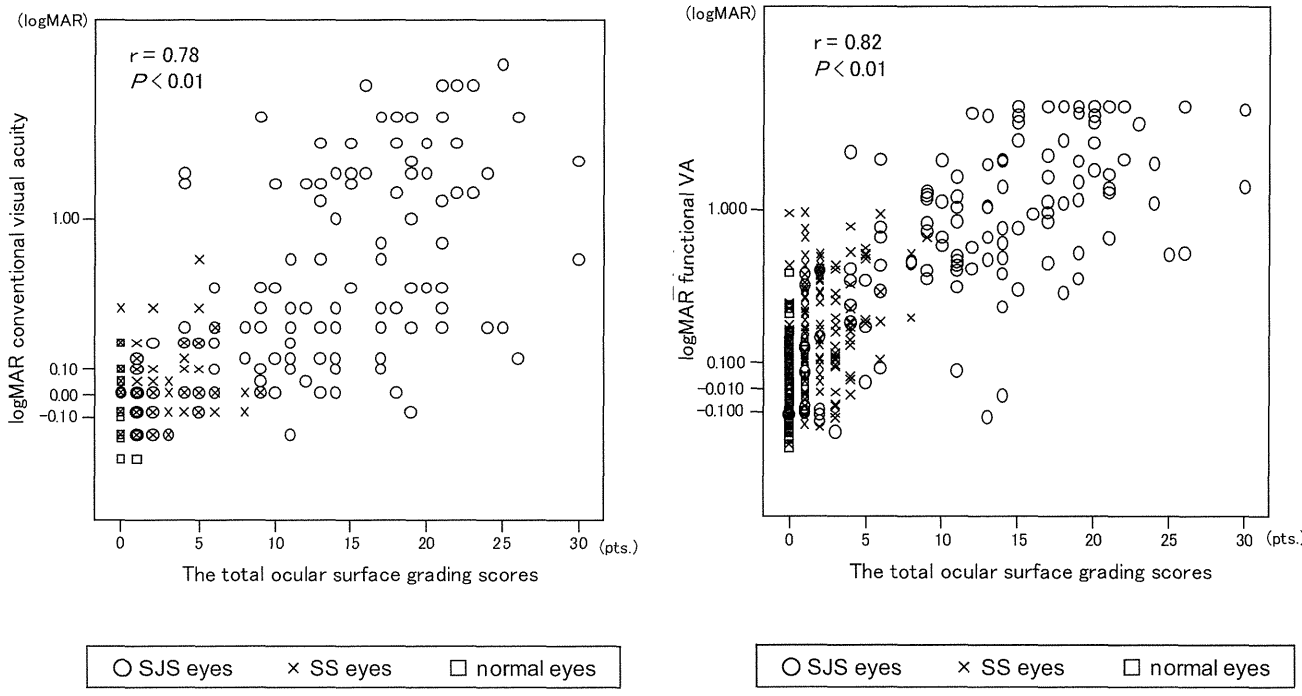

FIGURE 3. Correlation between visual function and ocular surface grading score. (Left) Correlation between logMAR conventional visual acuity scores and total ocular surface grading scores. (Right) Correlation between logarithm of minimal angle of resolution (logMAR) functional visual acuity scores and total ocular surface grading scores. SJS: Stevens-Johnson syndrome; SS: Sjögren syndrome.

TABLE 4. Multivariable Regression Analyses Between Ocular Surface Grading Score, logMAR Conventional Visual Acuity, and logMAR Functional Visual Acuity

	logMAR Conventional Visual Acuity[a]		logMAR Functional Visual Acuity[b]	
Complication	Standard Partial Regression	*P* value	Standard Partial Regression	*P* value
Neovascularization	0.509	<.001	0.229	<.001
Opacification	0.385	<.001	0.308	<.001
Keratinization	−0.088	.002	−0.131	<.001
SPK	0.054	.061	0.193	<.001
Symblepharon	0.059	.103	0.162	<.001
Conjunctivalization	−0.058	.262	0.168	<.001
Corneal epithelial defect	0.034	.198	0.027	.319

logMAR = logarithm of minimal angle of resolution; SPK = superficial punctuate keratopathy.

[a]Conditioned multiple correlation coefficient for logMAR conventional visual acuity =0.81.

[b]Conditioned multiple correlation coefficient for logMAR functional visual acuity =0.84.

higher compared to SS patients (Figure 2, Top). The mean visual maintenance ratios in SJS patients with and without aqueous tear deficiency and SS patients were 0.86 ± 0.10, 0.83 ± 0.14, and 0.91 ± 0.07, respectively. The visual maintenance ratios in SJS patients both with and without aqueous tear deficiency were significantly lower compared to SS patients (Figure 2, Top).

• CLINICAL FINDINGS: Table 3 shows the mean ocular surface grading scores in SS and SJS patients and normal subjects. The mean ocular surface grading scores in all 12 components of clinical findings was significantly higher in SJS patients compared to SS patients and normal subjects ($P < .05$).

The mean ocular surface grading scores in SJS patients with and without aqueous tear deficiency and SS patients were 12.0 ± 8.1, 12.8 ± 5.7, and 2.3 ± 1.8, respectively. The total ocular surface grading scores in SJS both with and without aqueous tear deficiency were significantly higher compared to SS patients (Figure 2, Middle).

• CORRELATION BETWEEN VISUAL FUNCTION AND CLINICAL FINDINGS: Figure 3 shows the correlation between visual function and ocular surface grading score in SJS patients, SS patients, and normal subjects overall. A strong significant correlation was observed between total ocular-surface grading scores and best-corrected logMAR Landolt conventional visual acuities ($r = 0.78$, $P < .001$), as well as best-corrected logMAR Landolt functional visual acuities ($r = 0.82$, $P < .001$).

Table 4 shows the correlation of visual function and ocular surface grading scores. The results of multiple linear regression analysis between the clinical findings and log-

TABLE 5. Correlations Between Ocular Complications and Visual Function or the Composite National Eye Institute Visual Function Questionnaire Scores in Stevens-Johnson Syndrome Patients With Aqueous Tear Deficiency and Sjögren Syndrome Patients

	SJS With Aqueous Tear Deficiency				SS			
	Pearson CC				Pearson CC			
	Log Conventional Visual Acuity	Log Functional Visual Acuity	Visual Maintenance Ratio	NEI VFQ-25	Log Conventional Visual Acuity	Log Functional Visual Acuity	Visual Maintenance Ratio	NEI VFQ-25
Trichiasis	0.09	0.08	0.16	−0.02	—	—	—	—
Symblepharon	0.43[b]	0.53[b]	−0.30[a]	−0.46[a]	0.08	0.15	−0.07	−0.12
Punctal involvement	0.55[b]	0.57[b]	−0.28	−0.49[b]	0.09	0.13	−0.13	0.01
MG involvement	0.48[b]	0.44[b]	−0.23	−0.55[b]	0.06	0.07	−0.04	−0.42[b]
MJ involvement	0.25	0.33[b]	−0.26	−0.39[a]	−0.06	0.20[a]	−0.32[a]	−0.05
Conjunctival hyperemia	0.28[a]	0.31[a]	−0.23	−0.48[b]	0.22[b]	0.11	0.01	−0.24
Keratinization	0.06	0.09	−0.03	−0.03	—	—	—	—
Conjunctivalization	0.53[b]	0.52[b]	−0.19	−0.53[b]	0.29[b]	0.41[b]	−0.28[b]	−0.22
Opacification	0.59[b]	0.67[b]	−0.47[b]	−0.69[b]	0.17	0.20	−0.19	—
Corneal epithelial defect	0.06	0.16	−0.15	−0.11	—	—	—	—
Neovascularization	0.64[b]	0.63[b]	−0.20	−0.63[b]	0.21[a]	0.23[b]	−0.14	−0.2[a]
SPK	0.35[a]	0.35[a]	−0.08	−0.41[a]	0.04	0.212	−0.11	−0.22
Total ocular complications	0.55[b]	0.58[b]	−0.26	−0.61[b]	0.12	0.25[b]	−0.15	−0.40[b]

CC = correlation coefficient; MG = meibomian gland; MJ = mucocutaneous junction; NEI VFQ-25 = National Eye Institute visual function questionnaire; SJS = Stevens-Johnson syndrome; SPK = superficial punctuate keratopathy; SS = Sjögren syndrome.

[a] $P < .05$.

[b] $P < .01$.

TABLE 6. Correlations Between Visual Function and Ocular Surface Grading Scores or Composite National Eye Institute Visual Function Questionnaire Scores in Good, Intermediate, or Poor Conventional Visual Acuity Group of Stevens-Johnson Syndrome Patients

	All Groups		Good Conventional Visual Acuity Group		Intermediate Conventional Visual Acuity Group		Poor Conventional Visual Acuity Group	
	Pearson CC	*P* Value	Pearson CC	*P* Value	Pearson CC	*P* Value	Pearson CC	*P* Value
Conventional visual acuity vs clinical finding scores	0.59[b]	.001	0.37	.08	0.24	.15	0.40[b]	.001
Functional visual acuity vs clinical finding scores	0.63[b]	.001	0.56[b]	.005	0.49[b]	.002	0.34[b]	.007
Conventional visual acuity vs composite NEI VFQ-25 scores	−0.74[b]	.001	−0.44	.06	−0.25	.25	−0.56[a]	.03
Functional visual acuity vs composite NEI VFQ-25 scores	−0.74[b]	.001	−0.55[b]	.02	−0.20	.37	−0.57[a]	.03

CC = correlation coefficient; NEI VFQ-25 = National Eye Institute Visual Function Questionnaire.

[a] $P < .05$.

[b] $P < .01$.

MAR conventional visual acuity showed a significant and strong correlation with neovascularization, opacification, and keratinization grades. Clinical findings such as SPK, symblepharon, and conjunctivalization also had a significant and strong correlation with the functional visual acuities. The multiple regression equation of logMAR conventional visual acuity was expressed as follows: logMAR conventional visual acuity = −0.084 + neovascularization × 0.509 + opacification × 0.385 + keratinization × −0.088. Likewise, the multiple regression equation of logMAR functional visual

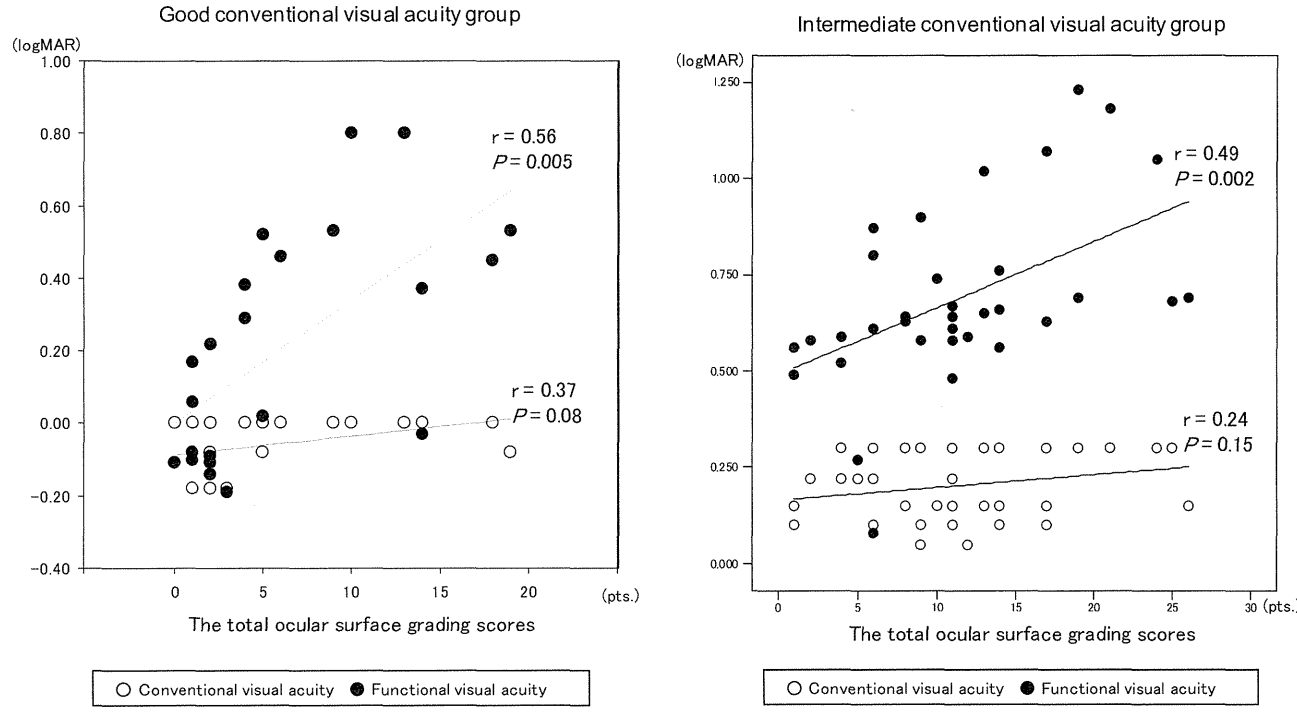

FIGURE 4. Correlations between visual function and ocular surface grading score in the good and intermediate conventional visual acuity group of Stevens-Johnson syndrome patients. (Left) Correlation in the good conventional visual acuity group of Stevens-Johnson syndrome patients. (Right) Correlation in the intermediate conventional visual acuity group of Stevens-Johnson syndrome patients. logMAR = logarithm of minimal angle of resolution.

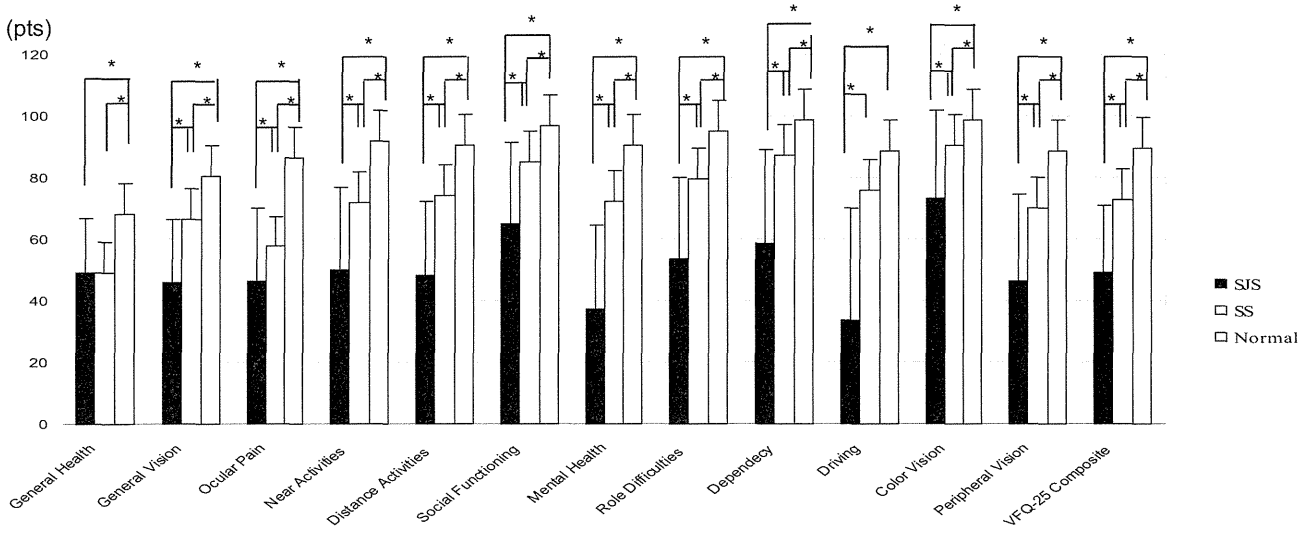

FIGURE 5. Visual Function Questionnaire-25 results in patients with Stevens-Johnson syndrome (SJS), patients with Sjögren syndrome (SS), and healthy normal subjects.

acuity was expressed as follows: logMAR functional visual acuity = −0.061 + neovascularization × 0.229 + opacification × 0.308 + keratinization × −0.131 + SPK × 0.193 + symblepharon × 0.162 + conjunctivalization × 0.168.

Table 5 shows the correlation between ocular complications and visual function in SJS patients with aqueous tear deficiency and SS patients. Strong significant correlations were observed between total ocular surface grading score and logMAR conventional visual acuities or logMAR functional visual acuities in SJS patients with aqueous tear deficiency, and similar strong significant correlations in SJS patients without aqueous tear defi-

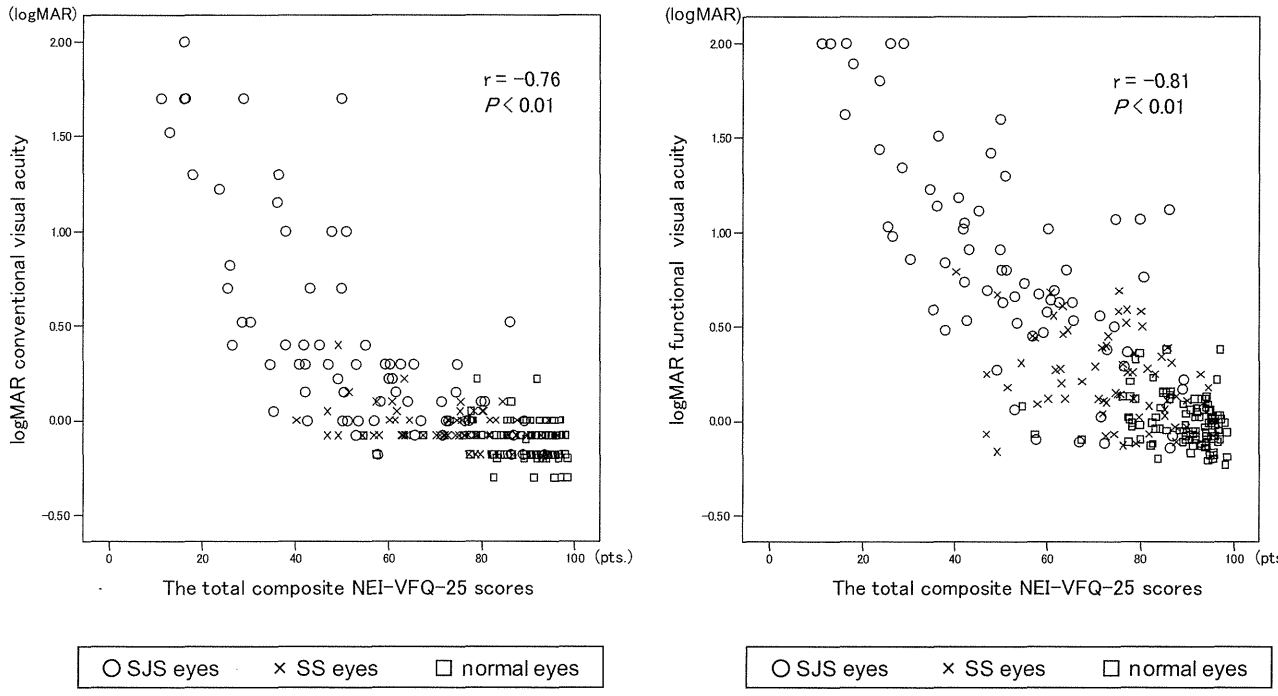

FIGURE 6. Relation between visual function and composite NEI VFQ-25 scores. (Left) Correlation between logMAR conventional visual acuity scores and total composite NEI VFQ-25 scores. (Right) Correlation between logarithm of minimal angle of resolution (logMAR) functional visual acuity scores and total composite NEI VFQ-25 scores. SJS: Stevens-Johnson syndrome; SS: Sjögren syndrome.

ciency (r = 0.66, $P < .001$) (data not shown), while significant correlations were observed only between total ocular surface grading score and logMAR functional visual acuities in SS patients (Table 5).

Table 6 shows the correlations between visual function and ocular surface grading scores in the good, intermediate, and poor conventional visual acuity groups of SJS patients. A strong positive significant correlation was observed between total ocular surface grading scores and logMAR Landolt functional visual acuities in the good conventional visual acuity group (r = 0.56, $P = .005$) and intermediate conventional visual acuity group (r = 0.49, $P = .002$), while no correlation was observed between total ocular surface grading scores and logMAR Landolt conventional visual acuities in these groups (Figure 4).

• VISUAL FUNCTION QUESTIONNAIRE-25: Mean subscale and composite NEI VFQ scores for SJS and SS patients and normal subjects are presented in Figure 5. All 12 subscale NEI VFQ scores were significantly lower in the SJS patients compared to the normal subjects ($P < .05$). Likewise, all subscale scores were significantly lower in the SS patients compared to the normal subjects ($P < .05$). The subscale of "ocular pain" was remarkably low in SS patients, while all subscale scores were remarkably lower in SJS patients. The mean composite NEI VFQ scores of the 12 subscales were 49.1 ± 21.6 in SJS patients, 72.8 ± 12.8 in SS patients, and 89.4 ± 8.1 in the normal subjects.

The mean total composite NEI VFQ score in SJS patients with severe corneal complications was 45.2 ± 20.9. The mean total composite NEI VFQ scores in SJS patients with minimal corneal complications and SS patients were 62.2 ± 19.8 and 73.0 ± 12.8, respectively. The total composite NEI VFQ scores in SJS patients were significantly lower compared to SS patients (Figure 1, Right).

The mean total composite NEI VFQ scores in SJS patients with and without aqueous tear deficiency were 51.6 ± 20.2, 49.4 ± 23.5, and 72.8 ± 12.8, respectively. The total composite NEI VFQ scores in SJS both with and without aqueous tear deficiency were significantly lower compared to SS patients (Figure 2, Right).

• CORRELATION OF VISUAL FUNCTION AND NEI VFQ-25 SCORES: Figure 6 shows the correlation between visual function and the composite NEI VFQ-25 scores in SJS patients, SS patients, and normal subjects overall. A strong negative correlation was detected between the composite NEI VFQ-25 scores and best-corrected logMAR Landolt conventional visual acuities (r = −0.76, $P < .01$), and best-corrected logMAR Landolt functional visual acuities (r = −0.81, $P < .01$).

Table 6 shows the correlations between visual function and the composite NEI VFQ-25 scores in the good, intermediate, and poor conventional visual acuity groups in SJS patients. A positive significant correlation was

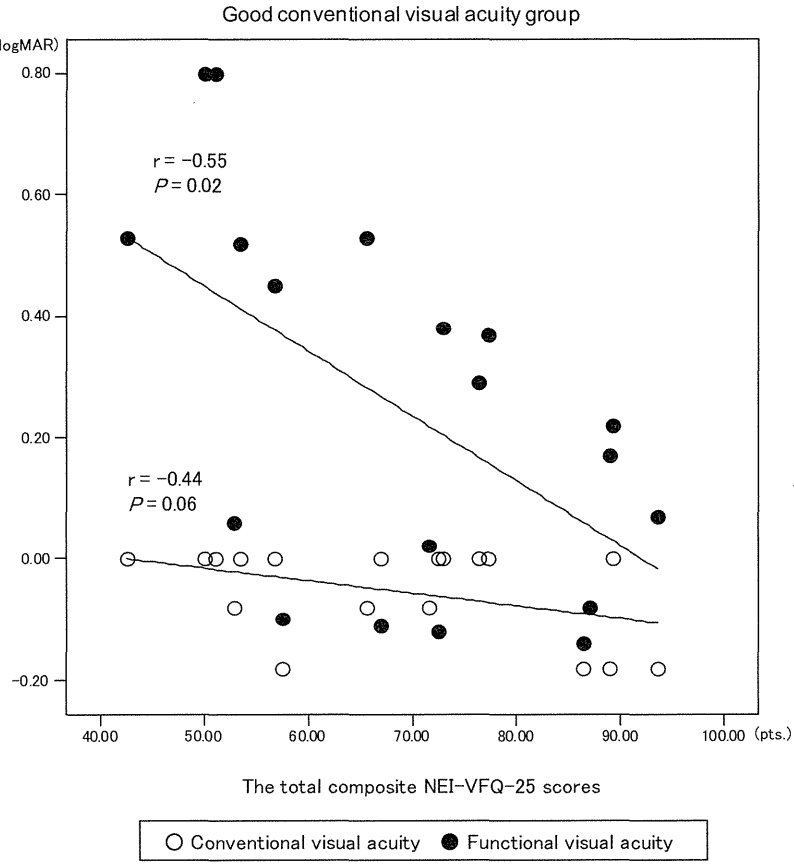

FIGURE 7. Correlations between visual function and the total composite NEI VFQ-25 scores in the good conventional visual acuity group of Stevens-Johnson syndrome patients. logMAR = logarithm of minimal angle of resolution.

observed between the composite NEI VFQ-25 scores and logMAR Landolt functional visual acuities in the good conventional visual acuity group (r = 0.55, P = .02), while no correlation was observed between the composite NEI VFQ-25 scores and logMAR Landolt conventional visual acuities in this group (r = 0.44, P = .06) (Figure 7).

• CORRELATION BETWEEN NEI VFQ-25 SCORES AND CLINICAL FINDINGS: Table 5 shows the correlation between ocular complications and the total composite NEI VFQ-25 scores in SJS patients with aqueous tear deficiency and SS patients. Strong significant correlations were observed between total ocular surface grading score and the composite NEI VFQ-25 scores in SJS patients with aqueous tear deficiency and SS patients (Table 5), and similarly in SJS patients without aqueous tear deficiency (r = 0.51, P = .002) (data not shown).

DISCUSSION

SEVERE OCULAR SURFACE DISEASE ASSOCIATED WITH SJS has been reported to cause visual deterioration. However, quantifying visual acuity in SJS patients has not been assessed, although an interest in the quantitative interpretation of visual function has been rising over the last few years, especially in the fields of refractive surgery, cataract, and dry eyes, through analyses by contrast sensitivity, contrast visual acuity, and wavefront analysis.[9–19] In this report, we measured the functional visual acuity in addition to conventional visual acuity testing and evaluated the relations between visual functions, ocular surface clinical findings, and the vision-related quality of life in SJS patients. We chose functional visual acuity testing for the assessment of the visual function, which has been shown to be efficient in the detection of "masked impairment of visual function" in dry eye patients, since SJS is known to be associated with severe dry eyes.[20–24]

Visual function testing revealed several interesting findings. First, visual acuities measured by conventional Landolt visual acuity testing were low in the SJS patients as compared with SS patients and normal subjects. When we focused on the visual function of the SS patients and normal subjects, the functional visual acuity scores in the SS patients were significantly lower than in the normal subjects, although there were no differences in the conventional visual acuities. In addition, the mean visual maintenance ratios in the SJS patients were significantly lower than in the SS patients, indicating that ability to

maintain the best visual acuity in SJS patients had deteriorated more than in the SS patients.

The functional visual acuity examination has been shown to be useful for the assessment of visual function related to dry eyes in our previous reports.[20–22] A previous report has also suggested the possibility that the functional visual acuity examination might reflect the effect of ocular surface findings and dry eye states on visual functions.[8] In this report, we analyzed the relation of ocular surface findings with visual function and quality of life in detail by grading the severity of ocular surface findings. The clinical severity scores of the examined ocular surface findings were much higher in the SJS patients. We analyzed whether visual disturbance and quality of life were similarly affected in SJS and SS patients without corneal complications or only with minimal corneal complications. Interestingly, we observed that visual function and quality of life were deteriorated in SJS patients with minimal corneal complications compared with SS patients. Moreover, we noted more visual dysfunction and declined quality of life in SJS patients with similar aqueous tear deficiency compared to SS patients. According to the multiple linear regression analysis, neovascularization, opacification, and keratinization involving the optical axis appeared to have a significant effect on the logMAR conventional visual acuities. SPK, symblepharon, and conjunctivalization also had a significant effect on the logMAR functional visual acuities.

Our findings that a stronger correlation existed between ocular surface grading score and logMAR functional visual acuity compared to logMAR conventional visual acuity suggest that functional visual acuity testing can indeed reflect the effect of clinical complications of ocular surface disease on visual function in SJS. In the correlations between visual function and ocular surface grading scores in the good, intermediate, and poor conventional visual acuity groups in SJS patients, a strong positive significant correlation was observed between total ocular surface grading scores and logMAR Landolt functional visual acuities in the good conventional visual acuity group and intermediate conventional visual acuity group, while no correlation was observed between total ocular surface grading scores and logMAR Landolt conventional visual acuities in these groups. These results suggest that functional visual acuity reflects the effect of ocular surface complications on visual function more sensitively in SJS patients with good and intermediate conventional visual acuities. The strong correlation of logMAR functional visual acuity with ocular surface grading score also suggests that functional visual acuity may be detecting the effect of ocular surface disease severity on other visual functions such as contrast, glare, or higher-order aberrations (compared to conventional visual acuity testing), which needs to be investigated in future studies employing the above-mentioned methodologies in conjunction with functional visual acuity testing.

The mean of all VFQ-25 subscale scores was remarkably worse in the SJS patients compared to normal subjects. Likewise, the mean of all subscale scores in SS patients was significantly lower than in the normal subjects. When analyzed in detail, only the subscale scores of "general health" and "ocular pain" were worse, without marked changes in other subscale scores. In SJS patients, as compared with normal subjects, all VFQ-25 subscale scores, especially "ocular pain," "near activities," "distance activities," "mental health," "role difficulties," and "driving," were very low. These findings suggest that SJS patients suffer from an actual limitation of vision-related daily activity rather than a sense of decreased visual performance and health decline.

A strong negative correlation was observed in the relation between logMAR conventional visual acuities and the VFQ-25 composite scores in this study, with a strong correlation detectable for the relation between the VFQ-25 composite scores and the logMAR functional visual acuities.

We had noteworthy observations that patients with SJS had significantly worse dry eye and visual symptom scores compared to SS patients. We believe these observations owe to the presence of a higher incidence of ocular surface complications in SJS such as symblepharon, corneal opacification, and SPK.

One of the weak points of the current study is that SJS patients, SS patients, and normal subjects were not age-matched. However, it was actually difficult to recruit subjects with age matching in the current study. In fact, the age of onset of SS is usually beyond middle age, while individuals have a risk to be involved with SJS at any age. Moreover, recruitment of elderly individuals with normal tear functions as normal control subjects is another challenging task. It should be noted that the VFQ-25 subscale scores might have been affected by sex and age differences. Another weakness was the lack of definitive diagnosis of SJS/TEN by skin biopsy. We diagnosed SJS or TEN on the history of the presence of cryptogenic fever and acute inflammation of mucosal membranes, most commonly after taking cold remedies, antibiotics, or anti-inflammatory drugs, and on the presence of the chronic ocular surface complications.

Overall, although standard visual acuity testing is a good measurement of one aspect of visual function, the functional visual acuity examination provided other important and detailed information on visual functions related with clinical findings and vision-related quality of life. In conclusion, SJS patients with good or intermediate visual acuity scores measured by conventional visual acuity testing were found to suffer from lower vision-related quality of life, as assessed by functional visual acuity testing and VFQ scores.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF Interest. None of the authors received lecture fees or equity payments from Nidek. Drs Kazuo Tsubota and Minako Kaido both hold patent rights for the method and the apparatus for the measurement of functional visual acuity (US patent no: 7470026). All study centers received and shared an official grant from the Japanese Ministry of Health and Welfare, Tokyo, Japan during the conduct of the study. Involved in conception and design (M.K., C.S., S.K., K.T.); analysis and interpretation (M.K., C.S., K.T.); writing the article (M.K.); critial revision of the article (M.Y., C.S., S.K., J.S., Y.T., Y.H., T.C., K.T.); final approval of the article (M.K., M.Y., C.S., S.K., J.S., Y.T., Y.H., T.C., K.T.); data collection (M.K., M.Y., C.S., J.S., Y.T., Y.H., T.C.); provision of materials, patients, or resources (M.K., M.Y., C.S., J.S., Y.T., Y.H., T.C.); statistical expertise (M.K.); obtaining funding (M.K., M.Y., C.S.); lierature search (M.K., C.S.); and administrative, technical, or logistical support (C.S., S.K., K.T.). Ethics committee approvals for the examination procedures and study protocol were obtained at each center for this prospective study (IRB approval number: 17–129, Keio University School of Medicine, 20. 6, 2006). Written informed consent was obtained from each patient to participate in this study.

REFERENCES

1. Arsujo OE, Floweres FP. Steven-Johnson syndrome. *J Emerg Med* 1984;2(2):129–135.
2. Tsubota K, Toda I, Saito H, Shinozaki N, Shimazaki J. Reconstruction of the corneal epithelium by limbal allograft transplantation for severe ocular surface disorders. *Ophthalmology* 1995;102(10):1486–1496.
3. Power WJ, Ghoraishi M, Merayo-Llovers J, Neves RA, Foster CS. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectum. *Ophthalmology* 1995; 102(11):1669–1676.
4. Puangsricharern V, Tseng SCG. Cytologic evidence of corneal disease with limbal stem cell deficiency. *Ophthalmology* 1995;102(19):1476–1485.
5. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med* 1999;340(22):1697–1703.
6. Kaido M, Goto E, Dogru M, Tsubota K. Punctal occlusion in the management of chronic Stevens-Johnson syndrome. *Ophthalmology* 2004;111(5):895–900.
7. Kaido M, Dogru M, Yamada M, et al. Functional visual acuity in Stevens-Johnson syndrome. *Am J Ophthalmol* 2006; 142(6):917–922.
8. Sotozono C, Ueta M, Koizumi N, et al. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. *Ophthalmology* 2009; 116(4):685–690.
9. Rieger G. The importance of the precorneal tear film for the quality of topical imaging. *Br J Ophthalmol* 1992;76(3):157–158.
10. Niesen U, Businger U, Hartmann P, Senn P, Schipper I. Glare sensitivity and visual acuity after excimer laser photorefractive for myopia. *Br J Ophthalmol* 1997;81(2):136–140.
11. Ghaith AA, Daniel J, Stulting RD, Thompson KP, Lynn M. Contrast sensitivity and glare disability after radial keratotomy and photorefractive keratectomy. *Arch Ophthalmol* 1998; 116(1):12–18.
12. Roland M, Iester M, Maci A, Calabria G. Low spatial-contrast sensitivity in dry eyes. *Cornea* 1998;17(4):376–379.
13. Liu Z, Pflugfelder SC. Corneal surface regularity and the effect of artificial tears in aqueous tear deficiency. *Ophthalmology* 1999;106(5):939–943.
14. Nakamura K, Bissen-Miyajima H, Toda I, Hori Y, Tsubota K. Effect of laser in situ keratomileusis correction on contrast visual acuity. *J Cataract Refract Surg* 2001;27(3):357–361.
15. Huang FC, Tseng SH, Shih MH, Chen FK. Effect of artificial tears on corneal surface regularity, contrast sensitivity, and glare disability in dry eyes. *Ophthalmology* 2002;109(10): 1934–1940.
16. Bron AJ, Tifffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res* 2004;78(3):347–360.
17. Kojima T, Ishida R, Dogru M, et al. A new noninvasive tear stability analysis system for the assessment of dry eyes. *Invest Ophthalmol Vis Sci* 2004;45(5):1369–1374.
18. Puell MC, Benitez-del-Castillo JM, Martinez-de-la-Casa J, et al. Contrast sensitivity and disability glare in patients with dry eye. *Acta Ophthalmol Scand* 2006;84(4):527–531.
19. Koh S, Maeda N, Hirohara Y, et al. Serial measurements of high-order aberrations after blinking in patients with dry eye. *Invest Ophthalmol Vis Sci* 2008;49(1):133–138.
20. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired functional visual acuity of dry eye patients. *Am J Ophthalmol* 2002;133(2):181–186.
21. Goto E, Yagi Y, Kaido M, Matsumoto Y, Konomi K, Tsubota K. Improved functional visual acuity after punctal occlusion in dry eye patients. *Am J Ophthalmol* 2003;135(5):704–705.
22. Ishida R, Kojima T, Dogru M, et al. The application of a new continuous functional visual acuity measurement system in dry eye syndrome. *Am J Opthalmol* 2005;139(2):253–258.
23. Goto E, Ishida R, Kido M, et al. Optical aberrations and visual disturbances associated with dry eye. *Ocul Surf* 2006; 4(4):207–213.
24. Kaido M, Ishida R, Dogru M, Tamaoki T, Ysubota K. Efficacy of punctum plug treatment in short break-up time dry eye. *Optom Vis Sci* 2008;85(8):758–763.
25. Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV. Sjogren's syndrome: proposed criteria for classification. *Arthritis Rheum* 1986;29(5):577–585.
26. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. *Ophthalmology* 2007; 114(7):1294–1302.
27. Van Bijsterveld OP. Diagnostic tests in the Sicca syndrome. *Arch Ophthalmol* 1969;82(1):10–14.
28. Suzukamo Y, Oshika T, Yuzawa M, et al. Psychometric properties of the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25), Japanese version. *Health Qual Life Outcome* 2005;3:65.
29. Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctuate keratopathy magnitude and its correlation with corneal epithelial permeability. *Arch Ophthalmol* 2003;121(11):1537–1539.
30. Bron AJ, Benjamin L, Snibson GR. Meibomian gland diseases. Classification and grading of lid changes. *Eye* 1991; 5(Pt 4)395–411.

Biosketch

Minako Kaido graduated from the Medical University of Occupational and Environmental Health, Fukuoka, Japan in 1991. She joined Dr Tsubota's dry eye and cornea team at Tokyo Dental College Ichikawa Hospital in 1995 and has been a pivotal member of Dr Tsubota's team at Keio University School of Medicine, Tokyo Japan since 2004. She received her PhD degree in 2012. Dr Kaido's work is focused on the treatment of dry eyes and functional visual acuity technology.

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ORIGINAL ARTICLE

A whole-genome association study of major determinants for allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients

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[9]The members of the Japan Pharmacogenomics Data Science Consortium are listed in the Appendix.

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Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are severe, cutaneous adverse drug reactions that are rare but life threatening. Genetic biomarkers for allopurinol-related SJS/TEN in Japanese were examined in a genome-wide association study in which Japanese patients ($n=14$) were compared with ethnically matched healthy controls ($n=991$). Associations between 890 321 single nucleotide polymorphisms and allopurinol-related SJS/TEN were analyzed by the Fisher's exact test (dominant genotype mode). A total of 21 polymorphisms on chromosome 6 were significantly associated with allopurinol-related SJS/TEN. The strongest association was found at rs2734583 in *BAT1*, rs3094011 in *HCP5* and GA005234 in *MICC* ($P=2.44\times10^{-8}$; odds ratio = 66.8; 95% confidence interval, 19.8–225.0). rs9263726 in *PSORS1C1*, also significantly associated with allopurinol-related SJS/TEN, is in absolute linkage disequilibrium with *human leukocyte antigen-B*5801*, which is in strong association with allopurinol-induced SJS/TEN. The ease of typing rs9263726 makes it a useful biomarker for allopurinol-related SJS/TEN in Japanese.
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Keywords: allopurinol; Stevens–Johnson syndrome; toxic epidermal necrolysis; human lymphocyte antigen; single nucleotide polymorphism; genome-wide association study

Introduction

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid to reduce plasma uric acid levels to a normal range. It is the most frequently used anti-hyperuricemic agent in the world due to its long-term pharmacological effect.[1] However, allopurinol is also one of the most frequent causes of a variety of delayed severe cutaneous adverse drug reactions (SCARs).[2] According to spontaneous reports of severe adverse drug reactions to the Ministry of Health, Labor, and Welfare of Japan, allopurinol-related SCARs accounted for about 11% of all reported SCAR cases in Japan in 2008.[3] Allopurinol-related SCARs include the drug-induced hypersensitivity syndrome, Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[4] SJS/TEN are characterized by high fever, malaise and rapid development of blistering exanthema, with macules and target-like lesions, accompanied by mucosal involvement.[5] Even though the incidence of SJS/TEN is extremely low, the mortality rate of TEN can be as high as 26%.[5] Therefore, SJS/TEN is a serious problem in allopurinol therapy, in spite of the ideal anti-hyperuricemic effect of allopurinol.

Although previous works have suggested that the development of SJS/TEN depends on an immune mechanism involving a drug-dependent cytotoxic cell response against epidermal cells,[5,6] the pathophysiology of SJS/TEN remains largely unknown. Susceptibility to such idiosyncratic reactions is thought to be genetically determined, and familial predisposition to allopurinol-induced SJS/TEN has been reported.[6] Therefore, the exploratory studies for genetic risk factors related to SJS/TEN are needed. A strong association has been observed between allopurinol-induced SCAR and the human lymphocyte antigen (*HLA*) allele B variant (*HLA-B*5801*) in the Han Chinese in Taiwan[7] and in the Thai population.[8] These studies showed that the *HLA-B*5801* allele is present in all patients with allopurinol-induced SCAR (51/51 of Han Chinese and 27/27 of Thai patients) and in only 12–15% of tolerant patients (20/135 and 7/54, respectively). The odds ratio (OR) was 580 (95% confidence interval , 34–9781; $P = 4.7 \times 10^{24}$) for the Han-Chinese data[7] and 348.3 (95% confidence interval, 19.2–6336.9; $P = 1.61 \times 10^{13}$) for the Thai study.[8] Although the association was confirmed in both Caucasian and Japanese subjects,[9,10] the OR in the Han-Chinese and Thai populations were much higher than those in the Caucasian (OR = 80) and Japanese (OR = 40) groups. These reports indicated that *HLA-B*5801* is the valid genetic biomarker for allopurinol-induced SJS/TEN in various ethnic groups, but the mechanisms by which *HLA-B*5801* is specifically involved in allopurinol-induced SJS/TEN progression and the strength of the association showed ethnic differences are unknown.

Currently, genotyping by high-density array scanning of the whole genome allows discovery of previously unsuspected genetic risk factors that influence the pathogenesis of serious adverse drug reactions.[11–13] Genome-wide association studies (GWASs) provide opportunities to uncover polymorphisms that influence susceptibility to allopurinol-induced SJS/TEN free of mechanistic hypotheses. Therefore, in addition to *HLA-B* typing as shown in our previous study,[10] we further conducted a retrospective pharmacogenetic case–control study using whole-genome single nucleotide polymorphism (SNP) data from high-density DNA microarrays in order to identify new and effective genetic biomarkers for allopurinol-related SJS/TEN in Japanese patients.

Materials and methods

Recruitment of study subjects

A total of 141 Japanese SJS/TEN patients from unrelated families were recruited from July 2006 to April 2010 from participating institutes of the Japan Severe Adverse Reactions (JSAR) research group and through a nationwide blood-sampling network system in Japan for SJS/TEN onset patients, operated by the National Institute of Health Sciences.[10] In all, 121 of these patients were diagnosed as defined SJS or TEN by JSAR research group's dermatological experts based on diagnostic criteria[4] that are currently used in Japan. Information was collected using a standardized case report form that includes medical records, co-administrated drug records, disease progress and involvement of systemic complications, as well as SJS/TEN treatment. Among the 141 SJS/TEN patients, 20 were diagnosed as probable SJS due to atypical or mild symptoms. TEN and SJS were defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema and high fever. SJS was defined as skin detachment of 10% or less of the body surface area, and TEN as skin detachment of more than 10%, excluding staphylococcal scaled skin syndrome.[5] In all enrolled cases defined as SJS or TEN, allopurinol was regarded as the drug responsible for SJS or TEN if the onset of SJS/TEN symptoms occurred within the first 2 months of allopurinol exposure. For the retrospective pharmacogenetic case–control study, 991 healthy, ethnically matched subjects in the Tokyo metropolitan area were used as the control group. Healthy subjects were used as the control group instead of allopurinol-tolerant patients because the incidence of SJS/TEN is extremely low (0.4–6 per million per year).[3]

The ethics committees of the National Institute of Health Sciences, each participating institute of the JSAR research group and the Japan Pharmacogenomics Data Science Consortium (JPDSC) approved this study. Written informed consent was obtained from all cases and ethnically matched controls.

Whole-genome genotyping of SNPs

Genome-wide genotyping of the 14 allopurinol-related SJS/TEN patients and 991 ethnically matched controls was conducted using the Illumina Human 1M-Duo BeadChip (Illumina, San Diego, CA, USA), which contained 11 63 218 SNPs. SNPs were discarded from case–control association analysis if they exhibited a minor allele frequency <0.001 in the control group (2 37 890 SNPs), a call rate <0.95 for each SNP (32 640 SNPs) or a *P*-value <0.001 in the test of Hardy–Weinberg equilibrium among controls (2 368 SNPs). These quality control steps removed a total of 2 72 897 SNPs. All samples had a call rate for each microarray above 0.99. Sample duplicates and hidden relatedness were investigated on the basis of pairwise identity-by-state analysis via PLINK;[14] however, there was no duplicate or hidden relatedness in the samples. This quality-control procedure ensured reliable genotyping data.

HLA genotyping and TaqMan genotyping of SNPs on chromosome 6

HLA A, *B* and *Cw* types were determined using sequencing-based methods, as described previously.[10] Representative SNPs of 6p21 (rs2734583, rs3099844, rs9263726 and rs3131643) were re-genotyped using TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA) (ID; C__27465749_10, C__27455402_10, C__30352071_10, C__26778946_20) according to the manufacturer's instruction using 5 ng of genomic DNA. We did not genotype rs9267445 and rs1634776 because TaqMan SNP genotyping assays for these SNPs were not available. Measurement of the linkage disequilibrium (LD) coefficient was performed using

the *HLA* types and 6p21 SNPs of the 141 Japanese SJS/TEN cases and an additional 65 Japanese individuals (non-SJS/TEN patients). The LD coefficient was calculated as previously described.[15,16]

Association analysis

Genome-wide SNPs data from allopurinol-related SJS/TEN cases and ethnically matched controls were used for association analysis using the Fisher's exact test based on the dominant genotype mode and minor allele frequencies of each SNP. Because there are no homozygotes of minor alleles of SNPs, which have significantly related to allopurinol-related SJS/TEN except rs3099844 and rs3131643 in 'Case group', other association analysis models such as trend test (Cochran–Armitage analysis) or recessive model analysis were not applied in this study. All association analyses were carried out with PLINK.[14] *P*-values were corrected for multiple testing according to the Bonferroni's correction. *P*-values $<5.62\times10^{-8}$ were regarded as statistically significant.

Results

Characteristics of study subjects

A total of 14 allopurinol-treated Japanese patients, who were diagnosed with definite SJS/TEN were recruited for the whole-genome association study (IDs 1–14 in Table 1). Patients, IDs 1, 2, 3, 9, 10, 13 and 14 were reported in our previous paper.[10] After the GWAS, an additional four allopurinol-treated Japanese SJS/TEN patients were recruited for *HLA* typing (IDs 15–18). Therefore, a total of 18 allopurinol-treated Japanese SJS/TEN patients participated in the study (Table 1). In all, 12 of 18 patients were male and 6 were female, and the average age was 72.3 ± 10.0 (mean $\pm$ s.d.) years. In all, 12 of 18 cases showed systemic complications of liver and/or renal dysfunction, and most patients had high fever. The average period of SJS/TEN onset after allopurinol treatment was 21.7 ± 11.9 days. Drug-induced lymphocyte stimulation tests were examined in 13 of 18 patients to determine the causative agent; however, in these tests, only two cases (IDs 1 and 5) were positive for allopurinol and only one (ID 16) was positive for oxipurinol, a metabolite of allopurinol. The patient (ID 1) who was positive for the drug-induced lymphocyte stimulation test for allopurinol was also positive for other co-administrated drugs (Table 1). On the other hand, patients who received a patch test showed positive reactions for allopurinol although only two patients were examined (ID 4, 10). The patient who was patch test positive for allopurinol (ID 4) was also patch test positive for other co-administrated drugs (Table 1). Four patients (ID 1, 2, 4 and 14) were co-administrated non-steroidal anti-inflammatory drugs, four (ID 7, 8, 11 and 15) were co-administrated angiotensin II receptor antagonists and three (ID 4, 7 and 17) were co-administrated statin anti-hyperlipemic agents.

Whole-genome association study of major determinants for allopurinol-related SJS/TEN

A total of 14 allopurinol-related SJS/TEN patients (IDs 1–14), who were diagnosed with definite SJS/TEN, and 991 ethnically matched controls, were genotyped with the use of the Illumina Human 1M-Duo BeadChip containing 11 63 218 SNPs. A series of quality-control steps resulted in the elimination of 2 72 897 polymorphisms. For each SNP, Fisher's exact tests were performed to compare the dominant genotype distributions and minor allelic frequencies in the allopurinol-related SJS/TEN patients (the case group) versus those in the ethnically matched healthy control group. The resulting *P*-values were adjusted with the Bonferroni's correction ($P<5.62\times10^{-8}$). The distribution of *P*-values from the Fisher's exact tests (dominant genotype mode) along each chromosome indicated that 21 SNPs were significantly associated with the cases, all of which were located on the chromosome 6: 6p21.3, 6p22.1 and 6p21.1 (Figures 1a and b). The quantile–quantile (Q–Q) plot for the distribution of *P*-values showed that observed *P*-values matched the expected *P*-values over the range of $0<-\log_{10}(p)<4.0$ (Figure 2). A departure was observed at the extreme tail ($-\log_{10}(p)>4.0$) of the distribution of test statistics for the allopurinol-related Japanese SJS/TEN, suggesting that the identified associations are likely due to true variants rather than potential biases such as genotyping error. These SNPs, with their associated genes, are described in Table 2. As is observed in all SNPs in Table 2, minor allele frequencies in the controls were quite small, ranging around 0.5–0.6%. The genotypic distributions of the case and control groups are identical among groups with the same *P*-value, suggesting that these SNPs might be linked. These SNPs also have ORs that are much higher than the ORs of SNPs commonly observed in sporadic cancer and other complex diseases, suggesting they are of higher penetrance. For example, the most significant SNPs (rs2734583, rs3094011 and GA005234) had an OR of 66.8 (95% confidence interval, 19.8–225.0), and the twentieth most significant SNPs (rs9263827 and rs1634776) had an OR of 60.9 (95% confidence interval, 18.3–202.5). Most SNPs in Table 2 are associated with known or predicted genes; of these, 13 are in known genes. Three SNPs (rs17190526, rs9263726 and rs2233945) were found in *PSORS1C1* (psoriasis susceptibility 1 candidate 1), which is considered as one of the potential psoriasis genes.[17–19] The *CCHCR1* (coiled coil α helical rod protein 1), which is a regulator of keratinocyte proliferation or differentiation and is over-expressed in keratinocytes in psoriatic lesions,[20–23] contained four SNPs (rs9263745, rs130077, rs9263781 and rs9263785). *HCP5* (HLA complex P5), which is involved in hypersensitivity to abacavir,[24–26] had three SNPs (rs3094011, rs3099844 and rs31431643). *TCF19* (transcription factor 19), which is a potential trans-activating factor that might play an important role in the transcription of genes required for the later stages of cell cycle progression,[27] contained two SNPs (rs9263794 and rs10448701). Two SNPs (rs9263796 and rs9263800) were also found in *POU5F1* (POU class 5 homeobox; alternative names for Oct4). *BAT1* (HLA-B

Table 1 Summary of clinical characteristics of Japanese patients with allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis

								Co-administered drugs	
Patient ID[a]	*ADR type*	*Sex/age (years)*	*Highest BT (°C)*	*Total area of blistering skin (%)*	*Systemic complications*	*DLST to allopurinol (PT)*	*Period of onset (days) by allopurinol*	*Drug name*	*DLST result/period of onset*
1	SJS	F/53	38.1	0.5	liver dysfunction renal dysfunction	+	26	loxoprofen clarithromycin	+/9 days +/26 days
2	TEN	M/58	37.1	15	neutropenia liver dysfunction	–	ca 10 days	loxoprofen levofloxacin	–/1 day –/1 day
3	SJS	M/77	unknown	unknown	none	not tested	16	none	
4	TEN	F/72	>37	20	none	–(PT+)	16	pitavastatin lansoprazole salicylamide, acetaminophen, caffeine, promethazine, methylenedisalicylate serrapeptase loxoprofen acetaminophen	–/16 days –/179 days –(PT+)/8 days –/1 day –/8 days (PT+)/8 days
5	TEN	M/82	39	35	none	+	52	none	
6	SJS	M/67	1	1	liver dysfunction	not tested	14	none	
7	SJS	M/76	38.8	unknown	GI tract disturbance liver dysfunction renal dysfunction	not tested	<26 days	losartan furosemide carbon atorvastatin	not tested/8 days not tested/3 days not tested/7 days not tested/8 days
8	SJS	M/83	>38	10	renal dysfunction	–	20	amlodipine olmesartan medoxomil	not tested/very long not tested/very long
9	TEN	M/75	>38	20	neutropenia liver dysfunction renal dysfunction	–	6	none	
10	SJS	M/75	38.4	6	neutropenia liver dysfunction renal dysfunction	–(PT+)	14	none	
11	SJS	M/74	37.8	8	neutropenia liver dysfunction renal dysfunction	–	38	cefazolin Furosemide Sodium polystyrene sulfonate olmesartan medoxomil	not tested/1 day not tested/53 day not tested/51 day not tested/59 day
12	SJS	M/67	38.9	2	liver dysfunction	not tested	17	none	
13	SJS	F/81	39.2	0.5	renal dysfunction	–	28	spironolactone	–/24 days
14	SJS	M/83	39	0	respiratory involvement	–	29	diclofenac	–/1 day
15	TEN	F/73	38	10	liver dysfunction renal dysfunction	–	27	valsartan epoetin β	–/18 days –/2 days
16	SJS	M/53	40	5	liver dysfunction	–(oxipurinol +)	19	none	
17	SJS	F/86	38	0	liver dysfunction renal dysfunction	–	30	rosuvastatin	–/43 days
18	TEN	F/66	37.8	15	none	not tested	2	none	

Abbreviations: ADR, adverse drug reaction; BT, body temperature; DLST; drug-induced lymphocyte stimulation test; F, female; M, male; PT, patch test; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

[a]Patients ID 1–14 were applied for whole genome analysis. ID 1–18 were for the *HLA* typing and the analysis of linkage disequilibrium.

Patients IDs 1, 2, 3, 9, 10, 13, and 14 were reported in our previous paper.[10]

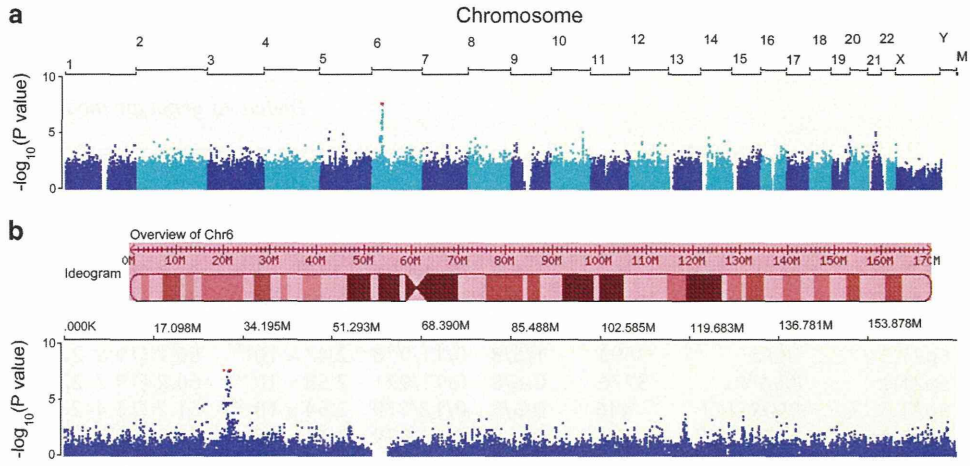

Figure 1 Genome-wide association study of allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis. Each dot represents a single nucleotide polymorphism (SNP). The *x* axis: the position of the SNP on chromosomes. The *y* axis: the $-\log_{10}$ of Fisher's exact test *P*-values (dominant genotype mode) of the SNP in the case–control association study. SNPs with *P*-values $<5.62\times10^{-8}$ are highlighted in red. (**a**) Whole genome. (**b**) Chromosome 6.

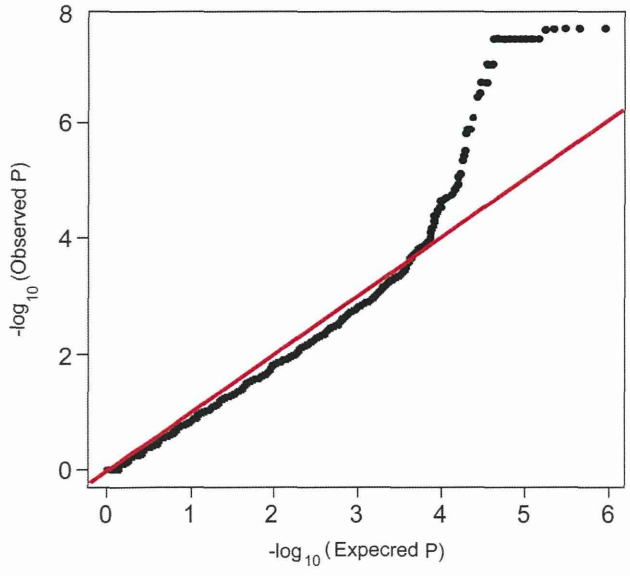

Figure 2 Quantile–quantile plot of Fisher's exact test statistics obtained from the genome-wide association study for allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis under dominant genotype mode. The solid red line represents the null model where observed Fisher's exact test values match the expected values. The dots represent observed versus the expected values from the case–control study.

associated transcript 1) and *PSORS1C3* each carried one SNP (rs2734583 and rs9263827). The SNPs, rs1634776 and rs4084090, were located in more than 10 kb away from the *HLA-B* and *HLA-C* genes, respectively. Two pseudo genes, *MICC* (major histocompatibility complex class I polypeptide-related sequence) and *PPIAP9* (peptidylprolyl isomerase A (cyclophilin A) pseudogene 9), had one SNP each (GA005234 and rs9267445). Previous report using Han-Chinese patients with allopurinol-induced SCAR indicated rs3117583 of *BAT3*, rs1150793 of *MSH5* and rs2855804 of *MICB*, which are located in *HLA* region, showed significant *P*-values ($P<1\times10^{-7}$).[7] In this study using Japanese patients, both rs3117583 and rs1150793 showed $P=6.34\times10^{-3}$ (allele frequency mode) and $P=6.14\times10^{-3}$ (dominant genotype mode). There was no data of rs2855804 in the Illumina Human 1M-Duo BeadChip.

HLA types of allopurinol-related SJS/TEN patients

Classical class I *HLA* types (*A*, *B* and *Cw*) of allopurinol-related SJS/TEN patients were determined because the *HLA-B*5801* type is associated with allopurinol-related SCARs in Han Chinese,[7] Caucasians[9] and Japanese[10] (Table 3). In this analysis, four patients with allopurinol-related SJS/TEN (IDs 15–18), who were recruited after BeadChip analysis, joined the case group (total of 18 allopurinol-related SJS/TEN patients). Eight cases of *HLA-A*3303* (allele frequency = 22.2%), 10 cases of *HLA-B*5801* (allele frequency = 27.8%) and 10 cases of *HLA-Cw*0302* (allele frequency = 27.8%) were found in 18 allopurinol-related SJS/TEN patients (Table 3). By comparison, the allelic frequencies of *HLA-A*3303*, *HLA-B*5801* and *HLA-Cw*0302* were 7.9%, 0.6% and 0%, respectively in Japanese general population (Tables 4a–c). The OR of *HLA-A*3303* was calculated as 3.32 (Table 4a). The OR of *HLA-B*5801* was calculated as 62.8 (Table 4b), which was a little larger than the previously reported OR in Japanese patients.[10] *HLA-Cw*0302* also showed significant association with allopurinol-related SJS/TEN (Table 4c). *HLA-A*3303* and *HLA-Cw*0302* are in LD with *HLA-B*5801* in the Japanese although the general frequency of *HLA-A*3303* is higher than other two types. Other *HLA-A*, *B* and *Cw* types, which were not listed in Tables 4a–c, showed very low frequencies in the general Japanese population, or were not found in 18 allopurinol-related SJS/TEN patients.

Table 2 The association of single nucleotide polymorphism with allopurinol-related Japanese patients with Stevens–Johnson syndrome or toxic epidermal necrolysis

							Dominant genotype mode		*Allelic frequency mode*	
Order	*SNP*	*Chromosome*	*Closest gene*	*Distance to gene (bp)*	*Case*[a]	*Control*[a]	P	*Odds ratio (95% CI)*	P	*MAF (%)*
1	rs2734583	6p21.3	*BAT1*	0	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
1	rs3094011	6p21.3	*HCP5*	6553	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
1	GA005234	6p22.1	*MICC*	0	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
4	rs3099844	6p21.3	*HCP5*	3693	1/5/8	0/11/978	2.47×10^{-8}	66.7 (19.8–224.5)	1.33×10^{-9}	0.56
5	rs9267445	6p21.1	*PPIAP9*	3776	0/6/8	0/11/971	2.58×10^{-8}	66.2 (19.7–222.9)	4.87×10^{-8}	0.56
6	rs17190526	6p21.3	*PSORS1C1*	−446	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263726	6p21.3	*PSORS1C1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs2233945	6p21.3	*PSORS1C1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263733	6p21.3	*POLR2LP*	139	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263745	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs130077	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263781	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263785	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263794	6p21.3	*TCF19*	0	0/6/8	0/12/979	2.47×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs1044870	6p21.3	*TCF19*	0	0/6/8	0/12/979	2.58×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263796	6p21.3	*POU5F1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263800	6p21.3	*POU5F1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs4084090	6p21.3	*HLA-C*	17691	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
19	rs3131643	6p21.3	*HCP5*	0	1/5/8	0/12/977	3.68×10^{-8}	61.1 (18.4–203.1)	2.08×10^{-9}	0.61
20	rs9263827	6p21.3	*PSORS1C3*	−3369	0/6/8	0/12/974	3.75×10^{-8}	60.9 (18.3–202.5)	7.07×10^{-8}	0.61
20	rs1634776	6p21.3	*HLA-B*	12661	0/6/8	0/12/974	3.75×10^{-8}	60.9 (18.3–202.5)	7.07×10^{-8}	0.61

Abbreviations: CI, confidence interval; MAF; minor allelic frequency; SNP, ingle nucleotide polymorphism.
[a]Number of subjects in minor homo/hetero/major homo.

Table 3 *HLA* types and representative genotypes in 6p21 of allopurinol-related Japanese patients with Stevens–Johnson syndrome or toxic epidermal necrolysis

ID	HLA-A		HLA-B		HLA-Cw		*rs2734583*	*rs3099844*	*rs9267445*	*rs9263726*	*rs3131643*	*rs1634776*
1	2402	3303	4002	**5801**	0302	0304	T/**C**	C/**A**	G/**C**	G/A	C/**T**	G/**A**
2	2402	3101	1501	5601	0303	0401	T/T	C/C	G/G	G/G	C/C	G/G
3	2402	3101	5201	**5801**	0302	1202	T/**C**	C/**A**	G/**C**	G/A	C/**T**	G/**A**
4	1101	1101	4801	**5801**	0302	0803	T/**C**	**A**/**A**	G/**C**	G/A	**T**/**T**	G/**A**
5	2402	2602	4006	5101	0801	1402	T/T	C/C	G/G	G/G	C/C	G/G
6	0201	1101	1518	3501	0401	0801	T/T	C/C	G/G	G/G	C/C	G/G
7	2402	3303	5201	**5801**	0302	1202	T/**C**	C/**A**	G/**C**	G/A	C/**T**	G/**A**
8	0201	2402	1527	4003	0304	0401	T/T	C/C	G/G	G/G	C/C	G/G
9	2402	2402	3501	5201	0303	1202	T/T	C/C	G/G	G/G	C/C	G/G
10	0210	1101	4002	4006	0401	0801	T/T	C/C	G/G	G/G	C/C	G/G
11	0207	2402	4601	5101	0102	1402	T/T	C/C	G/G	G/G	C/C	G/G
12	2402	3101	3901	4001	0304	0702	T/T	C/C	G/G	G/G	C/C	G/G
13	0207	3303	4601	**5801**	0102	0302	T/**C**	C/**A**	G/**C**	G/A	C/**T**	G/**A**
14	3101	3303	3901	**5801**	0302	0702	T/**C**	C/**A**	G/**C**	G/A	C/**T**	G/**A**
15	2402	3303	5101	**5801**	0302	1402	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA
16	0201	3303	3802	**5801**	0302	0702	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA
17	2402	3303	0702	**5801**	0302	0702	T/**C**	C/**A**	NA	G/**A**	C/**T**	NA
18	2402	3303	5101	**5801**	0302	0304	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA

Abbreviations: HLA, human leukocyte antigen; NA, not available.
Single nucleotide polymorphisms data of rs2734583, rs3099844, rs9263726 and rs3131643 are from BeadChip analysis and TaqMan genotyping analysis. Single nucleotide polymorphisms data of rs9267445 and rs1634776 are from BeadChip analysis.
Underlines of HLA types mean that these types are in linkage disequilibrium. HLA-B*5801s are expressed by bold types.
Bold types of the nucleotide mean the variant allele.

Table 4a Association between *HLA-A* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

HLA-A allele	*Number of alleles detected (allele frequency)*		P	*Odds ratio (95% CI)*
	Case, n = *36 (%)*	*General population control* (n = *986*)[a] *(%)*		
0201	3 (8.3)	10.9	0.7895	
0206	0 (0)	10.4	0.0426	
0207	2 (5.6)	3.4	0.3650	
0210	1 (2.8)	0.1	0.0692	
1101	4 (11.1)	8.1	0.5299	
2402	13 (36.1)	35.6	1.000	1.02 (0.51–2.04)
2601	0 (0)	9.8	0.0417	
2602	1 (2.8)	2.2	0.5657	
3101	4 (11.1)	7.7	0.5195	
3303	8 (22.2)	7.9	0.0077	3.32 (1.46–7.54)

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-A* types of which the allele frequencies in the Japanese population are more than 9% or which were detected in this study.
[a]General population control data are cited from Tanaka *et al.*[40]

Table 4b Association between *HLA-B* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

HLA-B allele	*Number of alleles detected (allele frequency)*		P	*Odds ratio (95% CI)*
	Case, n = *36 (%)*	*General population control* (n = *986*)[a] *(%)*		
0702	1 (2.8)	5.2	1.000	
1501	1 (2.8)	7.2	0.5076	
1518	1 (2.8)	0.9	0.3025	
1527	1 (2.8)	0	0.0352	
3501	2 (5.6)	8.6	0.7621	
3802	1 (2.8)	0.3	0.1338	
3901	2 (5.6)	4.0	0.6520	
4001	1 (2.8)	5.1	1.0000	
4002	2 (5.6)	8.2	0.7620	
4003	1 (2.8)	1.1	0.3512	
4006	2 (5.6)	5.3	0.7150	
4403	0 (0)	6.9	0.1648	
4601	2 (5.6)	3.8	0.6441	
4801	1 (2.8)	2.7	1.0000	
5101	4 (11.1)	7.9	0.5244	
5201	3 (8.3)	13.7	0.4624	
5401	0 (0)	6.5	0.1620	
5601	1 (2.8)	1.0	0.3273	
5801	10 (27.8)	0.6	5.388×10^{-12}	62.8 (21.2–185.8)

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-B* types of which the allele frequencies in the Japanese population are more than 6.5% or which were detected in this study.
[a]General population control data are cited from Tanaka *et al.*[40]

*LD of HLA-B*5801 with SNPs on chromosome 6*

We compared the genotypic distributions of six SNPs, which were significantly associated with SJS/TEN (Table 2), with *HLA* types because these SNPs are located near the *HLA-B* gene. These 6 SNPs listed in Table 3 represent 21 SNPs in Table 2 because the other 15 SNPs are in absolute LD with 1 of the 6 SNPs. Representative six variants of the significant SNPs on chromosome 6 were found in all of the SJS/TEN patients who carried the *HLA-B*5801* (10 patients) (Table 3). Therefore, in order to evaluate LD in the Japanese

Table 4c Association between *HLA-Cw* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

	Number of alleles detected (allele frequency)			
HLA-Cw allele	*Case, n = 36 (%)*	*General population control (n = 234)[a] (%)*	P	*Odds ratio (95% CI)*
0102	2 (5.6)	17.0	0.0859	
0302	10 (27.8)	0	5.303×10^{-10}	
0303	2 (5.6)	7.8	1.000	
0304	4 (11.1)	11.3	1.000	
0401	4 (11.1)	6.5	0.2961	
0702	4 (11.1)	11.3	1.000	
0801	3 (8.3)	10.9	0.7777	
0803	1 (2.8)	2.6	1.000	
1202	3 (8.3)	10.4	1.000	
1402	3 (8.3)	5.7	0.4559	
1403	0 (0)	12.2	0.0192	

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-Cw* types of which the allele frequencies in the Japanese population are more than 10% or which were detected in this study.
[a]General population control data are cited from Tokunaga *et al.*[41]

Table 5 The linkage disequilibrium between *HLA* types and representative single nucleotide polymorphisms on 6p21 of 206 Japanese individuals

HLA	*rs3099844*	*rs3131643*	*rs2734583*	*rs9267445*	*rs9263726*	*rs1634776*
A	0.821	0.621	0.835	0.798	0.847	0.803
B	0.973	0.873	1.000	1.000	1.000	0.996
Cw	0.984	0.773	1.000	1.000	1.000	0.909

Abbreviation: HLA, human leukocyte antigen.
Data are expressed in D'.

Table 6 The linkage disequilibrium between representative single nucleotide polymorphisms on 6p21 and *HLA-B*5801* of 206 Japanese individuals

SNP	*D'*	r^2
rs3099844	0.930	0.866
rs3131643	0.929	0.674
rs2734583	1.000	0.931
rs9267445	1.000	0.896
rs9263726	1.000	1.000
rs1634776	1.000	0.905

Abbreviation: SNP, single nucleotide polymorphism.

population, LD coefficients (D') were calculated between classical class 1 *HLA* types and six representative SNPs at 6p21, using the *HLA*-type and SNPs genotype data of 206 Japanese individuals, including 141 SJS/TEN cases and an additional 65 non-SJS/TEN Japanese subjects. As shown in Tables 5 and 6 representative SNPs on chromosome 6 showed LD for the *HLAs*. In particular, three SNPs (rs2734583, rs9267445 and rs9263726) showed a strong linkage with *HLA-B* and *Cw* alleles (Table 5). LD between six representative SNPs in 6p21 and *HLA-B*5801* are shown in Table 6. A novel observation was the absolute LD ($D' = 1$, $r^2 = 1$) between rs9263726 in *PSORS1C1* and the *HLA-B*5801* allele.

Discussion

In order to explore new genetic biomarkers associated with the occurrence of allopurinol-related SJS/TEN Japanese patients, we conducted a GWAS using 890321 SNPs from patients with allopurinol-related SJS/TEN and an ethnically matched control group. The GWAS data indicated that most SNPs significantly associated with allopurinol-related SJS/TEN are located on or close to genes that overlap the 6p21 region, especially the genes neighboring *HLA-B*. There was no significantly associated SNP in any other region of the genome (Figures 1 and 2 and Table 2), indicating that the 6p21 region has the most important role in the progress of allopurinol-related SJS/TEN. We expected to find SJS/TEN-associated SNPs, which are unrelated to *HLA-B*5801* from this GWAS study because the association of *HLA-B*5801* with SJS/TEN is incomplete (10/18) in Japanese patients in contrast to Han Chinese[7] and Thai patients.[8] However, most

of significant SNPs were closely linked with *HLA-B*5801* (Table 6). Previous studies have indicated that a SNP (rs2395029) in the *HCP5*, which is on 6p21.3, is strongly associated with human immunodeficiency virus-1 set points,[28–30] abacavir-induced hypersensitivity[24–26] and flucloxacillin-induced liver injury.[31] This SNP is in strong LD with *HLA-B*5701* in Caucasians.[25] Another SNP in 6p21 in *PSORS1C1*, a psoriasis-susceptibility candidate gene, was related with psoriasis in Swedish and Canadian populations[17,18] and exhibits LD with *HLA-Cw*0602* in Canadian populations.[18] These reports suggest that SNPs located in 6p21 link with a specific type of classical class I *HLA* that could be an alternative biomarker for the physiological phenomenon. Therefore, we examined the LD between these SNPs, shown in Table 2, and *HLA-B*5801*, which has been regarded as a genetic biomarker of SJS/TEN not only in Han Chinese,[7] but also in Caucasians[9] and Japanese.[10] We found that all of the Japanese patients with the allopurinol-related SJS/TEN who had the *HLA-B*5801* (10 patients) also had variant SNPs of genes that are located in 6p21, including *BAT1*, *HCP5*, *PPIAP9*, *PSORS1C1* and *HLA-B* (Table 3). The analysis of the LD coefficients between SNPs located in 6p21 and *HLA* types in the Japanese population indicated that these SNPs are in strong LD with *HLA* types (Table 5), and an absolute LD between rs9263726 in *PSORS1C1* and *HLA-B*5801* was observed in the Japanese population (Table 6). These results mean that all subjects (14 individuals including 10 with allopurinol-related SJS/TEN) who carry *HLA-B*5801* are in complete accord with all subjects with minor A allele of rs9263726 in the Japanese population. Therefore, rs9263726 in *PSORS1C1* is an alternative biomarker for *HLA-B*5801* in the Japanese population. Conventional genotyping of rs9263726 based on allelic discrimination offers several advantages over *HLA-B* typing, which is determined by genotyping of several SNPs forming the *HLA-B*5801* haplotype. Various broadly used technologies (for example, TaqMan genotyping) allow the standardized identification of two distinct alleles in one reaction tube, limiting the risk of contamination and allowing high-throughput genotyping with high sensitivity and specificity. In addition, the test is largely independent of both the performance of and interpretation by laboratory personnel. SNP genotyping is also less time consuming and cheaper than sequence-based *HLA* typing, and it does not require specialized laboratories. Therefore, the easy detection of these SNPs has a practical and economical advantage in clinical application for predicting the onset of allopurinol-related SJS/TEN. Although the previous report revealed that three SNPs in *HLA* region strongly associated with allopurinol-related SCAR in Han Chinese,[7] the two SNPs analyzed by the Illumina Human 1M-DUO BeadChip showed only weak association in the Japanese. This ethnic difference might be due to the difference of LD.

The functional analysis of genes that carry these SNPs—including *HCP5*, *BAT1*, *PSORS1C1*, *CCHCR1*, *TCF19* and *POU5F1*—in the pathogenesis of allopurinol-related SJS/TEN might be useful for determining their relevance. *CCHCR1* is a regulator of keratinocyte proliferation or differentiation and is overexpressed in keratinocytes in psoriatic lesions.[20–23] *TCF19* is a potential trans-activating factor that could play an important role in the transcription of genes required for the later stages of cell cycle progression.[27] Possible psoriasis candidate genes near *HLA-B* include *PSORS1C1*,[17–19] *CCHCR1*,[22,23] and *POU5F1*.[32,33] Mutations in *BAT1* may be associated with rheumatoid arthritis.[34–36] *HCP5* encodes an endogenous retroviral element mainly that is expressed in immune cells and there is evidence that the SNP in this gene is protective against human immunodeficiency virus-1 infection.[37–39] The functions and relevance of these genes suggest that the pathogenesis of allopurinol-related SJS/TEN might involve not only an immune system disorder, but also processes of cell proliferation and differentiation.

In conclusion, the results of this GWAS of allopurinol-related SJS/TEN in Japanese patients show that SNPs in genes located in 6p21, which are in LD with *HLA-B*5801*, are strongly associated with the cutaneous adverse reaction. Therefore, these SNPs, especially rs9263726, prove to be predictors for allopurinol-related SJS/TEN in Japanese, and their genes might be involved in the pathogenesis of allopurinol-related SJS/TEN. The OR of rs9263726 is extremely high from this case–control study and the typing cost of SNP is much cheaper than that of *HLA* typing. Moreover, the SJS/TEN has a very severe adverse reaction of allopurinol, which is high mortality. Therefore, we believe that the screening of rs9263726 genotype before allopurinol administration is necessary to prevent SJS/TEN in allopurinol-treated Japanese patients, although its allele frequency is very low in the Japanese. Association analyses of other ethnic populations are needed for confirming and comparing the results obtained in this study. *In vitro* functional studies of these genes are also necessary for identification of the physiological and molecular pathways leading to allopurinol-related SJS/TEN.

Conflict of interest

The authors declare no conflict of interest except one member of JPDSC, Mitsubishi Tanabe Pharma, which is a distributor of allopurinol in Japan.

Acknowledgments

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References

1 Wortmann RL. Gout and hyperuricemia. *Curr Opin Rheumatol* 2002; **14**: 281–286.
2 Chung WH, Hung SI, Chen YT. Human leukocyte antigens and drug hypersensitivity. *Curr Opin Allergy Clin Immunol* 2007; **7**: 317–323.
3 Tohkin M, Ishiguro A, Kaniwa N, Saito Y, Kurose K, Hasegawa R. Prediction of severe adverse drug reactions using pharmacogenetic biomarkers. *Drug Metab Pharmacokinet* 2010; **25**: 122–133.

4 Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch Dermatol* 1993; **129**: 92–96.
5 French LE. Toxic epidermal necrolysis and Stevens Johnson syndrome: our current understanding. *Allergol Int* 2006; **55**: 9–16.
6 Bowman C, Delrieu O. Immunogenetics of drug-induced skin blistering disorders. Part I: perspective. *Pharmacogenomics* 2009; **10**: 601–621.
7 Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP *et al.* HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci USA* 2005; **102**: 4134–4139.
8 Tassaneeyakul W, Jantararoungtong T, Chen P, Lin PY, Tiamkao S, Khunarkornsiri U *et al.* Strong association between HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. *Pharmacogenet Genomics* 2009; **19**: 704–709.
9 Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S *et al.* A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 2008; **18**: 99–107.
10 Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K *et al.* HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics* 2008; **9**: 1617–1622.
11 Wilke RA, Lin DW, Roden DM, Watkins PB, Flockhart D, Zineh I *et al.* Identifying genetic risk factors for serious adverse drug reactions: current progress and challenges. *Nat Rev Drug Discov* 2007; **6**: 904–916.
12 Nakamura Y. Pharmacogenomics and drug toxicity. *N Engl J Med* 2008; **359**: 856–858.
13 Daly AK, Day CP. Genetic association studies in drug-induced liver injury. *Semin Liver Dis* 2009; **29**: 400–411.
14 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
15 Zhao JH, Curtis D, Sham PC. Model-free analysis and permutation tests for allelic associations. *Hum Hered* 2000; **50**: 133–139.
16 Zhao JH. 2LD, GENECOUNTING and HAP: computer programs for linkage disequilibrium analysis. *Bioinformatics* 2004; **20**: 1325–1326.
17 Holm SJ, Carlen LM, Mallbris L, Stahle-Backdahl M, O'Brien KP. Polymorphisms in the SEEK1 and SPR1 genes on 6p21.3 associate with psoriasis in the Swedish population. *Exp Dermatol* 2003; **12**: 435–444.
18 Rahman P, Butt C, Siannis F, Farewell VT, Peddle L, Pellett FJ *et al.* Association of SEEK1 and psoriatic arthritis in two distinct Canadian populations. *Ann Rheum Dis* 2005; **64**: 1370–1372.
19 Zhang XJ, He PP, Wang ZX, Zhang J, Li YB, Wang HY *et al.* Evidence for a major psoriasis susceptibility locus at 6p21(PSORS1) and a novel candidate region at 4q31 by genome-wide scan in Chinese hans. *J Invest Dermatol* 2002; **119**: 1361–1366.
20 Suomela S, Elomaa O, Skoog T, Ala-aho R, Jeskanen L, Parssinen J *et al.* CCHCR1 is up-regulated in skin cancer and associated with EGFR expression. *PLoS One* 2009; **4**: e6030.
21 Tiala I, Wakkinen J, Suomela S, Puolakkainen P, Tammi R, Forsberg S *et al.* The PSORS1 locus gene CCHCR1 affects keratinocyte proliferation in transgenic mice. *Hum Mol Genet* 2008; **17**: 1043–1051.
22 Suomela S, Kainu K, Onkamo P, Tiala I, Himberg J, Koskinen L *et al.* Clinical associations of the risk alleles of HLA-Cw6 and CCHCR1*WWCC in psoriasis. *Acta Derm Venereol* 2007; **87**: 127–134.
23 Tiala I, Suomela S, Huuhtanen J, Wakkinen J, Holtta-Vuori M, Kainu K *et al.* The CCHCR1 (HCR) gene is relevant for skin steroidogenesis and downregulated in cultured psoriatic keratinocytes. *J Mol Med* 2007; **85**: 589–601.
24 Hughes AR, Mosteller M, Bansal AT, Davies K, Haneline SA, Lai EH *et al.* Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics* 2004; **5**: 203–211.
25 Colombo S, Rauch A, Rotger M, Fellay J, Martinez R, Fux C *et al.* The HCP5 single-nucleotide polymorphism: a simple screening tool for prediction of hypersensitivity reaction to abacavir. *J Infect Dis* 2008; **198**: 864–867.
26 Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J *et al.* HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008; **358**: 568–579.
27 Teraoka Y, Naruse TK, Oka A, Matsuzawa Y, Shiina T, Iizuka M *et al.* Genetic polymorphisms in the cell growth regulated gene, SC1 telomeric of the HLA-C gene and lack of association of psoriasis vulgaris. *Tissue Antigens* 2000; **55**: 206–211.
28 Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L *et al.* HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci USA* 2000; **97**: 2709–2714.
29 Altfeld M, Addo MM, Rosenberg ES, Hecht FM, Lee PK, Vogel M *et al.* Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *AIDS* 2003; **17**: 2581–2591.
30 Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M *et al.* A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007; **317**: 944–947.
31 Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A *et al.* HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* 2009; **41**: 816–819.
32 Oka A, Tamiya G, Tomizawa M, Ota M, Katsuyama Y, Makino S *et al.* Association analysis using refined microsatellite markers localizes a susceptibility locus for psoriasis vulgaris within a 111 kb segment telomeric to the HLA-C gene. *Hum Mol Genet* 1999; **8**: 2165–2170.
33 Chang YT, Hsu CY, Chou CT, Lin MW, Shiao YM, Tsai CY *et al.* The genetic polymorphisms of POU5F1 gene are associated with psoriasis vulgaris in Chinese. *J Dermatol Sci* 2007; **46**: 153–156.
34 Okamoto K, Makino S, Yoshikawa Y, Takaki A, Nagatsuka Y, Ota M *et al.* Identification of I kappa BL as the second major histocompatibility complex-linked susceptibility locus for rheumatoid arthritis. *Am J Hum Genet* 2003; **72**: 303–312.
35 Kilding R, Iles MM, Timms JM, Worthington J, Wilson AG. Additional genetic susceptibility for rheumatoid arthritis telomeric of the DRB1 locus. *Arthritis Rheum* 2004; **50**: 763–769.
36 Quinones-Lombrana A, Lopez-Soto A, Ballina-Garcia FJ, Alperi-Lopez M, Queiro-Silva R, Lopez-Vazquez A *et al.* BAT1 promoter polymorphism is associated with rheumatoid arthritis susceptibility. *J Rheumatol* 2008; **35**: 741–744.
37 van Manen D, Kootstra NA, Boeser-Nunnink B, Handulle MA, van't Wout AB, Schuitemaker H. Association of HLA-C and HCP5 gene regions with the clinical course of HIV-1 infection. *AIDS* 2009; **23**: 19–28.
38 Catano G, Kulkarni H, He W, Marconi VC, Agan BK, Landrum M *et al.* HIV-1 disease-influencing effects associated with ZNRD1, HCP5 and HLA-C alleles are attributable mainly to either HLA-A10 or HLA-B*57 alleles. *PLoS One* 2008; **3**: e3636.
39 Han Y, Lai J, Barditch-Crovo P, Gallant JE, Williams TM, Siliciano RF *et al.* The role of protective HCP5 and HLA-C associated polymorphisms in the control of HIV-1 replication in a subset of elite suppressors. *AIDS* 2008; **22**: 541–544.
40 Tanaka H, Akaza T, Juji T. Report of the Japanese Central Bone Marrow Data Center. *Clin Transpl* 1996; **9**: 139–144.
41 Tokunaga K, Ishikawa Y, Ogawa A, Wang H, Mitsunaga S, Moriyama S *et al.* Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. *Immunogenetics* 1997; **46**: 199–205.

Appendix

Japan Pharmacogenomics Data Science Consortium (JPDSC)

The Japan Pharmacogenomics Data Science Consortium is composed of Astellas Pharma, Otsuka Pharmaceutical, Daiichi Sankyo, Taisho Pharmaceutical, Takeda Pharmaceutical and Mitsubishi Tanabe Pharma, and is chaired by Ichiro Nakaoka (Takeda Pharmaceutical).

Visual Improvement after Cultivated Oral Mucosal Epithelial Transplantation

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Purpose: To report the effectiveness, disease-specific outcomes, and safety of cultivated oral mucosal epithelial sheet transplantation (COMET), with the primary objective of visual improvement.

Design: Noncomparative, retrospective, interventional case series.

Participants: This study involved 46 eyes in 40 patients with complete limbal stem cell deficiency (LSCD) who underwent COMET for visual improvement. These LSCD disorders fell into the following 4 categories: Stevens-Johnson syndrome (SJS; 21 eyes), ocular cicatricial pemphigoid (OCP; 10 eyes), thermal or chemical injury (7 eyes), or other diseases (8 eyes).

Methods: Best-corrected visual acuity (BCVA) and ocular surface grading score were examined before surgery; at the 4th, 12th, and 24th postoperative week; and at the last follow-up. Data on COMET-related adverse events and postoperative management were collected. The outcomes in each disease category were evaluated separately.

Main Outcome Measures: The primary outcome was the change in median logarithm of the minimum angle of resolution (logMAR) BCVA at the 24th postoperative week. The secondary outcome was the ocular surface grading score.

Results: Median logMAR BCVA at baseline was 2.40 (range, 1.10 to 3.00). In SJS, logMAR BCVA improved significantly during the 24 weeks after surgery. In contrast, the BCVA in OCP was improved significantly only at the 4th postoperative week. In 6 of the 7 thermal or chemical injury cases, logMAR BCVA improved after planned penetrating keratoplasty or deep lamellar keratoplasty. Grading scores of ocular surface abnormalities improved in all categories. Of 31 patients with vision loss (logMAR BCVA, >2) at baseline, COMET produced improvement (logMAR BCVA, ≤2) in 15 patients (48%). Visual improvement was maintained with long-term follow-up (median, 28.7 months). Multivariate stepwise logistic regression analysis showed that corneal neovascularization and symblepharon were correlated significantly with logMAR BCVA improvement at the 24th postoperative week ($P = 0.0023$ and $P = 0.0173$, respectively). Although postoperative persistent epithelial defects and slight to moderate corneal infection occurred in the eyes of 16 and 2 patients, respectively, all were treated successfully with no eye perforation.

Conclusions: Long-term visual improvement was achievable in cases of complete LSCD. Cultivated oral mucosal epithelial sheet transplantation offered substantial visual improvement even for patients with end-stage severe ocular surface disorders accompanying severe tear deficiency. Patients with corneal blindness such as SJS benefited from critical improvement of visual acuity.

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Corneal renewal and repair are mediated by corneal epithelial stem cells situated mainly in the limbus, the narrow region between the cornea and the bulbar conjunctiva.[1] Damage or depletion of the corneal epithelial stem cells, known as limbal stem cell deficiency (LSCD), leads to conjunctival invasion that results in vascularization and scarring of the cornea with an associated profound loss of vision.[1] Limbal stem cell deficiency can be caused by Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), and thermal or chemical injury, which are all characterized by the loss of corneal epithelial stem cells. Such LSCD may cause severe ocular surface diseases (OSDs) in which cicatrization resulting from conjunctival fibrosis, symblepharon, and severe dry eye greatly disrupt visual function and can progress gradually with chronic inflammation.[2–4] To date, few effective medical or surgical treatments for severe OSDs have been available.[5–15]

Since 1998, the authors have used amniotic membrane transplantation to treat severe OSDs. Amniotic membrane exhibits an anti-inflammatory effect and also acts as a substrate for epithelialization.[16] The results of previous studies have shown that amniotic membrane transplantation alone[17,18] or amniotic membrane transplantation combined with limbal transplantation[6,19,20] promoted epithelialization, reduced pain, reconstructed the fornix, and minimized inflammation of the ocular surface to a remarkable degree in

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patients with severe OSDs. Based on these promising results, novel methods have been developed for the cultivation of allogeneic corneal[7,8,21] or autologous oral mucosal[22–25] epithelial cells on a denuded amniotic membrane. Immunologic rejection and increased risk of infection or systemic adverse effects associated with the long-term immunosuppressive therapy accompanying allograft transplantation[6] encouraged changing to autologous cultivated oral mucosal epithelial transplantation (COMET) in patients with severe OSDs in 2002.[10,11,23,26]

To clarify the effectiveness, disease-specific outcomes, and safety of COMET, all of the clinical data from all 72 patients that the authors treated with COMET since 2002 were analyzed. The objective of this present study was to summarize the long-term clinical outcomes of 40 of those 72 patients who underwent COMET with the primary objective of visual improvement between June 2002 and December 2008.

Patients and Methods

Patients

Autologous COMET was performed on consecutive patients who were diagnosed with total LSCD based on the complete disappearance of the palisades of Vogt and 360° of conjunctivalization.[1] The COMET treatment protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine, Kyoto, Japan, in 2002. The final decision to perform COMET was made by the university's team of corneal specialists. Before the surgery, written informed consent was obtained from all patients in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. The current retrospective study used an itemized data collection form, and the medical records of all patients who underwent COMET between June 2002 and December 2008 were examined retrospectively. This retrospective study protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine in 2009. In this study, 40 of the 72 patients who underwent COMET were analyzed with the primary objective of visual improvement.

Cell Culture

All of the COMET sheets were prepared at the good manufacturing practices–graded Cell Processing Center at Kyoto Prefectural University of Medicine as previously described.[23,26] Autologous oral mucosal epithelial cells were obtained from a 6-mm–diameter biopsy specimen obtained from each patient's buccal mucosa, and the cells then were cultured on an amniotic membrane spread on the bottom of a culture insert and were cocultured with mitomycin C-inactivated 3T3 fibroblasts (NIH-3T34; RIKEN Cell Bank, Tsukuba, Japan). The cultured cells were submerged in medium for approximately 1 week and then were exposed to air by lowering the medium level (airlifting) for 1 to 2 days. All amniotic membrane was obtained from caesarean sections according to the preparation method described previously.[23] Although fetal bovine serum initially was used as a culture medium, autologous serum was used in later cultures to reduce the risk of transmitting nonhuman pathogens.[26]

Transplantation and Postoperative Management

The surgical procedure (see the Supplemental Video, available at http://aaojournal.org) and postoperative management have been described previously.[24,25] In patients with severe symblepharon or a large area of bare sclera exposed during surgery, amniotic membrane was transplanted onto the bare sclera to reconstruct conjunctival fornices.[18] In patients with a cataract, phacoemulsification and aspiration plus intraocular lens implantation were performed simultaneously with COMET. No penetrating keratoplasty or deep lamellar keratoplasty was performed simultaneously with COMET. For patients with severe corneal stromal opacity, a 2-step surgical approach was planned, with the first step being COMET and the second step being either penetrating or deep lamellar keratoplasty.[25]

Systemic corticosteroid (betamethasone, 1 mg/day) and cyclosporine (2 to 3 mg/kg daily) were administered to prevent postoperative inflammation and immunologic response and then were tapered, depending on the clinical findings. Dexamethasone (0.1%) and antibiotic eye drops were instilled 4 times daily. Dry-eye patients were administered artificial tears. A therapeutic soft contact lens was used for at least 1 month to protect transplanted epithelium from mechanical ablation.

Postoperative Follow-up and Outcomes

Best-corrected visual acuity (BCVA) was converted to the logarithm of the minimum angle of resolution (logMAR). Ocular surface conditions including corneal appearance (epithelial defects, clinical conjunctivalization, neovascularization, opacification, keratinization, and symblepharon) were graded by at least 2 ophthalmologists (C.S., T.I., and T.N.) on a scale from 0 to 3 according to their severity, in accordance with a previously reported grading system.[27] Severe OSDs are characterized by an associated loss of conjunctival stem cells, and the severity of conjunctival involvement affects the visual prognosis. Therefore, findings on upper and lower fornix shortening were added to evaluate the grade of conjunctival appearance. Fornix shortening was graded from 0 to 3 based on the following clinical features: normal depth (grade 0), shortened by less than one quarter (grade 1), shortened by one quarter to one half (grade 2), and shortened by more than one half (grade 3). Upper and lower fornix shortenings were graded separately. The sum of each grading score was defined as the ocular surface grading score (maximum, 24).

Each patients logMAR BCVA, ocular surface grading score, and data on adverse events related to COMET or postoperative management were collected from the medical records at these specific time points: before surgery; at the 4th, 12th, and 24th postoperative weeks; and at the last follow-up examination. The primary outcome was the change in logMAR BCVA at the 24th postoperative week. Because other ocular diseases can affect this visual outcome, a secondary outcome, the ocular surface grading score, also was defined.

Statistical Analysis

The change in BCVA and ocular surface grading score from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except for other diseases. Multivariate stepwise logistic regression analysis was used to determine the factors influencing visual improvement.

This study defined the critical visual improvement rate as the proportion of patients in whom BCVA at the 24th postoperative week had improved to at least 0.01, as a percentage of the patients with a BCVA of less than 0.01 at baseline. Patients with a visual acuity of 0.01 or more can read and walk using vision aids. Thus, an improvement to at least 0.01 indicates a capacity for independence in daily life. If data were missing from the 24th postoperative week, data from follow-up at the last visit were substituted.