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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.

S tevens-Johnson syndrome (SJS) and its severe variant, toxic epidermal necrolysis (TEN) with spots, are acute inflammatory vesiculobullous reactions of the skin and mucous membranes such as the ocular surface, oral cavity, and genitals. They are rare but often associated with inciting drugs and/or infectious agents[1–3].

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 patients[6], 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	HLA genotype	Carrier frequency (%)		Dominant model analysis			Gene frequency (%)		Dominant model analysis		
		CM-SJS/TEN with SOC	Control	P	Pc	Odds ratio (95% CI)	CM-SJS/TEN with SOC	Control	P	Pc	Odds ratio (95% CI)
Indian	A*02:06	1/20 (5.0%)	3/55 (5.5%)	0.938	-	0.91 (0.09–9.31)	1/39 (2.5%)	3/110 (2.7%)	0.939	-	0.91 (0.09–9.06)
	B*44:03	12/20 (60.0%)	6/55 (10.9%)	1.07.E-05	2.14.E-05	12.25 (3.57–42.01)	17/40 (42.5%)	7/110 (6.4%)	9.37.E-08	1.87.E-07	10.88 (4.04–29.3)
Brazilian	A*02:06	0/39 (0.00%)	0/134 (0.00%)				0/78 (0.00%)	0/268 (0.00%)			
	B*44:03	10/39 (25.6%)	15/134 (11.2%)	0.0239	0.0478	2.74 (1.12–6.71)	11/78 (14.1%)	15/268 (5.60%)	0.0121	0.0242	2.77 (1.22–6.31)
Korean	A*02:06	11/31 (35.5%)	14/90 (15.6%)	0.0181	0.0362	3.00 (1.18–7.57)	12/62 (19.4%)	16/180 (8.9%)	0.0263	0.0526	2.46 (1.09–5.54)
	B*44:03	6/31 (19.4%)	18/90 (20.0%)	0.938	-	0.96 (0.34–2.69)	7/62 (11.3%)	19/180 (10.6%)	0.872	-	1.07 (0.43–2.70)

P: P values obtained with the χ^2-test (Pearson), CI: Confidence interval.
Pc: P values corrected for the multiplicity of testing by the number of comparisons 2 (HLA-A*02:06 + HLA-B*44:03).
CM-SJS/TEN: cold medicine-related SJS/TEN, SOC: severe ocular surface complications.

induced SJS/TEN in Taiwanese Han Chinese patients[7] and *HLA-A*31:01* was strongly associated with carbamazepine-induced SCARs including SJS/TEN in Japanese-[8] and European patients[9]. We recently reported that cold medicine-related 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 Japanese patients[10].

The ophthalmologists Mondino et al.[11] and the dermatologists Roujeau et al.[12,13] reported that *HLA-B12* (*HLA-Bw44*) was significantly increased in Caucasian SJS patients many of whom developed SJS/TEN after taking non-steroidal anti-inflammatory drugs (NSAIDs). *HLA-B12* is primarily coded by *HLA-B*44:02* or *HLA-B*44:03* (http://www.allelefrequencies.net/). The significant association between HLA-B12 and SJS/TEN in Caucasian patients may be attributable to their genetic background.

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*.

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[1,14,15]. They defined patients with SOC as those who manifested a pseudomembrane and an epithelial defect on the ocular surface in the acute stage, and as patients with ocular sequelae such as dry eye, trichiasis, symblepharon, and conjunctival invasion into the cornea in the chronic stage.

As in our previous study, we focused on SJS/TEN with SOC suspected of having been induced by cold medicines such as multi-ingredient cold medications and NSAIDs. As we found earlier that the genetic predisposition might be different between SJS/TEN with and without severe mucosal involvement including SOC[10] we focused on patients from different ethnic groups who presented with SJS/TEN with SOC.

Samples from Indian patients with CM-SJS/TEN were collected at the LV Prasad Eye Institute (n = 20; 12 males, 8 females; age range 7 to 63 years; median age 27.1 ± 13.4 (SD) years). Their age at onset ranged from 3 to 42 years (median age at onset, 19.2 ± 12.2 (SD) years; in 8 patients the age at onset was unknown). The drugs administered to these patients and the HLA type (A and B) of patients with CM-SJS/TEN with SOC 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 drugs administered, the ethnicity, and the HLA type (A and B) of these CM-SJS/TEN patients with SOC are shown in Supplemental Table 2. Healthy volunteers (n = 134; 55 males, 79 females; median age 41.2 ± 12.8 years) were the Brazilian controls (ethnicity: pardo, n = 66; white, n = 62; black, n = 4, Indian plus white, n = 2).

Samples from Korean patients with CM-SJS/TEN were collected at the Seoul National University College of Medicine, Chonnam National University, Yonsei 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). Their age at SJS/TEN onset ranged from 3 to 63 years (median age at onset, 23.0 ± 16.1 years). The drugs used and the HLA type (A and B) of these patients with SOC are presented in Supplemental Table 3. The specific drug(s) were not known in all patients. Healthy volunteers (n = 90; 35 males, 55 females; median age 31.7 ± 7.9 years) were the Korean controls.

Samples from Indian subjects were obtained by extracting DNA from whole peripheral blood with the phenol chloroform method. For Brazilian samples, DNA was extracted from whole peripheral blood using the PAX gene blood DNA kit (Qiagen, Hilden, Germany) or from saliva using Oragene DNA (Kyodou International, Kanagawa, Japan). To obtain the samples from Korean subjects, DNA was extracted from whole peripheral blood using the PAXgene Blood DNA kit (Qiagen).

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,

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 phycoerythrin-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 χ^2-test (Pearson) (JMP 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 = 1.07 × 10^{-5}, odds ratio (OR) = 12.25, gene frequency: p = 9.37 × 10^{-8}, OR = 10.88) 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 = 0.0239, 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*02:06* 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 90 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*02:06* and *HLA-B*44:03*[10]. 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[16] 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*02:06* 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[11–13]. 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*02:06* was strongly associated with the Japanese CM-SJS/TEN with SOC, and the Korean and Japanese population is genetically close[16], in Korean patients CM-SJS/TEN with SOC was not strongly associated with *HLA-A*02:06*. To determine whether *HLA-A*02:06* 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[10]. We used Cochran-Mantel-Haenszel statistics and found that both HLA-A*02:06 and HLA-B*44:03 are significantly associated with CM-SJS/TEN with SOC (Supplemental 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-[4], Caucasian-[5], and Japanese patients[6]. 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[7] and *HLA-A*31:01* in Japanese-[8] and European patients[9].

In CM-SJS/TEN with SOC, the associated alleles we identified are *HLA-A*02:06* 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

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Additional information