Independent strong association of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement

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Stevens-Johnson syndrome (SJS) and its severe variant, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes. Cold medicines including non-steroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient cold medications are reported to be important inciting drugs. We used two sample sets of Japanese patients to investigate the association between HLA genotypes and cold medicine-related SJS/TEN (CM-SJS/TEN), including acetaminophen-related SJS/TEN (AR-SJS/TEN) with severe mucosal involvement such as severe ocular surface complications (SOC). HLA-A*02:06 was strongly associated with CM-SJS/TEN with SOC and AR-SJS/TEN with SOC. HLA-B*44:03 was also detected as an independent risk allele for CM-, including AR-SJS/TEN with SOC. Analyses using data obtained from CM-SJS/TEN patients without SOC and patients with CM-unrelated SJS/TEN with SOC suggested that these two susceptibility alleles are involved in the development of only CM-SJS/TEN with SOC patients.
allopurinol and anticonvulsants such as carbamazepine are the main inciting drugs for SJS/TEN; we and others found that cold medicines including non-steroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient cold medications are also major causative drugs for SJS/TEN. However, there have been no reports on the association between HLA genotypes and cold medicines in patients with SCAR.

Many SJS/TEN survivors suffer severe sequelae such as visual disturbance due to severe ocular surface complications (SOC) in the acute phase of the disease. In our earlier study of 71 Japanese SJS/TEN patients we reported the strong association between HLA-A*0206 and SJS/TEN with SOC. We found that a considerable number of these patients used cold medicines to treat the common cold. Therefore, in this study we focused on a possible association between HLA genotypes and cold medicine (NSAIDs and analgesics)-related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement including SOC.

Results

HLA-type associated with CM-SJS/TEN with SOC. First we compared the carrier frequencies of HLA alleles in the 131 CM-SJS/TEN with SOC patients and in 419 controls. The results are summarized in Table 2.

| Explanation of subjects | Group 1 (KPUM) | Group 2 (NHS) |
|-------------------------|----------------|--------------|
| a | Number of SJS/TEN patients with SOC who had taken cold medicines for treatment of common cold (CM-SJS/TEN with SOC group) | 131 | 20 |
| Female/Male | 80/51 | 14/6 |
| Age of onset (years, mean ± SD) | 26.6 ± 17.5 | 54.0 ± 17.7 |
| b (which are included in a) | Number of SJS/TEN patients with SOC who had taken acetylsalicylic acid for treatment of common cold (Acetylsalicylic-SJS/TEN with SOC group) | 37/22 | 9/5 |
| Female/Male | 31.1 ± 15.8 | 35.2 ± 16.9 |
| Patients with SJS/TEN without SOC who had taken cold medicines for treatment of common cold (CM-SJS/TEN without SOC group) | 14 | 38 |
| Female/Male | 11/3 | 19/19 |
| Age of onset (years, mean ± SD) | 44.8 ± 19.3 | 57.4 ± 23.1 |
| d | the samples excluded because of drug unrelated or detail unknown | 17 | - |
| Controls | Healthy volunteers | 419 | 74 |
| Female/Male | 350/69 | 220 |
| Age (years, mean ± SD) | 25.0 | 35.5 ± 11.0 |

As we observed the same tendency in Groups 1a and 2a, we combined the 151 CM-SJS/TEN with SOC patients (Group 1a, n = 131; Group 2a, n = 20) to compare the carrier frequencies of HLA-A*0206 and HLA-B*4403 with the frequencies in the 639 combined healthy controls. (Group 1, n = 419; Group 2, n = 220). The combined data revealed a strong association of HLA-A*0206 and HLA-B*4403 with CM-SJS/TEN with SOC (HLA-A*0206, p = 2.7 × 10⁻⁶; OR = 5.6; HLA-B*4403, p = 1.25 × 10⁻³, OR = 1.99) (Table 4a).

Comparison between CM-SJS/TEN with and without SOC. Among 16 CM-SJS/TEN without SOC patients (Group 2c), 2 carried HLA-A*0206 and none carried HLA-B*4403 (Table 4b). These carrier frequencies did not differ significantly from the Group 2 controls (p = 1.000 and p = 0.2324, respectively). These results suggest that HLA-A*0206 and HLA-B*4403 are not common risk factors for both CM-SJS/TEN with and without SOC, but were risk factors for only CM-SJS/TEN with SOC.

For further confirmation we compared the carrier frequency of both HLA alleles in the 151 combined CM-SJS/TEN with SOC patients (Group 1a, n = 131, Group 2a, n = 20) and in the 16 CM-SJS/TEN without SOC patients in Group 2c. The carrier frequencies of both alleles were significantly higher in the CM-SJS/TEN with SOC (Group 1a + Group 2a) than in the CM-SJS/TEN without
Analysis of CM unrelated-SJS/TEN with SOC. As shown in Table 1, Group 1d contained 14- and Group 2d contained 38 patients with CM unrelated (other medicine related) -SJS/TEN with SOC. Among the 14 CM unrelated-SJS/TEN with SOC patients from Group 1d, 3 carried HLA-A*02:06 and 4 carried HLA-B*44:03. Among the 38 CM unrelated SJS/TEN with SOC patients from Group 2d, 4 manifested HLA-A*02:06 and 2 had HLA-B*44:03. To obtain higher power, we combined the data from the 52 CM unrelated -SJS/TEN with SOC patients from Groups 1d (n = 14) and 2d (n = 38) and compared their carrier frequency with that of combined healthy volunteers (n = 639). As shown in Table 4c, the carrier frequencies of HLA-A*02:06 and HLA-B*44:03 were comparable in the 2 groups (52 CM unrelated -SJS/TEN with SOC patients and 639 controls) and the difference was not statistically significant.

Analysis of acetaminophen-SJS/TEN with SOC (AR-SJS/TEN with SOC). Acetaminophen is contained as an analgesic in most cold medicines. At least 59 patients in Group 1b and 14 in Group 2b were known to have taken acetaminophen for a few ~ several days before the onset of SJS/TEN. Therefore we examined the association of HLA-A*02:06 and HLA-B*44:03 with acetaminophen-related SJS/TEN (AR-SJS/TEN) with SOC using the combined data (73 AR-SJS/TEN with SOC from 59 in Group 1b and 14 in Group 2b). In all 73

Table 2 | Results of association analysis for HLA types and CM-SJS/TEN with SOC in Group 1 (KPUM)

| HLA genotype | Carrier frequency (%) | Dominant model analysis |
|--------------|-----------------------|-------------------------|
|              | Case (n = 131) | Control (n = 419) | P | Pc | Odds ratio (95% CI) |
| HLA-A        |             |                        |   |    |                      |
| A*02:06      | 62/131 (47.3%) | 57/419 (13.60%) | 2.79E-16 | 4.75E-15 | 5.71 (3.666-8.881) |
| A*03:01      | 5/131 (3.82%)  | 4/419 (0.95%)   | 0.0242 | 0.412 | 4.12 (1.089-15.564) |
| A*11:01      | 10/131 (7.6%)  | 71/419 (16.95%) | 8.67E-03 | 0.147 | 0.405 (0.202-0.811) |
| A*24:02      | 57/131 (43.5%) | 256/419 (61.10%)| 3.89E-04 | 6.60E-03 | 0.490 (0.330-0.730) |
| HLA-B        |              |                        |   |    |                      |
| B*13:01      | 10/131 (7.6%)  | 13/419 (3.10%)   | 0.0237 | 0.807 | 2.58 (1.104-6.032)  |
| B*15:01      | 11/131 (8.4%)  | 69/419 (16.47%)  | 0.0222 | 0.755 | 0.465 (0.238-0.908) |
| B*44:02      | 5/131 (3.82%)  | 5/419 (1.91%)    | 0.0498 | 1.69  | 3.29 (0.936-11.532) |
| B*44:03      | 31/131 (23.7%) | 66/419 (15.75%)  | 0.0381 | 1.29  | 1.66 (0.242-6.682)  |
| B*46:01      | 22/131 (16.8%) | 38/419 (9.07%)   | 0.0133 | 0.453 | 2.02 (1.148-3.566)  |
| B*52:01      | 12/131 (9.2%)  | 79/419 (18.85%)  | 9.16E-03 | 0.311 | 0.434 (0.228-0.825) |
| B*54:01      | 10/131 (7.6%)  | 61/419 (14.56%)  | 0.0391 | 1.33  | 0.485 (0.241-0.976) |
| HLA-C        |              |                        |   |    |                      |
| C*03:04      | 42/131 (32.1%) | 98/419 (23.39%)  | 0.0467 | 0.841 | 1.55 (1.00-2.38)    |
| C*05:01      | 5/131 (3.82%)  | 5/419 (1.91%)    | 0.0498 | 0.897 | 3.29 (0.936-11.532) |
| C*12:02      | 13/131 (9.9%)  | 80/419 (19.09%)  | 0.0145 | 0.262 | 0.467 (0.251-0.870) |

P: P values obtained with z-tests.
Pc: P values corrected for the multiplicity of testing by the number of comparisons (17, 34, and 18 for HLA-A, HLA-B and HLA-C, respectively).
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: confidence interval.

Table 3 | Results of association analysis between HLA types and CM-SJS/TEN with SOC in Group 2 (NIHS)

| HLA genotype | Carrier frequency (%) | Dominant model analysis |
|--------------|-----------------------|-------------------------|
|              | Case (n = 20) | Control (n = 220) | P | Pc | Odds ratio (95% CI) |
| HLA-A        |             |                        |   |    |                      |
| A*02:06      | 9/20 (45.0%) | 30/220 (13.6%) | 0.0014 | 0.00560 | 5.18 (1.98-13.56) |
| A*03:01      | 0/20 (0%)   | 19/220 (8.6%)   | 0.3804 |         |                    |
| A*11:01      | 2/20 (10.0%) | 39/220 (17.7%)  | 0.5408 |         |                    |
| A*24:02      | 14/20 (70.0%)| 132/220 (60.0%)| 0.4770 |         |                    |
| HLA-B        |             |                        |   |    |                      |
| B*13:01      | 2/20 (10%)  | 6/220 (2.7%)   | 0.1364 |         |                    |
| B*15:01      | 2/20 (10%)  | 39/220 (17.7%)  | 0.5408 |         |                    |
| B*44:02      | 0/20 (0%)   | 4/220 (1.8%)   | 1.0000 |         |                    |
| B*44:03      | 8/20 (40.0%)| 30/220 (13.6%)  | 0.0058 | 0.0406 | 4.22 (1.59-11.19) |
| B*46:01      | 2/20 (10%)  | 18/220 (8.2%)  | 0.6764 |         |                    |
| B*52:01      | 1/20 (5.0%) | 48/220 (21.8%) | 0.0857 |         |                    |
| B*54:01      | 5/20 (25%)  | 33/220 (15.0%) | 0.3316 |         |                    |
| HLA-C        |             |                        |   |    |                      |
| C*03:04      | 6/20 (30%)  | 43/220 (19.5%)  | 0.2573 |         |                    |
| C*05:01      | 0/20 (0%)   | 4/220 (1.8%)   | 1.0000 |         |                    |
| C*12:02      | 1/20 (5.0%) | 47/220 (21.4%) | 0.1388 |         |                    |

P: p-values obtained by Fisher's exact tests are shown.
Pc: p-values corrected for the multiplicity of testing by the number of comparisons: [4, 7 and 3 for HLA-A, HLA-B and HLA-C, respectively].
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: confidence interval.
Discussion

In this study we examined possible HLA risk factors for CM-SJS/TEN with SOC using two independently collected data sets of Japanese SJS/TEN patients. The carrier frequency of HLA-A*02:06, which we reported to have a very strong association with causative drug-unspecified SJS/TEN with SOC\(^{11,13}\), was significantly higher in CM-SJS/TEN with SOC patients than in the healthy controls. This significant association was maintained in AR-SJS/TEN with SOC.

On the other hand, the carrier frequency of HLA-A*02:06 in the 16 CM-SJS/TEN without SOC patients of Group 2c and the 52 CM-unrelated SJS/TEN with SOC patients from Groups 1d and 2d did not significantly differ from that in our healthy controls. These results suggest that HLA-A*02:06 is a risk factor for CM-SJS/TEN with SOC but not for CM-SJS/TEN without SOC or CM-unrelated SJS/TEN with SOC.

Moreover, HLA-A*02:06 and HLA-B*44:03 might not be primarily associated with only infection related SJS/TEN, because drug-unrelated SJS/TEN with SOC in KPUM, which seemed to be only infectious agents-related SJS/TEN, was not associated with HLA-A*02:06 and HLA-B*44:03 in our preliminary study (Supplemental Table 1).

Table 4 | Results of association analyses using combined SJS/TEN patients’ data

| HLA genotype | CM-SJS/TEN with SOC (Group 1a and Group 2a) | CM-SJS/TEN without SOC (Group 2c) | Control (Combined healthy controls) | Dominant model analysis |
|--------------|--------------------------------------------|----------------------------------|-------------------------------------|------------------------|
|              | Carrier frequency (%)                       | Odds ratio (95% CI)              | p                                   |                        |
| **A*02:06**  | 71/151 (47.0%)                              | 1.26 (0.92–1.71)                 | 0.13                                 | 2.72E-20               |
| **B*44:03**  | 39/151 (25.8%)                              | 0.75 (0.44–1.28)                 | 0.29                                 | 0.00125                |

a. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and combined healthy volunteers’ data

| HLA genotype | CM-SJS/TEN with SOC (Group 1a and Group 2a) | CM-SJS/TEN without SOC (Group 2c) | Control (Combined healthy controls) | Dominant model analysis |
|--------------|--------------------------------------------|----------------------------------|-------------------------------------|------------------------|
|              | Carrier frequency (%)                       | Odds ratio (95% CI)              | p                                   |                        |
| **A*02:06**  | 71/151 (47%)                                | 1.26 (0.92–1.71)                 | 0.13                                 | 2.72E-20               |
| **B*44:03**  | 39/151 (25.8%)                              | 0.75 (0.44–1.28)                 | 0.29                                 | 0.00125                |

b. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and without SOC (Group 2c)

c. Comparison of CM unrelated SJS/TEN with SOC and combined healthy volunteers’ data

| HLA genotype | CM unrelated-SJS/TEN with SOC (Group 1d and Group 2d) | Control (Combined healthy controls) | Dominant model analysis |
|--------------|-------------------------------------------------------|-------------------------------------|------------------------|
|              | Carrier frequency (%)                                  | Odds ratio (95% CI)                 | p                      |
| **A*02:06**  | 7/52 (13.5%)                                           | 0.514                               |                        |
| **B*44:03**  | 6/52 (11.5%)                                           | 0.514                               |                        |

d. Comparison between Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b) and combined healthy volunteers’ data

| HLA genotype | Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b) | Control (Combined healthy controls) | Dominant model analysis |
|--------------|-------------------------------------------------------|-------------------------------------|------------------------|
|              | Carrier frequency (%)                                  | Odds ratio (95% CI)                 | p                      |
| **A*02:06**  | 37/73 (50.7%)                                          | 2.54E-15                            | 0.0059                 |
| **B*44:03**  | 20/73 (27.4%)                                          | 2.16 (1.27–3.78)                    |                        |

*Woolf’s correction
P: P values obtained by \( \chi^2 \)-tests.
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: Confidence interval.

The carrier frequency of HLA-A*02:06 in all of our healthy controls was 13.6% (Tables 2 and 3), indicating that HLA-A*02:06 is a very common allele in the Japanese. However, as it is very rare in Caucasians and less frequent in Southern Han Chinese\(^{21}\), in these populations, this allele might not be a major risk factor for CM-SJS/TEN with SOC. We also found a significant association between HLA-B*44:03 and CM-SJS/TEN with SOC (including AR-SJS/TEN with SOC). This association was not detected in CM-SJS/TEN without SOC patients nor in CM-unrelated SJS/TEN with SOC patients. This again suggests HLA-B*44:03 as a risk factor for CM-SJS/TEN with SOC. Data on our controls (Tables 2 and 3) indicate that HLA-B*44:03 is a common HLA-B type in the Japanese population. Unlike HLA-A*02:06, HLA-B*44:03 is observed in Asians, Caucasians and Africans\(^{21}\). Reports from the USA\(^{22}\) and France\(^{12,13}\) showed that the HLA-B12 (HLA-Bw44) antigen was significantly increased in Caucasian SJS/TEN patients. The HLA-B12 antigen is mainly coded by HLA-B*44:02 or HLA-B*44:03 (http://www.allelefrequencies.net/).

Cold medicines were reported to be major causative drugs in SJS/TEN in Europe\(^6\) and in its drug safety communications, the U.S. Food and Drug Administration (http://www.fda.gov/Drugs/DrugSafety/ucm363041.htm) alerted to the possibility of serious skin reactions to acetaminophen. The significant association of HLA-B*44:03 with SJS/TEN in European patients may be attributable to their genetic backgrounds. To determine whether HLA-B*44:03 is a common risk
factor for CM-SJS/TEN with SOC in various populations, independent association studies in divergent ethnic groups are needed.

Because HLA-A*02:06 is rarely a haplotype with HLA-B*44:03 (http://www.allelefrequencies.net/), these two HLA alleles might be independent genetic risk factors that render the host susceptible to severe mucosal disorders and to severe sequelae such as visual disturbance when SJS/TEN develops after the administration of cold medicines including NSAIDs. In our study, 96 of 151 patients (63.6%) with CM-SJS/TEN with SOC (group 1, n = 131; group 2, n = 20) harbored either HLA-A*02:06 or HLA-B*44:03. On the other hand, only 177 of our 639 controls (27.7%) had one of these HLA alleles.

Forman et al.18 and Leaute-Labreze17 reported other infectious agents as triggers of SJS/TEN. Elsewhere27 we showed that rs775296T/T, a SNP of Toll-like receptor 3 (TLR3), was a risk factor for SJS/TEN with SOC and that the interaction between rs775296T/T and HLA-A*02:06 exerted more than additive effects. TLR3 is a pattern-recognition receptor related to innate immunity after viral infections that often produce common cold symptoms. Moreover, cold medicines such as acetaminophen and NSAIDs, including ibuprofen and loxoprofen, commonly down-regulate the production of prostanoid including PGE2. We also reported earlier that in our study population, EP3, which is one of the PGE2 receptors, polymorphisms were strongly associated with SJS/TEN with SOC44 and that the EP3 protein levels were much lower in the conjunctival epithelial cells of SJS/TEN patients than in the control subjects45,46. It is noteworthy that in our earlier study of SJS/TEN with SOC patients44 about 80% had CM-SJS/TEN with SOC. It might be possible that not only cold medicine but cold medicine with infectious agent could cause CM-SJS/TEN with SOC, because the patients develop CM-SJS/TEN with SOC by taking cold medicines after having common cold induced by infectious agents. We believe that interactions between HLA risk factors detected in the current study and TLR3, and/or EP3 might be keys in the pathogenesis of CM-SJS/TEN with SOC.

In summary, we reported the association between certain HLA types and CM-SJS/TEN with SOC. We propose that HLA-A*02:06 and HLA-B*44:03 be considered as strong risk factors for CM-SJS/TEN with SOC. Our findings may help to elucidate the pathogenesis of CM-SJS/TEN with SOC.

**Methods**

Our study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan, the National Institute of Health Sciences, Tokyo, Japan, and the Faculty of Medicine, University of Tokyo, Tokyo, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Helsinki Declaration. The purpose of the study and the experimental protocols were explained to all participants and their prior written informed consent was obtained.

**Patients and controls.** Japanese SJS/TEN patients (n = 228) were independently recruited at Kyoto Prefectural University of Medicine (KPU/M) (Group 1, n = 162) and by the Japan Severe Adverse Reactions Research Group, mainly conducted by the National Institute of Health Sciences (NIHS) (Group 2, n = 74). Between October 2004 and May 2013, 162 SJS/TEN with SOC were treated at Kyoto Prefectural University of Medicine; of these, 71 were included in our previous study17. The diagnosis of SJS/TEN with SOC was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and the involvement of at least 2 mucosal sites including the oral cavity and ocular surface. Some of the patients had developed SJS/TEN many years before recruitment for this study. Of the 162 patients in Group 1, 131 patients had taken cold medicines such as NSAIDs and multi-ingredient cold medications for a few ~ several days before disease onset for common-cold symptoms; they were classified as CM-SJS/TEN with SOC (Group 1a). Although the specific drugs were not identified by all 131 CM-SJS/TEN with SOC patients, 59 of 131 CM-SJS/TEN with SOC patients (45%) reported taking medicines containing acetaminophen (AR-SJS/TEN with SOC, Group 1b). Among the 162 SJS/TEN with SOC patients (Group 1), 14 patients (Group 1d) were classified as CM-SJS/TEN with SOC because they manifested anticonvulsants-related SJS/TEN with SOC (n = 10) or SJS/TEN with SOC after being treated with antimalarial-, anticonvulsant-, or anti-depressive agents or steroids (n = 4). We also excluded 17 patients; in 9 SJS/TEN with SOC the drugs were unknown and in 8 SJS/TEN with SOC were not related to drugs.

Group 2 (n = 74) consisted of patients with newly-developed SJS/TEN; they were recruited between June 2006 and May 2013 by participating institutes or via a nationwide blood sampling network operated by the NIHS in cooperation with the Ministry of Health, Labour and Welfare, the Pharmaceutical and Medical Devices Agency, and the Federation of Pharmaceutical Manufacturers’ Association of Japan. The criteria proposed by Bastuji-Garin et al.20 were used for a diagnosis of SJS/TEN in this group. Ocular surface complications were judged to be severe ocular complications (SOS) when pseudo-membrane formation and/or conjunctival or corneal epithelial defects were observed in the acute phase. As shown in Table 1, Group 2 (n = 74) consisted of 20 patients with CM-SJS/TEN with SOC (Group 2a), all 6 of these presented with AR-SJS/TEN with SOC (Group 2b). Group 2 also included 16 patients with CM-SJS/TEN without SOC (Group 2c), and 38 patients with CM-unrelated-SJS/TEN with SOC (Group 2d). The background of the 236 patients with SJS/TEN in group1 and group2 is summarized in Table 1.

Japanese volunteers (n = 639) served as the controls. They were independently recruited by the University of Tokyo (n = 419)20 and by Kyoto Prefectural University of Medicine (n = 220)20 and served for comparison studies of patient groups 1 and 2, respectively. In this study we enrolled only mainland Japanese.

**HLA genotyping.** We analyzed HLA-A, -B, and -C of all 162 group 1 patients, which consist of 131 CM-SJS/TEN with SOC (group 1a), 14 CM-unrelated (other medicine related) SJS/TEN with SOC (group 1d), and 17 SJS/TEN with SOC excluded because of being drug-unrelated and detail unknown. We performed polymerase chain reaction (PCR) assays followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial bead-based typing kits (Wakunaga, Hiroshima, Japan). In group 2 (n = 74) we performed high-resolution HLA typing with a sequence-based method using ScCoRe-A, -B, and -C, locus sequencing kits (Invitrogen Corp., Brown Deer, WI, USA) and ABI 3730 and 3130 DNA sequencers (Applied Biosystems, Foster City, CA, USA). HLA genotypes were assigned using Assign SBT- or Assign ATP software (versions 3.2.7b and 1.0.2.4; respectively, Conexio Genomics, Western Australia, Australia). We also genotyped all volunteers for HLA-A, -B, and -C using PCR-SSO and commercial bead-based typing kits (Wakunaga or One Lambda, CA, USA).

**Statistical analysis.** We compared the carrier frequency of individual HLA alleles between our patients and controls based on the dominant model using the χ²-test (Labo Server software/World Fusion, Tokyo, Japan) or Fisher’s exact test (JMP version 7.0.1 software; SAS Institute Japan Ltd., Tokyo, Japan). Significance levels were corrected with the Bonferroni correction for multiple comparisons.

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