DNA polymerase (Roche Diagnostics Korea, Seoul, Korea) was added to the DNA sample (5 μL). The same volume of master mix was added to 5 μL of deionized water as a negative control. The DNA amplification was performed according to the program parameters in an ABI 9700 thermal cycler (Applied Biosystems Inc., Foster City, CA, USA). After the primary amplification, the PCR products were sequenced using 3 separate sequencing primers for exon 2 of DRB1 (forward, reverse, and codon 86), and 2 separate sequencing primer pairs for DQB1 (forward and reverse of both exon 2 and exon 3). After 25 cycles of the sequencing reaction, the DNA was purified, denatured, and sequenced using an ABI 3130 genetic analyzer (Applied Biosystems). Sequence data were analyzed using Assign-SBT™ 3.5+ software (Conexio Genomics, Fremantle, Western Australia, Australia).

Genotyping of the DQA1 locus was performed using the method previously described by Voorter et al. [14]. In total, 16 separate sequencing and amplification primers (forward and reverse of exons 1-4) were used. DNA amplification was performed in an ABI 9700 thermal cycler (Applied Biosystems). For the sequencing reactions, a solid phase sequencing approach was performed using an Autoread Sequencing kit (Amersham Biosciences, Roosendaal, Netherlands) according to the supplier’s protocol. Sequence data were analyzed using Pharmacia Typing Software (Pharmacia Biotech, Uppsala, Sweden).

4. High-resolution PCR-SSP
High-resolution PCR-SSP was performed using AllSet+™ Gold SSP Typing Kits (Invitrogen Corp., Madison, WI, USA). High-resolution PCR-SSP provided allelic-level genotyping of DRB1*15/16 and locus-specific allelic-level genotyping of DQB1 and DQA1. A master mix containing dNTPs, gel loading buffer, and Taq DNA polymerase was added to the contamination control tube and the wells of the AllSet+™ Gold SSP tray containing allele- and/or group-specific primers (23, 28, and 31 pairs for DRB1*15/16, DQA1 and DQB1, respectively) with a control primer pair matching the non-allelic sequence. Genomic DNA (2 μL) was added to each well, and DNA amplification was performed according to the program parameters using an ABI 9700 thermal cycler. The PCR products were transferred to 1.8% agarose gels containing ethidium bromide, and electrophoresed for 10 min at 15 V/cm. The positive lanes were visually determined and analyzed using UniMatch Software 4.0 (Invitrogen Corp., Madison, WI, USA).

5. Statistical analysis
Data were analyzed by sorting patients according to the presence or absence of cataplexy, sleep paralysis, hypnagogic hallucinations, the results of polysomnography and MSLT based on ICSD-1 and -2 criteria [11], and HLA types. Categorical variables were used for the frequency analysis. Significance testing was conducted using Fisher’s exact test for independence. P-values were corrected using the Bonferroni method (Pc) by multiplying the probability value by the number of alleles compared. P values less than 0.05 were considered statistically significant. The frequency and concordance for HLA alleles were compared for both the SBT and high-resolution PCR-SSP methods. The HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotype, sleep paralysis, hypnagogic hallucinations, and a mean REM sleep latency less than 20 min and a mean sleep latency less than 5 min based on ICSD-1 and -2 criteria were included as confounding variables in the multiple logistic regression. Haplotypes were constructed by giving priority to combinations that showed higher association in previously reported Korean data [15]. The test for Hardy-Weinberg equilibrium (HWE) in control subjects was performed with a permutation test using SAS/Genetics version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS
1. The frequency of HLA phenotypes in patients with narcolepsy
Among the 67 patients, there were 51 males and 16 females. The median age of the subjects was 34.8 yr (range: 13-69 yr). Of the 67 patients, 44 (65.7%) were diagnosed to have narcolepsy with cataplexy, and 23 (34.3%) were diagnosed to have narcolepsy without cataplexy based on the ICSD-2 criteria.

Phenotype frequencies of HLA alleles in controls and narcolepsy patients are shown in Table 1. HLA-DRB1*1501, DQA1*0102, and DQB1*0602 were present in 46 (68.7%), 48 (71.6%), and 46 (68.7%) patients with narcolepsy, respectively, regardless of having cataplexy. All patients with DRB1*1501 were also typed as DQB1*0602, and DRB1*1501 and DQB1*0602 were more frequently detected in patients with cataplexy than in patients without cataplexy (86.4% vs. 34.8%, P<0.003 for DRB1*1501 and P<0.001 for DQB1*0602). The frequency of DQA1*0102 was also significantly higher in patients with cataplexy than in patients without cataplexy (86.4% vs. 43.5%, P<0.014). In healthy controls, the frequencies of DRB1*1501 (14.8%) and DQB1*0602 (14.1%) were significantly lower than the frequen-
Table 1. Phenotype frequencies of HLA class II alleles in controls and narcolepsy patients with or without cataplexy

| Allele    | Control, N = 467 | Narcolepsy with cataplexy, N = 44 | Narcolepsy without cataplexy, N = 23 |
|-----------|------------------|-----------------------------------|-------------------------------------------|
|           | N (%)            | N (%)                             | Pc-value†                                  |
| DRB1*0101 | 58 (12.4)        | 1 (2.3)                           |                                           |
| DRB1*0301 | 28 (6.0)         | 2 (4.5)                           | 1 (4.3)                                   |
| DRB1*0401 | 7 (1.5)          | 0 (0)                             | 1 (4.3)                                   |
| DRB1*0403 | 29 (6.2)         | 1 (2.3)                           | 1 (4.3)                                   |
| DRB1*0404 | 19 (4.1)         | 0 (0)                             | 1 (4.3)                                   |
| DRB1*0405 | 81 (17.3)        | 3 (6.8)                           | 3 (13.0)                                  |
| DRB1*0406 | 38 (8.1)         | 2 (4.5)                           | 1 (4.3)                                   |
| DRB1*0407 | 6 (1.3)          | 1 (2.3)                           | 0 (0)                                     |
| DRB1*0408 | 1 (0.2)          | 0 (0)                             | 1 (4.3)                                   |
| DRB1*0410 | 12 (2.6)         | 1 (2.3)                           | 0 (0)                                     |
| DRB1*0701 | 62 (13.3)        | 2 (4.5)                           | 3 (13.0)                                  |
| DRB1*0802 | 18 (3.9)         | 0 (0)                             | 2 (8.7)                                   |
| DRB1*0803 | 73 (15.6)        | 2 (4.5)                           | 4 (17.4)                                  |
| DRB1*0901 | 83 (17.8)        | 6 (13.6)                          | 2 (8.7)                                   |
| DRB1*1001 | 14 (3.0)         | 0 (0)                             | 0 (0)                                     |
| DRB1*1101 | 41 (8.8)         | 2 (4.5)                           | 1 (4.3)                                   |
| DRB1*1106 | 1 (0.2)          | 0 (0)                             | 0 (0)                                     |
| DRB1*1201 | 30 (6.4)         | 5 (11.4)                          | 4 (17.4)                                  |
| DRB1*1202 | 30 (6.4)         | 3 (6.8)                           | 1 (4.3)                                   |
| DRB1*1205 | 0 (0)            | 1 (2.3)                           | 0 (0)                                     |
| DRB1*1301 | 9 (1.9)          | 0 (0)                             | 1 (4.3)                                   |
| DRB1*1302 | 78 (16.7)        | 6 (13.6)                          | 2 (8.7)                                   |
| DRB1*1307 | 0 (0)            | 1 (2.3)                           | 0 (0)                                     |
| DRB1*1401 | 28 (6.0)         | 3 (6.8)                           | 1 (4.3)                                   |
| DRB1*1404 | 0 (0)            | 1 (2.3)                           | 0 (0)                                     |
| DRB1*1405 | 32 (6.9)         | 0 (0)                             | 1 (4.3)                                   |
| DRB1*1406 | 7 (1.5)          | 0 (0)                             | 0 (0)                                     |
| DRB1*1407 | 2 (0.4)          | 0 (0)                             | 1 (4.3)                                   |
| DRB1*1410 | 2 (0.4)          | 0 (0)                             | 0 (0)                                     |
| DRB1*1412 | 2 (0.4)          | 0 (0)                             | 0 (0)                                     |
| DRB1*1501 | 69 (14.8)        | 38 (86.4)                         | <0.001                                    |
| DRB1*1502 | 32 (6.9)         | 2 (4.5)                           | 2 (8.7)                                   |
| DRB1*1602 | 6 (1.3)          | 1 (2.3)                           | 0 (0)                                     |
| DQA1*0101 | 63 (13.5)        | 1 (2.3)                           | 0 (0)                                     |
| DQA1*0102 | 142 (30.4)       | 38 (86.4)                         | <0.001                                    |
| DQA1*0103 | 94 (20.3)        | 4 (9.1)                           | 6 (26.1)                                  |
| DQA1*0104 | 58 (12.4)        | 4 (9.1)                           | 3 (13.0)                                  |
| DQA1*0105 | 14 (3.0)         | 0 (0)                             | 0 (0)                                     |
| DQA1*0201 | 62 (13.3)        | 2 (4.5)                           | 3 (13.0)                                  |
| DQA1*0301 | 85 (18.2)        | 4 (9.1)                           | 3 (13.0)                                  |

*(Continued to the next page)*
cies among narcolepsy patients with cataplexy (both, \( P_c<0.001 \)). DQA1*0102 was detected in 30.4% of the controls, which was also significantly lower than the percentage observed in narcolepsy patients with cataplexy (\( P_c<0.001 \)). Compared with the control group, higher frequencies of DRB1*1501, DQA1*0102, and DRB1*0602 were observed among narcolepsy patients without cataplexy; however, this effect did not reach statistical significance. In addition, differences in the frequencies of all other alleles except DRB1*1501, DQB1*0602, and DQA1*0102 were also observed among the groups; however, none of these differences reached statistical significance between the controls and narcolepsy patients with or without cataplexy (Table 1).

Table 1. (Continued from the previous page) Phenotype frequencies of HLA class II alleles in controls and narcolepsy patients with or without cataplexy

|                | Control*, N = 467 | Narcolepsy with cataplexy, N = 44 | Narcolepsy without cataplexy, N = 23 |
|----------------|------------------|-----------------------------------|-------------------------------------|
|                | N (%)            | N (%)                             | \( P_c\)-value\*† OR\* (95% CI)     | N (%)                             | \( P_c\)-value\*‡ OR\* (95% CI)     |
| DQA1*0302      | 93 (19.9)        | 6 (13.6)                          |                                    | 3 (13.0)                          |                                    |
| DQA1*0303      | 110 (23.6)       | 4 (9.1)                           |                                    | 5 (21.7)                          |                                    |
| DQA1*0401      | 15 (3.2)         | 1 (2.3)                           |                                    | 2 (8.7)                           |                                    |
| DQA1*0501      | 28 (6.0)         | 2 (4.5)                           |                                    | 1 (4.3)                           |                                    |
| DQA1*0503      | 11 (2.4)         | 1 (2.3)                           |                                    | 0 (0)                             |                                    |
| DQA1*0505      | 55 (11.8)        | 6 (13.6)                          |                                    | 2 (8.7)                           |                                    |
| DQA1*0506      | 1 (0.2)          | 1 (2.3)                           |                                    | 1 (4.3)                           |                                    |
| DQA1*0507      | 6 (1.3)          | 0 (0)                             |                                    | 0 (0)                             |                                    |
| DQA1*0508      | 7 (1.5)          | 0 (0)                             |                                    | 1 (4.3)                           |                                    |
| DQA1*0601      | 40 (8.6)         | 3 (6.8)                           |                                    | 1 (4.3)                           |                                    |
| DB1*0201       | 28 (6.0)         | 2 (4.5)                           |                                    | 1 (4.3)                           |                                    |
| DB1*0202       | 57 (12.2)        | 2 (4.5)                           |                                    | 3 (13.0)                          |                                    |
| DB1*0301       | 116 (24.8)       | 11 (25.0)                         |                                    | 7 (30.4)                          |                                    |
| DB1*0302       | 92 (19.7)        | 4 (9.1)                           |                                    | 4 (17.4)                          |                                    |
| DB1*0303       | 99 (21.2)        | 6 (13.6)                          |                                    | 3 (13.0)                          |                                    |
| DB1*0401       | 78 (16.7)        | 3 (6.8)                           |                                    | 3 (13.0)                          |                                    |
| DB1*0402       | 34 (7.3)         | 2 (4.5)                           |                                    | 2 (8.7)                           |                                    |
| DB1*0501       | 75 (16.1)        | 1 (2.3)                           |                                    | 2 (8.7)                           |                                    |
| DB1*0502       | 21 (4.5)         | 2 (4.5)                           |                                    | 1 (4.3)                           |                                    |
| DB1*0503       | 44 (9.4)         | 3 (6.8)                           |                                    | 2 (8.7)                           |                                    |
| DB1*0601       | 87 (18.6)        | 4 (9.1)                           |                                    | 6 (26.1)                          |                                    |
| DB1*0602       | 66 (14.1)        | 38 (86.4)                         | \(<0.001\)                         | 38.5 (8.8-300.6)                  | 8 (34.8)                          |
| DB1*0603       | 9 (1.9)          | 0 (0)                             |                                    | 0 (0)                             |                                    |
| DB1*0604       | 45 (9.6)         | 3 (6.8)                           |                                    | 2 (8.7)                           |                                    |
| DB1*0609       | 35 (7.5)         | 3 (6.8)                           |                                    | 1 (4.3)                           |                                    |

\*Pc-values greater than 0.05 are not displayed.

*The control data were obtained from previously published studies in Korea [12-13]; †Pc-value and OR from comparison of narcolepsy with cataplexy group to the control group; ‡Pc-value and OR from comparison of narcolepsy with cataplexy group to narcolepsy without cataplexy group.

Abbreviations: OR, odds ratio; Pc-value, Bonferroni corrected \( P \) value.

All individuals who were homozygous for DRBI*1501 or DQB1*0602 were DRBI*1501-DQBI*0602 homozygotes, and all DRBI*1501-DQBI*0602 homozygotes were also DQA1*0102 homozygotes. There were 5 patients (7.5%) who were homozygous for both DRBI*1501 and DQBI*0602, and 4 of these patients (80%) had cataplexy. There were 13 patients (19.4%) who were homozygous for DQA1*0102, and these patients also had cataplexy more frequently than DQA1*0102 heterozygotes, although this difference did not reach statistical significance (25.0% vs. 8.7%, \( P=0.191 \)). Only 2 control individuals (0.4%) were DRBI*1501-DQA1*0102-DQBI*0602 homozygotes.

In the 69 control subjects with DRBI*1501-DQA1*0102-DQBI*0602...
2. High-resolution molecular typing of narcolepsy-associated HLA alleles

There were no discordant results observed in the DRB1*15/16 and DQB1 typing of the 67 patients and the DQA1 typing of 33 patients using the SBT and high-resolution PCR-SSP methods. Using the high-resolution PCR-SSP technique, DRB1*1501, DRB1*1502, and DRB1*1602 alleles were easily distinguished according to their band patterns. A total of 14 DQB1 and 15 DQA1 alleles, including DQB1*0602 and DQA1*0102, which have been shown to be associated with narcolepsy, were correctly identified. During an initial interpretation, one SBT and one PCR-SSP result for DQA1 were incorrectly assigned; however, these initial assignments were corrected after a final interpretation. In one case, DQA1*0401 was assigned as DQA1*0102 during the initial reading because a weak positive band was ignored in the PCR-SSP. The other was an incorrect assignment of DQA1*0506 as DQA1*0503, due to an overlapped peak not recognized in the SBT. In addition to the comparable performance of PCR-SSP and SBT, the PCR-SSP method saved not only significant time, since the testing time is less than 3 hr compared to more than 6 hr for the SBT method, but also resources, such as an expensive sequencing analyzer and labor for testing and interpretation.

3. Clinico-physical and somnographic characteristics of narcolepsy patients

Sleep paralysis and hypnagogic hallucinations were observed in 52.2% and 37.3% of patients, respectively. The frequencies of sleep paralysis and hypnagogic hallucinations were significantly higher among patients with cataplexy than among patients without cataplexy (63.6% vs. 30.4%, P=0.010 for sleep paralysis;
TABLE 3. Demographic, clinical, and somnographic characteristics of narcolepsy patients according to HLA types

|                  | DRB1*1501* | DQA1*0102* |
|------------------|------------|------------|
|                  | Pos | Neg | P value | OR (95% CI) | Pos | Neg | P value | OR (95% CI) |
| Total, N (%)     | 46 (68.7) | 21 (31.3) | 48 (71.6) | 19 (28.4) |
| M : F ratio      | 34 : 12 | 17 : 4 | 0.758 | 0.67 (0.19-2.38) | 35 : 13 | 16 : 3 | 0.526 | 0.51 (0.13-2.02) |

Clinical features

|                  | DRB1*1501* | DQA1*0102* |
|------------------|------------|------------|
| Sleep paralysis, N (%) | 29 (63.0) | 6 (28.6) | 0.017 | 4.27 (1.39-13.07) | 30 (62.5) | 5 (26.3) | 0.014 | 4.67 (1.44-15.13) |
| Hypnagogic hallucinations, N (%) | 23 (50.0) | 2 (9.5) | 0.002 | 9.50 (1.98-45.55) | 23 (47.9) | 2 (10.5) | 0.005 | 7.82 (1.63-37.62) |
| Nocturnal polysomnography | Mean REM sleep latency < 20 min | 42 (91.3) | 18 (85.7) | 0.669 | 1.75 (0.36-8.63) | 44 (91.7) | 16 (84.2) | 0.395 | 2.06 (0.42-10.24) |
| Sleep latency < 10 min | 35/45 (77.8) | 5/19 (26.3) | < 0.001 | 10.18 (3.03-34.19) | 35/47 (74.5) | 5/17 (29.4) | 0.003 | 7.54 (2.26-25.11) |
| Multiple sleep latency test MSL < 5 min | 42 (91.3) | 15 (71.4) | 0.060 | 4.20 (1.04-16.96) | 44 (91.7) | 13 (68.4) | 0.025 | 5.08 (1.24-20.76) |
| MSL ≤ 8 min | 45 (97.8) | 19 (90.5) | 0.229 | 4.74 (0.41-55.42) | 47 (97.9) | 17 (89.5) | 0.192 | 5.53 (0.47-64.96) |
| SOREMP ≥ 2 times | 45 (97.8) | 20 (95.2) | 0.532 | 2.25 (0.13-37.80) | 47 (97.9) | 18 (94.7) | 0.490 | 2.61 (0.16-44.01) |

*Identical to DQB1*0602, that is, all individuals were DRB1*1501-DQA1*0102-DQB1*0602. Abbreviations: Pos, positive; Neg, negative; OR, odds ratio; CI, confidence interval; REM, rapid eye movement; MSL, mean sleep latency; SOREMP, sleep-onset REM periods.

DISCUSSION

HLA-DRB1*15/*16, DQA1, and DQB1 typing was performed in narcolepsy patients using both the SBT and high-resolution PCR-SSP methods. The SBT method is used for the identification of sequence variations, as well as the direct detection of new polymorphisms [16]. However, this method is demanding in terms of the resources, technique, time, and labor required. PCR-SSP is a powerful method for detecting genetic variability, including single base pair mismatches. It is a relatively quick and easy technique, and the post-amplification processing is simple, rapid, and consists only of determining whether amplification has occurred [17, 18]. Additionally, HLA typing using PCR-SSP has been shown to be a relatively easy method with a higher detection rate than both PCR-SSO and PCR-restriction fragment length polymorphism (PCR-RFLP) methods [16-18]. Furthermore, when the PCR-SSP method was used, the results of the HLA typing were quickly obtained at a relatively low cost.

In this study, when comparing the results using the high-resolution PCR-SSP and SBT methods, there were no discordant results observed for the typing of DRB1*15/*16, DQA1, and DQB1. However, there were potential errors in both methods during the interpretation process. The weak intensity of the positive bands in the PCR-SSP method, and the small or overlapped peaks in

52.3% vs. 8.7%, P < 0.001 for hypnagogic hallucinations, Table 2). The difference in the frequency of a mean REM sleep latency less than 20 min between patients with and without cataplexy was statistically significant (72.7% vs. 40.0%, P = 0.024). In multivariable analyses, only the HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotype still maintained statistical significance (P = 0.033).

A majority of the subjects (70.5%) had onset of disorder before adolescence. Although the frequency of cataplexy was lower, and the frequencies of sleep paralysis and hypnagogic hallucinations were slightly higher among patients with onset before adolescence, these differences did not reach statistical significance (67.4% vs. 83.3%, P = 0.207 for cataplexy; 58.1% vs. 50.0%, P = 0.559 for sleep paralysis; 44.2% vs. 33.3%, P = 0.432 for hypnagogic hallucinations). The frequencies of polysomnography and the MLST diagnostic criteria were similar across groups (data not shown).

In the context of HLA type, the frequency of sleep paralysis and hypnagogic hallucinations was significantly higher among patients with DRB1*1501-DQB1*0602, (63.0% vs. 28.6%, P = 0.017 for sleep paralysis; 50.0% vs. 9.5%, P = 0.002 for hypnagogic hallucinations; Table 3). Similar results were observed among patients typed as DQA1*0102 (62.5% vs. 26.3%, P = 0.014 for sleep paralysis; 47.9% vs. 10.5%, P = 0.005 for hypnagogic hallucinations). According to the polysomnography findings, a mean REM sleep latency of less than 20 min was observed more frequently among DRB1*1501-DQB1*0602 (77.8% vs. 26.3%, P < 0.001) and DQA1*0102 (74.5% vs. 29.4%, P = 0.003) positive patients than among negative patients; furthermore, DQA1*0102 was associated with a mean sleep latency of less than 5 min (91.7% vs. 68.4%, P = 0.025).
Use of PCR-SSP HLA typing in narcolepsy

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In this study, 5 patients (7.5%) were diagnosed with narcolepsy with cataplexy when compared to the control group. Other HLA types were also suggested as being associated with narcolepsy, such as the presence of cataplexy. Previous research conducted in Korea by Hong et al. revealed significant differences in DQA1*0103 and DQB1*0301 as susceptibility alleles, and in DQA1*0103 and DQB1*0601 as protective alleles in the context of a DQA1*0102 or DQB1*0602 bearing haplotype [8]. In our study, we compared the haplotype combinations for all alleles carried in trans of DRB1*1501-DQA1*0102-DQB1*0602 in the control subjects and narcolepsy patients. However, the differences in the frequencies did not reach statistical significance. Therefore, additional large-scale studies would be needed in order to evaluate the difference in the haplotype distribution according to the presence or absence of cataplexy.

Sleep paralysis and hypnagogic hallucinations were frequently observed among narcolepsy patients with cataplexy, and a significant number of these patients had DRB1*1501, DQA1*0102, and DQB1*0602. However, we could not confirm the significance of these findings in a multivariate analysis between the presence or absence of cataplexy and other categorized variables. The clinical features associated with narcolepsy, such as sleep paralysis and hypnagogic hallucinations, have been considered to have low diagnostic value. These criteria was removed from the ICSD-2 diagnostic criteria due to insufficient specificity for narcolepsy [11, 23]. Previous studies have shown that the frequency of hypnagogic hallucinations and sleep paralysis does not appear to differ significantly between patients with and without cataplexy [20, 22, 24]. In this study, a mean REM sleep latency of less than 20 min in nocturnal polysomnography was frequently observed among narcolepsy patients with DRB1*1501, DQA1*0102, and DQB1*0602. Watson et al. has reported the associations between somnographic findings and DQB1*0602 [22]. They showed that sleep latency was significantly shorter in patients with DQB1*0601/DQB1*0501 were detected in only a few patients (3 and 1 patients, respectively); therefore, we could not assess the clinical significance. A total of 18 patients (26.9%) had DQB1*0301, which was similar to the previously reported frequency in Korean patients (27.8%); and this proportion was relatively high compared to that observed among Japanese, European-American, and African-American patients (10% to 14.9%) [1, 8]. However, there was no significant difference observed in the frequency of DQB1*0301 between the control and patient groups. With the exception of the DRB1*1501, DQB1*0602, and DQA1*0102, no significant differences in the frequency of other HLA alleles were observed between the patient and control groups, or among the patients according to the presence or absence of cataplexy.

This study provides additional population data that confirms the association of the HLA-DRB1*1501, DQA1*0102, and DQB1*0602 alleles with narcolepsy and cataplexy among Korean patients [8, 10]. In previous reports, DRB1*1501-DQB1*0602 and DQA1*0102-DQB1*0602 have been shown to display a high degree of linkage disequilibrium in the Korean population [12, 13, 15]. There were some observed differences in the frequency of DRB1*1501, DQA1*0102, and DQB1*0602 among previous studies on narcolepsy patients [2, 8, 9]. These differences have been proposed to be due to differences in the diagnostic criteria, including the presence of cataplexy, the ethnic origin of the patient groups, and the resolution of the HLA-typing method [2, 19-21]. Even the frequency of DQB1*0602 in Korean narcoleptic patients with cataplexy from previous reports and this study varied from 86.4% to 100% [9]. The relatively lower frequencies of DRB1*1501 and DQB1*0602 observed in this study compared to that observed by Hong et al. [8] and Roh et al. [9] might be due to the differences in conducting the polysomnography and the MLST or distinguishing between atypical and typical cataplexy.

Among Japanese subjects, it was determined that a significant proportion of patients with narcolepsy were typed as DRB1*1501-DQA1*0102-DQB1*0602 homozygotes (15.2% of the patients). Other HLA types were also suggested as being associated with narcolepsy, DQB1*0301 as a susceptibility allele, and DQB1*0601/DQB1*0602 and DQB1*0601/DQB1*0501 heterozygotes as having protective effects [1, 8]. In addition, the DQB1*0602 homozygote had a 2- to 4-fold increased risk of developing narcolepsy with cataplexy when compared to the heterozygote. In this study, 5 patients (15% of 33) were DRB1*1501-DQA1*0102-DQB1*0602 homozygotes, and among these patients, 4 presented with cataplexy. Heterozygous DQB1*0601/DQB1*0602 alleles with narcolepsy and cataplexy among Korean patients.
0602 than in patients without DQB1*0602 but did not evaluate mean REM sleep latency. In this study, we categorized and analyzed the somnographic findings according to ICSD-1 and -2 criteria, whereas in the previous study these were analyzed as continuous variables. Although we considered that differences in sleep latency may exist between patients with and without DRB1*1501, DQA1*0102, and DQB1*0602, this study might be not sufficient to confirm the significance of these differences due to limitation in number of patients. The relationship of HLA alleles with clinical and somnographic findings in narcolepsy patients should be confirmed through further studies.

In summary, this study confirmed the association between specific HLA alleles and narcolepsy among Korean patients using both the high-resolution PCR-SSP and SBT methods. Differences in the clinical and somnographic findings according to the presence of particular HLA alleles, as well as the presence or absence of cataplexy, were shown. In addition to the high level of concordance with SBT methods for identifying DRB1*15/16, DQA1, and DQB1 alleles, the high-resolution PCR-SSP was simple and cost-effective method for the testing of HLA-DRB1, -DQA1, and -DQB1 alleles known to be related with narcolepsy.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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