ARTICLE

HLA-A*02:06 and PTGER3 polymorphism exert additive effects in cold medicine-related Stevens–Johnson syndrome with severe ocular complications

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We previously reported that PTGER3 (prostaglandin E receptor 3 (subtype EP3)) single-nucleotide polymorphisms (SNPs) were associated with Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) with severe ocular complications (SOC). We also documented that approximately 80% of our SJS/TEN patients had taken cold medicines within several days before disease onset, and we thus designated them cold medicine-related SJS/TEN (CM-SJS/TEN) patients. Moreover, we reported that HLA-A*02:06 with TLR3 polymorphisms exerted more than additive effects in SJS/TEN with SOC. In this study, we focused on CM-SJS/TEN with SOC and analyzed the association with PTGER3 SNPs and an interactive effect between PTGER3 SNPs and HLA-A*02:06 in not only the Japanese but also the Korean population. In the Japanese population, PTGER3 SNP rs1327464 was most significantly associated with CM-SJS/TEN with SOC (G versus A; odds ratio (OR) = 0.232, \( P = 7.92 \times 10^{-10} \)), and we found an interaction with additive effects between HLA-A*02:06 and the high-risk genotypes PTGER3 rs1327464 GA or AA (OR = 10.8, \( P = 2.56 \times 10^{-7} \)). We also found a significant association between Korean CM-SJS/TEN with SOC and PTGER3 SNP rs1327464 GG versus GA+AA, OR = 0.246, \( P = 0.00101 \), and we detected an additive effect between HLA-A*02:06 and the high-risk genotypes PTGER3 rs1327464 GA or AA (OR = 14.2, \( P = 5.58 \times 10^{-6} \)).

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INTRODUCTION

Stevens–Johnson syndrome (SJS) and some toxic epidermal necrolysis (TEN) are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, including the ocular surface, oral cavity and genitals, which are often associated with inciting drugs and infectious agents.1–6 These reactions carry high mortality rates of 3% for SJS and 27% for TEN,7 and surviving patients often experience severe sequelae such as vision loss due to severe ocular surface complications (SOC),8 although incidences are rare (one to six cases per million persons).6,9

We previously reported that PTGER3 (prostaglandin E receptor 3 (subtype EP3)) SNPs were associated with SJS/TEN with SOC. Our genome-wide association study showed associations between six single-nucleotide polymorphisms (SNPs) in the prostaglandin E receptor 3 gene (PTGER3) and SJS/TEN with SOC,8 and our subsequent analysis using the DigiTag2 assay showed that 20 of the 38 SNPs of PTGER3 were associated with SJS/TEN with SOC.10

We also documented that approximately 80% of our SJS/TEN patients had taken cold medicines within several days before disease onset and accordingly designated them cold medicine-related SJS/TEN (CM-SJS/TEN) patients.1 Moreover, we reported that HLA-A*02:06 with TLR3 polymorphisms exerted more than additive effects in SJS/TEN with SOC.11 We also reported that HLA-A*02:06 was strongly associated with CM-SJS/TEN with SOC in Japanese individuals1 and was significantly associated with CM-SJS/TEN with SOC in Korean individuals.2

In this study, we focused on CM-SJS/TEN with SOC and analyzed the association with PTGER3 SNPs. We also asked whether there is an interactive effect between PTGER3 SNPs and HLA-A*02:06 in not only Japanese but also Korean populations.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review boards of Kyoto Prefectural University of Medicine and the University of Tokyo as well as by the institutional review boards of Seoul National University College of Medicine, Yonsei University College of Medicine, Chonnam National University Medical School and College of Medicine and the Catholic University of Korea.

All experimental procedures were conducted in accordance with the principles of the Declaration of Helsinki. The purpose of the research and the experimental protocols were explained to all the participants; all gave their written informed consent before their participation in this study.

The diagnosis of SJS/TEN by the Japanese and Korean ophthalmologists was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions and the involvement of at least two mucosal sites including the ocular surface.1,2 Because ophthalmologists usually encounter SJS/TEN patients in the chronic rather than the
### Table 1. Japanese CM-SJS/TEN with SOC patients

**a. Association with PTGER3 SNPs**

| RS number of SNP | Frequency of genotypes (%) | Allele 1 versus allele 2 | Genotype 11 versus 12+22 | Genotype 11+12 versus 22 |
|------------------|-----------------------------|--------------------------|--------------------------|--------------------------|
|                  | Genotypes                  | Cases (Cases/Controls)   | Corrected P value        | Corrected P value        | Corrected P value        |
|                  | Allele 1: major allele      |                          |                         |                          |                          |
|                  | Allele 2: minor allele      |                          |                          |                          |                          |
| rs755865         | 11 C/C                     | 56/132 (42.4) 105/219 (47.9) | 0.106                    | 0.315                    | 0.8772                   |
| rs17131450       | 11 C/C                     | 97/132 (73.5) 194/221 (87.8) | 1.22 x 10^{-4}           | 6.36 x 10^{-4}           | 0.0792                   |
| rs702            | 11 C/C                     | 87/131 (66.4) 109/221 (49.3) | 0.0028                   | 1.81 x 10^{-3}           | 0.628                    |
| rs1325949        | 11 A/A                     | 91/132 (68.9) 105/221 (47.5) | 1.94 x 10^{-3}           | 8.66 x 10^{-5}           | 0.959                    |
| rs2421805        | 11 T/T                     | 44/130 (33.8) 104/216 (48.1) | 1.93 x 10^{-3}           | 9.21 x 10^{-3}           | 0.0128                   |
| rs7543182        | 11 G/G                     | 92/132 (69.7) 112/221 (50.7) | 9.29 x 10^{-3}           | 4.64 x 10^{-4}           | 0.591                    |
| rs755874         | 11 G/G                     | 91/132 (68.9) 112/221 (50.7) | 0.0309                   | 1.59 x 10^{-3}           | 0.959                    |
| rs4147115        | 11 A/A                     | 45/128 (35.2) 54/212 (25.5) | 0.0603                   | 0.0569                   | 0.291                    |
| rs465093         | 11 C/C                     | 84/132 (63.6) 113/220 (51.4) | 0.129                    | 0.0247                   | 0.486                    |
| rs17131478       | 11 T/T                     | 11/132 (8.33) 15/221 (6.79) | 1.63 (1.13–2.36)         | 1.90 (1.06–3.53)         | 0.839                    |
| rs17131479       | 11 C/C                     | 87/132 (65.9) 135/217 (62.2) | 0.485                    | 0.486                    | 0.744                    |
| rs7521005        | 11 A/A                     | 84/132 (63.6) 114/221 (51.6) | 0.138                    | 0.0273                   | 0.479                    |
| rs7541092        | 11 G/G                     | 86/131 (65.6) 136/218 (62.4) | 0.488                    | 0.540                    | 0.608                    |
| rs1327464        | 11 G/G                     | 83/131 (63.4) 194/219 (88.6) | 7.92 x 10^{-10}          | 1.90 x 10^{-8}           | 1.23 x 10^{-3}           |
| rs1409161        | 11 G/G                     | 57/132 (43.2) 68/221 (30.8) | 0.0988                   | 0.0183                   | 0.958                    |
| rs34885906       | 11 T/T                     | 120/132 (90.9) 189/221 (85.5) | 0.152                    | 0.138                    | —                        |
Table 1. (Continued)

a. Association with PTGER3 SNPs

| rs number of SNP | Genotypes | Allele 1: major allele | Allele 2: minor allele | Cases | Controls | P valueᵃ | Corrected Pᵇ OR (95% CI) | P valueᵃ | Corrected Pᵇ OR (95% CI) | P valueᵃ | Corrected Pᵇ OR (95% CI) |
|------------------|------------|-----------------------|-----------------------|-------|----------|----------|--------------------------|----------|--------------------------|----------|--------------------------|
| rs2817864        | 11         | T/T                   | 77/132 (58.3)         | 118/221 (53.4) | 0.146   | 0.367    | 0.0352                   | 26/131 (19.9) | 21/218 (9.63)             | 4.40     | (0.983–19.7)             |
|                  | 12         | T/G                   | 53/132 (40.2)         | 89/221 (40.3)  | —       | —        | 0.634                    | 21/218 (9.63) | 89/221 (40.3)             | —        | —                        |
|                  | 22         | G/G                   | 2/132 (1.52)          | 14/221 (6.33)  | —       | —        | 4.40 (0.983–19.7)        | 21/218 (9.63) | 89/221 (40.3)             | —        | —                        |
| rs1409981        | 11         | G/G                   | 37/131 (28.2)         | 60/218 (27.5)  | 0.874   | 0.884    | 0.664                    | 21/218 (9.63) | 89/221 (40.3)             | —        | —                        |
|                  | 12         | G/A                   | 65/131 (49.6)         | 114/218 (52.3) | —       | —        | 0.32                    | 12 G/A | 65/131 (49.6)             | —        | —                        |
|                  | 22         | A/A                   | 29/131 (22.1)         | 44/218 (20.2)  | —       | —        | 0.154 (0.0956–0.249)     | 22 A/A | 29/131 (22.1)             | —        | —                        |

Abbreviations: CI, confidence interval; CM-SJS, cold medicine-related Stevens-Johnson syndrome; OR, odds ratio; SOC, severe ocular complication; SNP, single-nucleotide polymorphism; TEN, toxic epidermal necrolysis.ᵃP value for allele or genotype frequency. Comparison was between patients and controls using the chi-square test (Pearson).ᵇCorrected P values corrected for the multiplicity of testing by the number of comparisons (n = 18). In table 1a, bold values denote P values that are significant after correction for the multiplicity of testing by the number of comparisons. In table 1b, bold value denotes OR with additive effect.

RESULTS

Associations with PTGER3 SNPs in Japanese population

In Japanese patients, 7 of 18 SNPs previously reported to be associated with SJ/TEN were significantly associated with CM-SJS/TEN with SOC after Bonferroni correction (Table 1a). PTGER3 SNP rs1327464 (G versus A) was most significantly associated with CM-SJS/TEN with SOC; the odds ratio (OR) for the major allele was 0.232 (P = 7.92 × 10⁻¹¹).
Interaction between HLA-A*02:06 and PTGER3 gene SNPs in Japanese population

As we had found earlier that HLA-A*02:06 was strongly associated with CM-SJS/TEN with SOC in the Japanese, we then looked for interactive effects between these seven SNPs of the PTGER3 gene and HLA-A*02:06 (Supplementary Table 1). We found an interaction with additive effects between HLA-A*02:06 and the high-risk genotypes PTGER3 rs1327464 GA or AA (OR = 10.8, P = 2.56 × 10^{-7}; Table 1b) but not with other SNPs (Supplementary Table 2).

Associations with PTGER3 SNPs in Korean population

We then analyzed those seven SNPs using Korean samples because we had detected the same association between HLA-A*02:06 and CM-SJS/TEN with SOC in Korean patients. Although the number of Korean cases (n = 30) was small, we again found a significant association between CM-SJS/TEN with SOC and PTGER3 SNP rs1327464 (GG versus GA+AA, OR = 2.46, P = 0.00101; Table 2a) but not with other SNPs (Supplementary Table 2).

Interaction between HLA-A*02:06 and the PTGER3 SNP in Korean population

When we tested for possible interactive effects between the SNPs of the PTGER3 gene and HLA-A*02:06, we detected an additive effect (HLA-A*02:06 with PTGER3 rs1327464 risk genotypes GA or AA; OR = 14.2, P = 5.58 × 10^{-6}; Table 2b).

DISCUSSION

We now document that several SNPs of PTGER3 are significantly associated with CM-SJS/TEN with SOC and that the association with PTGER3 SNP rs1327464 (cases, n = 131; controls, n = 219, OR (major allele) = 0.232, P = 7.92 × 10^{-15}) is much stronger than that we previously reported regarding SJS/TEN with SOC, which included not only cold medicine-related but also other drug-related cases (cases, n = 116; controls, n = 221; OR (major allele) = 0.46, P = 0.0043). The association with the other six SNPs was the same as or slightly stronger than we previously reported. This result might suggest that CM-SJS/TEN with SOC is a purer phenotype than SJS/TEN with SOC.

Earlier, we reported that HLA-A*02:06 is significantly associated with CM-SJS/TEN with SOC in Japanese and Korean populations; in this study, we also found a significant association between HLA-A*02:06 and Japanese or Korean CM-SJS/TEN with SOC (Supplementary Table 3). We also reported that HLA-A*02:06 with TLR3 polymorphisms exerted more than additive effects in SJS/TEN with SOC. Our current study shows that HLA-A*02:06 with TLR3 SNP rs3775296 T/T also exerts more than additive effects in CM-SJS/TEN with SOC (Supplementary Table 4). HLA-A*02:06 with PTGER3 rs1327464 GA/AA also exerts an additive effect in CM-SJS/TEN with SOC. After removing samples with both HLA-A*02:06 and TLR3 SNP rs3775296 T/T, the additive effect between HLA-A*02:06 and PTGER3 rs1327464 GA/AA persisted in CM-SJS/TEN with SOC (Supplementary Table 5; OR = 10.6, P = 4.34 × 10^{-4}), suggesting that these interactions are independent of each other. In the Japanese population, although HLA-A*02:06 alone showed OR = 5.46 and P = 1.39 × 10^{-11}, and PTGER3 rs1327464 GA/AA alone showed OR = 4.48 and P = 1.90 × 10^{-8}, the combination of HLA-A*02:06 and PTGER3 rs1327464 GA/AA showed a higher OR (OR = 10.8, P = 2.56 × 10^{-7}) than each allele alone. Moreover, in the Korean population, the combination of HLA-A*02:06 and PTGER3 rs1327464 GA/AA showed a higher OR (OR = 14.2, P = 5.58 × 10^{-9}) than each allele alone, although HLA-A*02:06 alone showed OR = 2.50 and P = 0.0412, and PTGER3 rs1327464 GA/AA alone showed OR = 4.07 and P = 0.00101. These findings might show that using the combination of these two polymorphisms could improve genetic testing compared with using only one susceptibility gene.

In the Japanese population, combined genotyping for HLA-A*02:06, TLR3 rs3775296 T/T and PTGER3 rs1327464 GA or AA may help to predict the risk for CM-SJS/TEN with SOC.

On the basis of our previous and current observations, we suggest that, in addition to microbial infections and cold medicines, the combination of multiple gene polymorphisms and their interactions contribute strongly to the onset of CM-SJS/TEN with SOC.

### Table 2. Korean CM-SJS/TEN with SOC patients

**a. Association with PTGER3 SNP**

| rs number of SNP | Frequency of genotypes (%) Allele 1: major allele Allele 2: minor allele | Genotypes | Cases | Controls | P value \(^a\) OR (95% CI) |
|------------------|---------------------------------------------------------------------------------|--------------------|-------|---------|-----------------------------|
| rs1327464        |                                                                                  |                    |       |         |                             |
| 11               | G/G 17/30 (56.7)                                                               | 101/120 (84.2)     |       |         | **0.00203** 1.96 (0.83–4.67) |
| 12               | G/A 13/30 (43.3)                                                               | 19/120 (15.8)      | 0.311 (0.144–0.673) | 0.246 (0.103–0.589) |
| 22               | A/A 0/30 (0.00)                                                                | 0/120 (0.00)       |       |         |                             |

**b. Interaction between HLA-A*02:06 and PTGER3 rs1327464 GA/AA**

| HLA-A*02:06 | rs1327464 GA or AA | CM-SJS/TEN with SOC | Controls | P value \(^a\) OR (95% CI) |
|-------------|--------------------|---------------------|----------|-----------------------------|
| +           | +                  | 8/30 (26.7%)        | 3/120 (2.50%) | 5.58 × 10^{-6} 14.2 (3.49–57.7) |
| +           | –                  | 2/30 (6.67%)        | 17/120 (14.2%) | 0.269 0.433 (0.0943–1.99) |
| –           | +                  | 5/30 (16.7%)        | 16/120 (13.3%) | 0.638 1.30 (0.435–3.89) |
| –           | –                  | 15/15 (50.0%)       | 84/120 (70.0%) | 0.0386 0.429 (0.190–0.968) |

### Abbreviations:

CI: confidence interval; CM-SJS, cold medicine-related Stevens-Johnson syndrome; OR, odds ratio; SOC, severe ocular complication; SNP, single-nucleotide polymorphism; TEN, toxic epidermal necrolysis. \(^a\) value for allele or genotype frequency. Comparison was between patients and controls using the chi-square test (Pearson). In table 1a, bold values denote OR values that are significant after correction for the multiplicity of testing by the number of comparisons. In table 1b, bold value denotes OR with additive effect.
In conclusion, this study clarified the following: (1) SNPs of PTGER3 are significantly associated with CM-SJS/TEN with SOC, and the association with PTGER3 SNP rs1327464 (OR (major allele) = 0.232, \( P = 7.92 \times 10^{-10} \)) is much stronger than that we previously reported regarding SJS/TEN with SOC, suggesting that CM-SJS/TEN with SOC might be a purer phenotype than SJS/TEN previously reported regarding SJS/TEN with SOC, and (2) in CM-SJS/TEN with SOC, HLA-A*02:06 with PTGER3 rs1327464 GA/AA also exerts an additive effect.

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AUTHOR CONTRIBUTIONS

MU and KT wrote the main manuscript text and prepared the tables. MU, KT and HS contributed to the analysis of the research findings and reviewed the manuscript. MU, CS, K-CY, MKK, KYS, C-KJ, KT and SK contributed materials to the research study and reviewed the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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Interactive effect of PTGER3 SNPs and HLA-A*02:06 in CM-SJS/TEN

M Ueta et al.

Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv).