drug-specific $CD8^+$ T cells were found to predominantly proliferate during the acute stages of SJS/TEN.[54] While drug-specific $CD4^+$ T cells are essential in drug-mediated immune reaction, $CD8^+$ T cells are critical to the development of SJS/TEN.

If SJS/TEN is the ultimate adverse drug reaction on the spectrum of erythema multiforme, which are non-severe adverse drug reactions, then regulation of the immunological reaction could influence the severity of the reactions. For example, immunocompromised individuals, such as patents with AIDS or malignancies or those who undergo immunosuppressive therapy, tend to be prone to SJS/TEN.[3,55]

REGULATORY T CELL

Regulatory T cells maintain self-tolerance and suppress immune responses. Treg have been reported to be involved in the pathogenesis of SJS/TEN.[56,57] Treg function is profoundly impaired in TEN, even though the cells are present at normal frequency.[57] These functional defects in TEN are restored upon recovery. These findings indicate that transitory impairment in their function during the acute stage of TEN may relate to severe epidermal damage, while a gradual loss of their function after resolution of DIHS may increase the subsequent risk of autoimmune disease occurrence.[57] Indeed, in an animal model of TEN, Treg cells were found to prevent experimentally induced epidermal injury mimicking TEN.[56,58]

$CD4^+Foxp3^+$ Treg exists in heterogeneous subpopulations divided by the level of CD45RA expression: $CD4^+CD45RA^+$ Foxp3low resting Treg (rTreg), $CD4^+CD45RA^-$Foxp3high activated Treg (aTreg) and cytokine-secreting $CD4^+CD45RA^-$Foxp3low non-suppressive T cells (non-Treg).[59] Particularly, non-Treg lacks immunosuppressive activity and has the potential to secrete pro-inflammatory cytokines such as γ-interferon and IL-17.[59] The relative frequencies of the three subtypes varies in disease conditions.[60–62] For example, the number of aTreg cells is low and the number of non-Treg cells is high in active systemic lupus erythematosus.[59] The relative frequencies of the three Treg subpopulations and the cytokine-secreting activity are found to differ between SJS/TEN patients and healthy controls.[63] These results indicate that the imbalance of Treg subpopulations is involved in the pathogenesis of SJS/TEN.

T-HELPER (TH)17 CELLS

T-helper 17 cells, a recently described effector $CD4^+$ T-cell subset that produces IL-17 and IL-22, have been implicated in the pathogenesis of various autoimmune and allergic diseases.[64] The proportion of circulating IL-17-producing $CD4^+$ T cells but not $CD8^+$ T cells is significantly higher in patients with SJS/TEN than in patients with erythema multiforme, as well as in healthy subjects.[65] IL-17-producing $CD4^+$ T cells in a CLA^+CCR4^+ subset with skin-homing properties are found at a significantly higher proportion in this subset of patients with SJS/TEN.[65] The proportion of circulating Th17 cells decreases significantly after disease improvement. Collectively, these results suggest that skin-homing Th17 cells are involved in the pathogenesis of SJS/TEN. Th17 cells may be involved in inflammation and tissue damage in patients with SJS/TEN through regulation of the recruitment of neutrophils and other inflammatory leukocytes.

The percentages of Th17 tend to be high in SJS/TEN (2–6 days after onset) as compared to normal subjects and MPE patients.[66]

CD94/NKG2C$^+$ CTL

Cytotoxic T lymphocytes with NK-like activity (NK-CTL) have been shown to express TCR restricted by the HLA-Ib molecule HLA-E.[67] Alternatively, the HLA-E-specific activating receptor CD94/NKG2C can trigger TCR-independent cytotoxicity in CTL. In SJS/TEN lesions, keratinocytes from affected skin express HLA-E, which sensitizes keratinocytes to killing by CD94/NKG2C$^+$ CTL.[68] The frequencies of CD94/NKG2C$^+$ peripheral blood T and NK cells were found to be increased in patients with SJS/TEN during the acute phase. The Morel report indicates that CD94/NKG2C may be involved in triggering cytotoxic lymphocytes in patients with SJS/TEN.

CONCLUSION

The SJS/TEN pathomechanism involves immunological phenomena, including antigen (causative drug) presentation and cytotoxic signaling, and the pathomechanism involves immune molecule and T lymphocyte subtypes. Recent advances in the genetics and immunology of SJS/TEN provide promising targets for SJS/TEN disease prevention and therapies.

CONFLICT OF INTEREST: None.

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Predictive Factors Associated With Acute Ocular Involvement in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

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• PURPOSE: To suggest an objective score for grading the acute ocular severity of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and to determine predictive factors for severe acute ocular involvement such as ocular surface epithelial defect and/or pseudomembrane formation.

• DESIGN: Retrospective cohort study.

• METHODS: The medical records of SJS (n = 87) and TEN (n = 48) patients between 2005 and 2007 were reviewed. An acute ocular severity score was determined on a scale from 0 to 3 (none, mild, severe, and very severe) according to the existence of hyperemia, corneal or conjunctival epithelial defect, and pseudomembrane formation. The associations between the severe acute ocular involvement and factors such as patient age, exposed drugs, systemic severity, and the prevalence of ocular sequelae were examined.

• RESULTS: The number of cases with score grade 0, 1, 2, and 3 was 19 (21.8%), 31 (35.6%), 22 (25.3%), and 15 (17.2%) in 87 SJS cases and 12 (25.0%), 11 (22.9%), 17 (35.4%), and 8 (16.7%) in 48 TEN cases. Multivariate logistic regression analysis revealed that patient age (odds ratio [OR], 0.98; 95% confidence interval [CI], 0.96–0.99; $P = .007$) and nonsteroidal anti-inflammatory drugs NSAIDs or cold remedies (OR, 2.58; 95% CI, 1.26–5.29; $P = .010$) were predictive factors for severe acute ocular involvement. The prevalence of visual disturbance and eye dryness increased according to the increase of acute ocular severity ($P = .001$ and $P = .007$ in SJS; $P = .007$ and $P = .014$ in TEN, respectively).

• CONCLUSIONS: At the onset of SJS/TEN, strict attention should be paid to ocular involvement in young patients and in patients exposed to NSAIDs or cold remedies. (Am J Ophthalmol 2015;160(2):228–237. © 2015 by Elsevier Inc. All rights reserved.)

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STEVENS-JOHNSON SYNDROME (SJS) AND ITS MORE severe variant, toxic epidermal necrolysis (TEN), are acute inflammatory disorders of the skin and mucous membranes that predispose patients to life-threatening complications such as sepsis, respiratory dysfunction, and multiple organ failure.[1–4] Both diseases are rare, yet can affect anyone, regardless of age, and usually as a consequence of adverse drug reactions. A variety of drugs including antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and antiepileptic medications (ie, many if not most of the popularly used over-the-counter drugs) have been reported to cause severe drug reactions and induce SJS or TEN.[5,6] Ocular surface inflammation develops rapidly at the acute stage of the disease, and acute conjunctivitis occur prior to, or simultaneously with, skin eruptions.[7] Extensive inflammation of the ocular surface is often accompanied by corneal and/or conjunctival epithelial defects. Common signs after the acute stage include persistent epithelial defects, ulceration, and perforation, ultimately developing into corneal, conjunctival, and eyelid cicatricial changes such as neovascularization, opacification, keratinization, and symblepharon.[7,8] Visual impairment and severe dryness of the eye continue lifelong as ocular sequelae.[7,8] Although we previously reported both surgical[9–14] and nonsurgical[15] therapeutic methods to treat chronic-stage SJS/TEN, it remains impossible to restore the ocular surface to its normal healthy state (ie, that of before disease onset).

In a previous report from Power and associates,[16] the authors categorized acute ocular severity into the grades of mild, severe, or very severe. In that grading system, mild

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involvement was defined as mild conjunctival injection, lid edema, and/or chemosis; moderate involvement consisted of membrane formation, corneal epithelial loss of more than 30%, corneal ulceration, and/or corneal infiltrates; and severe involvement consisted of fornix shortening, symblepharon formation, and/or visual loss. Considering the fact that SJS and TEN are characterized by epidermal necrosis with detachment and erosion of the mucous membranes,[2,17] corneal or conjunctival epithelial defects are prominent disease-related factors at the onset. In addition, it is reported that extreme inflammation develops on the ocular surface accompanying pseudomembrane formation at the acute stage.[16,18,19] Thus, we hypothesized that a simple grading system based on the presence of conjunctivitis, corneal or conjunctival epithelial defect, and pseudomembrane formation might be appropriate to evaluate acute ocular severity of SJS/TEN.

Owing to the high mortality rate of patients with SJS and TEN (1%～5% and 20%～30%, respectively), investigations of these diseases have been intensively focused on systemic severity and general treatment.[6,20–22] Unfortunately, even in the large-scale survey of the severe adverse drug reactions conducted in Europe,[4,21–23] the ocular involvement was not explored. Moreover, the predictive features of ocular involvement at the acute stage of SJS/TEN remain unclear.

The aim of this study was to construct a simpler and more practical score for grading the acute ocular severity of SJS and TEN, as well as to determine predictive factors having severe or very severe (as opposed to none or mild) acute ocular involvement in SJS/TEN patients based on a Japanese nationwide retrospective study of SJS and TEN patients in which both dermatologists and ophthalmologists participated.[24] Furthermore, correlation between the severe acute ocular involvement and the prevalence of ocular sequelae (visual disturbance and dryness of the eye) was examined.

METHODS

THIS STUDY WAS APPROVED BY THE ETHICS COMMITTEE and Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan (RBMR-E-215), and was carried out in accordance with the tenets set forth in the Declaration of Helsinki.

• **PATIENTS:** In order to investigate ocular involvement in SJS/TEN patients as a first step, we retrospectively reviewed SJS and TEN patients who were diagnosed in the period from January 2005 to December 2007, with the medical records of those patients being obtained from dermatologists.[24]

• **DATA COLLECTION:** To identify SJS and TEN patients, a questionnaire was first sent to dermatologists at 607 medical institutions, and dermatologists from 212 medical institutions responded. Secondly, case report forms (CRFs) were sent to dermatologists. Based on the medical records in each institution, patients matching the diagnostic criteria for SJS and TEN, which was made by the Japanese Ministry of Health, Labour and Welfare in 2005 (Table 1) (diagnostic criteria and systemic severity index score for Stevens-Johnson syndrome available at http://www.nanbyou.or.jp/entry/3680, accessed April 21, 2015), were determined. The CRF was structured as follows: (1) diagnostic criteria,

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
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

(2) patient information (ie, sex, age, past medical history and accompanying systemic diseases), (3) exposed drugs, (4) clinical symptoms, (5) systemic findings (ie, existence of a high fever and/or respiratory problem, the quality and location of a rash, and involvement of the lips, mouth, or other mucous membrane), (6) laboratory findings, and (7) a histopathologic examination of a skin biopsy. The results by this study for systemic and dermatologic findings had already been reported.[24]

Then, for registration of this survey, CRFs for investigating ocular involvement were sent to ophthalmologists, to whom patients were referred by dermatologists, and the patients who were treated by ophthalmologists were selected as this study population. This ophthalmologic CRF consisted of (1) ocular surface findings at first appearance, at the day of worst severity, and at last appearance, with the sketches of epithelial defect and pseudomembrane formation being included; (2) pre-existing ocular diseases; (3) ocular sequelae at the chronic stage (ie, visual disturbance and eye dryness); and (4) exposed drug and therapeutic drugs. Ocular surface findings at the acute stage were graded as an acute ocular severity score, as described below (Table 2). Ocular findings at last appearance (at the chronic stage) were graded as per the previously described methods.[8] Exposed drugs were categorized to NSAIDs, cold remedies, antibiotics, anticonvulsants, and others. The records of steroid pulse therapy, high-dose steroids, intravenous immunoglobulin, plasmapheresis as systemic therapy, and topical antibiotics and steroids were collected.

• ACUTE OCULAR SEVERITY SCORE, DEFINITION AND MEASUREMENT: In this study, ocular surface findings at the acute stage were graded as an acute ocular severity score ranging from grade 0 to 3, which were termed as "none," "mild," "severe," and "very severe" (Table 2). We considered that ocular surface inflammation and epithelial necrosis or apoptosis might be the initial ocular pathologic processes of SJS/TEN. Conjunctival hyperemia, which indicates ocular surface inflammation, was assessed as grade 1. Eyes with accompanying pseudomembrane formation or ocular surface epithelial defect were assessed as grade 2. Eyes with both pseudomembrane formation and ocular surface epithelial defect were assessed as grade 3. In addition, we checked all of the sketches of ocular surface appearances contained within the ophthalmologic CRF.

The acute stage was defined as the first 2 months from onset, because both SJS and TEN are self-limited until 6–8 weeks after onset. In addition, the acute ocular severity scores at the day of worst severity during the acute stage were documented, because ocular severity at the acute stage often worsens, even during medical treatment.[16,18] In cases where the acute ocular severity differed between both eyes, the more severe eye was chosen as the eye to be evaluated.

The systemic severity index score is a summed score, shown in the Supplemental Table (available at AJO.com), with the maximum score being 14. Moreover, the systemic severity index subscore is defined as a summed score that includes the score for surface area of skin lesions on the body, except for the score for ocular lesion, with the maximum score being 11.

TABLE 2. Grading Scores for Acute Ocular Severity of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Acute Ocular Manifestations	Grade
No ocular involvement	0 (none)
Conjunctival hyperemia	1 (mild)
Either ocular surface epithelial defect or pseudomembrane formation	2 (severe)
Both ocular surface epithelial defect and pseudomembrane formation	3 (very severe)

• STATISTICAL ANALYSIS: Acute ocular severity at the day of worst severity was compared to various factors such as the patient's age, sex, exposed drugs, and the systemic severity index subscore (Supplemental Table). In addition, predictive factors at diagnosis associated with acute ocular severity were examined. Finally, correlation between the acute ocular severity score and the prevalence of ocular sequelae (visual disturbance and eye dryness) was examined.

To summarize the data, the mean (standard deviation [SD]) for continuous variables and frequencies (%) for categorical variables were used. To examine whether or not the increase of acute ocular severity affected the linear trend for each factor, the Jonckheere-Terpstra test of trend for continuous variables and the Cochran-Armitage test of trend for categorical variables were used. In addition, the logistic regression model was used to determine predictive factors for categories of the acute ocular severity score, which included the cases with no or mild ocular involvement (acute ocular severity score grades 0 and 1, as reference) and the cases with severe or very severe ocular involvement (acute ocular severity score grades 2 and 3). The candidate predictive factors were selected with a P value of $<.05$ for the Wald test in univariate analysis, and the predictive factors were selected from those factors by using the reverse method with a P value of $<.05$ for each factor in multivariate analysis. The odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) based on the Wald test were then estimated. All analyses were performed by SAS version 9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

• PATIENT CHARACTERISTICS AND THERAPY: In the current study, 87 SJS patients and 48 TEN patients were

TABLE 3. Relationship Between Factors and Acute Ocular Severity Score in the Patients With Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Variable	Acute Ocular Severity Score				*P* Value
	0 (None)	1 (Mild)	2 (Severe)	3 (Very Severe)	
Stevens-Johnson syndrome (N = 87)	N = 19	N = 31	N = 22	N = 15	
Demographic variables					
Sex, n (%)					
Male	7 (36.8%)	16 (51.6%)	8 (36.4%)	5 (33.3%)	.567
Age at onset					
Mean ± SD, y	57.1 ± 18.2	52.3 ± 18.7	54.4 ± 19.1	39.2 ± 19.2	.050
<50, n (%)	7 (36.8%)	11 (35.5%)	8 (36.4%)	11 (73.3%)	.054
Systemic severity index subscore					
Mean ± SD	4.5 ± 1.6	3.9 ± 1.8	4.0 ± 1.6	4.8 ± 1.4	.579
Median (range)	5.0 (1–7)	4.0 (1–8)	4.0 (1–7)	5.0 (2–7)	
Exposed drug, n (%)					
NSAIDs					
Yes	9 (47.4%)	10 (32.3%)	9 (40.9%)	9 (60.0%)	.394
Cold remedies					
Yes	1 (5.3%)	3 (9.7%)	3 (13.6%)	4 (26.7%)	.062
Antibiotics					
Yes	4 (21.1%)	6 (19.4%)	3 (13.6%)	1 (6.7%)	.212
Anticonvulsants					
Yes	4 (21.1%)	14 (45.2%)	6 (27.3%)	2 (13.3%)	.370
Toxic epidermal necrolysis (N = 48)	N = 12	N = 11	N = 17	N = 8	
Demographic variables					
Sex, n (%)					
Male	5 (41.7%)	6 (54.5%)	8 (47.1%)	4 (50.0%)	.794
Age at onset					
Mean ± SD, y	62.0 ± 14.5	64.6 ± 25.5	47.9 ± 19.0	39.6 ± 14.6	.062
<50, n (%)	2 (16.7%)	2 (18.2%)	9 (52.9%)	6 (75.0%)	.002
Systemic severity index subscore					
Mean ± SD	7.1 ± 1.7	8.3 ± 2.2	8.0 ± 1.5	8.0 ±1.3	.335
Median (range)	7.5 (4–9)	9.0 (3–11)	9.0 (4–10)	8.5 (6–9)	
Exposed drug, n (%)					
NSAIDs					
Yes	3 (25.0%)	2 (18.2%)	9 (52.9%)	4 (50.0%)	.079
Cold remedies					
Yes	0 (0.0%)	0 (0.0%)	6 (35.3%)	2 (25.0%)	.015
Antibiotics					
Yes	0 (0.0%)	5 (45.5%)	4 (23.5%)	1 (12.5%)	.578
Anticonvulsants					
Yes	2 (16.7%)	1 (9.1%)	4 (23.5%)	2 (25.0%)	.463

NSAIDs = nonsteroidal anti-inflammatory drugs.

The Jonckheere-Terpstra test of trend for continuous variables and the Cochran-Armitage test of trend for categorical variables were used.

identified from institutions throughout Japan. Of those patients, 36 SJS patients (41.4%) and 23 TEN patients (47.9%) were men. In addition, the mean age (SD) for SJS and TEN patients was 51.6 (19.4) and 53.9 (20.8), respectively. Systemic steroid pulse therapy was administered in 40 SJS cases (46.0%) and in 28 TEN cases (58.3%). Intravenous immunoglobulin was administered in 8 SJS cases (9.2%) and in 21 TEN cases (43.3%). Plasmapheresis was performed in 3 SJS cases (3.4%) and in 10 TEN cases (20.8%). Of the 104 cases with ocular involvement, antibiotics and steroids were administered in 74 cases (71.2%) and 85 cases (81.7%), respectively. Topical steroids were administered in all 23 cases with acute ocular severity score grade 3 (very severe).

• ACUTE OCULAR SEVERITY SCORE: Of the 87 SJS cases, the acute ocular severity score was grade 0 in 19 cases (21.8%), grade 1 in 31 cases (35.6%), grade 2 in 22 cases

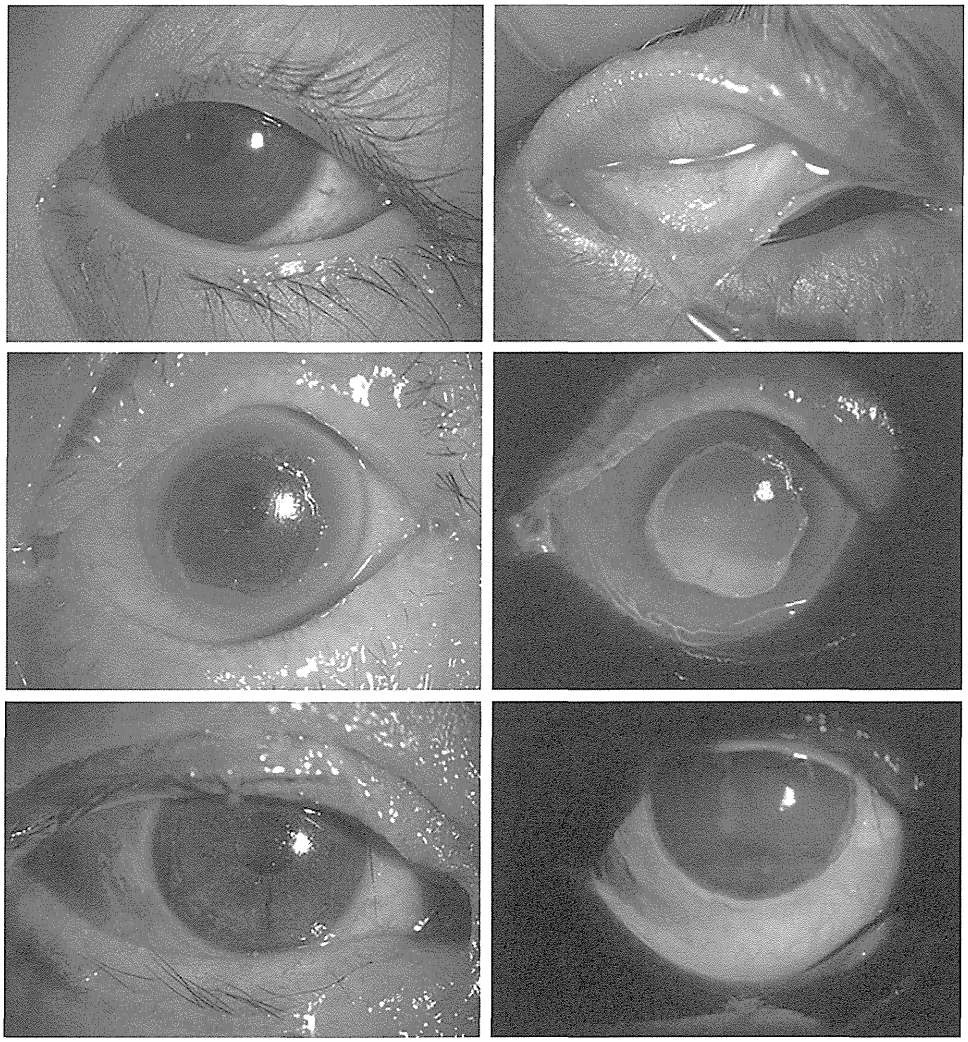

FIGURE 1. Representative slit-lamp appearances of Stevens-Johnson syndrome (SJS)- and toxic epidermal necrolysis (TEN)-associated ocular involvement at the acute stage. Bilateral conjunctivitis with hyperemia (Top left) is the most frequently associated ocular finding. Pseudomembrane formation (Top right) is seen in cases accompanying extensive ocular surface inflammation. Ocular surface corneal (Middle left) or conjunctival (Bottom left) epithelial defects can easily be seen by fluorescein staining (Middle right and Bottom right).

(25.3%), and grade 3 in 15 cases (17.2%) (Table 3). Of the 48 TEN cases, the score was grade 0 in 12 cases (25.0%), grade 1 in 11 cases (22.9%), grade 2 in 17 cases (35.4%), and grade 3 in 8 cases (16.7%) (Table 3). The typical findings observed in such cases are shown in Figure 1.

• ASSOCIATION BETWEEN CHARACTERISTICS AT DIAGNOSIS AND ACUTE OCULAR SEVERITY SCORE: The acute ocular severity scores in male subjects were similar to those in female subjects in both SJS ($P = .603$) and TEN ($P = .940$). Patient age at disease onset tended to be younger in the group with severe or very severe ocular involvement than in the group with no or mild ocular involvement in both disease categories (SJS: $P = .050$; TEN: $P = .062$) (Figure 2 and Table 3). In addition, the systemic severity index subscores did not differ among the 4 groups according to the acute ocular severity score in both SJS and TEN (SJS: $P = .579$; TEN: $P = .335$) (Table 3).

NSAIDs were the exposed drugs in more than 40.0% of the SJS and TEN cases with an acute ocular severity score grade 2 and 3; however, the use of NSAIDs was found to not be associated with the acute ocular severity (SJS: $P = .394$; TEN: $P = .079$) (Table 3). In addition, the use of cold remedies (as the exposed drugs) was found to be increased according to the worsening of acute ocular severity in TEN patients ($P = .015$), but not in SJS patients ($P = .062$) (Table 3). The use of antibiotics was found to not be associated with the acute ocular severity (SJS: $P = .212$; TEN: $P = .578$) (Table 3). Furthermore, the use of anticonvulsants was found to not be associated

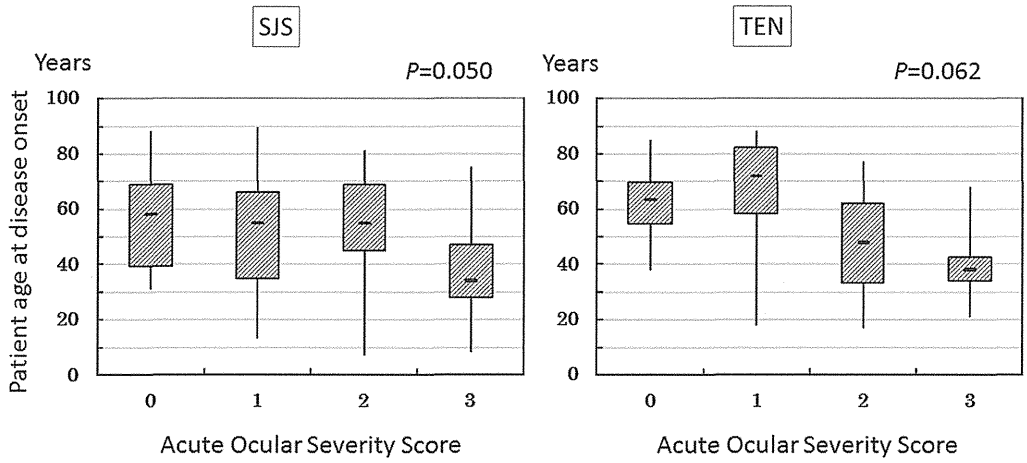

FIGURE 2. Relationship between the acute ocular severity score and patient age in the Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) cases. The horizontal line within each shaded box represents the median value, and the bottom and top lines of each box represent the 25th and 75th percentiles, respectively. The vertical lines extending from the top and bottom of each box represent the lowest and highest values, respectively (or are located 1.5 times the interquartile range away from the box). The Jonckheere-Terpstra test was used to determine whether or not patient age was related to the severity scores.

TABLE 4. Logistic Regression Analysis of the Association Between Variables at Onset and Acute Ocular Severity in Patients With Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

	Univariate Logistic Regression			Multivariate Logistic Regression		
Variable at Onset N = 135	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Disease: TEN (vs SJS)	1.47	0.72–2.98	.287			
Sex: male (vs female)	0.78	0.39–1.54	.466			
Age at onset (y)	0.97	0.96–0.99	.004	0.98	0.96–0.99	.007
Age at onset (y): >50 (vs 50≤)	0.36	0.18–0.72	.004			
NSAIDs	2.04	1.02–4.1	.045			
Cold remedies	5.51	1.72–17.62	.004			
NSAIDs or cold remedies	2.68	1.33–5.38	.006	2.58	1.26–5.29	.010
Antibiotics	0.66	0.27–1.63	.363			
Anticonvulsants	0.72	0.33–1.58	.415			

CI = confidence interval; NSAIDs = nonsteroidal anti-inflammatory drugs; OR = odds ratio; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis.

with the acute ocular severity (SJS: $P = .370$; TEN: $P = .463$) (Table 3).

• PREDICTIVE FACTORS AT DIAGNOSIS ASSOCIATED WITH ACUTE OCULAR SEVERITY: A total of 135 patients were analyzed. Univariate logistic regression analysis revealed that disease (TEN or SJS) was not associated with acute ocular severity (grade 2 and 3 vs grade 0 and 1, OR: 1.47, 95% CI: 0.72–2.98, $P = .287$). In contrast, patient age (OR: 0.97, 95% CI: 0.96–0.99, $P = .004$) and NSAIDs (OR: 2.04, 95% CI: 1.02–4.10, $P = .045$), cold remedies (OR: 5.51, 95% CI: 1.72–17.6, $P = .004$), and NSAIDs or cold remedies (OR: 2.68, 95% CI: 1.33–5.38, $P = .006$) were associated with acute ocular severity (grade 2 and 3 vs grade 0 and 1) as the candidate predictive factors (Table 4). Further, in multivariate logistic regression analysis, patient age (OR: 0.98, 95% CI: 0.96–0.99, $P = .007$) and NSAIDs or cold remedies (OR: 2.58, 95% CI: 1.26–5.29, $P = .010$) were identified as predictive factors for acute ocular severity (grade 2 and 3 vs grade 0 and 1).

• CORRELATION BETWEEN ACUTE OCULAR SEVERITY SCORE AND PREVALENCE OF OCULAR SEQUELAE: As ocular sequelae, visual disturbance (defined as best-corrected visual acuity [BCVA] worse than 20/20) was observed in 15 SJS patients (15/87, 17.2%) and in 6 TEN patients (6/48, 12.5%) (Table 5). In both SJS and TEN, the prevalence of visual disturbance (BCVA worse than

TABLE 5. Prevalence of Ocular Sequelae Stratified by Acute Ocular Severity Score in Patients With Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Variable	Category	Acute Ocular Severity Score				*P* Value[a]
		0 (None)	1 (Mild)	2 (Severe)	3 (Very Severe)	
Stevens-Johnson syndrome		N = 19	N = 31	N = 22	N = 15	
Visual disturbance	Better than 20/20 of BCVA	18 (94.7%)	29 (93.5%)	16 (72.7%)	9 (60.0%)	.001
	20/20 - 20/200 of BCVA	1 (5.3%)	1 (3.2%)	6 (27.3%)	5 (33.3%)	
	Worse than 20/200 of BCVA	0 (0.0%)	1 (3.2%)	0 (0.0%)	1 (6.7%)	
Severity of dry eye	None	17 (89.5%)	24 (77.4%)	11 (50.0%)	7 (46.7%)	.001
	Mild	2 (10.5%)	6 (19.4%)	6 (27.3%)	6 (40.0%)	
	Moderate	0 (0.0%)	1 (3.2%)	4 (18.2%)	1 (6.7%)	
	Severe	0 (0.0%)	0 (0.0%)	1 (4.5%)	1 (6.7%)	
Toxic epidermal necrolysis		N = 12	N = 11	N = 17	N = 8	
Visual disturbance	Better than 20/20 of BCVA	12 (100.0%)	11 (100.0%)	14 (82.4%)	5 (62.5%)	.007
	20/20 - 20/200 of BCVA	0 (0.0%)	0 (0.0%)	2 (11.8%)	3 (37.5%)	
	Worse than 20/200 of BCVA	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	
Severity of dry eye	None	11 (91.7%)	6 (54.5%)	9 (52.9%)	3 (37.5%)	.014
	Mild	1 (8.3%)	4 (36.4%)	7 (41.2%)	3 (37.5%)	
	Moderate	0 (0.0%)	1 (9.1%)	0 (0.0%)	2 (25.0%)	
	Severe	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	

BCVA = best-corrected visual acuity.

[a]*P* values of Cochran-Armitage trend test were calculated by 2 categories of better or worse than 20/200 of BCVA in visual disturbance and 2 categories of none or more than mild severity of dry eye.

20/20) increased according to the increase of acute ocular severity (SJS: $P = .001$; TEN: $P = .007$) (Figure 3 and Table 5). In addition, dry eye (as ocular sequelae) was observed in 28 of 87 SJS patients (32.2%) and in 18 of 48 TEN patients (37.5%) (Table 5). Furthermore, in both SJS and TEN, the prevalence of more than mild dry eye increased according to the increase of acute ocular severity (SJS: $P = .001$; TEN: $P = .014$) (Figure 3 and Table 5).

DISCUSSION

SJS AND TEN ARE RARE, YET POTENTIALLY FATAL, SEVERE skin mucosal disorders. Owing to the serious general symptoms and high mortality rates associated with these diseases, the ocular involvement is often easily overlooked. In addition, owing to the low incidence of acute SJS/TEN, most ophthalmologists are unfamiliar with the findings of acute ocular involvement. However, SJS/TEN patients with ocular sequelae (which can be associated with acute ocular severity) need ophthalmic treatment for an extended period of time and the quality of life of these patients is extremely poor.[25,26] Furthermore, the findings of this current study show that the chronic ocular sequelae more frequently occurred in patients with severe or very severe ocular involvement (grades 2 and 3 of the acute ocular severity score) than in patients with no or mild ocular involvement. Thus, strict attention must be paid to ocular involvement at the acute stage.

On the other hand, the diagnostic criteria for SJS and TEN produced by the Japanese Ministry of Health, Labour and Welfare (2005) (Table 1) were determined by a research group consisting of dermatologists and ophthalmologists who specialize in severe adverse drug reactions. In these diagnostic criteria, the acute ocular severity was included as accompanying findings. The systemic severity index score, which is often referred to by dermatologists, also includes the ocular surface findings as accompanied findings (Supplemental Table, available at AJO.com). Recognition of the acute ocular severity (and the corresponding proper treatment) can work not only to reduce the rate of ocular sequelae but also to produce an adequate differential diagnosis.[27] We believe that all dermatologists, as well as all physicians who are not ophthalmologists, should pay strict attention to ocular findings at disease onset in SJS/TEN cases. Hence, from the aspect of the judgment of acute ocular severity in SJS/TEN cases, this current study provided a common platform.

The proposed acute ocular severity score included only the initial ocular pathologic process. To the best of our knowledge, at the onset of both SJS and TEN, it is vital to elucidate the distinctive appearances of corneal and/or conjunctival epithelial defects and pseudomembrane formation. These ocular surface inflammation and epithelial necrosis or apoptosis are part of the initial ocular pathologic processes of SJS/TEN, and the secondary processes include

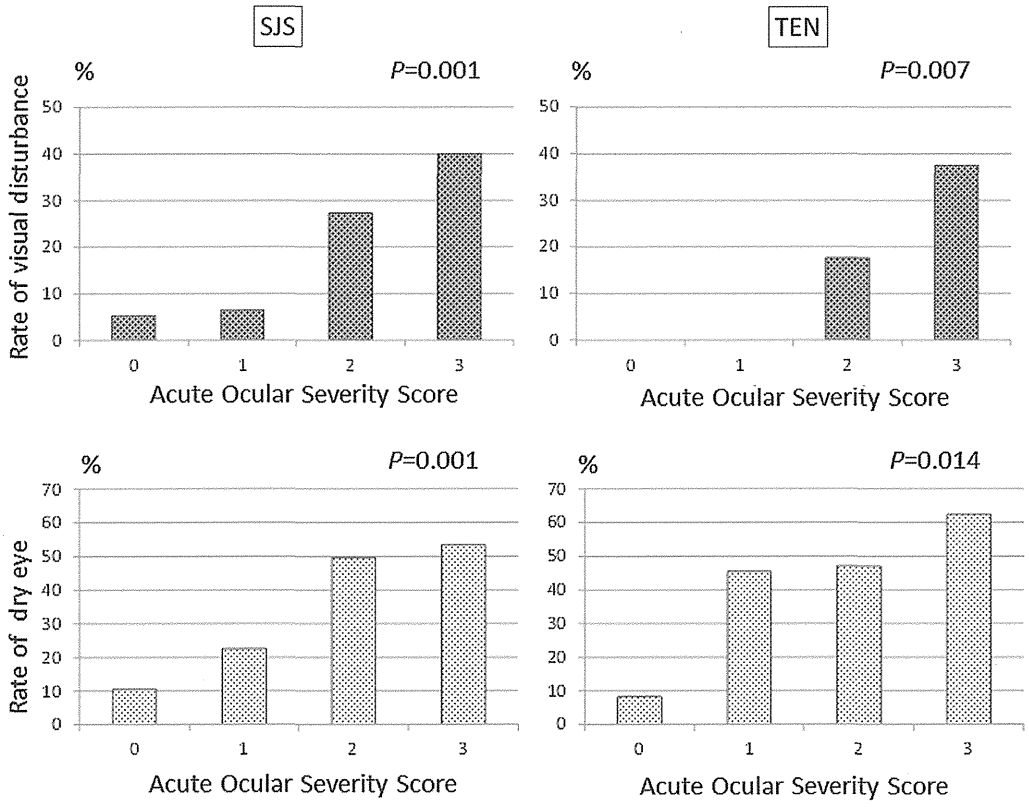

FIGURE 3. Relationship between the acute ocular severity score and ocular sequelae in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The percentage rate of the presence of visual disturbance (Top 2 charts) and eye dryness (Bottom 2 charts) in the SJS and TEN cases, respectively, compared to the acute ocular severity score. The Cochran-Armitage test was used to determine whether or not acute ocular severity was related to ocular sequelae.

persistent epithelial defects, ulceration and perforation, fornix shortening, symblepharon formation, and vision loss. Although the previously reported grading system included the secondary ocular findings,[16,28] our suggested acute ocular severity scoring system included only the initial ocular pathologic process to find the early progression of ocular involvement.

We previously reported in a retrospective analysis that visual prognoses were significantly better in the group receiving topical steroids at the acute stage compared with the no-treatment group.[7] Based on our prospective study, we have shown the therapeutic importance of a corticosteroid pulse therapy and topical administration of betamethasone at disease onset for reducing the degree of ocular sequelae.[18] At disease onset, a severe cytokine storm arises on the ocular surface and was found to be related to ocular severity.[19] Thereby, we believe that prompt diagnosis of ocular involvement and early intervention by ophthalmologists might improve the patient's visual prognosis.

The findings of this present study clearly demonstrated that patient age is closely related to acute ocular severity. Previous reports from ophthalmologists have described the mean age of SJS/TEN patients as being around 25 years.[7,8] On the other hand, the mean patient age reported from dermatologists is around 45 years.[6] Along with those findings from the previous reports, our results suggest that susceptibility to SJS/TEN with ocular involvement differs depending on the patient's age.

The findings of this study demonstrated that NSAIDs or cold remedies as the exposed drugs are predictive factors for the increase of acute ocular severity. In addition, those drugs can be associated with the prevalence of ocular sequelae, since acute ocular severity was found to be associated with ocular sequelae in this study. In SJS/TEN with ocular sequelae, common flu symptoms (general malaise, fever, sore throat, etc) reportedly precede skin eruptions in 80% of the cases.[7] Following the ingestion of cold medicine for such prodromal symptoms, skin eruptions, high fever, and bilateral conjunctivitis occurred with extreme inflammation on the ocular surface. Hence, the ocular symptoms of SJS/TEN can be assumed to be a drug-induced inflammatory disorder.

The findings of our recent reports have elucidated the participation of a genetic involvement in SJS/TEN.[29–41] In one report, we showed that a strong association exists between HLA-A*02:06 and SJS/TEN with severe ocular complication.[31] SJS/TEN with severe ocular complication signifies the cases with acute ocular severity score graded 2

and 3 at the acute stage or the cases with severe ocular sequelae at the chronic stage. In addition, we also clarified that HLA-A*02:06 and HLA-B*44:03 are susceptible alleles that increase the risk of cold medicine–related SJS/TEN with severe ocular complication.[41] This present study clearly demonstrated the strong relations between NSAIDs or cold remedies and SJS/TEN with severe ocular involvement (grades 2 and 3). Most cold remedies include acetaminophen or NSAIDs such as aspirin, ibuprofen, or others. Reportedly, there are statistically significant differences in the single nucleotide polymorphisms of toll-like receptor 3 (TLR3),[30] a pattern-recognizing receptor related to innate immunity following viral infections. Polymorphisms of EP3, one of the prostaglandin E2 (PGE2) receptors, are reportedly strongly associated with SJS/TEN with severe ocular complication.[36] Cold medicines and NSAIDs, including ibuprofen and loxoprofen, commonly downregulate the production of prostanoids, including PGE2. Thus, we believe that interactions between HLA risk factors, TLR3, and/or EP3 might be keys in the pathogenesis of cold medicine–related SJS/TEN with severe ocular complication.[34,37]

It should be noted that this present study did have some limitations, such as the sample size being too small to perform statistical validation analysis for the simple scoring of acute ocular severity. Thus, we were not able to analyze the relations of the treatment.

In conclusion, we proposed simple criteria of acute ocular involvement in patients with SJS and TEN, and demonstrated that acute ocular severity was significantly associated with patient age and exposed drugs. Further studies are needed to validate our findings, yet based on the identification of predictive factors associated with ocular severity at the acute stage SJS/TEN, we emphasize that strict attention must be paid to younger-age patients and patients exposed to NSAIDs or cold remedies.

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Biosketch

Dr. Chie Sotozono graduated from the Kyoto Prefectural University of Medicine, Kyoto, Japan in 1986, and subsequently completed her residency training at the Department of Ophthalmology of that same prestigious university. Dr. Sotozono received her Ph.D. from the Kyoto Prefectural University of Medicine at 1995. At present, Dr. Sotozono is an Assistant Professor in the Department of Ophthalmology at Kyoto Prefectural University of Medicine, and specializes in clinical research and cornea-related diseases and regenerative medicine.

SUPPLEMENTAL TABLE. Systemic Severity Index Score for Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

Evaluated Condition	Score
1. Mucous lesions	
Ocular lesions	
Pseudomembrane formation	1
Ocular surface epithelial defect (Ocular surface erosive lesions)	1
Bilateral acute keratoconjunctivitis	1
Labial and/or oral lesions	
Oral diffuse erosive lesions with bloody scales	1
Labial erosive lesions with bloody scales alone	1
Oral or labial erosive lesions alone	1
Genital involvement	1
2. Body surface area of skin lesions (select 1 from the 3)	
≥30%	3
10%–30%	2
<10%	1
3. Fever: ≥38.0 °C	1
4. Respiratory dysfunction	1
5. Epidermal detachment	1
6. Liver dysfunction (ALT ≥ 100 IU/L)	1

ALT = alanine aminotransferase.

The summed score ≥6 was treated as severe and summed scores ≤5 was treated as moderate. Each of the following conditions is evaluated as severe regardless of the summed score: (1) pseudomembrane formation and/or ocular surface epithelial defect, (2) respiratory dysfunction associated with Stevens-Johnson syndrome/toxic epidermal necrolysis, (3) toxic epidermal necrolysis extends with diffuse erythema.

IKZF1, a new susceptibility gene for cold medicine–related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement

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Background: Stevens-Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, including the ocular surface, oral cavity, and genitals. These reactions are very rare but are often associated with inciting drugs, infectious agents, or both.

Objective: We sought to identify susceptibility loci for cold medicine–related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement (SMI).

Methods: A genome-wide association study was performed in 808 Japanese subjects (117 patients with CM-SJS/TEN with SMI and 691 healthy control subjects), and subsequent replication studies were performed in 204 other Japanese subjects (16 cases and 188 control subjects), 117 Korean subjects (27 cases and 90 control subjects), 76 Indian subjects (20 cases and 56 control subjects), and 174 Brazilian subjects (39 cases and 135 control subjects).

Results: In addition to the most significant susceptibility region, *HLA-A*, we identified *IKZF1*, which encodes Ikaros, as a novel susceptibility gene (meta-analysis, rs4917014 [G vs T]; odds ratio, 0.5; $P = 8.5 \times 10^{-11}$). Furthermore, quantitative ratios of the *IKZF1* alternative splicing isoforms Ik1 and Ik2 were significantly associated with rs4917014 genotypes.

Conclusion: We identified *IKZF1* as a susceptibility gene for CM-SJS/TEN with SMI not only in Japanese subjects but also in Korean and Indian subjects and showed that the Ik2/Ik1 ratio might be influenced by *IKZF1* single nucleotide polymorphisms, which were significantly associated with susceptibility to CM-SJS/TEN with SMI. (J Allergy Clin Immunol 2015;135:1538-45.)

Key words: *Stevens-Johnson syndrome, toxic epidermal necrolysis, cold medicine, severe mucosal involvement, genome-wide association study,* IKZF1, *alternative splicing*

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Abbreviations used
CM-SJS/TEN: Cold medicine–related SJS/TEN
GWAS: Genome-wide association study
IRF: Interferon regulatory factor
KPUM: Kyoto Prefectural University of Medicine
NSAID: Nonsteroidal anti-inflammatory drug
OR: Odds ratio
SJS: Stevens-Johnson syndrome
SMI: Severe mucosal involvement
SNP: Single nucleotide polymorphism
TEN: Toxic epidermal necrolysis
TLR: Toll-like receptor

Stevens-Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, including the ocular surface, oral cavity, and genitals. These reactions are often associated with inciting drugs and infectious agents.[1-3] Although they are rare, with an annual incidence of 1 to 6 cases per million persons,[3,4] these reactions carry high mortality rates of 3% for SJS and 27% for TEN,[5] and surviving patients often experience severe sequelae, such as vision loss caused by severe ocular surface complications.[6]

HLA genotypes are associated with SJS/TEN. In the Taiwanese Han Chinese the *HLA-B*15:02* allele exhibited a strong significant association with carbamazepine-induced SJS/TEN (cases, n = 44; control subjects [tolerant], n = 101; odds ratio [OR], 2504; $P_{corrected} = 3.1 \times 10^{-27}$).[7] Similarly, in Japanese (cases, n = 77; control subjects [tolerant], n = 420; OR, 9.5; $P = 1.1 \times 10^{-16}$)[8] and European (cases, n = 145; control subjects [normal]; n = 257; OR, 15.0; $P = 3.5 \times 10^{-8}$) subjects,[9] the *HLA-A*31:01* allele was significantly associated with carbamazepine-induced cutaneous adverse reactions, including SJS/TEN, drug-induced hypersensitivity syndrome, and others. Allopurinol, a uric acid–decreasing drug that induces severe cutaneous adverse reactions, including SJS/TEN, was significantly associated with *HLA-B*58:01* in Han Chinese (cases, n = 51; control subjects [tolerant], n = 135; OR, 580; $P_{corrected} = 4.7 \times 10^{-24}$),[10] white (cases, n = 27; control subjects [normal], n = 1822; OR, 80; $P_{corrected} < 10^{-6}$),[11] and Japanese (cases, n = 36; control subjects [normal], n = 986; OR, 62.8; $P = 5.4 \times 10^{-12}$)[12] patients. Allopurinol and anticonvulsants, such as carbamazepine, are the main inciting drugs for SJS/TEN[13]; in addition we[1,14] and others[2,15] have cited cold medicines, including nonsteroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient medications, as causative drugs for SJS/TEN. We have also found that cold medicine–related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement (SMI), including severe ocular complications, was significantly associated with *HLA-A*02:06* (cases, n = 151; control subjects [normal], n = 639; OR, 5.6; $P = 2.7 \times 10^{-20}$) and significantly associated with *HLA-B*44:03* in Japanese subjects (cases, n = 151; control subjects [normal], n = 639; OR, 2.0; $P = 1.3 \times 10^{-3}$), and this *HLA* genotype was irrelevant to patients with CM-SJS/TEN without SMI.[16] Thus genetic predisposition, including *HLA* genotype, might be different between patients with SJS/TEN with and without SMI. We also reported that CM-SJS/TEN with SMI was significantly associated with *HLA-B*44:03* in Indian (cases, n = 20; control subjects [normal], n = 55; OR, 12.3; $P = 1.1 \times 10^{-5}$) and Brazilian (especially Brazilian white; cases, n = 15; control subjects [normal], n = 62; OR, 6.2; $P = 3.7 \times 10^{-3}$) subjects.[17]

Here we performed a genome-wide association study (GWAS) to identify genetic factors associated with CM-SJS/TEN with SMI; cold medicines included NSAIDs and multi-ingredient cold medications, and SMIs included severe ocular surface complications. We identified *IKZF1* as a susceptibility gene for CM-SJS/TEN with SMI not only in Japanese subjects but also in Korean and Indian subjects.

METHODS

Patients

This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine (KPUM), the University of Tokyo, and other collaborating research institutes (see the Methods section in this article's Online Repository at www.jacionline.org).

Diagnosis of SJS/TEN by ophthalmologists was based on a confirmed history of acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites, including the ocular surface.[1,14,16-23] Usually, ophthalmologists encounter patients with SJS/TEN in the chronic rather than acute stage, and therefore many of our patients had SJS/TEN many years before recruitment for this study. The samples from the National Institute of Health Sciences represented only patients with SJS/TEN in the acute stage, and the criteria proposed by Bastuji-Garin et al[24] were used for a diagnosis of SJS/TEN for these patients in the acute stage.

We defined patients with severe ocular complications as those who manifested pseudomembranes and epithelial defects on the ocular surface (cornea, conjunctiva, or both) in the acute stage[25] and as patients with ocular sequelae, such as severe dry eye, trichiasis, symblepharon, and conjunctival invasion into the cornea in the chronic stage.[6]

Moreover, we have focused here on CM-SJS/TEN, which can be induced by cold medicines, such as multi-ingredient cold medications and NSAIDs. The patients included in this study had taken cold medicines (eg, NSAIDs or multi-ingredient cold medications) after they had symptoms of the common cold a few to several days before disease onset; they were classified as having CM-SJS/TEN, although the specific drugs used were not named by each patient. We have also focused on patients with SJS/TEN with SMI because we previously found that the genetic predisposition might be different between patients with SJS/TEN with and without SMI.[16] Cases of NSAID-related SJS/TEN with SMI that did not involve symptoms of the common cold, such as involving rheumatoid arthritis or lumbago, were not included in this study. Detailed information on the patients with SJS/TEN with SMI and control subjects who were analyzed is shown in the Methods section in this article's Online Repository.

GWAS and single nucleotide polymorphism genotyping

In the GWAS we genotyped 820 samples, including 118 Japanese patients with SJS/TEN with SMI and 702 Japanese healthy control subjects (283 from KPUM and 419 from the University of Tokyo) by using the Affymetrix AXIOM Genome-Wide ASI 1 Array (Affymetrix, Santa Clara, Calif), according to the manufacturer's instructions. Because all genotyped samples passed the recommended sample quality control metric for the AXIOM arrays (Dish quality control > 0.82), we excluded 1 case sample with an overall call rate of less than 97%. We recalled the remaining 819 samples by using Genotype Console v4.1.4 software (Affymetrix). All samples used for GWASs passed a heterozygosity check, and 5 related samples were identified by using descendent testing. A principal component analysis found 6 outliers to be excluded by using the Smirnov-Grubbs test, and we showed that all cases (n = 117) and control subjects (n = 691) formed a single cluster with the HapMap Japanese (JPT) samples but not with the Chinese (CHB) samples (see Fig E1 in

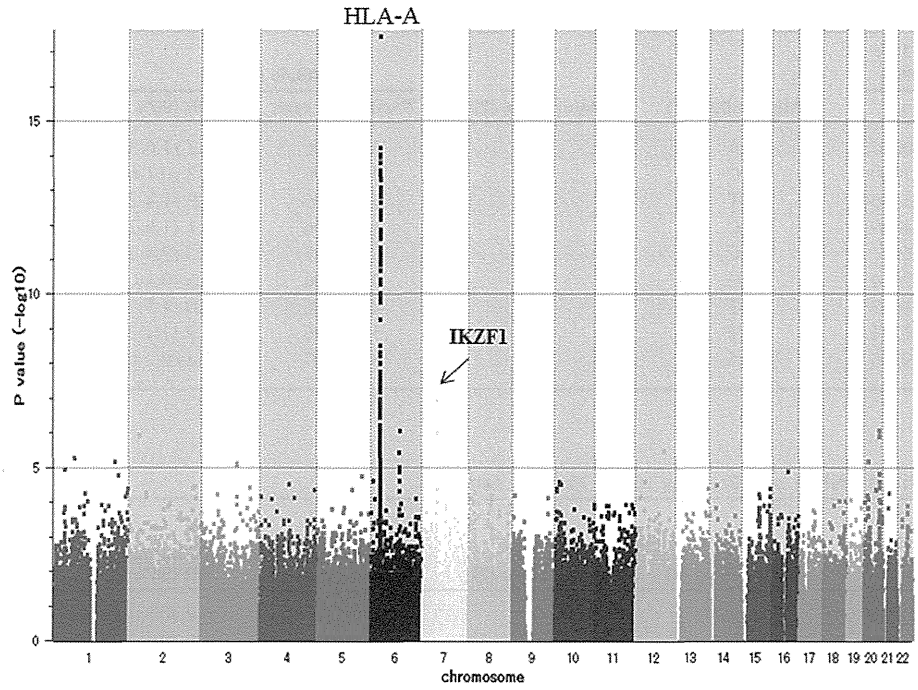

FIG 1. Results of the GWAS (Manhattan plot). *P* values were calculated with a χ^2 test for allele frequencies by using 117 cases and 691 control subjects.

this article's Online Repository at www.jacionline.org). The average overall call rate for the 117 patients with SJS/TEN and 691 control subjects reached 99.01% and 99.49%, respectively. We then applied the following threshold for single nucleotide polymorphism (SNP) quality control in data cleaning: SNP call rate of 95% or greater, minor allele frequency of 3% or greater, and Hardy-Weinberg equilibrium *P* value of .001 or greater in control subjects. A total of 449,205 SNPs on autosomal chromosomes passed the quality control filters and were used for the association study. A quantile-quantile plot of the distribution of test statistics for the comparison of genotype frequencies in cases and control subjects also showed that the inflation factor λ was 1.044 for all of the tested SNPs and decreased to 1.036 when SNPs in the *HLA* region were excluded (see Fig E2 in this article's Online Repository at www.jacionline.org). The χ^2 test was applied to an allele frequency model, all cluster plots for SNPs with *P* values of less than 10^{-3} were checked visually, and SNPs with ambiguous genotype calls were excluded.

The TaqMan SNP genotyping assay (Applied Biosystems, Foster City, Calif) or the DigiTag2[26,27] assay were used to confirm the genotypes at each SNP. For typing of validation by using the DigiTaq2 assay, 2 of the 691 control subjects were excluded because the quality of their data was insufficient. The remaining 689 subjects served as control subjects for typing by using the DigiTaq2 assay.

Some of the patients with CM-SJS/TEN and control subjects in the present study were subjects in our earlier studies. Fifty-seven candidate SNPs might have been associated with SJS/TEN ($P < 10^{-3}$). In the subsequent replication stage we selected 9 SNPs with *P* values of less than 10^{-5} from the results of the χ^2 test with allele frequency in the GWASs. SNP genotyping in 2 independent sets of samples (16 Japanese cases and 188 Japanese control subjects, 27 Korean cases and 90 Korean control subjects) was completed for the 8 SNPs for which functional TaqMan probes were available by using the TaqMan SNP genotyping assay, and 2 other independent sets of samples (20 Indian cases and 56 Indian control subjects, and 39 Brazilian cases and 135 Brazilian control subjects) were genotyped for the 4 IKZF1 SNPs. We used the Cochran-Mantel-Haenszel method as implemented in SAS (JMP Genomics; SAS Institute, Cary, NC) to conduct a meta-analysis.

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), and 40× TaqMan SNP Genotyping Assay probe (Applied Biosystems) for each SNP. The quantitative PCR thermal cycling program was 95°C for 20 seconds, followed by 50 cycles of 95°C for 3 seconds and 60°C for 20 seconds on the Applied Biosystems Step-one plus System.

For the replication study, a χ^2 test was applied to a 2 × 2 contingency table in the allele frequency.

Semiquantitative RT-PCR of *IKZF1* transcripts isoforms

Healthy volunteers were recruited from the University Hospital at KPUM. All subjects provided informed consent for genetic testing and quantification of gene transcripts under the approval of the ethics committee of KPUM. Venous blood samples were collected from the volunteers. DNA was extracted from whole blood samples by SRL (Tokyo, Japan). PureLine Total RNA Blood Purification Kits (Invitrogen, Carlsbad, Calif) were used to extract total RNA from whole blood samples.

Genotyping of rs4917014, rs10276619, and rs4917129 was performed with the TaqMan genotyping method. The ratios of Ik1 (full-length *IKZF1* isoform) and each *IKZF1* splicing isoform were estimated by using semiquantitative RT-PCR. RT-PCR was performed with the primer sets shown in Table E1 in this article's Online Repository at www.jacionline.org and Fast-Start Taq DNA polymerase (Roche, Mannheim, Germany) to detect Ik2, Ik3, and Ik4 isoforms (see the Methods section in this article's Online Repository). The nonparametric Jonckheere-Terpstra test and Kruskal-Wallis test were used for statistical analysis.

RESULTS

GWAS of CM-SJS/TEN with SMI in Japanese subjects

Fig 1 shows a genome-wide view of the SNP association data for 117 Japanese patients with CM-SJS/TEN with SMI and 691 control subjects, in which these association were based on allele frequencies. We found that the *HLA-A* region showed the strongest association with susceptibility to CM-SJS/TEN with

TABLE I. Nine SNPs associated with CM-SJS/TEN with SMI in Japanese patients ($P < 10^{-5}$)

Patients with SJS, n = 117; control subjects, n = 689				Allele (1 vs 2)		Minor allele frequency	
Gene symbol	rs no.	Minor allele (1)	Major allele (2)	*P* value (χ^2 test)	OR (95% CI)	Cases	Control subjects
LOC148709	rs10800873	A	C	4.24E-06	2.2 (1.6-3.2)	0.222	0.113
IGSF11	rs4687960	C	T	4.59E-06	0.2 (0.1-0.4)	0.030	0.135
FUT9	rs11153964	T	G	6.75E-06	1.9 (1.4-2.6)	0.385	0.244
FUT9	rs2294839	C	T	1.05E-06	2.1 (1.6-2.9)	0.323	0.184
IKZF1	rs897693	C	T	1.23E-07	4.3 (2.4-7.8)	0.085	0.021
IKZF1	rs4917014	G	T	2.12E-06	0.5 (0.4-0.7)	0.316	0.483
TMCC3	rs4761639	T	C	4.58E-06	2.0 (1.5-2.7)	0.355	0.217
SPTLC3	rs6041271	T	C	5.31E-06	1.9 (1.4-2.6)	0.616	0.455
TSHZ2	rs4809905	A	G	5.60E-07	0.4 (0.3-0.6)	0.188	0.355

TABLE II. Results of the 8 SNPs* analyzed by using Korean samples

Patients with SJS, n = 27; control subject, n = 90				Allele (1 vs 2)		Minor allele frequency	
Gene symbol	rs no.	Minor allele (1)	Major allele (2)	*P* value (χ^2 test)	OR (95% CI)	Cases	Control subjects
LOC148709	rs10800873	A	C	.574	0.8 (0.3-1.9)	0.130	0.161
IGSF11	rs4687960	C	T	.597	1.2 (0.6-2.6)	0.204	0.172
FUT9	rs11153964	T	G	.723	1.1 (0.5-2.4)	0.300	0.272
FUT9	rs2294839	C	T	.422	1.3 (0.7-2.7)	0.278	0.225
IKZF1	rs897693	C	T	.706	0.7 (0.1-5.8)	0.019	0.028
IKZF1	rs4917014	G	T	**3.97E-04**	**0.3 (0.1-0.6)**	0.222	0.494
TMCC3	rs4761639	T	C	.421	0.7 (0.4-1.6)	0.212	0.267
TSHZ2	rs4809905	A	G	.383	1.3 (0.7-2.6)	0.346	0.283

Values in boldface indicate statistical significance.
*The 8 SNPs showed *P* values of less than 10^{-5} in a GWAS with samples from Japanese subjects and for which functional TaqMan probes were available.

SMI ($P = 3.5 \times 10^{-18}$; OR, 4.4). This finding is consistent with findings from our previous studies, which showed strong association between SJS/TEN with SMI and *HLA-A*02:06*.[16,21-23]

Outside the *HLA* region, there were 57 SNPs with *P* values of less than 10^{-3} in allele frequency in the GWAS. Of the 57 SNPs, 45 had *P* values of less than 10^{-4}, and 9 of these 45 had *P* values of less than 10^{-5}. Although 2 loci, *IKZF1* and *TSHZ2*, among the 9 SNPs with *P* values of less than 10^{-5} showed relatively low *P* values (Table I), we could not find any associations that reached genome-wide significance in the GWAS.

Replication analysis with other Japanese and Korean subjects

Functional TaqMan probes were available for 8 of the 9 SNPs with *P* values of less than 10^{-5}. An independent set of 204 Japanese samples (16 Japanese patients with CM-SJS/TEN with SMI and 188 Japanese healthy control subjects) was used in a subsequent replication analysis to further evaluate these 8 SNPs. In this first replication study the SNPs had no significant association after applying Bonferroni correction because of the relatively small sample size (see Table E2 in this article's Online Repository at www.jacionline.org). However, the ORs of 7 of the 8 SNPs showed the same direction of association as those in the GWAS (rs11153964 [T vs G]: OR, 2.2 [$P = .029$] and rs2294839 [C vs T]: OR, 2.5 [$P = .013$] for *FUT9* and rs4917014 [G vs T]: OR, 0.5 [$P = .074$] for *IKZF1*).

Moreover, we genotyped these 8 SNPs in samples from the Korean population (27 patients with CM-SJS/TEN with SMI and 90 control subjects). Although the number of Korean cases was small, we found a significant association between Korean patients with CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.3; $P = 4.0 \times 10^{-4}$; Table II). Furthermore, the meta-analysis with Japanese and Korean samples showed a genome-wide significant association with *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 9.5 \times 10^{-10}$; see Table E3 in this article's Online Repository at www.jacionline.org).

Additional *IKZF1* SNP analysis with Japanese, Korean, Indian, and Brazilian subjects

Because the meta-analysis indicated that *IKZF1* rs4917014 was significantly associated with CM-SJS/TEN with SMI, we genotyped 2 additional *IKZF1* SNPs (rs10276619 and rs4917129); each of these SNPs was among the 57 SNPs with *P* values of less than 10^{-3} outside the *HLA* region in the GWAS. Notably, we could include additional samples in the analysis of 4 *IKZF1* SNPs (rs897693, rs4917014, rs4917129, and rs10276619); 16 additional samples from Japanese patients with CM-SJS/TEN with SMI collected at KPUM and 4 additional samples from Korean patients with CM-SJS/TEN with SMI were included. With all Japanese samples (149 from patients with SJS and 877 from control subjects), each of the 4 *IKZF1* SNPs (including rs10276619 and rs4917129) was significantly associated with CM-SJS/TEN with SMI (rs897693 [C vs T]: OR, 3.2; $P = 2.2 \times 10^{-6}$; rs4917014 [G vs T]: OR, 0.5; $P = 3.0 \times 10^{-8}$; rs4917129 [C vs T]: OR, 0.5; $P = 4.1 \times 10^{-6}$; and rs10276619 [G vs A]: OR, 1.8; $P = 1.3 \times 10^{-6}$; see

TABLE III. Meta-analysis of the 4 *IKZF1* SNPs using samples from Japanese, Korean, Indian, and Brazilian subjects

Patients with SJS, n = 239; control subjects, n = 1158		Minor	Major	Allele (1 vs 2)	
Gene symbol	rs number	allele (1)	allele (2)	*P* value*	OR (95% CI)
IKZF1	rs897693	C	T	7.98E-04	1.8 (1.3-2.5)
	rs4917014	G	T	**8.46E-11**	0.5 (0.4-0.6)
	rs4917129	C	T	**8.05E-09**	0.5 (0.4-0.7)
	rs10276619	G	A	**4.27E-09**	1.8 (1.5-2.3)

Values in boldface indicate statistical significance in the genome-wide association.
*Cochran-Mantel-Haenszel method.

Table E4 in this article's Online Repository at www.jacionline.org). With all Korean samples (31 from patients with SJS and 90 from control subjects), each of 3 of the 4 SNPs was significantly associated with CM-SJS/TEN with SMI (rs4917014 [G vs T]: OR, 0.4; $P = 1.2 \times 10^{-3}$; rs4917129 [C vs T]: OR, 0.4; $P = 4.3 \times 10^{-3}$; and rs10276619 [G vs A]: OR, 2.7; $P = 1.2 \times 10^{-3}$; see Table E5 in this article's Online Repository at www.jacionline.org). We also genotyped the 4 *IKZF1* SNPs in a set of Indian samples (20 from patients with CM-SJS/TEN with SMI and 56 from control subjects). Despite the small sample size, we found significant associations between Indian patients with CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.3; $P = .016$), although the result ceased to be significant when we corrected the *P* value for the number of alleles (ie, 4; see Table E6 in this article's Online Repository at www.jacionline.org). Furthermore, we also analyzed these 4 *IKZF1* SNPs in a set of Brazilian samples (39 from patients with CM-SJS/TEN with SMI and 135 from control subjects). There were no significant associations with these *IKZF1* SNPs; however, the ORs for these 4 SNPs with the Brazilian sample set showed the same direction of association as with Japanese samples (rs897693 [C vs T]: OR, 1.2; $P = .57$; rs4917014 [G vs T]: OR, 0.9; $P = .58$; rs4917129 [C vs T]: OR, 0.7; $P = .112$; and rs10276619 [G vs A]: OR, 1.5; $P = .12$; see Table E7 in this article's Online Repository at www.jacionline.org).

A meta-analysis that combined data from the Japanese, Korean, Indian, and Brazilian samples showed a significant genome-wide association between CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 8.5 \times 10^{-11}$; rs4917129 [C vs T]: OR, 0.5; $P = 8.1 \times 10^{-9}$; and rs10276619 [G vs A]: OR, 1.8; $P = 4.3 \times 10^{-9}$; Table III and see Fig E3 in this article's Online Repository at www.jacionline.org, which is a Forest plot summarizing the findings).

Association of *IKZF1* SNPs with the relative quantity of alternatively spliced isoforms

IKZF1 rs4917014 genotypes are not known to affect *IKZF1* mRNA expression levels.[28] Our analysis performed with the GENEVAR (Gene Expression Variation; Wellcome Trust Sanger Institute) database[29] also indicated that there was no relationship between the *IKZF1* rs4917014 genotype and gene expression levels (see Fig E4 in this article's Online Repository at www.jacionline.org; JPT, $P = .74$; Chinese, $P = .13$). Additionally, nonsynonymous substitutions, stop-gain, stop-loss, or splice site variants in the *IKZF1* gene region have never been registered in the Asian dbSNP database. Together, this evidence indicated that rs4917014, rs10276619, or other polymorphisms in strong linkage equilibrium with rs4917014 or rs10276619 did not influence *IKZF1* gene expression levels or functional amino acid replacements.

The human *IKZF1* gene encodes at least 11 protein isoforms through alternative mRNA splicing in human subjects. Although these isoforms share the C-terminus domain required to interact with other proteins, the number of N-terminus zinc-finger domains, which bind to DNA, differs among isoforms.[30-33] Ik1 (the full-length *IKZF1* isoform), Ik2 (lacking exon 4–encoded amino acid), Ik3 (lacking exon 6 and exon 7 amino acids), and Ik4 (lacking exon 4 and exon 6 amino acids) are reportedly abundantly expressed in human peripheral blood leukocytes,[32] and individual isoforms have 4, 3, 3, or 2 N-terminus zinc-finger domains, respectively (Fig 2, *A-D*). The location of rs4917014 and accession numbers of IKZF1 isoforms were shown in Fig E5 in this article's Online Repository at www.jacionline.org. Semiquantitative RT-PCR was performed to assess expression of these 4 *IKZF1* mRNA isoforms in each whole blood sample taken from 33 healthy subjects with different *IKZF1* SNP (rs4917014, rs10276619, and rs4917129) genotypes. The quantity of Ik2, Ik3, and Ik4 isoforms was normalized to the quantity of the Ik1 isoform for each subject to standardize the difference in the numbers of *IKZF1*-positive cells among individual samples. As shown in Fig 2, *E*, the Ik2/Ik1 ratio was significantly associated with the rs4917014 genotype ($P < .01$, Jonkheere-Terpstra test; $P < .05$, Kruskal-Wallis test). Notably, the rs10276619 and rs4917129 genotypes were also significantly associated with the Ik2/Ik1 ratio (Fig 2, *F* and *G*; $P < .05$, Jonkheere-Terpstra test; not significant by using the Kruskal-Wallis test). Probably because of the relatively low expression levels of Ik3 and Ik4 isoforms, associations of *IKZF1* SNPs with Ik3/Ik1 or Ik4/Ik1 ratios did not reach statistical significance (data not shown). These results indicated that the Ik2/Ik1 ratio might be influenced by *IKZF1* SNPs that are significantly associated with susceptibility to CM-SJS/TEN with SMI.

DISCUSSION

Here, we found that an *IKZF1* polymorphism was significantly associated with CM-SJS/TEN with SMI (including severe ocular surface complications) by performing a GWAS with Japanese samples and subsequent replication analyses with Korean, Indian, and Brazilian samples. We should acknowledge potential inflation because of population stratification in the replication studies, especially for Brazilian samples. Nevertheless, the data from individual population replication sets showed the same significant associations (Korean and Indian) or the same direction of association (Brazilian), and our meta-analysis that combined data from the Japanese, Korean, Indian and Brazilian replication sets showed a significant association between CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 8.5 \times 10^{-11}$; Table III).

In the GWAS the *HLA-A* region clearly showed the strongest association with susceptibility to CM-SJS/TEN with SMI. Regarding *IKZF1*, statistical power of the present GWAS sample size was calculated to be 86% with the following conditions: risk allele OR of 2.02, prevalence of 0.00001, and risk allele frequency in control subjects of 0.51. These findings indicated that *IKZF1*