METHODS

Patients

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine and the University of Tokyo, Graduate School of Medicine. All experimental procedures were conducted in accordance with the principles of the Helsinki Declaration. The purpose of the research and the experimental protocols were explained to all participants, and their prior written informed consent was obtained.

Diagnosis of SJS/TEN was based on a confirmed history of acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites, including the ocular surface.

In the acute stage patients with SJS/TEN manifest vesiculobullous lesions of the skin (Fig E1, *A*) and mucosa (especially that of the eyes and mouth), severe conjunctivitis (Fig E1, *B*), and persistent corneal epithelial defects caused by ocular surface inflammation. Oral involvement, including blisters, erosions, and bleeding of the mouth and lips (Fig E1, *C*), has been observed in all patients with SJS/TEN with severe ocular surface complications, and almost all such patients lose their fingernails in the acute or subacute stage as a result of paronychia (Fig E1, *D*). In the chronic stage, despite healing of the skin lesions, ocular surface complications, including conjunctival invasion into the cornea (Fig E1, *E*), dry eyes, symblepharon, and in some instances keratinization of the ocular surface persist.

To investigate 44 SNPs of the 13 genes along with alleles of HLA-A analyzed by means of direct sequencing, we enrolled 61 patients with SJS/TEN in the chronic or subacute phase; all presented with symptoms of ocular surface complications. The control subjects were 130 healthy volunteers. All participants and volunteers were Japanese residing in Japan. The average age of the 61 patients and 160 control subjects was 45.3 ± 18.1 (SD) and 36.8 ± 11.9 (SD) years, respectively. The male/female ratios in the patient and control groups were 26/35 and 49/81, respectively.

Furthermore, we added 55 subjects and 91 control subjects to obtain a total of 116 case samples and 221 control samples for analysis of LD block around *TLR3* and *PTGER3*. The average age of the 116 patients and 221 control subjects was 44.0 ± 18.0 (SD) and 35.6 ± 11.1 (SD) years, respectively. The male/female ratios in the patient and control groups were 46/70 and 89/132, respectively.

SNP genotyping

For a search of the 44 SNPs of 13 genes along with HLA-A alleles (listed in Table E2), SNP genotyping was performed by using PCR direct sequencing. Genomic DNA was isolated from human peripheral blood at SRL, Inc (Tokyo, Japan). For direct sequencing, PCR amplification was conducted with AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, Calif) for 35 cycles at 94°C for 1 minute and annealing at 60°C for 1 minute and 72°C for 1 minute on a commercial PCR machine (GeneAmp; PerkinElmer, Applied Biosystems). The PCR products were reacted with BigDye Terminator version 3.1 (Applied Biosystems), and sequence reactions were resolved on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

To obtain more detailed information of genetic variants in the *TLR3* and *PTGER3* regions, we genotyped 116 patients with SJS/TEN and 221 healthy control subjects for 42 SNPs by using the DigiTag2 and TaqMan SNP genotyping assays (Applied Biosystems). In the DigiTag2 assay we designed multiplex PCR primers for each of the 32 SNP sites. Multiplex PCR was performed in 10 μL of Multiplex PCR buffer containing 25 ng of genomic DNA, 25 nmol/L of each multiplex primer mix, 200 μmol/L of each deoxyribonucleoside triphosphate, 2.25 mmol/L $MgCl_2$, and 0.4 U of KAPA2G Fast HotStart DNA polymerase (Kapa Biosystems, Mowbray, South Africa). Cycling was performed at 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds and 68°C for 2 minutes. We used 36 SNPs covering a greater than 95% call rate for further analyses (Table E1). In the TaqMan SNP genotyping assay PCR amplification was performed in a 5-μL reaction mixture containing 1 μL of genomic DNA, 2.5 μL of ABsolute QPCR ROX Mix (Thermo Fisher Scientific, Inc, Waltham, Mass), and ×40 TaqMan SNP Genotyping Assay probe (Applied Biosystems) for each SNP. The QPCR thermal cycling program was 95°C for 15 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All samples subjected to the DigiTag2 assay and the TaqMan SNP genotyping assay were found to have a greater than 95% call rate. The 7 SNPs of *TLR3* and 6 SNPs of *PTGER3*, which we have reported previously, were examined by using PCR direct sequencing, as described above. The primers and probes used in this study are shown in Table E3.

HLA-A genotyping

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

Statistical analysis

A scan for epistatic interactions in data for multiple loci is associated with serious problems, such as computational burden and high dimensionality. The former restricts potential algorithms to those that are simple and fast, whereas the latter is a theoretic issue with no efficient and universal solution, being known as the "p>n or p>>n problem" or the "curse of dimensionality," causing standard methods of multivariate regression to break down and prohibitive conservation of alternate methods involving multiple univariate regression caused by necessary corrections of the heavy multiplicity. On the other hand, eclectic methods based on SNP filtering by *P* value could potentially miss interactions with no or only weak marginal effects. Instead of these current methods, we have therefore proposed the use of a model selection strategy for interaction analysis of high-dimensional data. Our new software, EPISIS, implements SIS (Fan and Lv 2008), followed by some iterative steps, which can be roughly regarded as a sophisticated analog of the classical forward-backward stepwise procedure suited to ultra–high-dimensional regression models. SIS has 2 major parts: screening and variable selection. In the screening part candidates are selected on the basis of feature ranking, and then subsequent variable selection from these candidates is carried out for interactions and main effects. As a variable selection algorithm for penalized regression, we use SCAD. We also use LASSO for comparison.

Statistical significance of the association with each SNP was assessed by using the Fisher exact test on 2 × 2 contingency tables. Haploview software (version 4.2) was used to infer the LD structure of specific genomic regions, to perform haplotype association testing, and to permute the data pertaining to their association.

Mice

BALB/c mice were purchased from CLEA (Tokyo, Japan) and used at 6 to 12 weeks of age for sensitization. TLR3 KO and *PTGER3* KO mice were generated, as described previously, and back-crossed for more than 7 generations to BALB/c mice. *TLR3/PTGER3* DKO mice were generated by interbreeding of *TLR3* KO and *PTGER3* KO mice at Kyoto Prefectural University of Medicine. They were subjected to EAC at 9 to 15 weeks of age, with age-matched, wild-type BALB/c mice as control animals. The mice were maintained on a 12-hour/12-hour light/dark cycle under specific pathogen-free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto Prefectural University of Medicine, Kyoto, Japan. All studies were performed in accordance with the Association for Research in Vision and Ophthalmology's "Statement for the use of animals in ophthalmic and vision research."

Eosinophil infiltration in a murine model of EAC

The experiments were conducted by using a protocol approved by the Institutional Animal Care and Use Committee of Kyoto Prefectural University of Medicine. Short RW was purchased from Polysciences, Inc (Warrington, Pa), and aluminum hydroxide (alum) was from Sigma-Aldrich (St Louis, Mo). The mice were immunized with an intracutaneous injection of RW adsorbed on alum (200 μg of RW and 2.6 mg of alum) into the left hind footpad on day 0. On day 7, they received an intraperitoneal injection of RW adsorbed on alum, and on day 18, their eyes were challenged with RW in PBS (500 μg in 5 μL per

eye) or with PBS alone (5 μL per eye). Their eyes, including the conjunctiva, were harvested 24 hours after the last challenge, fixed in 10% neutral-buffered formalin, and embedded in paraffin blocks for histologic analysis. Vertical 6-μm-thick sections were mounted on microscope slides, deparaffinized, and stained with Luna stain, which identifies erythrocytes and eosinophil granules. Using an entire section from the central portion of the eye, including the pupil and optic nerve head, we counted infiltrating eosinophils in the lamina propria mucosae of the tarsal conjunctiva. Cell counts were expressed as the number of infiltrating eosinophils per unit area (0.1 mm^2) measured with image software (Scion Corp, Frederick, Md).

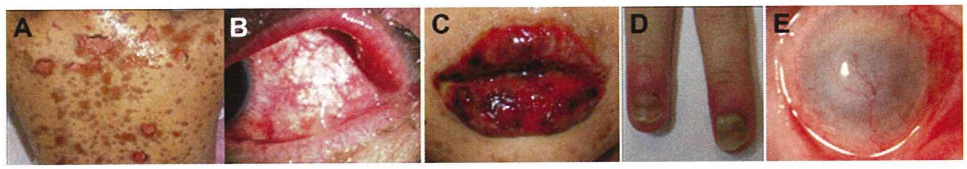

FIG E1. Features of patients with SJS/TEN with ocular complications. **A,** Vesiculobullous lesions of the skin in the acute stage. **B,** Severe conjunctivitis in the acute stage. **C,** Oral involvement, including blisters, erosions, and bleeding of the mouth and lips in the acute stage. **D,** Paronychia in the acute stage. **E,** Conjunctival invasion into the cornea in the chronic stage.

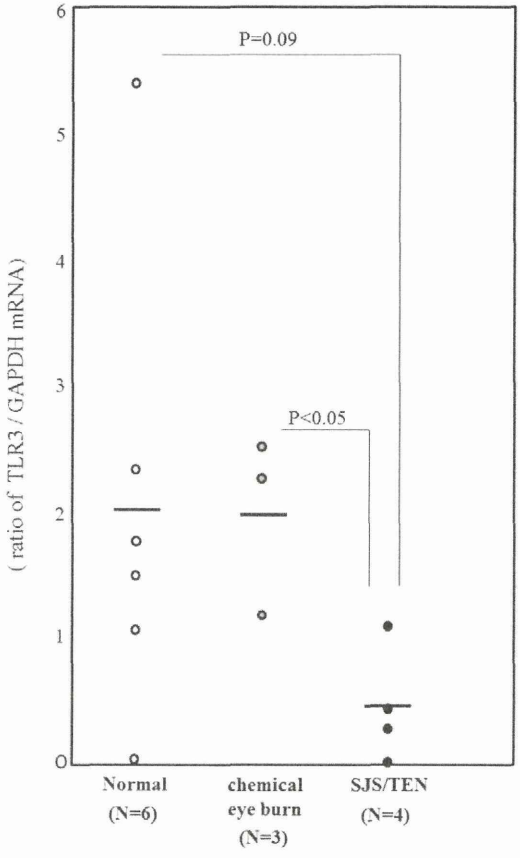

FIG E2. Expression of *TLR3* mRNA in conjunctival tissues from patients with SJS/TEN and chemical eye burn and the control subjects. Total RNA was isolated from conjunctival tissue sections by using the RNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The RT reaction was with the SuperScript preamplification kit (Invitrogen, Carlsbad, Calif). Quantitative RT-PCR was on an ABI-prism 7700 instrument (Applied Biosystems). The probes for human *TLR3* and human glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* were from Applied Biosystems. The results were analyzed with sequence detection software (Applied Biosystems). The quantification data were normalized to the expression of the housekeeping gene *GAPDH.*

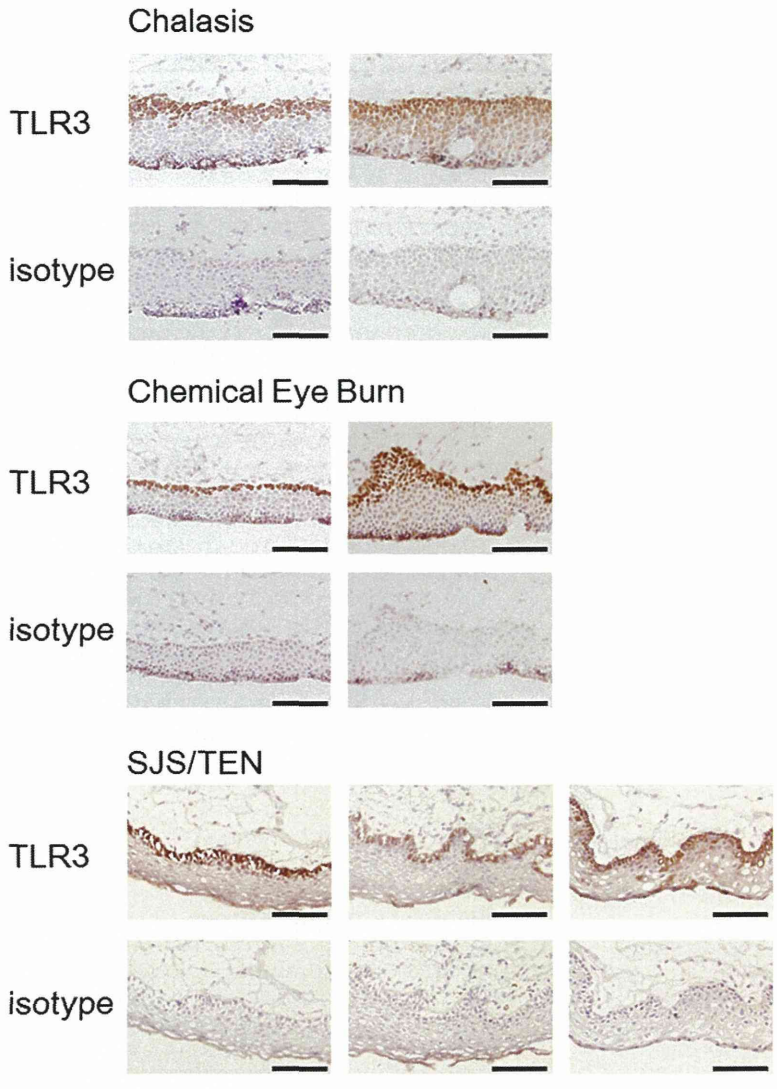

FIG E3. Immunohistologic analysis of *TLR3* in conjunctival epithelium of patients with SJS/TEN and chemical eye burn and control subjects. For TLR3 staining, we used rabbit polyclonal antibody to TLR3 (Abcam, Cambridge, Mass). The secondary antibody (Biotin-SP–conjugated AffiniPure F[ab′]$_2$ fragment donkey anti-rabbit IgG [H+L], 1:500 dilution; Jackson ImmunoResearch, Baltimore, Md) was applied for 30 minutes. The VECTASTAIN ABC reagent (Vector Laboratories, Burlingame, Calif) was used for increased sensitivity with peroxidase substrate solution (DAB substrate kit; Vector Laboratories) as a chromogenic substrate. Each *scale bar* represents 100 μm.

TABLE E1. Primers and probes used in SNP analysis of *TLR3* and *PTGER3*

Gene	rsID	Genotyping	Primer sequence or context sequence (VIC/FAM)
TLR3	rs4861699	DigiTag2	AACTTAAAGAAGTGAAAGACTTGAACACTGAAAACTATAA
			AGGTATTTAAACTAATTTGAGTGGATTTTTTGTGTATGGTGTA
	rs6822014	DigiTag2	ATAACTTGATGAGCTTGAAGACAAGTATACTTCTGTGAAA
			GGCATACACATACAATGGAATATTATTCTACCTTACAAAA
	rs4862632	DigiTag2	GCTTGATCTGCAAACATAAGTGACATACGCAAACATAATAA
			ACCATTTGTTTAGGTTCATAATATATTCATCGCATTACATA
	rs5743305	DigiTag2	ACTCACTTTTTTTCATTACAGATGTGCTATGATCTATTATA
			CAAGGCGCTCACAGAGAAGAAATCTTTTGAATATTAGTGAA
	rs11732384	DigiTag2	AAATTATTCCAGGTAAGTGTCAGGTAATAAAATCACCTTA
			GAGGGGTACATCTCACCTAAGCAAGGAGAATGTATTGTA
	rs7657186	DigiTag2	AGGCCAATACCACATTGTTCGGATTACTTTAGCTTTACAA
			CAACCACCACACACTTTTAAACGACCGAATCTCATAA
	rs6552950	DigiTag2	TGTTGCACCACCAACTTTCCTGACAACATTTGGTA
			ATTAACTTAGGAGAGGTCACACACCTTCACATAGAAGCTAA
	rs5743312	DigiTag2	TCCTATGAAGCAGAGTCATTATCACGCCCATTTGAAA
			AGTTGTCATCGAATCAAATTAAAGAGGTAAGAAGTAAGGTA
	rs7668666	DigiTag2	AGTGCCTTAACAGTGTGAATTTCAGTACAGTAAGAATTTAA
			GAGGGCTACGTGTCCTGGATCATGAAGACAGACTA
	rs3775292	DigiTag2	GAGAAAATCCGGGTGGAAAGACGAGAGGGAGAGCTA
			GACAGATTCCGAATGCTTGTGTTTGCTAATTCCAAACATA
	rs3775291	DigiTag2	AAGCAATATGTTCACAGGATTGATAAACCTGAAATACTTA
			TTGGCTATGTTGTTGTTGCTTAGATCCAGAATGGTCAA
	rs10025405	DigiTag2	AGCTATTCAGGTCTATTTCCAATAATCAGATTCTCTTTTATTGTA
			GTTTCAATGGGTATAATGCTATTTCCTTTGTAAAAGAGTA
	rs3775296	Direct sequencing	TTACCTTCTGCTTGACAAAGGG
			TGCATTTGAAAGCCATCTGC
	rs3775295	Direct sequencing	TCACATGGCTTATCAAACACACAG
	rs3775294		CATTGCTCTTCCTCAGATGCC
	rs3775293	Direct sequencing	CAGTTCTTTACTCCATCTCCGC
	rs3775292		CCAAGGCTCTGGTAAGGGTG
	rs3775291	Direct sequencing	TGGCTAAAATGTTTGGAGCA
	rs3775290		GAAGAGGCTGGAATGGTGAA
PTGER3	rs11803673	DigiTag2	GAAACACATCCTGGAGTCATTCTGATGGGATTGCTA
			GGACAGGGAGAGAGGGACGGAAAGAAAAAAATAATCAA
	rs7555865	DigiTag2	GAAACACATCCTGGAGTCATTCTGATGGGATTGCTA
			GGACAGGGAGAGAGGGACGGAAAGAAAAAAATAATCAA
	rs1327453	DigiTag2	AGTTACCATACACACAAATGGAAAACACACATAAACATGATGA
			TCAGTTCAACCTAACTGGTCTTCTACCCAGTCATTCATAA
	rs4320735	DigiTag2	AGACTTGAGTCTAGAATTGCTTTGTTGAGAGAGATAGGTA
			TGCAAGTGACAGAAATCAAAGTCAATTTAGCTTAGGTATA
	rs2182324	DigiTag2	ATAATGCCATCTTGCTACTATGTAGAGCAGAAACTTTCAA
			CCATAAGGGTTTAAAATCTTTATTCACTTCACTGATGTATA
	rs10443262	DigiTag2	GACAGCCCATCACAGGATCAAAGACCTAGGAAGGAA
			TAGTCTCATTTGACTCCATGCCCCATATGCTGGACA
	rs2421805	DigiTag2	AGATACGTAATGAAAGTGGTCTCTTGTTTGGTCTCTTCTTA
			ACACAGAGAGGCCTAACACTGAAAACCAATGACATAAAAA
	rs2225025	DigiTag2	GGAAGGTCACCTACAAAGGGAACCCTATCAAGCTAA
			TTTCCTTTCCATATTTAGCACTCCCTTAATGACTGTGCAA
	rs1409981	DigiTag2	TAGGGGATGTGAGAAGGAGGGTCCTGAAGATGAAA
			AAGCTGCCTTTACCCATACCTATTTTGAGTTACTCAGAAA
	rs6667891	DigiTag2	TAGGGGATGTGAGAAGGAGGGTCCTGAAGATGAAA
			AAGCTGCCTTTACCCATACCTATTTTGAGTTACTCAGAAA
	rs4147115	DigiTag2	CCTGAAACTCCATATTTTACAACCTCACCTCTGTGTATTTTTCA
			ATCCTAACCAGTTACTTTGTCTTATCAGTTTTAGCACTTA
	rs4650093	DigiTag2	CCTGAAACTCCATATTTTACAACCTCACCTCTGTGTATTTTTCA
			ATCCTAACCAGTTACTTTGTCTTATCAGTTTTAGCACTTA
	rs17131478	DigiTag2	CCTGAAACTCCATATTTTACAACCTCACCTCTGTGTATTTTTCA
			ATCCTAACCAGTTACTTTGTCTTATCAGTTTTAGCACTTA
	rs17131479	DigiTag2	CCTGAAACTCCATATTTTACAACCTCACCTCTGTGTATTTTTCA
			ATCCTAACCAGTTACTTTGTCTTATCAGTTTTAGCACTTA
	rs7521005	DigiTag2	ATGAACACTGACATATGAACTTAAAAGCTAGAATTTAACTTAAA
			CTTTTCTCAGATCCTCATTCTCCTCATGCAATATGCCATAA

(Continued)

TABLE E1. *(Continued)*

Gene	rsID	Genotyping	Primer sequence or context sequence (VIC/FAM)
	rs12039590	DigiTag2	AGAGTGTGGACTATCACTTGTCAAATATTTTGAGAAAATA
			AGGTATGTGAATCCTTATAACAGTCTTAAGGAGTAGACGTTA
	rs7541092	DigiTag2	AGGGAGATACAAATCAAACAAAAACATGTTGAAGTCAATA
			CATCTACAGGTCAAGAAATGCCTAGGAATGCCAGAAAA
	rs2068652	DigiTag2	AGGGAGATACAAATCAAACAAAAACATGTTGAAGTCAATA
			CATCTACAGGTCAAGAAATGCCTAGGAATGCCAGAAAA
	rs17131485	DigiTag2	CTGTGGAGAAGAATCTACCACCTTGATCTGGAGTTGTA
			GTTTGATTTGTATCTCCCTAAAATATCATCAGTTCTTCAAA
	rs11209710	DigiTag2	AGTATTGAAGAGTCTAATACTGAGTCATTGAAGGATATAGTA
			ATGCTTGAAGAATGCTCCAAGAAATGGACTATTCTCATATA
	rs1359835	DigiTag2	AGTATTGAAGAGTCTAATACTGAGTCATTGAAGGATATAGTA
			ATGCTTGAAGAATGCTCCAAGAAATGGACTATTCTCATATA
	rs1327464	DigiTag2	CTGCTTTGTAACTTGGGCTTGGAGAGGTTTATCCAA
			CTTCAATACTTCACTAGACTTTCTGGATGCAATGACTGTA
	rs12048245	TaqMan	TCTAGGAGATTCTGAGACAGGTGTT[C/T]GCTTCAAAAGGAAAAGCTTTTGAAA
	rs10889897	TaqMan	AGGAAAGGCATCAAAGGAATTGCAC[A/G]GGGTAGGAAAGTACAACGGATATTC
	rs1409161	TaqMan	CTTAATTTTAGGCTTCTGGTCTCCA[A/G]AACTGGAAGAAAATAGTTTTGGGTA
	rs12123324	TaqMan	GAAGCTCCTCAAGTGTTAGAGTTCA[C/T]AAGATGTTGGGTAACTGTACAGTTT
	rs6670616	TaqMan	TAAAGACGAATAAATACAGCTGTGT[A/T]TTGATTCCGCACTTTTCCTATGACA
	rs17090700	TaqMan	CATGACATTTGGGATTAAGTTCTGC[A/C]TTTTAGAGTACTCATTCATTGAAGT
	rs909848	TaqMan	TCATTAATAGTTCTTTCTGCTCACC[C/T]ACACTAGCTCACTAATTTATCCCCA
	rs11209733	TaqMan	ATTTGTAACTGTATATTAGCATTAA[C/G]TGTAGTCATCCTACAGGAGTATAGA
	rs17131450	Direct sequencing	TTTTATGCAGCTTTCGGTCA
			CCCCTCCAGGCTGATAACTC
	rs5702	Direct sequencing	CAAGTAGCAGTTGGCAGCAA
			TGCAATCAGACAGGCAAGAG
	rs1325949	Direct sequencing	AATTGCAAGTCCAGCTCAGG
			AGGCCTCAGGGAGCTTTTAC
	rs7543182	Direct sequencing	TGTGAGGCAAGAACCAGACA
			AGGACCTGGGAGGGAAGATA
	rs7555874	Direct sequencing	AAGCCAGCAAAGGACAAGAA
			TGTTGTGTGTCTGCCAGGTT
	rs4147114	Direct sequencing	TGCTGGAAGCTCATGGTCTA
			TGCATGGTTCGTCTAACCTTAT

TABLE E2. List of SNPs carried out in a statistical search for interactions

Symbol	Name	Chr	RefSeq allele	Note
PTGER3	Prostaglandin E receptor EP3	chr1		
	rs17131450		A/G	Genomic
	rs5702		C/T	Synonymous
	rs1325949		A/G	Intronic
	rs7543182		A/C	Intronic
	rs7555874		C/T	Intronic
	rs4147114		C/G	Intronic
	rs1327464		A/G	
IL13	Interleukin 13	chr5		
	rs1800925		C/T	Genomic
	rs20541		C/T	Missense
	rs1295685		C/T	3'UTR
TLR3	Toll-like receptor 3	chr4		
	rs3775296		G/T	Intronic
	rs3775295		C/T	Intronic
	rs3775294		C/T	Intronic
	rs3775293		C/T	Intronic
	rs3775292		C/G	Intronic
	rs3775291		A/G	Missense
	rs3775290		A/G	Intronic
FasL	Fas ligand	chr1		
	rs3830150		A/G	Intron of C1orf9
	rs2859247		C/T	Genomic
	rs2639614		A/G	Genomic
	rs929087		A/G	Intronic
IL4R	Interleukin 4 receptor	chr16		
	rs1805010		A/C/G/T	Missense
	rs1805015		C/T	Missense
	rs1801275		A/G	Missense
MAIL	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	chr3		
	rs3821727		C/G	Missense
	rs677011		A/G	Intronic
	rs595788		C/T	Intronic
	rs3217713		indel	Intronic
	rs14134		A/G	Synonymous
	rs622122		A/T	Intronic
	rs2305991		A/G	3'UTR
IL4	Interleukin 4	chr5		
	rs2243250		C/T	
IL1A	Interleukin 1, alpha	chr2		
	rs2071376		A/C	Intronic
	rs2071375		A/G	Intronic
	rs2071373		C/T	Intronic
	rs1894399		A/G	Intronic
	rs1609682		A/C	Intronic
TLR2	Toll-like receptor 2	chr4		
	rs3804100			Synonymous
	rs3804099			Synonymous
TLR5	Toll-like receptor 5	chr1		
	rs2072493		A/G	Missense
	rs5744168		A/C/G/T	STOP
PTGER4	Prostaglandin E receptor 4	chr5		
	rs1494558		A/C/G/T	Missense
Chr5p13	Genes in cytogenetic band chr5p13	chr5		
	rs6871834		A/G	Genomic
GNLY	Granulysin	chr2		
	rs3755007		A/C	Genomic

TABLE E3. Susceptible interactions between loci detected by using Interactive Sure Independence Screening

Locus 1	Locus 2	OR	95% CI	*P* value
***PTGER3* rs4147114 (GC)**	***TLR3* rs3775296 (TT)**	**25.3**	**3.2-203**	**.0000527**
PTGER3 rs4147114 (GC)	—	2.66	1.4-5.0	.0023
—	*TLR3* rs3775296 (TT)	5.35	2.0-14.1	.00025
HLA-A*02:06	***IL1A* rs1609682 (CA)**	**9.66**	**2.0-47.0**	**.00193**
*HLA-A*02:06*	—	3.46	1.8-6.8	.0002
—	*IL1A* rs1609682 (CA)	—	—	.31

Boldface text indicates the pairs with interactions.

TABLE E4. Association between *TLR3* SNPs and SJS/TEN with ocular complications

	Frequencies of genotypes (%)				Allele 1 vs allele 2	Genotype 11 vs 12+22	Genotype 11+12 vs 22
rs no. of SNP	Genotypes		Controls	Cases	*P* value* OR (95% CI)	*P* value* OR (95% CI)	*P* value* OR (95% CI)
rs4861699	11	G/G	39.8	58.6	.0018	.001	.17
	12	G/A	47.5	33.6	1.76 (1.2-2.5)	2.14 (1.4-3.4)	—
	22	A/A	12.7	7.8			—
rs6822014	11	A/A	61.9	50.5	.00071	.048	.00008
	12	A/G	33.9	32.4	0.54 (0.4-0.8)	0.63 (0.4-1.0)	0.21 (0.1-0.5)
	22	G/G	4.2	17.1			
rs11732384	11	G/G	51.6	65.5	.032	.014	.68
	12	G/A	41.2	28.4	1.52 (1.0-2.2)	1.78 (1.1-2.8)	—
	22	A/A	7.2	6.0			—
rs3775296†	11	G/G	51.6	44.0	.0046	.18	.00009
	12	G/T	43.0	37.1	0.61 (0.4-0.9)	—	0.25 (0.1-0.5)
	22	T/T	5.4	19.0		—	
rs5743312	11	C/C	54.1	46.6	.0059	.19	.0001
	12	C/T	41.4	36.2	0.62 (0.4-0.9)	—	0.23 (0.1-0.5)
	22	T/T	4.6	17.2		—	
rs7668666	11	C/C	39.4	30.4	.01	.11	.0069
	12	C/A	47.9	45.2	0.65 (0.5-0.9)	—	0.45 (0.3-0.8)
	22	A/A	12.7	24.3		—	
rs3775290†	11	G/G	38.5	34.5	.057	.47	.0069
	12	G/A	50.2	43.1	—	—	0.44 (0.2-0.8)
	22	A/A	11.3	22.4	—	—	

*P value for allele or genotype frequency comparison between cases and controls by using the χ^2 test.
†Italic rs numbers show previously reported SJS/TEN-associated SNPs.

TABLE E5. Association between *PTGER3* SNPs and SJS/TEN with ocular complications

	Frequencies of genotypes (%)				Allele 1 vs allele 2	Genotype 11 vs 12+22	Genotype 11+12 vs 22
rs no. of SNP	Genotypes		Controls	Cases	*P* value* OR (95% CI)	*P* value* OR (95% CI)	*P* value* OR (95% CI)
rs7555865	11	C/C	47.9	45.7	.10	.69	.0083
	12	C/T	42.5	34.5	—	—	0.43 (0.2-0.8)
	22	T/T	9.6	19.8	—	—	
rs17131450†	11	C/C	87.8	76.7	.00069	.0086	.0039
	12	C/T	11.8	18.1	0.41 (0.2-0.7)	0.46 (0.3-0.8)	0.08 (0.01-0.7)
	22	T/T	0.5	5.2			
rs5702†	11	C/C	49.3	64.7	.059	.0072	.6
	12	C/T	43.0	25.9	—	1.88 (1.2-3.0)	—
	22	T/T	7.7	9.5	—	—	—
rs1325949†	11	A/A	47.5	69.0	.0035	.00017	.88
	12	A/G	44.3	22.4	1.8 (1.2-2.6)	2.5 (1.5-3.9)	—
	22	G/G	8.1	8.6			—
rs2421805	11	T/T	48.1	33.6	.0014	.012	.0045
	12	T/G	44.4	48.7	0.58 (0.4-0.8)	0.55 (0.3-0.9)	0.37 (0.2-0.8)
	22	G/G	7.4	17.7			
rs7543182†	11	G/G	50.7	70.7	.0096	.00041	.54
	12	G/T	42.5	20.7	1.67 (1.1-2.5)	2.34 (1.5-3.8)	—
	22	T/T	6.8	8.6			—
rs.7555874†	11	G/G	50.7	69.8	.014	.00074	.54
	12	G/A	42.5	21.6	1.62 (1.1-2.4)	2.25 (1.4-3.6)	—
	22	A/A	6.8	8.6			—
rs1409981	11	G/G	84.7	73.3	.0021	.012	.040
	12	G/A	13.0	19.8	0.48 (0.3-0.8)	0.49 (0.3-0.9)	0.32 (0.1-1.0)
	22	A/A	2.3	6.9			
rs.4147114†	11	C/C	24.4	43.1	.0012	.00042	.10
	12	C/G	53.4	42.2	1.72 (1.2-2.4)	2.34 (1.5-3.8)	—
	22	G/G	22.2	14.7			—
rs4147115	11	A/A	25.5	39.5	.023	.0098	.34
	12	A/T	46.7	37.6	1.46 (1.1-2.0)	1.91 (1.2-3.1)	—
	22	T/T	27.8	22.9			—
rs4650093	11	C/C	51.4	65.5	.092	.013	.44
	12	C/T	42.3	25.9	—	1.8 (1.1-2.9)	—
	22	T/T	6.4	8.6	—	—	—
rs17131478	11	G/G	61.6	74.6	.035	.018	.79
	12	G/T	34.2	21.9	1.59 (1.0-2.5)	1.8 (1.1-3.0)	—
	22	T/T	4.1	3.5			—
rs17131479	11	C/C	62.2	75.0	.039	.018	.91
	12	C/G	34.1	21.6	1.58 (1.0-2.4)	1.8 (1.1-3.0)	—
	22	G/G	3.7	3.4			—
rs7521005	11	A/A	51.6	65.5	.10	.014	.44
	12	A/G	42.1	25.9	—	1.8 (1.1-2.8)	—
	22	G/G	6.3	8.6	—	—	—
rs7541092	11	G/G	62.4	74.8	.040	.023	.77
	12	G/A	33.5	21.7	1.57 (1.0-2.4)	1.8 (1.1-3.0)	—
	22	A/A	4.1	3.5			—
rs1359835	11	G/G	88.6	79.1	.0047	.019	.030
	12	G/C	10.9	17.4	0.45 (0.3-0.8)	0.49 (0.3-0.9)	0.13 (0.01-1.1)
	22	C/C	0.5	3.5			
rs1327464	11	G/G	88.2	78.4	.0043	.017	.031
	12	G/A	11.3	18.1	0.46 (0.3-0.8)	0.49 (0.3-0.9)	0.13 (0.01-1.1)
	22	A/A	0.5	3.4			
rs1409161	11	G/G	30.8	25.9	.040	.35	.014
	12	G/A	51.6	44.8	0.72 (0.5-1.0)	—	0.52 (0.3-0.9)
	22	A/A	17.6	29.3		—	
rs34885906	11	T/T	85.5	94.0	.026	.021	.0
	12	T/C	14.5	6.0	2.5 (1.1-5.8)	2.6 (1.1-6.2)	—
	22	C/C	0.0	0.0			—
rs2817864	11	T/T	53.4	61.2	.056	.17	.021
	12	T/G	40.3	37.9	—	—	7.8 (1.0-59.9)
	22	G/G	6.3	0.9	—	—	

*P value for allele or genotype frequency comparison between cases and controls by using the χ^2 test.
†Italic rs numbers show previously reported SJS/TEN-associated SNPs.

RESEARCH LETTERS

Downregulation of Monocyte Chemoattractant Protein 1 Expression by Prostaglandin E_2 in Human Ocular Surface Epithelium

Elsewhere, we reported that in the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of interleukin 6 (IL-6), IL-8, and monocyte chemoattractant protein 1 (MCP-1) were dramatically increased.[1] We also reported that Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications was associated with polymorphism of the prostaglandin E receptor 3 (EP_3) gene (*PTGER3*).[2]

Prostanoids are a group of lipid mediators that form in response to various stimuli. They include prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2. There are 4 subtypes of the PGE receptor: EP_1, EP_2, EP_3, and EP_4. We previously reported that PGE_2 suppresses polyinosine–polycytidylic acid (polyI:C)–stimulated cytokine production via EP_2 and/or EP_3 in human ocular surface epithelial cells.[3,4] PolyI:C is a ligand of Toll-like receptor 3, which is strongly expressed in ocular surface epithelium.[5] We found that PGE_2 suppresses the production of IL-6, chemokine (C-X-C motif) ligand 10, chemokine (C-X-C motif) ligand 11, and chemokine (C-C motif) ligand 5 but not IL-8 by epithelial cells on the human ocular surface[3]; it remains to be determined whether it also suppresses MCP-1 production. Monocyte chemoattractant protein 1 plays a significant role in the recruitment of monocytes and lymphocytes to the site of cellular immune reactions. In this study, we investigated whether PGE_2 downregulates polyI:C-induced MCP-1 production.

All experiments were conducted in accordance with the principles set forth in the Declaration of Helsinki. Enzyme-linked immunosorbent assay and quantitative real-time polymerase chain reaction were performed with primary human conjunctival epithelial cells and immortalized human corneal-limbal epithelial cells using previously described methods (eAppendix, http://www.archophthalmol.com).[3]

First, we examined whether PGE_2 downregulated the production and messenger RNA (mRNA) expression of MCP-1 induced by polyI:C stimulation in human conjunctival and corneal epithelial cells. We found that it significantly attenuated the production of MCP-1 (**Figure**, A). Quantitative real-time polymerase chain reaction confirmed that the mRNA expression of MCP-1 was significantly downregulated by PGE_2 (Figure, A).

Next, we examined which PGE_2 receptor(s) contributed to the downregulation of polyI:C-induced MCP-1. We used the EP_2 agonist ONO-AE-259, the EP_3 agonist ONO-AE-248, and the EP_4 agonist ONO-AE-329. Enzyme-linked immunosorbent assay showed that the EP_2 and EP_3 agonists significantly suppressed the polyI:C-induced production of MCP-1, while the EP_4 agonist did not exert suppression (Figure, B). Quantitative real-time polymerase chain reaction confirmed that the EP_2 and EP_3 agonists significantly downregulated the mRNA expression of MCP-1 (Figure, C). Thus, our results document that PGE_2 attenuated the mRNA expression and production of MCP-1 via both EP_2 and EP_3.

In human macrophages, PGE_2 attenuated the lipopolysaccharide-induced mRNA and protein expression of chemokines including MCP-1 through EP_4.[6] On the other hand, we demonstrated that in human ocular surface epithelial cells, PGE_2 attenuated the polyI:C-induced mRNA and protein expression of MCP-1 through EP_2 and EP_3 but not EP_4. Our findings suggest that EP_2 and EP_3 play important roles in the regulation of inflammation in epithelial cells, while EP_2 and EP_4 have important roles in immune cells such as macrophages.

In the tears and serum of patients with acute-stage Stevens-Johnson syndrome or toxic epidermal necrolysis, the levels of IL-6, IL-8, and MCP-1 were dramatically increased.[1] Although IL-8 was not regulated by PGE_2, IL-6 was regulated by PGE_2 via EP_3 in human ocular surface epithelial cells.[3] Herein, we demonstrated that MCP-1 could be regulated by PGE_2 via EP_2 and EP_3. The regulation of cytokine production by PGE_2 may be associated with the pathogenesis of Stevens-Johnson syndrome or toxic epidermal necrolysis with severe ocular complications because it was associated with polymorphism of the EP_3 gene (*PTGER3*), one of the PGE receptors (EP_1, EP_2, EP_3, EP_4).[2]

In summary, our results show that MCP-1 produced by human ocular surface epithelial cells could be downregulated by PGE_2 via EP_2 and EP_3.

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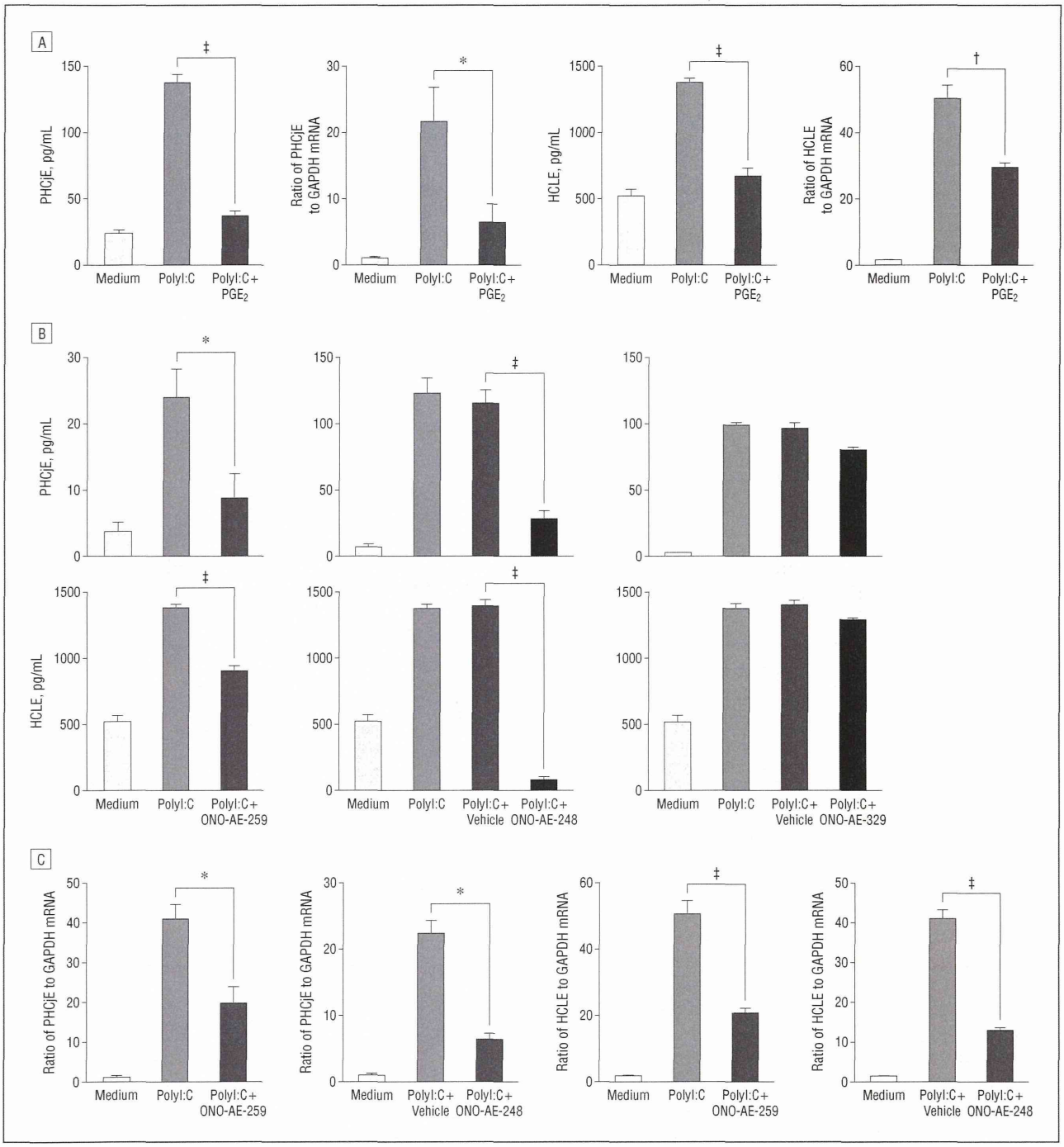

Figure. Prostaglandin E_2 (PGE_2) attenuated the messenger RNA (mRNA) expression and production of monocyte chemoattractant protein 1 via both prostaglandin E receptor 2 (EP_2) and EP_3. A, Primary human conjunctival epithelial cells (PHCjE) and human corneal-limbal epithelial cells (HCLE) were exposed to 10 μg/mL of polyinosine–polycytidylic acid (poly I:C) and 100 μg/mL of PGE_2 for 24 hours (enzyme-linked immunosorbent assay) or 6 hours (quantitative real-time polymerase chain reaction). GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase. B and C, The PHCjE and HCLE were exposed to 10 μg/mL of poly I:C and 10 μg/mL of the EP_2, EP_3, or EP_4 agonist for 24 hours (enzyme-linked immunosorbent assay) (B) or 6 hours (quantitative real-time polymerase chain reaction) (C). Data are representative of 3 separate experiments and are given as the mean (SEM) from 1 experiment carried out in 6 to 8 wells (enzyme-linked immunosorbent assay) (B) or 4 to 6 wells (quantitative real-time polymerase chain reaction) (C) per group. *$P<.05$; †$P<.005$; ‡$P<.001$.

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Author Contributions: Dr Ueta had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Online-Only Material: The eAppendix is available at http://www.archophthalmol.com.
Additional Contributions: Chikako Endo provided technical assistance.

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Depth Profile Study of Abnormal Collagen Orientation in Keratoconus Corneas

In a previous study,[1] we used femtosecond laser technology to cut ex vivo human corneas into anterior, mid, and posterior sections, after which x-ray scatter patterns were obtained at fine intervals over each specimen. Data analysis revealed the predominant orientation of collagen at each sampling site, which was assembled to show the variation in collagen orientation between central and peripheral regions of the cornea and as a function of tissue depth. We hypothesized that the predominantly orthogonal arrangement of collagen (directed toward opposing sets of rectus muscles) in the mid and posterior stroma may help to distribute strain in the cornea by allowing it to withstand the pull of the extraocular muscles. It was also suggested that the more isotropic arrangement in the anterior stroma may play a role in tissue biomechanics by resisting intraocular pressure while at the same time maintaining corneal curvature. This article, in conjunction with our findings of abnormal collagen orientation in full-thickness keratoconus corneas,[2,3] received a great deal of interest from the scientific community and prompted the following question: how does collagen orientation change as a function of tissue depth when the anterior curvature of the cornea is abnormal, as in keratoconus? Herein, we report findings from our investigation aimed at answering this question.

Methods. The Baron chamber used in our previous study[1] was adapted to enable corneal buttons to be clamped in place and inflated (by pumping physiological saline into the posterior compartment) to restore their natural curvature. A button diameter of 8 mm or larger was deemed necessary to ensure tissue stability during this process.

The next step, obtaining fresh, full-thickness, keratoconus buttons of sufficient diameter, proved to be problematic owing to the increasing popularity of deep anterior lamellar keratoplasty. Recently, however, the opportunity arose to examine an 8-mm full-thickness (300-340 µm minus epithelium) keratoconus corneal button with some central scarring and a mean power greater than 51.8 diopters (**Figure 1**). The tissue was obtained in accordance with the tenets of the Declaration of Helsinki and with full informed consent from a 31-year-old patient at the time of penetrating keratoplasty. Using techniques detailed previously,[1] the corneal button was clamped in the chamber and inflated. The central 6.3-mm region of the button was then flattened by the applanation cone and a single cut was made at a depth of 150 µm from the surface using an IntraLase 60-kHz femtosecond laser (Abbott Medical Optics Inc),[1] thus splitting the cornea into anterior and posterior sections of roughly equal thickness. Wide-angle x-ray scattering patterns were collected at 0.25-mm intervals over each cor-

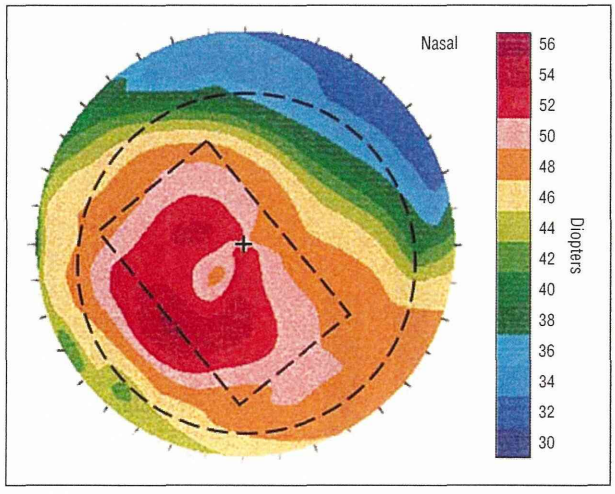

Figure 1. Corneal topography of the keratoconus cornea (recorded 12 years previously).[3] The broken lines show the 6.3-mm region of the cornea cut with the femtosecond laser (circle) and the region of greatest corneal steepening depicted in Figure 2 (rectangle).

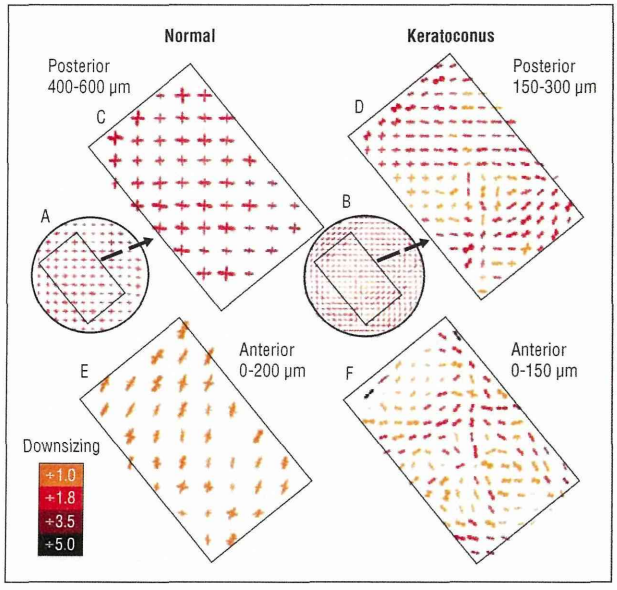

Figure 2. Collagen orientation in the normal (A) and keratoconus (B) posterior stroma (central 6.3 mm). The highlighted regions of the posterior (C and D) and anterior (E and F) stroma are expanded. Large vector plots showing high collagen alignment are downsized (key).

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.

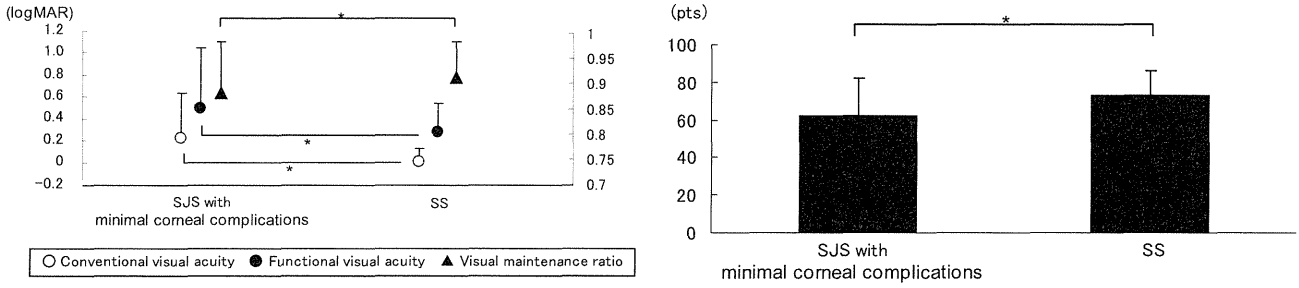

FIGURE 1. Visual function and Visual Function Questionnaire-25 in Stevens-Johnson syndrome (SJS) with minimal corneal complications and Sjögren syndrome (SS) patients. (Left) Conventional and functional visual acuity and visual maintenance ratio in Stevens-Johnson syndrome with minimal corneal complications 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.

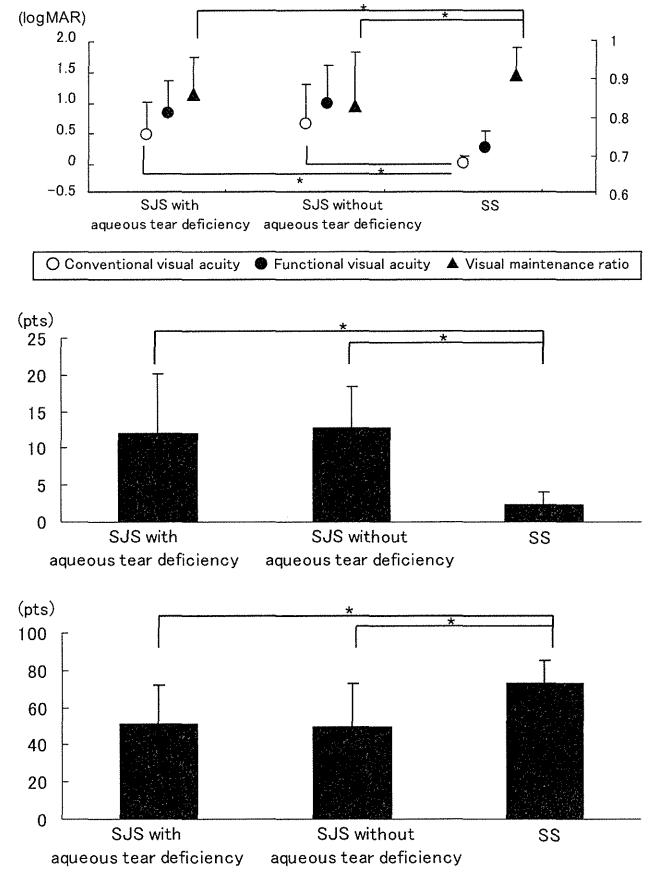

FIGURE 2. Visual function, ocular surface grading score, and Visual Function Questionnaire-25 in Stevens-Johnson syndrome (SJS) patients with and without aqueous tear deficiency and Sjögren syndrome (SS) patients. (Top) Conventional and functional visual acuity and visual maintenance ratio in Stevens-Johnson 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