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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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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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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 1 163 218 SNPs. SNPs were discarded from case–control association analysis if they exhibited a minor allele frequency <0.001 in the control group (237 890 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 272 897 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]

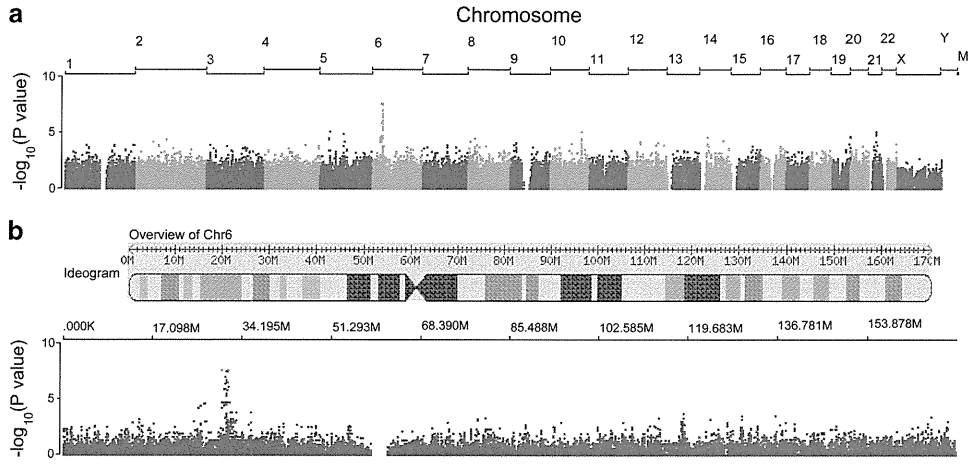

Figure 1 Genome-wide association study of allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis. Each dot represents a single nucleotide polymorphism (SNP). The *x* axis: the position of the SNP on chromosomes. The *y* axis: the $-\log_{10}$ of Fisher's exact test *P*-values (dominant 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.

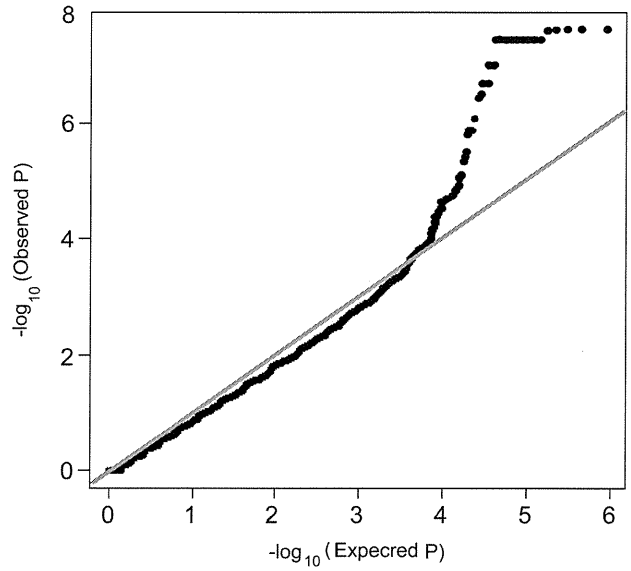

Figure 2 Quantile–quantile plot of Fisher's exact test statistics obtained from the genome-wide association study for allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis under dominant genotype mode. The solid red line represents the null model where observed Fisher's exact test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

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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).

The Relation Between Visual Performance and Clinical Ocular Manifestations in Stevens-Johnson Syndrome

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• PURPOSE: To investigate the relation between visual function, clinical findings, and visual symptoms in Stevens-Johnson syndrome (SJS) and to compare the results with Sjögren syndrome (SS) patients and normal subjects.

• DESIGN: Cross-sectional comparative study.

• METHODS: One hundred fifteen eyes of 59 consecutive patients with SJS and toxic epidermal necrolysis (TEN), 208 eyes of 104 healthy normal subjects, and 132 eyes of 66 SS patients were investigated in this multicenter study. All study subjects underwent tear function and ocular surface examinations, Landolt and functional visual acuity examinations, and the Japanese version of the NEI VFQ-25 (National Eye Institute Visual Function Questionnaire).

• RESULTS: The mean ocular surface grading scores were significantly higher and the mean score of all 12 NEI VFQ subscales was significantly lower in the SJS patients compared to the SS patients and the normal subjects ($P < .05$). The conventional and functional logarithm of minimal angle of resolution (logMAR) visual acuities in SJS patients with minimal corneal complications were significantly higher and the mean total composite NEI VFQ scores were lower compared to SS patients. The conventional and functional logMAR visual acuities and the mean ocular surface grading scores in SJS with aqueous deficiency were significantly higher and the mean total composite NEI VFQ scores were lower compared to SS patients. Strong correlations between best-corrected logMAR functional visual acuities and either ocular surface grading scores or the composite NEI VFQ-25 scores were observed.

• CONCLUSIONS: The functional visual acuity examination reflects the severity of clinical ocular surface findings and vision-related quality of life more than the standard conventional visual acuity in SJS. (Am J Ophthalmol 2012;154:499–511. © 2012 by Elsevier Inc. All rights reserved.)

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STEVENS-JOHNSON SYNDROME (SJS) IS AN ACUTE, self-limiting disease of the skin and mucous membranes associated with symblepharon, adhesive occlusion of the lacrimal puncta, and corneal opacification with conjunctivalization and severe dry eyes leading to worsening of the ocular surface health and poor quality of vision.[1–8]

Previous reports have demonstrated that contrast sensitivity, contrast visual acuity, glare disability, and wavefront aberrations are useful to detect quality of vision in everyday life.[9–19] Visual function assessment using these measurement methods has been reported to be useful in keratorefractive surgery, mild cataract, and dry eye diseases.[9–19] To the best of our knowledge, however, there are no reports about visual function assessment in patients with SJS except a previously published report by us.[8]

It has been our experience that SJS patients with good visual acuity and mild ocular surface morbidity may still complain of similar severe eye irritation and visual complaints as patients with Sjögren syndrome (SS). However, the differences in visual symptoms and conventional and dynamic visual acuity between these 2 entities have not been quantified and compared so far. In an attempt to investigate the visual function and ocular surface differences between SJS and SS, we performed this multicenter cross-sectional study, using a previously reported ocular surface morbidity severity questionnaire and functional visual acuity measurement.[20–24]

METHODS

• SUBJECTS: One hundred fifteen eyes of 59 consecutive patients (28 male, 31 female; mean age: 47.5 ± 16.0 years; range: 14–79 years) with SJS, including its more severe variant, toxic epidermal necrolysis (TEN), seen at the Cornea Subspecialty Outpatient Clinic of the Departments of Ophthalmology of Keio University, Tokyo Dental College, Tokyo Medical Center, Kyoto Prefectural University of Medicine, Hokkaido University, Ehime University, and Yamaguchi University were studied in this cross-sectional multicenter study. Clinicians participating

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TABLE 1. Clinical Severity Grading Criteria of the Ocular Surface Findings

	Grade 0	Grade 1	Grade 2	Grade 3	Comments
Assessment of Corneal Complications					
SPK	A1D1	A1D2, A2D1	A1D3, A2D2, A3D1	A2D3, A3D2, A3D3	Using fluorescein staining based on the area and density of the lesions as described by Miyata and associates[29]
Corneal epithelial defect	Absent	Less than 1/4 of the corneal surface	1/4 to 1/2 of the corneal surface	More than 1/2 of the corneal surface	
Conjunctivalization	Absent	Less than 1/4 of the corneal surface	1/4 to 1/2 of the corneal surface	More than 1/2 of the corneal surface	
Neovascularization	Absent	Confined to the corneal periphery	Extending beyond the pupil margin	Extending beyond the pupil margin into the central cornea	In eyes where significant opacification or extensive symblepharon formation made it difficult to evaluate corneal neovascularization, a grade of 3 was assigned
Corneal opacification	Clear cornea with easily visible iris details	Partial obscuration of the iris details	Iris details poorly seen with barely visible pupil margins	Complete obscuration of iris and pupil details	
Keratinization	Absent	Less than 1/4 of the corneal surface	1/4 to 1/2 of the corneal surface	More than 1/2 of the corneal surface	
Assessment of Conjunctival Complications					
Conjunctival hyperemia	Absent	Mild or sectoral engorgement of the conjunctival vessels	Moderate or diffuse engorgement of the conjunctival vessels	Severe or significant engorgement of the conjunctival vessels	
Symblepharon	Absent	Involving only the conjunctival surface	Less than 1/2 of the corneal surface	More than 1/2 of the corneal surface	
Assessment of Eyelid Complications					
Trichiasis	Absent	Less than 1/4 of the lid margin	1/4 to 1/2 of the lid margin	More than 1/2 of the lid margin	

Continued on next page

TABLE 1. Clinical Severity Grading Criteria of the Ocular Surface Findings *(Continued)*

MJ involvement	Normal MJ	Mild irregularity of MJ	Moderate irregularity of MJ	Severe irregularity of MJ	Bron grading was employed for the classification of MJ changes.[30]
					Fluorescein staining of the conjunctiva was performed for evaluating the MJ involvement.
					In eyes where significant keratinization of the lid margin or extensive symblepharon formation made it difficult to evaluate mucocutaneous junction involvement, Grade 3 was assigned.
MG involvement	Oily expressible secretion	Expressible yellowish-white oily secretion	Expressible thick cheesy material	Inability to express any secretion	The severity was determined clinically by the nature of the meibomian gland secretion expressed manually at the center of the upper lid.
Punctal involvement	Normal	Iatrogenic punctal occlusion	Either superior or inferior punctal occlusion	Both superior and inferior punctal occlusion	

A = area; D = density; MG = mucocutaneous gland; MJ = mucocutaneous junction; SPK = superficial punctuate keratopathy.

in the study received training to standardize the conduct of examinations performed at each center. The conduct of examinations was checked by trained coordinators at each center for consistency of the examination procedures. The diagnosis of SJS or TEN was based 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 chronic ocular surface complications such as symblepharon, entropion, trichiasis, xerophthalmia, and/or corneal vascularization.[1,3–5] Two hundred eight eyes of 104 healthy normal subjects (30 male, 74 female; mean age: 36.2 ± 12.0 years; range: 20–72 years) without dry eye disease and 132 eyes of 66 SS patients (66 female; mean age: 62.8 ± 11.1 years; range: 28–82 years) who were diagnosed according to Fox criteria were also investigated in this multicenter study.[25] Patients or control subjects with other systemic or ocular diseases, history of ocular surgery within 6 months, history of ocular cicatricial pemphigoid, or chemical, thermal, or radiation injury that would have adverse ocular surface effects were excluded according to the study exclusion criteria. SJS patients with a baseline best-corrected Landolt conventional visual acuity of less than 20/2000 attributable to cataract in both eyes, ocular surface keratinization, glaucoma, or posterior segment disease were excluded from this study, since the functional visual acuity measurement system cannot assess functional visual acuity at such low visual acuity levels.

• **SLIT-LAMP EXAMINATIONS:** All study subjects underwent slit-lamp examinations observing 12 components of 3 categories of ocular complications, such as corneal complications consisting of superficial punctuate keratopathy (SPK), epithelial defect, conjunctivalization, neovascularization, opacification, and keratinization; conjunctival complications consisting of hyperemia and symblepharon formation; and eyelid complications consisting of trichiasis, mucocutaneous junction involvement, meibomian gland involvement, and punctal damage. Each component was graded on a scale from 0 to 3, depending on the severity of involvement.[26]

The severity gradings and ocular surface tests were performed under the same single protocol by the researchers of all contributing study centers. Table 1 shows the clinical severity grading criteria of the ocular surface findings.

• **TEAR FUNCTION AND OCULAR SURFACE EXAMINATIONS:** The standard Schirmer test without topical anesthesia was performed as previously reported.[7] A vital staining severity grading was also assigned. A 2-μL volume of 1% fluorescein dye was instilled in the conjunctival sac by a micropipette. The minimum score for corneal fluorescein staining was 0 points and the maximum score was 9 points.[27]

• **STANDARD VISUAL ACUITY MEASUREMENTS:** Standard visual acuity testing using Landolt charts placed 5 m away from subjects was performed. Landolt visual acuity

TABLE 2. Standard Visual Acuity and Visual Parameters Assessed by Functional Visual Acuity Measurement System in Eyes of Patients With Sjögren Syndrome, Stevens-Johnson Syndrome Patients, and Healthy Normal Subjects

	SJS	SS	Normal
Conventional visual acuity			
logMAR	0.76 ± 0.76	−0.004 ± 0.13	−0.10 ± 0.10
Decimal	0.17	1.01	1.26
Functional visual acuity			
logMAR	0.98 ± 0.62[a]	0.28 ± 0.27[a]	−0.008 ± 0.13
Decimal	0.10	0.52	1.02
Maximal visual acuity			
logMAR	0.83 ± 0.65	0.10 ± 0.26	−0.15 ± 0.12
Decimal	0.15	0.79	1.41
Minimal visual acuity			
logMAR	1.19 ± 0.60	0.53 ± 0.36	0.17 ± 0.19
Decimal	0.06	0.30	0.68
Visual maintenance ratio	0.86 ± 0.12	0.91 ± 0.07[b]	0.98 ± 0.05[c,d]
Reaction time	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2
Blink number	11.2 ± 9.3	17.2 ± 9.6[b]	16.4 ± 8.7[c]

logMAR = logarithm of minimal angle of resolution; logMAR = logarithm of minimal angle of resolution; SJS = Stevens-Johnson syndrome; SS = Sjögren syndrome; VA = visual acuity.

[a] $P < .05$ between conventinal VA and functional VA.

[b] $P < .05$ between groups of SJS and SS.

[c] $P < .05$ between groups of SJS and Normal.

[d] $P < .05$ between groups of SS and Normal.

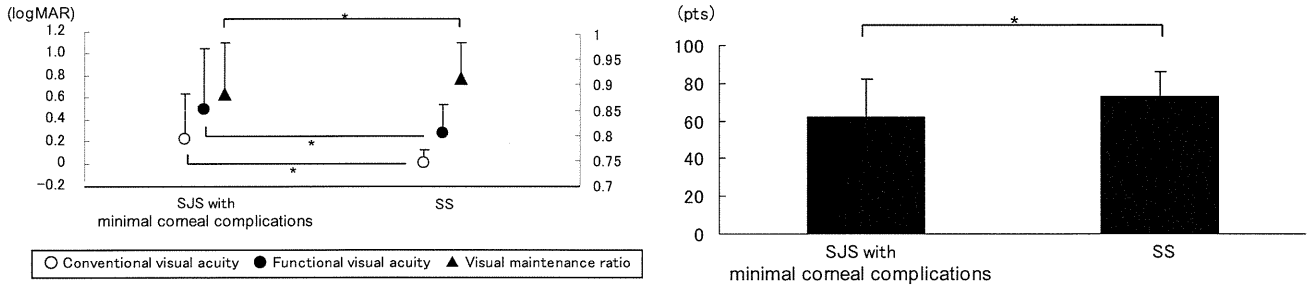

FIGURE 1. Visual function and Visual Function Questionnaire-25 in Stevens-Johnson syndrome (SJS) with minimal corneal complications and Sjögren syndrome (SS) patients. (Left) Conventional and functional visual acuity and visual maintenance ratio in Stevens-Johnson syndrome with minimal corneal complications and Sjögren syndrome patients. (Right) Total composite NEI VFQ-25 scores in Stevens-Johnson syndrome with minimal corneal complications and Sjögren syndrome patients. logMAR = logarithm of minimal angle of resolution.

was employed instead of Snellen chart since it is a standard test in Japan, and because the optotypes in Landolt and functional visual acuity testing are similar.

• FUNCTIONAL VISUAL ACUITY MEASUREMENTS: Continuous visual acuity testing during a 60-second period under natural blinking was performed as previously reported.[24]

• FUNCTIONAL VISUAL ACUITY INDICES: Briefly, the outcome parameters of the functional visual acuity measurement system were functional visual acuity (defined as the average of visual acuities measured during a 60-second testing), visual maintenance ratio (defined as the ratio of logMAR values of the functional visual acuities over the time frame for testing divided by the logarithm of minimal angle of resolution (logMAR) baseline visual acuity),[7] maximal corrected visual acuity, minimal corrected visual acuity, standard deviation of functional visual acuity, mean reaction time (defined as the mean of the response time taken by a subject to respond to an optotype), and blink numbers during a 60-second functional visual acuity test.

• VISUAL FUNCTION QUESTIONNAIRE-25: We used the Japanese version of the NEI VFQ-25 (National Eye Insti-

tute Visual Function Questionnaire 25) to evaluate the vision-related quality of life.[28] NEI VFQ-25 measures the following 12 vision-targeted subscales: general health, general vision, ocular pain, near activities, distant activities, social functioning, mental health, role difficulties, dependency, driving, color vision, and peripheral vision. A scale of 0 to 100 points is used for subscale scores. A score of 100 indicates the best possible score, while 0 indicates the worst possible score.

• STATISTICAL ANALYSIS: A 1-way ANOVA was performed for the comparison of conventional visual acuities, functional visual acuities, visual maintenance ratios, ocular surface grading scores, and VFQ-25 scores among SJS patients, SS patients, and normal control subjects. The Bonferroni test was used for further multiple comparisons. A paired t test was performed for the comparison between conventional and functional visual acuities in SJS patients, SS patients, and normal control subjects alone. To investigate whether the visual disturbance or quality of life are similarly affected in SJS patients compared to SS patients, conventional visual acuities, functional visual acuities, visual maintenance ratios, and VFQ-25 scores were compared among SJS patients with minimal corneal complications and SS patients by paired t test. Minimal corneal complication was defined as a grading score ≤4 points, in relation to keratinization, conjunctivalization, opacification, corneal epithelial defect, neovascularization, and SPK. Severe corneal complication was defined as a grading score >4 points. To investigate the effect of tear functions on the ocular surface complications, visual disturbance, or quality of life in SJS and SS patients, ocular surface grading scores, conventional visual acuities, functional visual acuities, visual maintenance ratios, and VFQ-25 scores were compared in SJS patients with and without aqueous tear deficiency by 1-way ANOVA. Aqueous tear deficiency was defined as a Schirmer test score ≤5 mm. The relation between ocular surface grading scores, conventional visual acuities, and functional visual acuities was analyzed by Pearson correlation analysis. The relation between ocular surface complications and conventional visual acuities, functional visual acuities, visual maintenance ratios, or VFQ-25 scores was also analyzed by Pearson correlation analysis in SJS patients with and without aqueous tear deficiency and SS patients. In the correlation analysis between ocular surface grading scores and conventional visual acuities or functional visual acuities in SJS patients, eyes were divided into 3 visual groups: good conventional visual acuity group (logMAR conventional visual acuity score ≤0), intermediate conventional visual acuity group (0 < logMAR conventional visual acuity ≤ 0.3), and poor conventional visual acuity group (0.3 < logMAR conventional visual acuity score ≤ 2.0). The relation between VFQ-25 score, conventional visual acuity, and functional visual acuity was analyzed by the same methodology, using the eye with better conventional visual acuity. The correlation between clinical findings, conventional visual acuities, and functional visual acuities was also investigated by multiple linear regression analysis. A probability level of $P < .05$ was considered statistically significant. SPSS (SPSS Inc, Chicago, Illinois, USA) was used as the statistical analysis software.

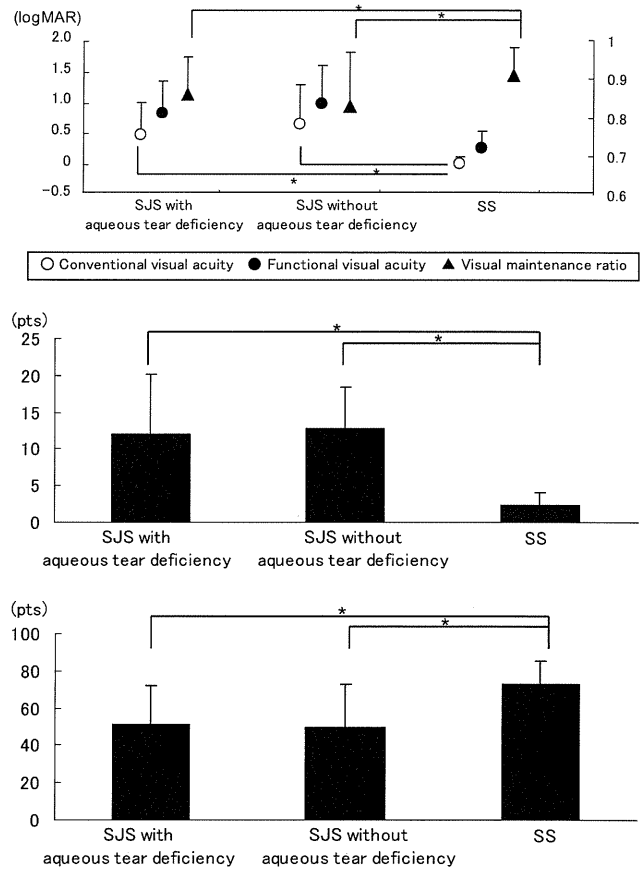

FIGURE 2. Visual function, ocular surface grading score, and Visual Function Questionnaire-25 in Stevens-Johnson syndrome (SJS) patients with and without aqueous tear deficiency and Sjögren syndrome (SS) patients. (Top) Conventional and functional visual acuity and visual maintenance ratio in Stevens-Johnson syndrome patients with and without aqueous tear deficiency and Sjögren syndrome patients. (Middle) Total ocular surface grading scores in Stevens-Johnson syndrome patients with and without aqueous tear deficiency and Sjögren syndrome patients. (Bottom) Total composite NEI VFQ-25 scores in Stevens-Johnson syndrome patients with minimal corneal complications and Sjögren syndrome patients. logMAR = logarithm of minimal angle of resolution.

RESULTS

• TEAR FUNCTION TESTS: The mean Schirmer test values were 9.1 ± 9.3 mm in SJS patients, 4.6 ± 4.5 mm in SS patients, and 18.6 ± 9.5 mm in healthy control subjects, respectively. The Schirmer test values were significantly higher in SJS patients compared to SS patients

TABLE 3. Percentages of Ocular Surface Grading Score in Sjögren Syndrome Patients, Stevens-Johnson Syndrome Patients, and Healthy Normal Subjects

	SJS				SS				Normal			
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 0	Grade 1	Grade 2	Grade 3	Grade 0	Grade 1	Grade 2	Grade 3
Assessment of corneal complications												
SPK	15.1%	25.9%	25.2%	33.8%	31.1%	25.6%	20.2%	23.3%	99.0%	1.0%	0	0
Corneal epithelial defect	92.2%	2.8%	0	5.0%	100%	0	0	0	100%	0	0	0
Conjunctivalization	32.1%	14.3%	12.9%	40.7%	95.4%	4.6%	0	0	100%	0	0	0
Neovascularizarion	25.7%	27.1%	24.3%	22.9%	97.7%	2.3%	0	0	98.1%	1.9%	0	0
Opacification	28.4%	50.4%	13.5%	7.8%	99.2%	0.8%	0	0		0	0	0
Keratinization	88.7%	4.3%	3.4%	3.4%	100%	0	0	0	100%	0	0	0
Assessment of conjunctival complications												
Conjunctival hyperemia	24.1%	58.9%	14.9%	2.1%	82.3%	16.9%	0.8%	0	100%	0	0	0
Symblepharon	38.8%	48.2%	7.9%	5.0%	99.2%	0.8%	0	0	100%	0	0	0
Assessment of eyelid complications												
Trichiasis	40.3%	25.2%	14.4%	20.1%	100%	0	0	0	99.0%	1.0%	0	0
MJ involvement	15.6%	42.6%	27.0%	14.9%	86.2%	12.3%	1.5%	0	100%	0	0	0
MG involvement	17.0%	22.0%	17.7%	43.3%	81.5%	14.6%	0	3.8%	100%	0	0	0
Punctal involvement	27.7%	19.9%	9.9%	42.6%	75.4%	23.1%	0.8%	0.8%	100%	0	0	0

MG = meibomian gland; MJ = mucocutaneous junction; SJS = Stevens-Johnson syndrome; SPK = superficial punctuate keratopathy; SS = Sjögren syndrome.

($P < .05$). A total of 49.6 % of the patients with SJS had Schirmer test values greater than 5 mm.

• STANDARD CONVENTIONAL VISUAL ACUITY: Table 2 shows the mean logMAR conventional visual acuity in SJS and SS patients and the normal subjects. The mean logMAR conventional visual acuity in SJS patients was significantly lower compared to the mean logMAR conventional visual acuity in SS patients and normal controls ($P < .05$).

The mean logMAR conventional visual acuity in SJS patients with severe corneal complications was 0.74 ± 0.57. The mean logMAR conventional visual acuity in SJS patients with minimal corneal complications and SS patients was 0.21 ± 0.42 and −0.001 ± 0.12, respectively. The logMAR conventional visual acuities in SJS patients were significantly higher compared to SS patients (Figure 1, Left).

The mean logMAR conventional visual acuity in SJS patients with and without aqueous tear deficiency and SS patients was 0.47 ± 0.53, 0.65 ± 0.63, and −0.004 ± 0.13, respectively. The logMAR conventional visual acuities in SJS patients were significantly higher compared to SS patients (Figure 2, Top).

• FUNCTIONAL VISUAL ACUITY INDICES: Table 2 shows the results of all indices measured by the functional visual acuity measurement system. The mean logMAR functional visual acuity was significantly lower compared to the mean logMAR conventional visual acuity in patients with SJS and SS ($P < .05$). The mean logMAR standard deviation of functional visual acuity was significantly greater in patients with SJS and SS compared to normal subjects ($P < .05$). The mean visual maintenance ratio in the SJS patients was significantly lower than in SS patients, and the mean visual maintenance ratio in SS patients was significantly lower than in normal subjects ($P < .05$). There were no significant differences in reaction times among SJS patients, SS patients, and normal subjects. The mean blink number in the SJS patients was significantly lower compared to SS patients and normal subjects ($P < .05$).

The mean logMAR functional visual acuity in SJS patients with severe corneal complications was 1.16 ± 0.45. The mean logMAR functional visual acuity in SJS and SS patients with minimal corneal complications was 0.50 ± 0.55 and 0.28 ± 0.27, respectively. The functional visual acuities in SJS patients were significantly higher compared to in SS patients (Figure 1, Left). The mean visual maintenance ratio in SJS and SS patients with minimal corneal complications was 0.88 ± 0.10 and 0.91 ± 0.07, respectively. Visual maintenance ratios in SJS patients were significantly lower compared to SS patients (Figure 1, Left).

The mean logMAR functional visual acuity in SJS patients with and without aqueous tear deficiency and SS patients was 0.83 ± 0.54, 0.99 ± 0.63, and 0.28 ± 0.27, respectively. The functional visual acuities in SJS patients with and without aqueous tear deficiency were significantly

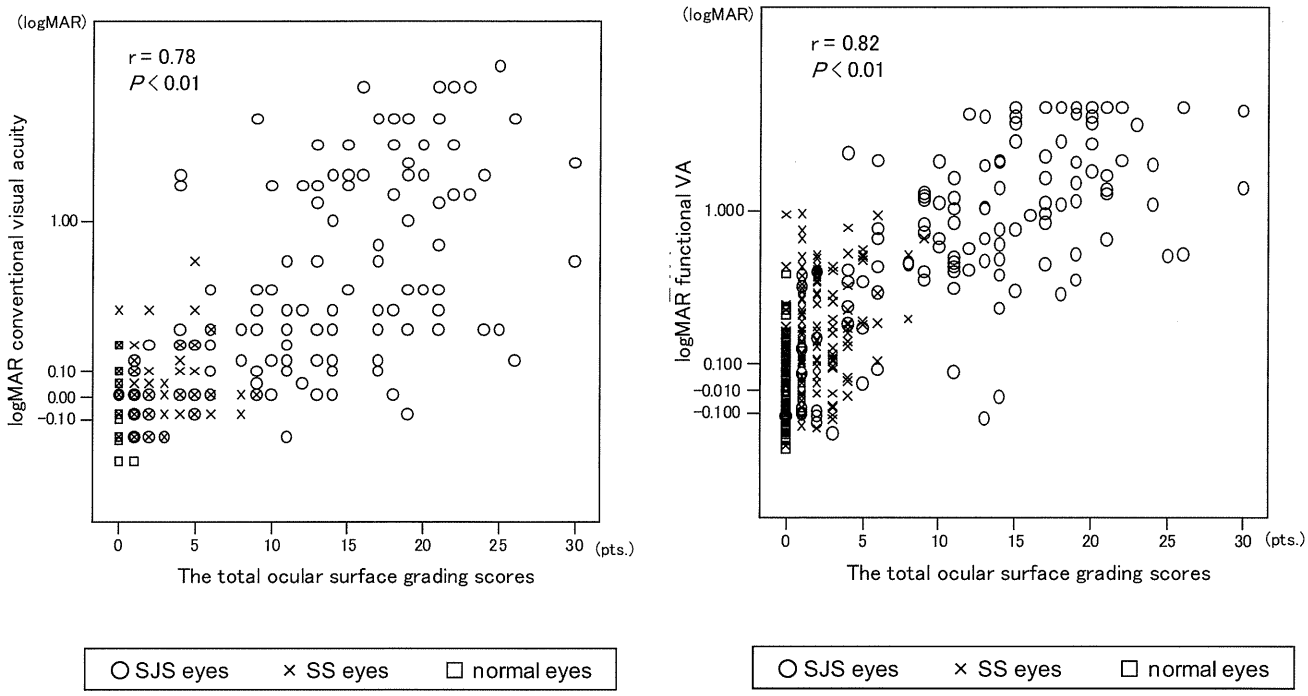

FIGURE 3. Correlation between visual function and ocular surface grading score. (Left) Correlation between logMAR conventional visual acuity scores and total ocular surface grading scores. (Right) Correlation between logarithm of minimal angle of resolution (logMAR) functional visual acuity scores and 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

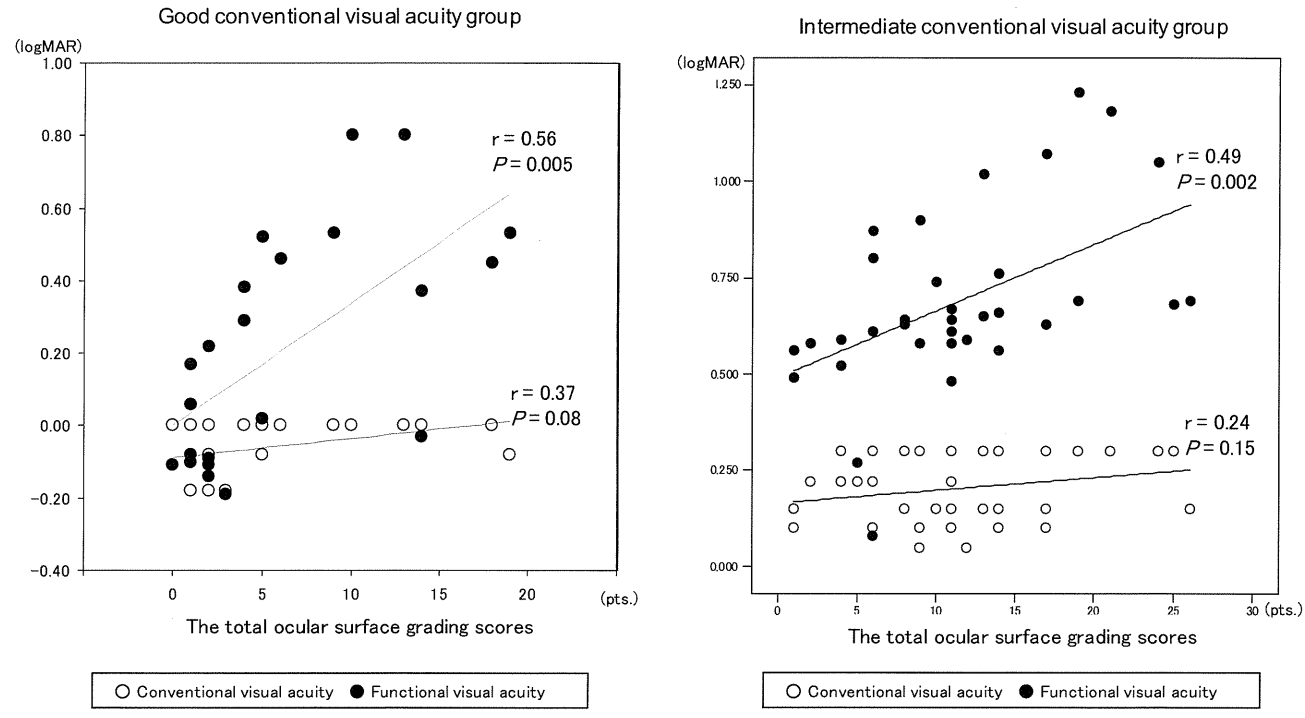

FIGURE 4. Correlations between visual function and ocular surface grading score in the good and intermediate conventional visual acuity group of Stevens-Johnson syndrome patients. (Left) Correlation in the good conventional visual acuity group of Stevens-Johnson syndrome patients. (Right) Correlation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

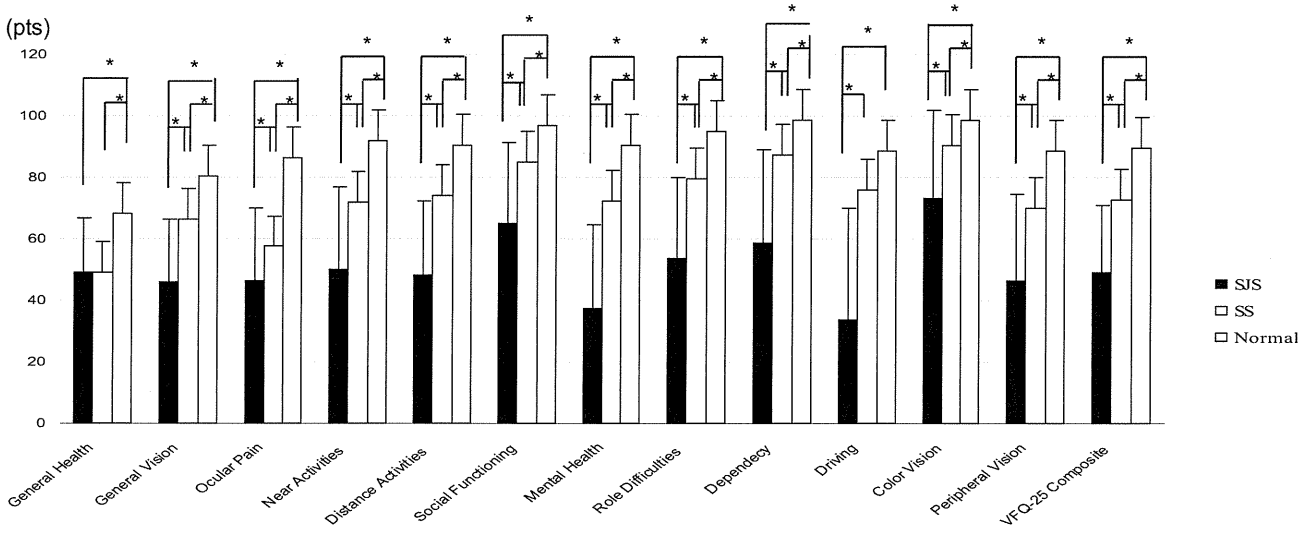

FIGURE 5. Visual Function Questionnaire-25 results in patients with Stevens-Johnson syndrome (SJS), patients with Sjögren syndrome (SS), and healthy normal subjects.

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-

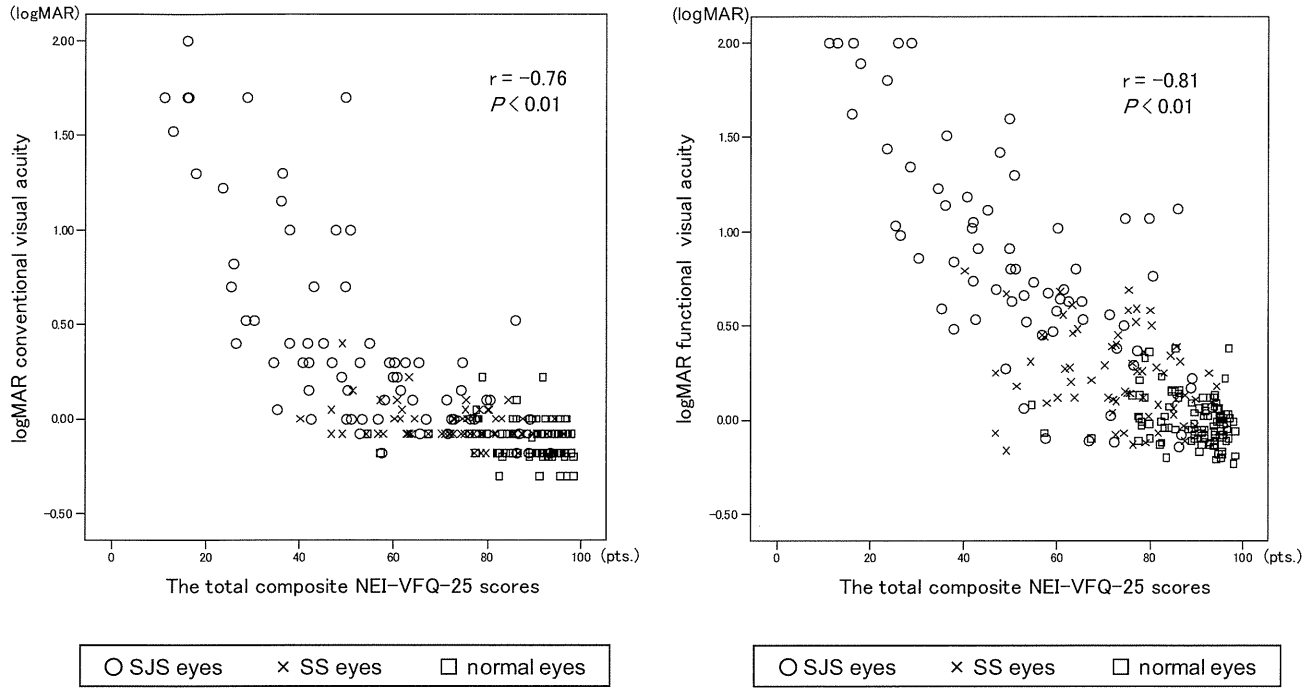

FIGURE 6. Relation between visual function and composite NEI VFQ-25 scores. (Left) Correlation between logMAR conventional visual acuity scores and total composite NEI VFQ-25 scores. (Right) Correlation between logarithm of minimal angle of resolution (logMAR) functional visual acuity scores and total 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