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ARTICLE

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

Mayumi Ueta[1,2,3], Katsushi Tokunaga[2], Chie Sotozono[3], Hiromi Sawai[2], Kyung-Chul Yoon[4], Mee Kum Kim[5], Kyoung Yul Seo[6], Choun-Ki Joo[7], Kei Tashiro[8] and Shigeru Kinoshita[1]

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 SJSTEN 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,[4] 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 individuals[1] 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

[1]Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [3]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [4]Department of Ophthalmology, Chonnam National University, Gwangju, Korea; [5]Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea; [6]Department of Ophthalmology, Severance Hospital, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Korea; [7]Department of Ophthalmology and Visual Science, Seoul St Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea and [8]Department of Genomic Medical Sciences, Kyoto Prefectural University of Medicine, Kyoto, Japan.
Correspondence: M Ueta (mueta@koto.kpu-m.ac.jp)
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Table 1. Japanese CM-SJS/TEN with SOC patients

a. Association with PTGER3 SNPs

rs number of SNP	*Frequency of genotypes (%) Allele 1: major allele Allele 2: minor allele*				*Allele 1 versus allele 2*	*Genotype 11 versus 12+22*	*Genotype 11+12 versus 22*
	Genotypes		*Cases*	*Controls*	P *value*[a] *Corrected* P[b] *OR (95% CI)*	P *value*[a] *Corrected* P[b] *OR (95% CI)*	P *value*[a] *Corrected* P[b] *OR (95% CI)*
rs7555865	11	C/C	56/132 (42.4)	105/219 (47.9)	0.106	0.315	0.0772
	12	C/T	55/132 (41.7)	93/219 (42.5)			
	22	T/T	21/132 (15.9)	21/219 (9.59)			
rs17131450	11	C/C	97/132 (73.5)	194/221 (87.8)	**1.22×10^{-4}**	**6.36×10^{-4}**	0.0190
	12	C/T	30/132 (22.7)	26/221 (11.8)	**2.20×10^{-3}**	**0.0114**	0.342
	22	T/T	5/132 (3.79)	1/221 (0.452)	**0.379** (0.228–0.630)	**0.386** (0.221–0.674)	0.115 (0.0133–0.999)
rs5702	11	C/C	87/131 (66.4)	109/221 (49.3)	0.0228	**1.81×10^{-3}**	0.628
	12	C/T	32/131 (24.4)	95/221 (43.0)	0.410	**0.0325**	
	22	T/T	12/131 (9.16)	17/221 (7.69)	1.52 (1.06–2.17)	**2.03** (1.30–3.18)	
rs1325949	11	A/A	91/132 (68.9)	105/221 (47.5)	**1.94×10^{-3}**	**8.86×10^{-5}**	0.950
	12	A/G	30/132 (22.7)	98/221 (44.3)	**0.0349**	**1.59×10^{-3}**	
	22	G/G	11/132 (8.33)	18/221 (8.14)	**1.77** (1.23–2.55)	**2.45** (1.56–3.86)	
rs2421805	11	T/T	44/130 (33.8)	104/216 (48.1)	**1.93×10^{-3}**	9.21×10^{-3}	0.0108
	12	T/G	65/130 (50.0)	96/216 (44.4)	**0.0348**	0.166	0.194
	22	G/G	21/130 (16.2)	16/216 (7.41)	**0.602** (0.436–0.831)	0.551 (0.351–0.865)	0.415 (0.208–0.829)
rs7543182	11	G/G	92/132 (69.7)	112/221 (50.7)	9.29×10^{-3}	**4.64×10^{-4}**	0.591
	12	G/T	29/132 (22.0)	94/221 (42.5)	0.167	**8.36×10^{-3}**	
	22	T/T	11/132 (8.33)	15/221 (6.79)	1.63 (1.13–2.36)	**2.24** (1.42–3.53)	
rs.7555874	11	G/G	91/132 (68.9)	112/221 (50.7)	0.0130	**7.85×10^{-4}**	0.591
	12	G/A	30/132 (22.7)	94/221 (42.5)	0.234	**0.0141**	
	22	A/A	11/132 (8.33)	15/221 (6.79)	1.59 (1.10–2.30)	**2.16** (1.37–3.40)	
rs4147115	11	A/A	45/128 (35.2)	54/212 (25.5)	0.0603	0.0569	0.291
	12	A/T	54/128 (42.2)	99/212 (46.7)			
	22	T/T	29/128 (22.7)	59/212 (27.8)			
rs4650093	11	C/C	84/132 (63.6)	113/220 (51.4)	0.129	0.0247	0.486
	12	C/T	37/132 (28.0)	93/220 (42.3)		0.445	
	22	T/T	11/132 (8.33)	14/220 (6.36)		1.66 (1.06–2.58)	
rs17131478	11	G/G	86/131 (65.6)	135/219 (61.6)	0.420	0.452	0.613
	12	G/T	41/131 (31.3)	75/219 (34.2)			
	22	T/T	4/131 (3.05)	9/219 (4.11)			
rs17131479	11	C/C	87/132 (65.9)	135/217 (62.2)	0.485	0.486	0.744
	12	C/G	41/132 (31.1)	74/217 (34.1)			
	22	G/G	4/132 (3.03)	8/217 (3.69)			
rs7521005	11	A/A	84/132 (63.6)	114/221 (51.6)	0.138	0.0273	0.479
	12	A/G	37/132 (28.0)	93/221 (42.1)		0.491	
	22	G/G	11/132 (8.33)	14/221 (6.33)		1.64 (1.06–2.56)	
rs7541092	11	G/G	86/131 (65.6)	136/218 (62.4)	0.488	0.540	0.608
	12	G/A	41/131 (31.3)	73/218 (33.5)			
	22	A/A	4/131 (3.05)	9/218 (4.13)			
rs1327464	11	G/G	83/131 (63.4)	194/219 (88.6)	**7.92×10^{-10}**	**1.90×10^{-8}**	**1.23×10^{-3}**
	12	G/A	40/131 (30.5)	24/219 (11.0)	**1.43×10^{-8}**	**3.41×10^{-7}**	**0.0221**
	22	A/A	8/131 (6.11)	1/219 (0.457)	**0.232** (0.142–0.381)	**0.223** (0.129–0.385)	**0.0705** (0.00872–0.571)
rs1409161	11	G/G	57/132 (43.2)	68/221 (30.8)	0.0988	0.0183	0.958
	12	G/A	52/132 (39.4)	114/221 (51.6)		0.329	
	22	A/A	23/132 (17.4)	39/221 (17.6)		1.71 (1.09–2.67)	
rs34885906	11	T/T	120/132 (90.9)	189/221 (85.5)	0.152	0.138	—
	12	T/C	12/132 (9.09)	32/221 (14.5)			
	22	C/C	0/132 (0.00)	0/221 (0.00)			

Table 1. (Continued)

a. Association with PTGER3 SNPs

rs number of SNP	*Frequency of genotypes (%) Allele 1: major allele Allele 2: minor allele*				*Allele 1 versus allele 2*	*Genotype 11 versus 12+22*	*Genotype 11+12 versus 22*
	Genotypes		*Cases*	*Controls*	P *value*[a] *Corrected* P[b] *OR (95% CI)*	P *value*[a] *Corrected* P[b] *OR (95% CI)*	P *value*[a] *Corrected* P[b] *OR (95% CI)*
rs2817864	11	T/T	77/132 (58.3)	118/221 (53.4)	0.146	0.367	0.0352
	12	T/G	53/132 (40.2)	89/221 (40.3)	—	—	0.634
	22	G/G	2/132 (1.52)	14/221 (6.33)	—	—	4.40 (0.983–19.7)
rs1409981	11	G/G	37/131 (28.2)	60/218 (27.5)	0.874	0.884	0.664
	12	G/A	65/131 (49.6)	114/218 (52.3)			
	22	A/A	29/131 (22.1)	44/218 (20.2)			

*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)*
+	+	22/131 (16.8%)	4/218 (1.83%)	2.56×10^{-7}	**10.8** (3.63–32.1)
+	−	39/131 (29.8%)	26/218 (11.9%)	3.38×10^{-5}	3.13 (1.80–5.45)
−	+	26/131 (19.9%)	21/218 (9.63%)	6.80×10^{-3}	2.32 (1.25–4.33)
−	−	44/131 (33.6%)	167/218 (76.6%)	1.74×10^{-15}	0.154 (0.0956–0.249)

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]*P* value for allele or genotype frequency. Comparison was between patients and controls using the chi-square test (Pearson). [b]Corrected *P*: *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.

acute stage, many of our patients had developed SJS/TEN many years before their recruitment for this study.

We defined patients with severe ocular complications (SOC) as patients who manifested pseudomembranes and ocular surface (cornea and/or conjunctiva) epithelial defects in the acute stage, and as ones with ocular sequelae such as severe dry eye, symblepharon, trichiasis and conjunctival invasion into the cornea in the chronic stage.[1,2]

In this study, we focused on cold medicine-related SJS/TEN (CM-SJS/TEN) that may have been elicited by cold medicines such as multi-ingredient cold medications and nonsteroidal anti-inflammatory drugs (NSAIDs). All the patients included in this study had taken cold medicines (e.g., NSAIDs, multi-ingredient cold medications) for symptoms of common cold a few to several days before disease onset.[1,2] They were designated CM-SJS/TEN patients, although not all were able to name the specific drugs used. We also focused on SJS/TEN patients with SOC because the genetic predisposition might be different between SJS/TEN with and without SOC.[1]

Samples from 132 patients with CM-SJS/TEN with SOC were collected at Kyoto Prefectural University of Medicine. Of these patients, 51 were male and 81 were female; their ages ranged from 6 to 92 years (mean 42.4 ± 17.4 (s.d.) years). The age at SJS/TEN onset ranged from 1 to 70 years (mean 24.7 ± 16.2 years). Some of the patients could not recall the specific drug(s) they used. The controls were 221 healthy Japanese volunteers recruited by Kyoto Prefectural University of Medicine. They consisted of 89 males and 132 females; their ages ranged from 11 to 77 years (mean 35.6 ± 11.1 years). Some of the CM-SJS/TEN patients and some of the controls had been included in our earlier studies.

Samples from 30 Korean patients with CM-SJS/TEN with SOC were collected at Seoul National University College of Medicine, Yonsei University, Chonnam National University and the Catholic University of Korea. These patients consisted of 11 males and 19 females, ranging in age from 4 to 71 years (mean 35.2 ± 18.9 years). Their ages at the onset of SJS/TEN ranged from 3 to 63 years (mean 23.7 ± 16.3 years). Healthy Korean volunteers ranging from 16 to 85 years old ($n = 120$; 48 males and 72 females; mean age 38.1 ± 15.6 years) served as the controls. Some of the CM-SJS/TEN patients and some of the controls had been included in our earlier studies.

Genomic DNA from the peripheral blood of Japanese patients and controls was isolated by SRL (Tokyo, Japan). The PAXgene blood DNA kit (Qiagen, Hilden, Germany) was used to extract DNA from whole peripheral blood of Korean patients and controls.

SNP genotyping

In the TaqMan SNP genotyping assay, PCR amplification was performed in a 10-μl reaction mixture containing 1 μl of genomic DNA, 5.0 μl of TaqMan GTXpress master mix (Applied Biosystems, Foster, CA, USA), and 40 × TaqMan SNP genotyping assay probe (Applied Biosystems) for each SNP. The QPCR thermal cycling program was 95 °C for 20 s, followed by 50 cycles of 95 °C for 3 s and 60 °C for 20 s on a step-one plus system (Applied Biosystems).

In the DigiTag2 assay,[10,12] multiplex PCR was performed in 10 μl multiplex PCR buffer containing 25 ng genomic DNA, 25 nM each multiplex primer mix, 200 μM each dNTP, 2.25 mM $MgCl_2$ and 0.4 U KAPA2G Fast HotStart DNA polymerase (Kapa Biosystems, Boston, MA, USA). Cycling was performed at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 68 °C for 2 min.

A chi-square test was applied to a two-by-two contingency table for the allele frequency and the dominant and recessive models.

HLA-A genotyping

For HLA-A genotyping, we performed PCR amplification followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial bead-based typing kits (WAK Flow, Wakunaga, Hiroshima, Japan) as described elsewhere.[1,2]

RESULTS

Associations with PTGER3 SNPs in Japanese population

In Japanese patients, 7 of 18 SNPs previously reported to be associated with SJS/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 ($P = 7.92\times10^{-10}$).

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*				*Allele 1 versus allele 2*	*Genotype 11 versus 12+22*	*Genotype 11+12 versus 22*
	Genotypes		*Cases*	*Controls*	P *value*[a] *OR (95% CI)*	P *value*[a] *OR (95% CI)*	P *value*[a] *OR (95% CI)*
rs1327464	11	G/G	17/30 (56.7)	101/120 (84.2)	**0.00203**	**0.00101**	—
	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]*P* value for allele or genotype frequency. Comparison was between patients and controls using the chi-square test (Pearson). 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.

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,[1] 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, $P = 2.56\times10^{-7}$; Table 1b).

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.[2] 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 = 0.246, $P = 0.00101$; 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, $P = 5.58\times10^{-6}$; 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, $P = 7.92\times10^{-10}$) 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, $P = 0.0043$).[10] 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;[2] 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.[11] 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, $P = 4.34\times10^{-7}$), suggesting that these interactions are independent of each other. In the Japanese population, although HLA-A*02:06 alone showed OR = 5.46 and $P = 1.39\times10^{-11}$, and *PTGER3* rs1327464 GA/AA alone showed OR = 4.48 and $P = 1.90\times10^{-8}$, the combination of *HLA-A*02:06* and *PTGER3* rs1327464 GA/AA showed a higher OR (OR = 10.8, $P = 2.56\times10^{-7}$) 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, $P = 5.58\times10^{-6}$) 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.

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