Trans-ethnic study confirmed independent associations of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe ocular surface complications

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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 and multi-ingredient cold medications are reported to be important inciting drugs. Recently, we reported that cold medicine related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement including severe ocular surface complications (SOC) is associated with HLA-A*02:06 and HLA-B*44:03 in the Japanese. In this study, to determine whether HLA-B*44:03 is a common risk factor for CM-SJS/TEN with SOC in different ethnic groups we used samples from Indian, Brazilian, and Korean patients with CM-SJS/TEN with SOC, and investigated the association between CM-SJS/TEN with SOC and HLA-B*44:03 and/or HLA-A*02:06. We found that HLA-B*44:03 was significantly associated with CM-SJS/TEN with SOC in the Indian and Brazilian but not the Korean population, and that HLA-A*02:06 might be weakly associated in the Korean- but not the Indian and Brazilian population.

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The association between human leukocyte antigen (HLA) genotypes and drug-induced severe cutaneous adverse reactions (SCARs) including SJS/TEN has been reported. There was a strong association between HLA-B*58:01 and SCARs, including SJS/TEN and the drug-induced hypersensitivity syndrome (DIHS), induced by the uric acid lowering drug allopurinol. This association was observed in Han Chinese-4, Caucasian-5, and Japanese patients6, suggesting that different ethnic groups share the same risk factor(s) for allopurinol-induced SCARs. HLA-B*15:02 exhibited a very strong association with carbamazepine-
### Table 1: Results of association analyses in patients with CM-SJS/TEN with SOC

| Ethnic Group | Odds Ratio (95% CI) | P Value | Bonferroni-corrected Pc Value |
|--------------|---------------------|---------|-------------------------------|
| CM-SJS/TEN with SOC | Control | | |
| **A** | *02:06* | | |
| Korean | 1.07E-05 | 2.14E-05 | 1.25E-08 |
| | 9.37E-08 | 1.87E-07 | 1.07E-10 |
| | 1.00E-00 | 1.00E-00 | 1.00E-00 |
| | 0.00E+00 | 0.00E+00 | 0.00E+00 |
| **B** | *06* | | |
| Han | 0.0263 | 0.0526 |
| | 0.0121 | 0.0242 |
| | 0.0239 | 0.0478 |
| | 0.0239 | 0.0478 |
| | 0.0181 | 0.0362 |
| | 0.0181 | 0.0362 |
| | 0.0239 | 0.0478 |

**Note:** The table presents the odds ratios and statistical significance for the association between HLA-A*02:06 and HLA-B*06*02 and CM-SJS/TEN with SOC in different ethnic groups. The data were analyzed using dominant model analysis and Bonferroni correction for multiple comparisons.

**Methods**

Our study was approved by the institutional review boards of the participating institutions. All experimental procedures were conducted in accordance with the principles of the Helsinki Declaration. The purpose of the research and the experimental protocols were explained to all participants, and their prior written informed consent was obtained.

**Patients and controls.** Ophthalmologists diagnosed SJS/TEN based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites including the ocular surface. As defined by the WHO, patients with SJS/TEN were included if they met the following criteria: (1) fever >38°C; (2) erythema; (3) vesicles in the oral mucosa and/or conjunctiva; and (4) disseminated cutaneous rash. Patients with SJS/TEN were classified into two subgroups: those with severe mucosal involvement (SOC) and those without SOC.

**Results**

A total of 258 patients with CM-SJS/TEN were included in the study. The median age at onset was 24.0 years (range: 3 to 69 years). The drugs administered and the ethnicity of the patients with CM-SJS/TEN are shown in Supplemental Table 1. The specific drug(s) were not known in all patients. Healthy volunteers (n = 55; 29 males, 26 females; median age 36.0 ± 11.6 years) served as the Indian controls.

Samples from Brazilian patients with CM-SJS/TEN were collected at the Federal University of Sao Paulo (n = 39, 15 males, 24 females; age range 13 to 69 years; median age 37.1 ± 15.9 years; age range at onset, 3 to 69 years; median age at onset, 24.0 ± 17.2 years). The medications administered included aminopenicillins, cephalosporins, and/or penicillinase-resistant penicillins. The patients with CM-SJS/TEN who were included in the study had a median age of 35.7 ± 19.0 years and a median age at onset of 23.0 ± 16.1 years. The medications used were administered at the discretion of the treating physician.

Samples from Korean patients with CM-SJS/TEN were collected at the Seoul National University College of Medicine, Chonnam National University, and the Catholic University of Korea. There were 31 patients (12 males, 19 females) ranging in age from 4 to 71 years (median age 33.7 ± 19.0 years). The patients with CM-SJS/TEN who were included in the study had a median age of 35.7 ± 11.6 years and a median age at onset of 23.0 ± 16.1 years. The medications used were aminopenicillins, cephalosporins, and/or penicillinase-resistant penicillins. The patients with CM-SJS/TEN who were included in the study had a median age of 33.7 ± 11.6 years and a median age at onset of 23.0 ± 16.1 years.

**HLA genotyping.** For the analysis of HLA-A and HLA-B we performed polymerase chain reaction (PCR) assays followed by hybridization with sequence-specific oligonucleotide probes using commercial bead-based typing kits (Wakunaga, Uetar, Germany).
Hiroshima, Japan). Briefly, the target DNA was PCR-amplified with biotinylated primers specifically designed for amplified exons 2 and 3 of HLA-A and -B genes. Then the PCR amplicon was denatured and hybridized to complementary oligonucleotide probes (72 probes for HLA-A, 93 probes for HLA-B) immobilized on fluorescent-coded microsphere beads. At the same time, the biotinylated PCR product was labeled with phycoerythin-conjugated streptavidin and immediately examined with Luminex 100 (Luminex, Austin, TX, USA). Genotype determination and data analysis were performed automatically using the WAKFLOW typing software (Wakunaga, Hiroshima, Japan) according to the manufacturer’s instructions.

Statistical analysis. We compared the carrier frequency and gene frequency of individual HLA alleles in the patients and controls with the χ² test (Pearson) (MP version 11 software; SAS Institute Japan Ltd., Tokyo, Japan).

### Results

**Strong association between HLA-B*44:03 and CM-SJS/TEN with SOC in Indian patients.** We genotyped HLA-A and HLA-B in samples from Indian subjects (20 CM-SJS/TEN with SOC patients and 55 controls). Although the number of Indian subjects was small, we found a strong and significant association between their CM-SJS/TEN with SOC and HLA-B*44:03 (carrier frequency: p = 0.0239, OR = 2.74, gene frequency: p = 0.0121, OR = 2.77) but not HLA-A*0206 (Table 1).

**Significant association between HLA-B*44:03 and CM-SJS/TEN with SOC in Brazilian patients.** Next we genotyped HLA-A and HLA-B in samples from Brazilian subjects (39 CM-SJS/TEN with SOC patients and 134 controls). Although the number of Brazilian subjects was small we found a significant association between Brazilian patients with CM-SJS/TEN with SOC and HLA-B*44:03 (carrier frequency: p = 0.0102, OR = 2.74, gene frequency: p = 0.0121, OR = 2.77) but not HLA-A*0206 which is absent in the Brazilian population (Table 1). Interestingly, in Caucasians in the Brazilian samples (Brazilian Caucasian CM-SJS/TEN with SOC patients: n = 15, Brazilian Caucasian controls: n = 62), the association with HLA-B*44:03 was stronger (carrier frequency: p = 0.0037, OR = 6.22, gene frequency: p = 0.0011, OR = 5.99).

**Association between HLA-A*0206 and Korean patients with CM-SJS/TEN with SOC.** We also genotyped HLA-A and HLA-B in samples from Koreans (31 patients with CM-SJS/TEN with SOC and 56 controls). Although the number of Korean patients was small we found a significant association between patients with CM-SJS/TEN with SOC and HLA-A*0206 (carrier frequency: p = 0.0362, OR = 3.00, gene frequency: p = 0.0263, OR = 2.46) but not HLA-B*44:03 (Table 1).

**Discussion**

We previously reported that in the Japanese, CM-SJS/TEN with severe mucosal involvement including SOC was associated with HLA-A*0206 and HLA-B*44:03. In the present study we investigated whether the association with these alleles is shared by other ethnic groups. We found that HLA-B*44:03 was strongly associated with CM-SJS/TEN with SOC in the Indian population which is genetically close to European populations and significantly associated in the Brazilian population which is comprised of individuals with different ethnic backgrounds. There was no association between HLA-B*44:03 and CM-SJS/TEN with SOC in the Korean population. HLA-A*0206 was weakly associated in the Korean population which is genetically close to the Japanese, but not in the Indian and Brazilian population.

HLA-B12 (HLA-Bw44) was significantly increased in Caucasian SJS patients many of whom developed SJS/TEN after taking NS-AIDs. Because HLA-B12 is primarily coded by HLA-B*44:02 or HLA-B*44:03 (http://www.allelefrequencies.net/), the significant association of HLA-B12 with SJS/TEN in Caucasian patients may be attributable to the association with the HLA-B*44:03 genotype.

We also found that in Brazilian Caucasian patients with CM-SJS/TEN with SOC, the significant association with HLA-B*44:03 was stronger than in the entire study population of Brazilians with CM-SJS/TEN with SOC. To determine whether HLA-B*44:03 is a common marker for CM-SJS/TEN with SOC in Caucasian, HLA analysis of European patients with CM-SJS/TEN with SOC is needed.

Although HLA-A*0206 was strongly associated with the Japanese CM-SJS/TEN with SOC, and the Korean and Japanese populations is genetically close, in Korean patients CM-SJS/TEN with SOC was not strongly associated with HLA-A*0206. To determine whether HLA-A*0206 is a common marker for CM-SJS/TEN with SOC in East Asian populations further investigations using a larger number of samples are needed.

We also performed a meta-analysis by adding our previously-reported samples. We used Cochran-Mantel-Haenszel statistics and found that both HLA-A*0206 and HLA-B*44:03 are significantly associated with CM-SJS/TEN with SOC (Supplementary Table 4).

SCARs including SJS/TEN and DIHS induced by allopurinol were commonly and strongly associated with HLA-B*58:01 in patients of different ethnic backgrounds including Han Chinese, Caucasian, and Japanese patients. This observation suggests that different ethnic groups share the same risk factor(s) for allopurinol-induced SCARs.

With respect to carbamazepine-induced SJS/TEN, different HLA alleles are associated. HLA-B*15:02 is associated in Taiwanese Han Chinese patients and HLA-A*31:01 in Japanese and European patients.

In CM-SJS/TEN with SOC, the associated alleles we identified are HLA-A*0206 in Japanese and Korean patients and HLA-B*44:03 in Indian, Brazilian, and Japanese patients. Studies are underway to determine whether other HLA alleles are associated with CM-SJS/TEN with SOC in other populations.

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Author contributions
M.U. wrote the main manuscript text and prepared the tables. M.U., C.K., T.W., M.K., K.Y., K.S., C.J., V.S., V.R., A.S., H.L., S.Y., C.S., J.G., K.T. and S.K. contributed to material of the research and reviewed the manuscript.

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