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Review article

Cold medicine-related Stevens–Johnson syndrome/toxic epidermal necrolysis with severe ocular complications—phenotypes and genetic predispositions

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ABSTRACT

Stevens–Johnson syndrome (SJS) is an acute inflammatory vesiculobullous reaction of the skin and mucosa, such as the ocular surface, oral cavity, and genitals. In patients with extensive skin detachment and a poor prognosis, the condition is called toxic epidermal necrolysis (TEN). Severe ocular complications (SOCs) appear in some—but not all—SJS/TEN patients who are diagnosed by dermatologists, and cold medicines including multi-ingredient cold medications and nonsteroidal anti-inflammatory drugs are the main causative drugs particularly for SJS/TEN with SOCs and all SJS and TEN. In this review, we focus on the genetic predisposition of cold medicine-related SJS/TEN (CM-SJS/TEN) with SOCs. CM-SJS/TEN with SOCs was strongly associated with *HLA-A*02:06* and significantly associated with *HLA-B*44:03* in Japanese individuals, significantly associated with *HLA-B*44:03* in Indian and Brazilian individuals, and associated with *HLA-A*02:06* in Korean individuals. In the first genome-wide association study (GWAS), we found an association between the prostaglandin E receptor 3 (*PTGER3*) gene and SJS/TEN with SOCs. In this study, we focused on CM-SJS/TEN with SOCs and found that the association of CM-SJS/TEN with SOCs became stronger than all SJS/TEN with SOCs. In the second GWAS, we found an association between the *IKZF1* gene and CM-SJS/TEN with SOCs not only in Japanese, but also in Korean and Indian populations. Moreover, we found that *TSHZ2* gene single nucleotide polymorphisms (SNPs) also showed especially low *p* values in the Japanese population; however, this association was not found in the Korean population. Furthermore, we investigated the interaction between susceptibility genes, and found multiplicative interactions of *HLA-A*02:06* and *TLR3* SNPs and additive interactions of *HLA-A*02:06* and *PTGER3* SNPs.

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1. Stevens–Johnson syndrome/toxic epidermal necrolysis in ophthalmology

Stevens–Johnson syndrome (SJS) is an acute inflammatory vesiculobullous reaction of the skin and mucosa, such as the ocular surface, oral cavity, and genitals. In patients with extensive skin detachment and a poor prognosis, the condition is called toxic epidermal necrolysis (TEN). Table 1 shows the diagnostic criteria for SJS and TEN in Japan.[1] A definite diagnosis of SJS requires mucosal lesions, whereas a definite diagnosis of TEN does not. Thus, SJS/TEN with mucosal lesions consists of SJS and a part of TEN. Moreover, not all cases of SJS/TEN with mucosal lesions have severe ocular lesions such as severe conjunctivitis with pseudomembrane and ocular surface epithelial defects in the acute stage (Figure 1). Figure 2 shows a case of severe conjunctivitis with pseudomembrane and ocular surface epithelial defects in the acute stage. It is reported that about 40% of SJS/TEN cases had severe ocular complications (SOCs) with pseudomembrane and ocular surface epithelial defects.[1]

Furthermore, dermatologists see SJS/TEN patients only in their acute stage, whereas ophthalmologists encounter such patients not only in the acute stage but also in the chronic stage. Therefore, for ophthalmologists, it is not easy to render a differential diagnosis of SJS or TEN when patients present in the chronic stage because the vesiculobullous skin lesions expressed in the acute stage have healed by the chronic stage.[2] Diagnosis of SJS/TEN in

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Table 1
Diagnostic criteria for Stevens–Johnson syndrome and toxic epidermal necrolysis by Japanese Ministry of Health, Labour, and Welfare (2005).

Diagnostic criteria
Stevens–Johnson syndrome
Clinical entity
A severe mucocutaneous disorder characterized by erythema, epidermal detachment, and enanthema accompanied by high fever
Essential criteria (required)
1. Severe hyperemic and/or hemorrhagic mucocutaneous lesions
2. Epidermal detachment involving <10% of the total body surface area
3. High-grade fever (>38.0°C) in the absence of antipyretic therapy
Supportive findings
1. Flat, atypical target lesions
2. Bilateral acute keratoconjunctivitis accompanied by ocular surface epithelial defect and/or pseudomembrane formation
3. Histologic evidence of epidermal necrosis
Diagnostic criteria
Toxic epidermal necrolysis
Clinical entity
A severe mucocutaneous disorder characterized by extensive erythema, epidermal detachment (including blisters and erosions), and enanthema accompanied by high fever. The extent of epidermal detachment is >10% of the total body surface area
Essential criteria (required)
1. Epidermal detachment involving >10% of the total body surface area
2. Exclusion of staphylococcal scalded skin syndrome
3. High-grade fever (>38.0°C) in the absence of antipyretic therapy
Supportive findings
1. Generalized macular or diffuse erythema
2. Enanthema including bilateral acute keratoconjunctivitis accompanied by ocular surface epithelial defect and/or pseudomembrane formation
3. Histologic evidence of marked epidermal necrosis

Note. From "Predictive factors associated with acute ocular involvement in Stevens–Johnson syndrome and toxic epidermal necrolysis," C. Sotozono, M. Ueta, E. Nakatani, A. Kitami, H. Watanabe, H. Sueki, et al., 2015, *Am J Ophthalmol*, *160*, p. 228–37 e2. Copyright 2015, *Elsevier Science*. Reproduced with permission.

ophthalmology was 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.[3–14] SJS/TEN patients with SOC in the acute stage often experience severe ocular sequelae, such as vision loss and very severe dry eye for which these patients would not have been able to lead a normal life.[15] Figure 3 shows severe ocular sequelae such as very severe dry eye, trichiasis, symbrepharon, scarring of lid conjunctiva, and conjunctival invasion of the cornea in the chronic stage. We defined patients with SOCs in the acute stage as those who manifested pseudomembranes and epithelial defects on the ocular surface (cornea and/or conjunctiva),[16] and in the chronic stage as patients with ocular sequelae such as severe dry eye, trichiasis, symblepharon, and conjunctival invasion into the cornea.[15] Thus, ophthalmologists tend to report both SJS and TEN with SOCs as "SJS" in a broad sense.[2]

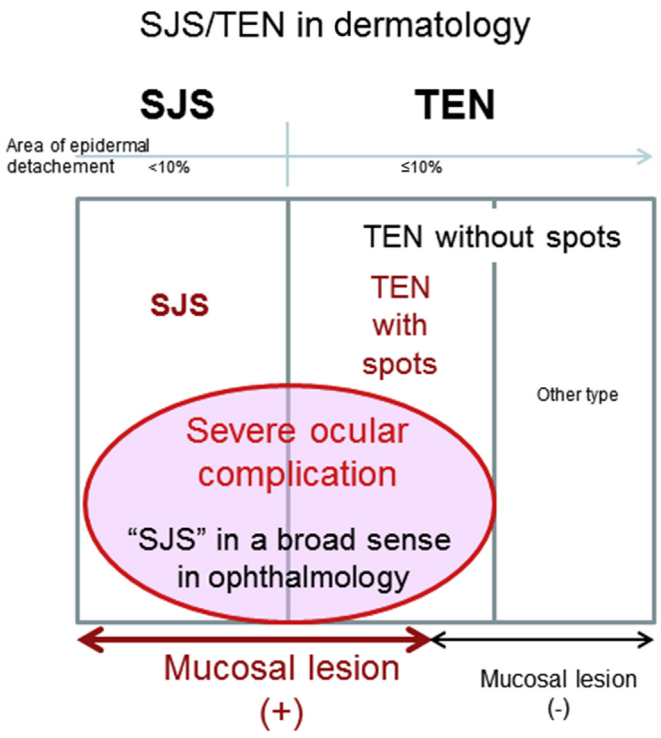

Figure 1. Ophthalmologists often report both SJS and TEN with severe ocular complications as SJS. SJS = Stevens–Johnson syndrome; TEN = toxic epidermal necrolysis. *Note.* From Ueta M. Genetic predisposition to Stevens–Johnson syndrome with severe ocular surface complications. *Cornea.* 2015;34(Suppl. 11):S158–S165. Reproduced with permission.

To summarize, SOCs appear in some—but not all—SJS/TEN patients who are diagnosed by dermatologists.

2. Causative drug and human leukocyte antigen analysis

SJS/TENs are rare with an annual incidence rate of 1–6 cases/1 million persons,[1,17,18] but SJS/TEN carry high mortality rates of 3% for SJS and 27% for TEN.[19] Moreover, they often associated with inciting drugs.[3–5,10,18,20–22] It was reported that allopurinol and anticonvulsants such as carbamazepine are the main inciting drugs for SJS/TEN[23]; however, we[2–5,10] and others[20,22] found that cold medicines including multi-ingredient cold medications and nonsteroidal anti-inflammatory drugs (NSAIDs) are also major causative drugs for SJS/TEN.

Furthermore, the association between human leukocyte antigen (HLA) genotypes and drug-induced severe cutaneous adverse reactions (SCARs) including SJS/TEN has been reported. Carbamazepine-induced SJS/TEN exhibited a very strong association with the *HLA-B*15:02* allele [case $n = 44$, control (tolerant) $n = 101$, odds ratio (OR) = 2504, $p_{corrected} = 3.1 \times 10^{-27}$] in Taiwanese Han Chinese patients.[24] The *HLA-A*31:01* allele was also reported to be strongly associated with carbamazepine-induced SCAR including SJS/TEN and drug-induced hypersensitivity syndrome (DIHS) in Japanese [case $n = 77$, control (tolerant) $n = 420$, OR = 9.5, $p = 1.1 \times 10^{-16}$][25] and European individuals [case $n = 145$, control (normal) $n = 257$, OR = 15.0, $p = 3.5 \times 10^{-8}$],[26] suggesting that different ethnic groups may have the different risk factors for carbamazepine-induced SCARs. Allopurinol, a uric acid-lowering drug, could also induce SCAR including SJS, TEN, and DIHS, and

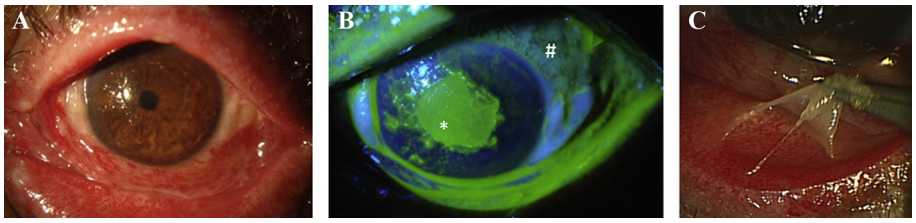

Figure 2. Severe conjunctivitis with pseudomembrane and ocular surface epithelial defects in the acute stage of Stevens–Johnson syndrome (SJS). Reproduced from Ueta with permission from Medical-Aoi Publications, Inc.

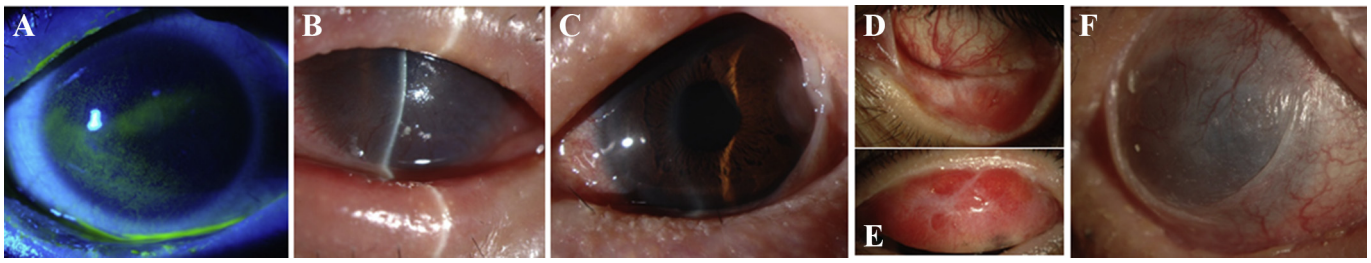

Figure 3. Severe ocular sequelae such as very severe dry eye, trichiasis, symbrepharon, scarring of lid conjunctiva and conjunctival invasion of cornea in the chronic stage of Stevens–Johnson syndrome (SJS). Reproduced from Ueta with permission from Medical-Aoi Publications, Inc.

allopurinol-induced SCARs were strongly associated with *HLA-B* 58:01* in Han Chinese [case $n = 51$, control (tolerant) $n = 135$, OR = 580, $p_{corrected} = 4.7 \times 10^{-24}$],[27] Caucasian [case $n = 27$, control (normal) $n = 1822$, OR = 80, $p_{corrected} < 10^{-6}$],[28] and Japanese patients (case $n = 36$, control (normal) $n = 986$, OR = 62.8, $p = 5.4 \times 10^{-12}$),[29] suggesting that different ethnic groups may share the same risk factors for allopurinol-induced SCARs.

We have also reported that cold medicine-related SJS/TEN (CM-SJS/TEN) with SOCs was strongly associated with *HLA-A*02:06* [case $n = 151$, control (normal) $n = 639$, OR = 5.6, $p = 2.7 \times 10^{-20}$] and significantly associated with *HLA-B*44:03* in Japanese individuals [case $n = 151$, control (normal) $n = 639$, OR = 2.0, $p = 1.3 \times 10^{-3}$], and these *HLA* genotypes were irrelevant to CM-SJS/TEN without SOCs.[3] Thus, genetic predisposition such as *HLA* genotype might be different between SJS/TEN with and without SOCs.[3] Moreover, *HLA-A*02:06* and *HLA-B*44:03* are not associated with cold medicine unrelated (other medicine related) SJS/TEN with SOCs,[3] suggesting that genetic predisposition, such as *HLA* genotype, might be different depending on their causative drugs.[3,5,30,31]

We also reported that CM-SJS/TEN with SOCs was significantly associated with *HLA-B*44:03* in Indian [case $n = 20$, control (normal) $n = 55$, OR = 12.3, $p = 1.1 \times 10^{-5}$] and Brazilian [especially Brazilian Caucasians; case $n = 15$, control (normal) $n = 62$, OR = 6.2, $p = 3.7 \times 10^{-3}$] individuals, and that *HLA-A*02:06* was associated with CM-SJS/TEN with SOC in Korean individuals [case $n = 31$, control (normal) $n = 90$, OR = 3.0, $p = 0.018$].[4]

We have likewise reported that cold medicines including multi-ingredient cold medications and NSAIDs were the main causative drugs for SJS/TEN with SOCs in all SJS and TEN.[1–3,5,10,31] About 80% of our SJS/TEN with SOC patients have developed SJS/TEN within several days after receiving treatment for common cold.[2,10]

Interestingly, allopurinol might induce SJS/TEN without SOCs,[30] and not all cases of carbamazepine-induced SJS/TEN have SOCs.[2]

In summary, genetic predisposition, such as *HLA* genotype, might be different depending on the causative drugs and their phenotype, for example, with and without SOCs (Figure 4).[2,3,5,31]

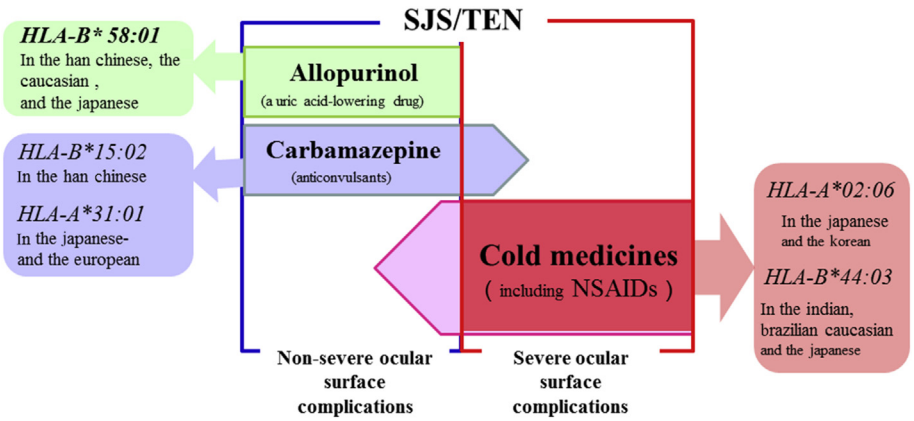

Figure 4. Associations between HLA type and the main causative drugs of SJS/TEN. HLA = human leukocyte antigen; NSAIDs = nonsteroidal anti-inflammatory drugs; SJS = Stevens–Johnson syndrome; TEN = toxic epidermal necrolysis. *Note.* From "IKZF1, a new susceptibility gene for cold medicine-related Stevens–Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement," by M. Ueta, H. Sawai, C. Sotozono C, et al., 2015, *J Allergy Clin Immunol*, *135*, p. 1538–1545. Copyright 2015, *Mosby*. Reproduced with permission.

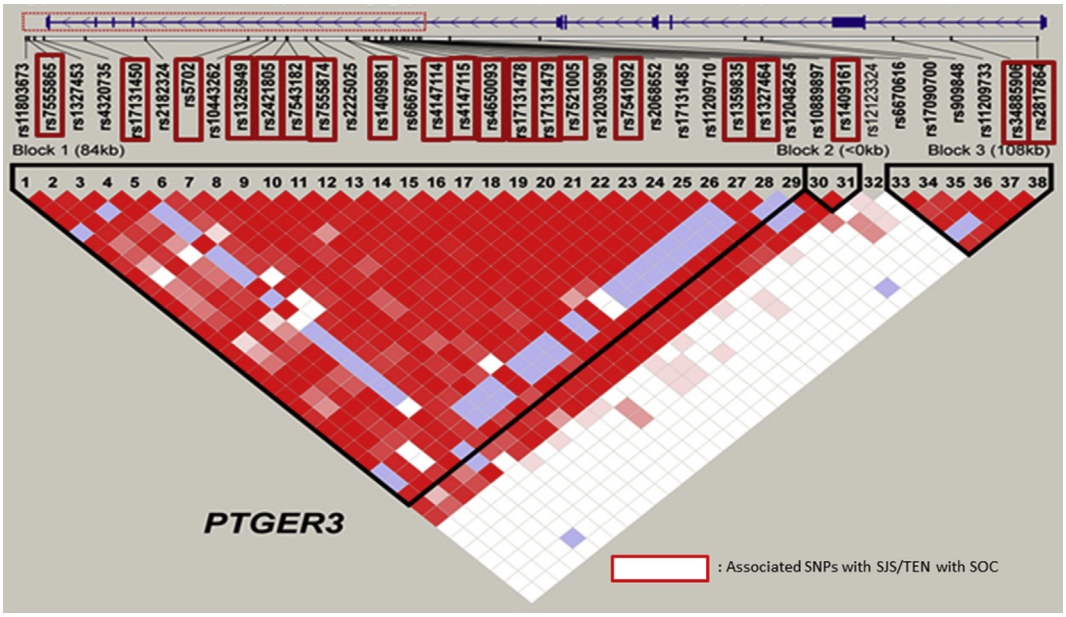

Figure 5. 20 SNPs of *PTGER3* associated with SJS/TEN with severe ocular complication. *PTGER3* = prostaglandin E receptor 3; SJS = Stevens–Johnson syndrome; SNP = single nucleotide polymorphism; TEN = toxic epidermal necrolysis. *Note*. From "Epistatic interaction between *Toll-like receptor 3 (TLR3)* and *prostaglandin E receptor 3 (PTGER3)* genes," by M. Ueta, G. Tamiya, K. Tokunaga, et al., 2012, *J Allergy Clin Immunol*, *129*, p. 1413–1416. Copyright 2012, *Mosby*. Reproduced with permission.

3. Genome wide association study

3.1. PTGER3: first genome-wide association study

We previously reported that a genome-wide association study (GWAS) using the Affymetrix GeneChip Mapping 500K Array Set (Affymetrix, Santa Clara, Calif) showed an association between *prostaglandin E receptor 3 (PTGER3)* gene and SJS/TEN with SOCs.[10] Moreover, we analyzed a total of 38 single nucleotide polymorphisms (SNPs) of the *PTGER3* gene using DigiTag2 assay[32,33] and found 20 SNPs associated with SJS/TEN with SOCs (Figure 5).[34] The protein of *PTGER3* gene is EP3. EP3 is one of the four receptors (EP1, EP2, EP3 and EP4) of prostaglandin E_2 (PGE2).[35]

Cold medicine such as NSAIDs (e.g., ibuprofen and loxoprofen) and cold medicine ingredients (e.g., acetaminophen) have the suppressive effect in the production of prostanoid, including PGE (Figure 6).[35]

It was reported that PGE_2 acts at EP3 in the airway epithelium and negatively regulates inflammation in the mouse asthma model.[36] We also reported that EP3 negatively regulated the inflammation of ocular surface in the mouse allergy conjunctivitis model[37] and skin in the mouse contact dermatitis model.[38] Because PGE_2 acts at EP3 and negatively regulates mucocutaneous inflammation,[36–38] we suggest that the suppression of PGE_2 production by cold medicine might contribute to the onset and pathogenesis of CM-SJS/TEN with SOCs.[2,10] Therefore, we turned to CM-SJS/TEN with SOCs. When we focused on CM-SJS/TEN with SOCs, the association with *PTGER3* gene became stronger than that in total SJS/TEN with SOCs; seven of 18 SNPs already reported to be associated with SJS/TEN were significantly associated with CM-SJS/TEN with SOCs after Bonferroni correction.[14] *PTGER3* SNP rs1327464 (G vs. A) was most significantly associated with CM-SJS/TEN with SOCs; the OR for the major allele was 0.232 ($p = 7.92 \times 10^{-10}$).[14] Figure 7 shows the p values

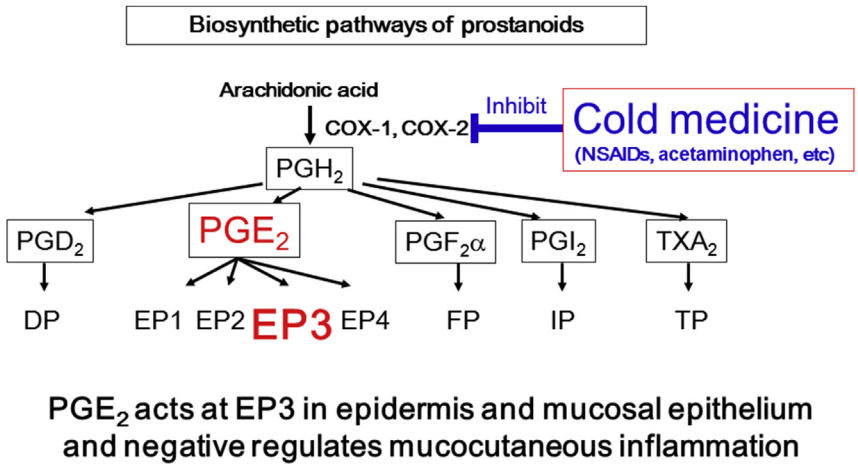

Figure 6. Cold medicine such as nonsteroidal anti-inflammatory drugs (NSAIDs) and cold medicine ingredients have the suppressive effect of the production of prostanoid, including PGE_2.

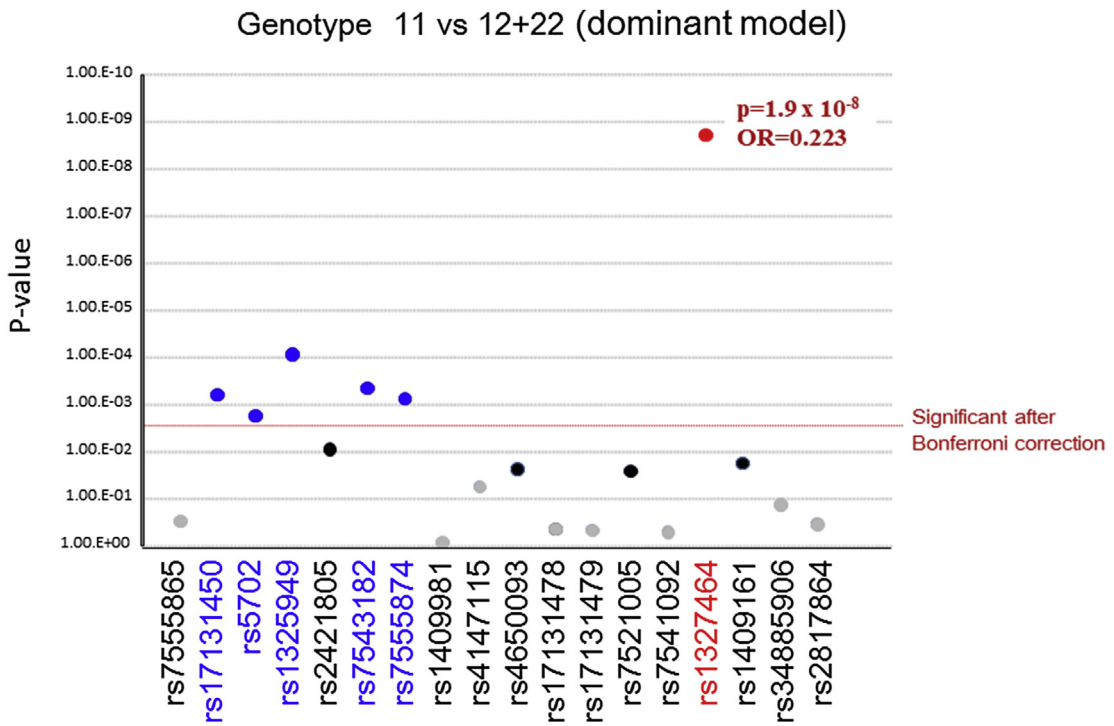

Figure 7. PTGER3 SNPs associated with CM-SJS/TEN with SOCs. PTGER3 SNP rs1327464 was most significantly associated with CM-SJS/TEN with SOCs

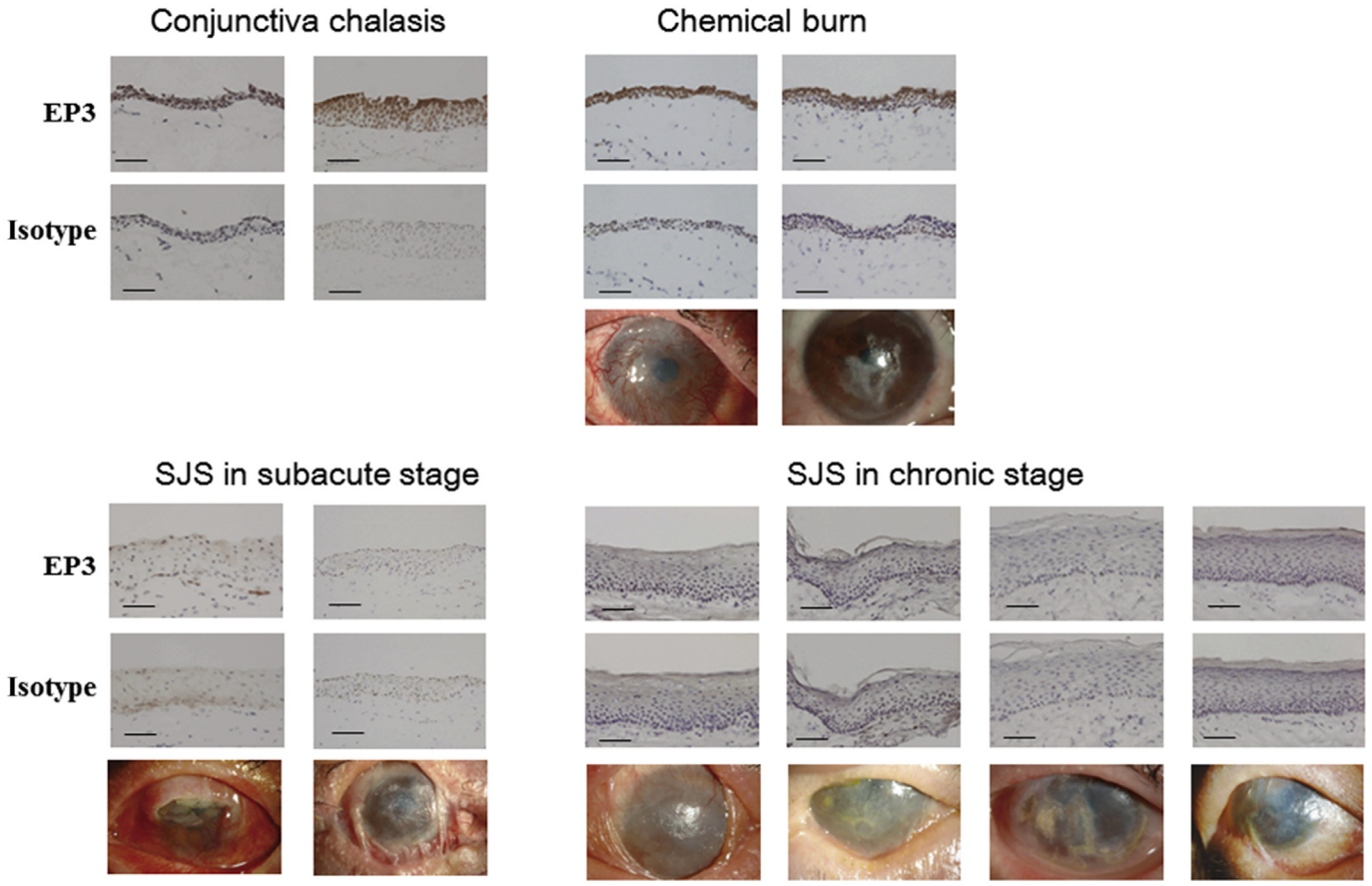

Figure 8. Downregulation of EP3 in the conjunctival epithelium of SJS/TEN with severe ocular complications. EP3 (brown stain) is strongly downregulated in the conjunctival epithelium of SJS compared with chemical burns. Controls consisted of isotype control antibodies instead of anti-EP3 antibodies. SJS = Stevens–Johnson syndrome; TEN = toxic epidermal necrolysis. *Note*. From "Prostaglandin E receptor subtype EP3 expression in human conjunctival epithelium and its changes in various ocular surface disorders," by M. Ueta, C. Sotozono, N. Yokoi, T. Inatomi, S. Kinoshita, 2011, *PLoS One*, *6*, p. e25209. Copyright 2011, *Public Library of Science*. Reproduced with permission.

of the 18 SNPs in the dominant models; *PTGER3* SNP rs1327464 (GG vs. GA + AA); $p = 1.90 \times 10^{-8}$, OR = 0.223.[14]

We also previously reported that EP3 protein levels are much lower in the conjunctival epithelium of patients with SJS/TEN with SOCs than control individuals such as conjunctival chalasis patients and chemical burn patients (Figure 8).[39] Thus, EP3 expression might be downregulated in the ocular surface of the SJS/TEN with SOCs patients.

Furthermore, because patients with CM-SJS/TEN with SOCs have developed this condition by taking cold medicines after having a common cold as a result of some viral or mycoplasma infections, we assume that not only cold medicine, but also some microbial infectious such as virus or mycoplasma, might be important and necessary to trigger the onset of SJS/TEN with SOCs.[2,31]

3.2. IKZF1: second GWAS

In the second GWAS, we focused on CM-SJS/TEN with SOCs, and performed a GWAS of Japanese 117 cases and 691 controls using the Affymetrix AXIOM Genome-Wide ASI 1 Array (Affymetrix, Santa Clara, Calif).[5] The Manhattan plot of the GWAS indicated that the HLA-A region showed the strongest association with susceptibility to CM-SJS/TEN with SOCs (Figure 9),[5] which was consistent with findings from our previous studies.[3,11,12]

Outside of the *HLA* region, there were 60 SNPs with $p < 10^{-3}$ (in the allele frequency, dominant model, or recessive model) in the GWAS.[5] Of the 60 SNPs, 47 were $p < 10^{-4}$, and 11 of these 47 were $p < 10^{-5}$.[5] Among the 11 SNPs of eight genes that were $p < 10^{-5}$, *IKZF1* showed especially low *p* values [rs897693: (C vs. T) OR = 4.3, $p = 1.2 \times 10^{-7}$; (CC + CT vs. TT), OR = 5.0, $p = 2.1 \times 10^{-8}$].[5]

Moreover, we genotyped the SNPs of the *IKZF1* gene with all Japanese samples (149 SJS, 877 controls) with 16 additional Japanese cases, Korean samples (31 SJS and 90 controls), Indian samples (20 CM-SJS/TEN with SOCs and 56 controls), and Brazilian samples (39 CM-SJS/TEN with SOCs and 135 controls), and then found that a meta-analysis from the Japanese, Korean, Indian, and Brazilian samples showed a significant genome-wide association between CM-SJS/TEN with SOCs and *IKZF1* [rs4917014 (G vs. T), OR = 0.5, $p = 8.5 \times 10^{-11}$].[5] These findings show that *IKZF1* may be a universal marker for susceptibility to CM-SJS/TEN with SOCs.[5]

Furthermore, we have performed a functional analysis for SNPs of the *IKZF1* gene and found that the Ik2/Ik1 (both are splicing isoforms) ratio may be influenced by *IKZF1* SNPs that are significantly associated with susceptibility to CM-SJS/TEN with SOCs.[5] To elucidate the role of *IKZF1* in the pathogenesis of CM-SJS/TEN with SOCs, we are going to do further investigations.

3.3. TSHZ2: second GWAS

Among the 11 SNPs of eight genes that were $p < 10^{-5}$ in the second GWAS, *TSHZ2* also showed especially low *p* values [rs4809905: (A vs. G), OR = 0.4, $p = 5.6 \times 10^{-7}$; (AA + AG vs. GG), OR = 0.3, $p = 1.5 \times 10^{-7}$].[5] Furthermore, we have examined a total of 17 SNPs of the near region of *TSHZ2* gene using 101 CM-SJS/TEN cases and 200 control samples, and found that seven SNPs (rs4811338, rs1555278, rs2092954, rs4371408, rs6021911, rs4809905, and rs6096940) showed a significant genome-wide association with CM-SJS/TEN with SOCs (Table 2). However, these SNPs were not associated with CM-SJS/TEN with SOCs in the Korean population (data not shown). *TSHZ2* might be a susceptibility gene for CM-SJS/TEN with SOCs in the Japanese population.

4. Interaction between susceptibility genes

4.1. HLA-A*02:06 and TLR3

SNPs have been widely used as genetic markers for identifying human disease susceptibility genes for the past decade. Moreover, it has recently become apparent that gene–gene interactions are meaningful in addition to major single-locus effects.[34] We previously reported that, in addition, *HLA-A*02:06*,[3,11,12] *PTGER3*,[10,34] and

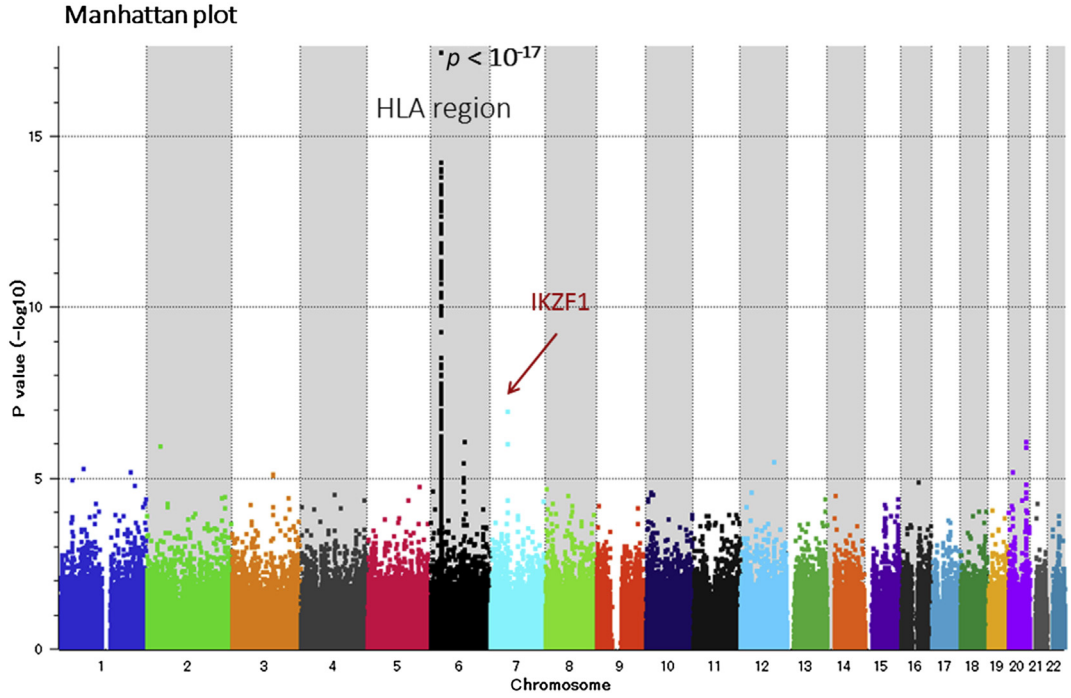

Figure 9. Manhattan plot of a genome-wide association study using the Affymetrix AXIOM Genome-Wide ASI 1 Array. HLA = human leukocyte antigen. *Note*. From "IKZF1, a new susceptibility gene for cold medicine-related Stevens–Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement," by M. Ueta, H. Sawai, C. Sotozono C, et al., 2015, *J Allergy Clin Immunol*, *135*, p. 1538–1545. Copyright 2015, *Mosby*. Reproduced with permission.

IKZF1,[5] *TLR3*[9,34] SNPs showed significant associations with SJS/TEN with SOCs, although the expression of TLR3 protein on the ocular surface was not different between SJS/TEN and controls such as conjunctival chalasis and chemical burn (Figure 10).[34]

When we focused on CM-SJS/TEN with SOCs, five of the seven SNPs, which were significantly associated with SJS/TEN with SOCs in our previous report,[13,34] were still associated with CM-SJS/TEN with SOCs (Table 3).

We previously also reported that *HLA-A*02:06* with *TLR3* SNPs exerted more than additive effects in SJS/TEN with SOCs.[13] When we focused on CM-SJS/TEN with SOCs, more than additive effects of *HLA-A*02:06* with *TLR3* SNPs in CM-SJS/TEN with SOCs were still observed. *HLA-A*02:06* and *TLR3* SNP rs3775296T/T or *TLR3* SNP rs5743312T/T exerted more than additive effects ($p < 0.00005$, OR = 37.7, Woolf's correction or $p < 0.00005$, OR = 37.5, Woolf's correction): 10 of 133 patients (7.5%) manifested both *HLA-A*02:06* and *TLR3* rs3775296T/T SNP or *TLR3* rs5743312T/T SNP, whereas none of the controls did (Table 4). There was a strong linkage disequilibrium (LD) between rs.3775296 and rs.5743312. Moreover, *HLA-A*02:06* and *TLR3* SNP rs3775290A/A exerted additive effects ($p < 0.00005$, OR = 9.9): 16 of 133 patients (12.0%) manifested both *HLA-A*02:06* and *TLR3* rs3775290A/A SNP, whereas only three of 220 controls (1.4%) did (Table 4).

By interaction analysis, *HLA-A*02:06* and *TLR3* SNP rs3775296T/T, which were in strong LD with *TLR3* SNP rs5743312T/T, manifested more than additive effects; therefore, the multiplicative

Table 2
Association between CM-SJS/TEN with SOCs and *TSHZ2* gene SNPs.

rs no. of SNP	Genotypes		Control	Case	Allele 1 vs. allele 2	Genotype 11 vs. 12 + 22	Genotype 11 + 12 vs. 22
					p[a] OR (95% CI)	p[a] OR (95% CI)	p[a] OR (95% CI)
rs7360046	11	G/G	1/200 (0.5)	0/101 (0.0)	0.28	0.48	0.31
	12	G/A	27/200 (13.5)	10/101 (9.9)	—	—	—
	22	A/A	172/200 (86.0)	91/101 (90.1)	—	—	—
rs4811338	11	G/G	29/200 (14.5)	5/101 (5.0)	**9.7×10^{-9}**	0.014	**7.0×10^{-10}**
	12	G/T	109/200 (54.5)	27/101 (26.7)	0.31	0.31	0.21
	22	T/T	62/200 (31.0)	69/101 (68.3)	(0.2–0.5)	(0.1–0.8)	(0.1–0.3)
rs1555278	11	T/T	19/196 (9.7)	5/100 (5.0)	**4.0×10^{-8}**	0.16	**7.7×10^{-10}**
	12	T/C	96/196 (49.0)	16/100 (16.0)	0.29	—	0.19
	22	C/C	81/196 (41.3)	79/100 (79.0)	(0.2–0.5)	—	(0.1–0.3)
rs6096922	11	G/G	1/200 (0.5)	0/101 (0.0)	0.28	0.48	0.31
	12	G/A	27/200 (13.5)	10/101 (9.9)	—	—	—
	22	A/A	172/200 (86.0)	91/101 (90.1)	—	—	—
rs4811342	11	G/G	1/200 (0.5)	0/101 (0.0)	0.28	0.48	0.31
	12	G/A	27/200 (13.5)	10/101 (9.9)	—	—	—
	22	A/A	172/200 (86.0)	91/101 (90.1)	—	—	—
rs2092954	11	C/C	29/200 (14.5)	5/101 (5.0)	**9.7×10^{-9}**	0.013	**7.0×10^{-10}**
	12	C/T	109/200 (54.5)	27/101 (26.7)	0.31	0.31	0.21
	22	T/T	62/200 (31.0)	69/101 (68.3)	(0.2–0.5)	(0.1–0.8)	(0.1–0.3)
rs4811343	11	C/C	1/200 (0.5)	0/101 (0.0)	0.22	0.48	0.26
	12	C/T	21/200 (10.5)	7/101 (6.9)	—	—	—
	22	T/T	178/200 (89.0)	94/101 (93.1)	—	—	—
rs4371408	11	G/G	29/200 (14.5)	5/101 (5.0)	**9.7×10^{-9}**	0.013	**7.0×10^{-10}**
	12	G/A	109/200 (54.5)	27/101 (26.7)	0.31	0.31	0.21
	22	A/A	62/200 (31.0)	69/101 (68.3)	(0.2–0.5)	(0.1–0.8)	(0.1–0.3)
rs6021911	11	G/G	20/200 (10.0)	5/101 (5.0)	**4.0×10^{-8}**	0.13	**9.8×10^{-10}**
	12	G/A	98/200 (49.0)	17/101 (16.8)	0.29	—	0.19
	22	A/A	82/200 (41.0)	79/101 (78.2)	(0.2–0.5)	—	(0.1–0.3)
rs4809905	11	A/A	28/200 (14.0)	5/101 (5.0)	**6.7×10^{-9}**	0.018	**5.3×10^{-10}**
	12	A/G	105/200 (52.5)	24/101 (23.8)	0.30	0.32	0.20
	22	G/G	67/200 (33.5)	72/101 (71.3)	(0.2–0.5)	(0.1–0.9)	(0.1–0.3)
rs8123906	11	A/A	3/200 (1.5)	0/101 (0.0)	0.0010	0.21	0.0011
	12	A/G	49/200 (24.5)	10/101 (9.9)	0.33	—	0.31
	22	G/G	148/200 (74.0)	91/101 (90.1)	(0.2–0.7)	—	(0.2–0.6)
rs6096940	11	G/G	28/200 (14.0)	5/101 (5.0)	**9.3×10^{-9}**	0.018	**9.1×10^{-10}**
	12	G/A	104/200 (52.0)	24/101 (23.8)	0.30	0.32	0.21
	22	A/A	68/200 (34.0)	72/101 (71.3)	(0.2–0.5)	(0.1–0.9)	(0.1–0.3)
rs968162	11	A/A	20/200 (10.0)	5/101 (5.0)	5.5×10^{-8}	0.13	**1.6×10^{-9}**
	12	A/G	97/200 (48.5)	17/101 (16.8)	0.30	—	0.20
	22	G/G	83/200 (41.5)	79/101 (78.2)	(0.2–0.5)	—	(0.1–0.3)
rs6021974	11	A/A	3/200 (1.5)	0/101 (0.0)	4.8×10^{-4}	0.22	5.0×10^{-4}
	12	A/G	49/200 (24.5)	9/101 (8.9)	0.29	—	0.28
	22	G/G	148/200 (74.0)	92/101 (91.1)	(0.1–0.6)	—	(0.1–0.6)
rs1474794	11	T/T	31/200 (15.5)	5/101 (5.0)	1.2×10^{-6}	0.0077	1.1×10^{-6}
	12	T/C	105/200 (52.5)	34/101 (33.7)	0.39	0.28	0.30
	22	C/C	64/200 (32.0)	62/101 (61.4)	(0.3–0.6)	(0.1–0.8)	(0.2–0.5)
rs6068248	11	C/C	30/200 (15.0)	5/100 (5.0)	2.3×10^{-6}	0.011	1.6×10^{-6}
	12	C/T	106/200 (53.0)	34/100 (34.0)	0.40	0.30	0.30
	22	T/T	64/200 (32.0)	61/100 (61.0)	(0.3–0.6)	(0.1–0.8)	(0.2–0.5)
rs6068268	11	C/C	27/199 (13.6)	33/101 (32.7)	5.7×10^{-6}	9.2×10^{-5}	4.4×10^{-4}
	12	C/T	97/199 (48.7)	50/101 (49.5)	2.2	3.1	2.8
	22	T/T	75/199 (37.7)	18/101 (17.8)	(1.6–3.1)	(1.7–5.5)	(1.6–5.0)

CI = confidence interval; CM-SJS = cold medicine-related Stevens–Johnson syndrome; OR = odds ratio; SNP = single nucleotide polymorphism; SOC = severe ocular complication; TEN = toxic epidermal necrolysis.
Bold shows the genome wide significance ($p < 5 \times 10^{-8}$).
[a] p value for allele or genotype frequency comparison between case and control using the chi-square test.

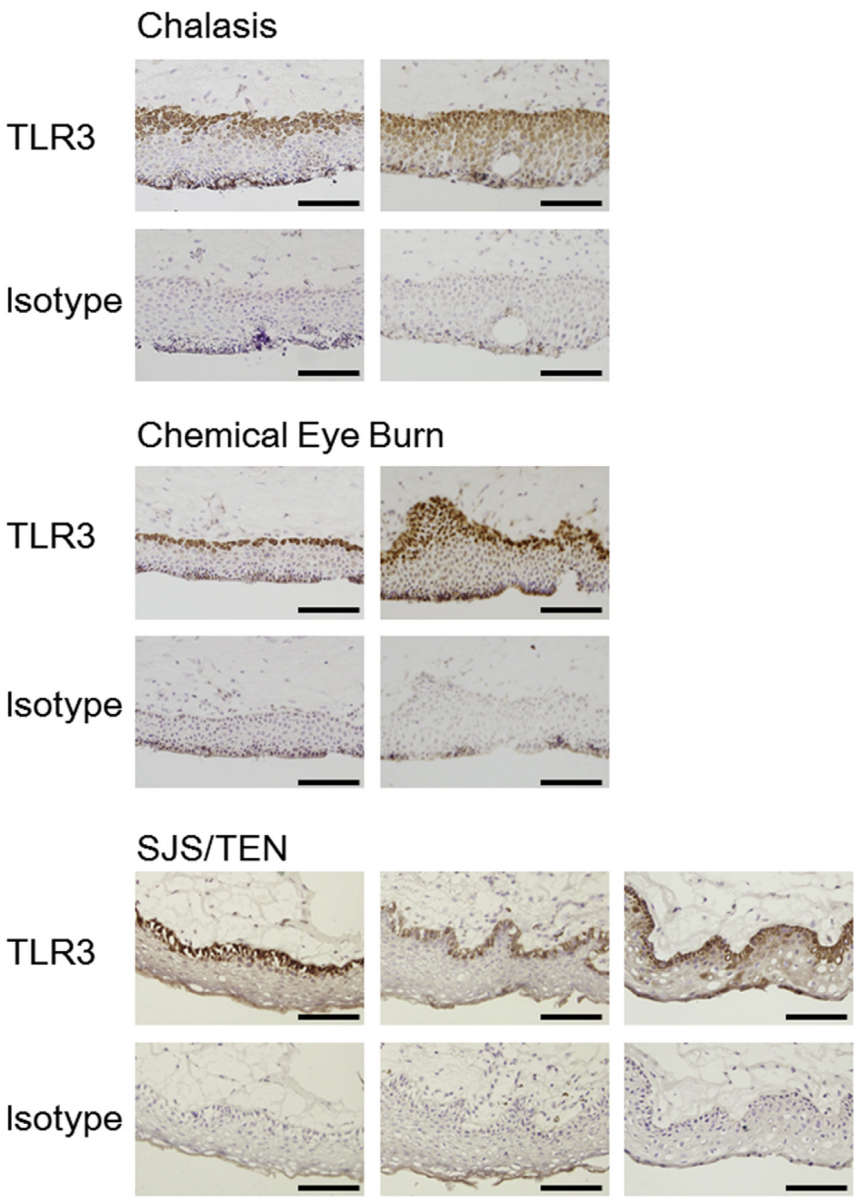

Figure 10. TLR3 expression in the conjunctival epithelium of SJS/TEN with severe ocular complications. The expression of TLR3 protein (brown stain) on the ocular surface was not different between SJS/TEN and controls such as conjunctival chalasis and chemical burn. Controls consisted of isotype control antibodies instead of anti-EP3 antibodies. SJS = Stevens–Johnson syndrome; TEN = toxic epidermal necrolysis. *Note*. From "Epistatic interaction between Toll-like receptor 3 (TLR3) and prostaglandin E receptor 3 (PTGER3) genes," by M. Ueta, G. Tamiya, K. Tokunaga, et al., 2012, *J Allergy Clin Immunol*, *129*, p. 1413–1416. Copyright 2012, *Mosby*. Reproduced with permission.

interactions of *HLA-A* and *TLR3* gene might be required for the onset of CM-SJS/TEN with SOCs.

HLA-A is a component of HLA Class I, which resides on the surface of all nucleated cells and alerts the immune system that the cell may be infected by a virus, thereby targeting the cell for destruction, and TLR3 recognizes viral double-stranded RNA.[40]

As the onset of SJS/TEN was associated not only with the administration of drugs but also with putative microbial infection, multiplicative interactions of *HLA-A* and *TLR3* gene might contribute to the characteristic and pathogenic immune response to the microbial infection.

4.2. *HLA-A*02:06* and *PTGER3*

Next, we looked for interactive effects between these seven SNPs of the *PTGER3* gene and *HLA-A*02:06* in CM-SJS/TEN with SOCs, because the *PTGER3* SNPs were significantly associated with the patients.[14] 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, $p = 2.56 \times 10^{-7}$; Table 5).[14] In the Japanese population, although *PTGER3* rs1327464 GA/AA alone showed OR = 4.48 and $p = 1.90 \times 10^{-8}$, and HLA-A*02:06 alone showed OR = 5.46 and $p = 1.39 \times 10^{-11}$, the combination of *PTGER3* rs1327464 GA/AA and *HLA-A*02:06* could show a higher OR (OR = 10.8, $p = 2.56 \times 10^{-7}$) than each allele alone.

Moreover, we found that even in Korean populations, there was an additive effect between the SNPs of the *PTGER3* gene and *HLA-A*02:06* (Table 6).[14] In the Korean population, the combination of *PTGER3* rs1327464 GA/AA and *HLA-A*02:06* could show a higher OR (OR = 14.2, $p = 5.58 \times 10^{-6}$) than each allele alone, although *PTGER3* rs1327464 GA/AA alone showed OR = 4.07 and $p = 0.00101$, and *HLA-A*02:06* alone showed OR = 2.50 and $p = 0.0412$.

Table 3
Association with TLR3 SNPs in CM-SJS/TEN with SOC in Japanese individuals.

rs no. of SNP	Frequencies of genotypes (%)				Allele 1 vs. allele 2	Genotype 11 vs. 12 + 22	Genotype 11 + 12 vs. 22
	Genotypes		Control	Case	p[a] OR (95% CI)	p[a] OR (95% CI)	p[a] OR (95% CI)
rs4861699	11	A/A	28/221 (12.7)	8/86 (9.3)	0.0156	0.4102	**0.0066**
	12	A/G	105/221 (47.5)	29/86 (33.7)	0.62	0.71	0.50
	22	G/G	88/221 (39.8)	49/86 (57.0)	(0.4–0.9)	(0.3–1.6)	(0.3–0.8)
rs6822014	11	G/G	9/214 (4.2)	13/131 (9.9)	0.0673	**0.0349**	0.2377
	12	G/A	72/214 (33.6)	45/131 (34.4)	1.40	2.51	1.30
	22	A/A	133/214 (62.1)	73/131 (55.7)	(1.0–2.0)	(1.0–6.0)	(0.8–2.0)
rs11732384	11	A/A	16/220 (7.3)	9/133 (6.8)	0.1737	0.8575	0.1081
	12	A/G	91/220 (41.4)	44/133 (33.1)	0.78	0.93	0.70
	22	G/G	113/220 (51.4)	80/133 (60.2)	(0.6–1.1)	(0.4–2.2)	(0.5–1.1)
rs3775296	11	T/T	12/220 (5.5)	18/133 (13.5)	0.0604	**0.0083**	0.3436
	12	T/G	94/220 (42.7)	53/133 (39.8)	1.37	2.71	1.23
	22	G/G	114/220 (51.8)	62/133 (46.6)	(1.0–1.9)	(1.3–5.8)	(0.8–1.9)
rs5743312	11	T/T	10/219 (4.6)	17/133 (12.8)	**0.0492**	**0.0050**	0.3195
	12	T/C	90/219 (41.1)	51/133 (38.3)	1.40	3.06	1.24
	22	C/C	119/219 (54.3)	65/133 (48.9)	(1.0–2.0)	(1.4–6.9)	(0.8–1.9)
rs7668666	11	A/A	27/212 (12.7)	26/133 (19.5)	0.0986	0.0876	0.2795
	12	A/C	101/212 (47.6)	62/133 (46.6)	1.30	1.66	1.28
	22	C/C	84/212 (39.6)	45/133 (33.8)	(1.0–1.8)	(0.9–3.0)	(0.8–2.0)
rs3775290	11	A/A	25/220 (11.4)	26/133 (19.5)	0.1554	**0.0340**	0.6324
	12	A/G	110/220 (50.0)	59/133 (44.4)	1.25	1.90	1.11
	22	G/G	85/220 (38.6)	48/133 (36.1)	(0.9–1.7)	(1.0–3.4)	(0.7–1.7)

CI = confidence interval; CM-SJS = cold medicine-related Stevens–Johnson syndrome; OR = odds ratio; SNP = single nucleotide polymorphism; SOC = severe ocular complication; TEN = toxic epidermal necrolysis.
Bold shows $p < 0.05$.
[a] p value for allele or genotype frequency comparison between case and control using the chi-square test.

Table 4
Interaction between *HLA-A*02:06* and *TLR3* SNPs in CM-SJS/TEN with SOCs.

HLA-A*02:06 and TLR3 rs3775296					
HLA-A*02:06	rs3775296	感冒薬SJS	Controls	p[a]	OR (95% CI)
+	+	10/133 (7.52%)	0/220 (0.00%)	3.69×10^{-5}	37.6* (2.19–647.7)
+	−	52/133 (39.1%)	30/220 (13.6%)	4.04×10^{-8}	4.07 (2.42–6.83)
−	+	8/133 (6.02%)	12/220 (5.45%)	0.8253	1.11 (0.441–2.79)
−	−	63/133 (47.4%)	178/220 (80.9%)	5.34×10^{-11}	0.212 (0.132–0.343)
HLA-A*02:06 and TLR3 rs5743312					
HLA-A*02:06	rs5743312	感冒薬SJS	Controls	p[a]	OR (95% CI)
+	+	10/133 (7.52%)	0/219 (0.00%)	3.84×10^{-5}	37.5[b] (2.18–644.7)
+	−	52/133 (39.1%)	30/219 (13.7%)	4.61×10^{-8}	4.04 (2.41–6.80)
−	+	7/133 (5.26%)	10/219 (4.57%)	0.7674	1.16 (0.431–3.13)
−	−	64/133 (48.1%)	179/219 (81.7%)	3.75×10^{-11}	0.207 (0.128–0.336)
HLA-A*02:06 and TLR3 rs3775290					
HLA-A*02:06	rs3775290	感冒薬SJS	Controls	p[a]	OR (95% CI)
+	+	16/133 (12.0%)	3/220 (1.36%)	1.68×10^{-5}	9.89 (2.82–34.6)
+	−	46/133 (34.6%)	27/220 (12.3%)	5.28×10^{-7}	3.78 (2.21–6.48)
−	+	10/133 (7.52%)	22/220 (10.0%)	0.4314	0.732 (0.335–1.60)
−	−	61/133 (45.9%)	168/220 (76.4%)	6.00×10^{-9}	0.262 (0.165–0.416)

CI = confidence interval; CM-SJS = cold medicine-related Stevens–Johnson syndrome; OR = odds ratio; SNP = single nucleotide polymorphism; SOC = severe ocular complication; TEN = toxic epidermal necrolysis.
[a] p value for allele or genotype frequency comparison between case and control using the chi-square test.
[b] Woolf's correction.

Table 5
Interaction between HLA-A*02:06 and PTGER3 rs1327464 GA/AA among Japanese individuals.

HLA-A*02:06	rs1327464 GA or AA	CM-SJS/TEN with SOC	Controls	p[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)

CM-SJS = cold medicine-related Stevens–Johnson syndrome; HLA = human leukocyte antigen; OR = odds ratio; PTGER3 = prostaglandin E receptor 3; SOC = severe ocular complication; TEN = toxic epidermal necrolysis.
Note. From "HLA-A*02:06 and PTGER3 polymorphism exerts additive effects in cold medicine-related Stevens–Johnson syndrome with severe ocular complications," by M. Ueta, K. Tokunaga, C. Sotozono, H. Sawai, K.C. Yoon, M.K. Kim, et al., 2015, *Hum Genome Var*, *2*, p. 15023. Copyright 2015, *Nature Publishing Group*. Reproduced with permission.

Table 6
Interaction between HLA-A*02:06 and PTGER3 rs1327464 GA/AA among Korean individuals.

HLA-A*02:06	rs1327464 GA or AA	CM-SJS/TEN with SOC	Controls	p^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)

CM-SJS = cold medicine-related Stevens–Johnson syndrome; HLA = human leukocyte antigen; OR = odds ratio; PTGER3 = prostaglandin E receptor 3; SOC = severe ocular complication; TEN = toxic epidermal necrolysis.
Note. From "HLA-A*02:06 and PTGER3 polymorphism exerts additive effects in cold medicine-related Stevens–Johnson syndrome with severe ocular complications," by M. Ueta, K. Tokunaga, C. Sotozono, H. Sawai, K.C. Yoon, M.K. Kim, et al., 2015, *Hum Genome Var*, *2*, p. 15023. Copyright 2015, *Nature Publishing Group*. Reproduced with permission.
[a] *p* value for allele or genotype frequency. Comparison was between patients and controls using the chi-square test (Pearson).

Thus, the interaction between *HLA-A*02:06* and *PTGER3* SNP might strongly contribute to the onset of CM-SJS/TEN with SOCs.

5. Application strategy in future

Because CM-SJS/TEN is a rare condition that probably has a complex genetic background, it is reasonable to posit multiplicative interactions of genes such as *HLA-A* and *TLR3*,[13] and *HLA-A* and *PTGER3*.[14]

Notably, 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 SOCs, suggesting that the interactions are independent of each other.[14]

These findings might show that the combinations of the two CM-SJS/TEN with SOCs associated polymorphisms, such as *HLA-A*02:06* and *TLR3 SNP* and *HLA-A*02:06* and *PTGER3 SNP*, could give improvements for a genetic testing compared with using only one susceptibility gene.[14]

Based on all of our previous 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 SOCs.[14]

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