Table 1. Human leukocyte antigen (HLA)-B genotypes of patients

HLA-B	Patients (n = 22)	Population (n = 371)†	HLA-B	Patients (n = 22)	Population (n = 371)†	HLA-B	Patients (n = 22)	Population (n = 371)†
B*0702	1 (2.2%)	48 (6.5%)	B*4001	2 (4.5%)	31 (4.2%)	B*5101	7 (15.9%)	57 (7.7 %)
B*1501	3 (6.8%)	65 (8.7%)	B*4002	3 (6.8%)	64 (8.6%)	B*5201	7 (15.9%)	79 (10.7%)
B*1518	2 (4.5%)	11 (1.5%)	B*4006	1 (2.2%)	29 (3.9%)	B*5401	2 (4.5%)	57 (7.7%)
B*3501	4 (9%)	56 (7.6%)	B*4403	1 (2.2%)	65 (8.7%)	B*5502	1 (2.2%)	14 (1.9%)
B*3901	2 (4.5%)	33 (4.4%)	B*4601	3 (6.8%)	27 (3.6%)	B*5901	1 (2.2%)	13 (1.8%)
B*3902**	2 (4.5%)	4 (0.5%)	B*4801	2 (4.5%)	22 (3%)	B*1502	0 (0%)	1 (0.1%)

†Allele count of the population was obtained from.[7] **$P < 0.05$.

Table 2. Human leukocyte antigen (HLA)-A genotypes of patients

HLA-A	Patients (n = 22)	Population (n = 371)†
A*0201	2 (4.5%)	85 (11.5%)
A*0206	2 (4.5%)	57 (7.7%)
A*0207	3 (6.8%)	16 (2.2%)
A*1101	3 (6.8%)	61 (8.2%)
A*2402	18 (41%)	281 (38%)
A*2601	1 (2.2%)	60 (8.1%)
A*2603	3 (6.8%)	18 (2.4%)
A*3101**	11 (25%)	53 (7.1%)
A*3303	1 (2.2%)	71 (9.7%)

†Allele count of the population was obtained from [7]. **$P < 0.01$.

replicate the result.[8–10] However, instead of achieving a simple association, they have revealed a complex spectrum of the association between this marker and cADR. The original group have shown that carmabazepine-induced non-SJS/TEN cADR do not associate with this marker.[10] Thus, our result that B*1502 is absent in our non-SJS/TEN cADR cases appears to be along the same line. However, we did not find B*1502 in two of our SJS cases. The HLA-B of these SJS patients were HLA-B*3501, B*5901 (case 11) and B*1518, B*4002 (case 13). It has been found that the HLA-B*1502 allele is much rarer in Japanese subjects (allele frequency, 0.1%),[7] compared to Han-Chinese subjects in Taiwan (allele frequency, 8.6%)[5] and people in north-eastern Thailand (allele frequency, 52.5%).[11] Thus, it appears that this biomarker has limited clinical use in predicting SJS in Japanese patients.

Recently, Ueta *et al.* studied 40 cases of SJS who had suffered ocular symptoms in Japan.[12] Although the causative drug was not carmabazepine alone, they also did not detect any B*1502 allele in their subjects. Instead, they found an overall association with HLA-A*0206. We also found two cases possessing HLA-A*0206 but these were not associated with severe cADR in our study population (Table 2).

In conclusion, we performed HLA genotyping in subjects in whom carmabazepine-induced cADR had occurred and who needed hospitalization, including two SJS cases. We could not find HLA-B*1502 in any of these subjects. It is unlikely that HLA-B*1502 will be a satisfactory genetic marker of carbamazepine-induced cADR in Japanese subjects. We found an association with HLA-A*3101, but whether this is a risk allele warrants further study in a larger sample size.

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REFERENCES

1 Ferraro TN, Buono RJ. The relationship between pharmacology of antiepileptic drugs and human gene variation. *Epilepsy Behav* 2005; **7**: 18–36.
2 Aihara Y, Ito SI, Kobayashi Y *et al.* Carmabazepine-induced hypersensitivity syndrome associated with transient hypogammmaglobulinemia and reactivation of human herpes 6 infection demonstrated by real-time quantitative polymerase chain reaction. *Brit J Dermatol* 2003; **149**: 165–169.
3 Ranzey B, Correia O, Kelly JP *et al.* Risks of Stevens-Johnson Syndrome and Toxic epidermal necrosis during first weeks of antiepileptic therapy: a case-control study. *Lancet* 1999; **353**: 2190–2194.

4 Rzany B, Mockenhuupt M, Baur S *et al*. Epidemiology of erythema exsudativum multiforme majus, Stevens-Johnson Syndrome, and toxic epidermal necrolysis in Germany (1990–1992): structure and results of a population-based registry. *J Clin Epidem* 1996; **49**: 769–773.
5 Chung WH, Hung SI, Hong HS *et al*. Medical genetics; a marker for Stevens-Johnson syndrome. *Nature* 2004; **8**: 486.
6 Lonjou C, Thomas T, Borot N *et al*. A marker for Stevens-Johnson syndrome ethnicity matters. *Pharmacogenomics J* 2006; **6**: 265–268.
7 Saito S, Ota S, Yamada E *et al*. Allele frequencies and haplotypic associations defined by alleiic DNA typing at HLA class I and class II loci in the Japanese population. *Tissue Antigens* 2000; **56**: 522–529.
8 Alfirevic A, Jorgensen AL, Williamson PR *et al*. HLA-B locus in Caucasian patients with carbamazepine hypersensitivity. *Pharamacogenomics* 2006; **7**: 813–818.
9 Man CB, Kwan P, Baum L *et al*. Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 2007; **48**: 1015–1018.
10 Hung SL, Chung WH, Jee SH *et al*. Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenetics Genomics* 2006; **16**: 297–306.
11 Romphruk A, Phongaen K, Chotechai J *et al*. HLA-B*15 subtypes in the population of north-eastern Thailand. *Eur J Immuno Genet* 2003; **30**: 153–158.
12 Ueta M, Sotozono C, Tokunaga K *et al*. Strong association between HLA-A*0206 and Stevens-Johnsons Syndrome in the Japanese. *Am J Opthal* 2007; **143**: 367–368.

Association of Combined IL-13/IL-4R Signaling Pathway Gene Polymorphism with Stevens-Johnson Syndrome Accompanied by Ocular Surface Complications

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Purpose. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute-onset mucocutaneous diseases induced by infectious agents or inciting drugs. The authors previously reported an association between SJS/TEN and *IL-4R* gene polymorphism that is essential for IL-4 and IL-13 signaling. To examine *IL-4* and *IL-13* gene polymorphisms and the combination of these polymorphisms with *IL-4R* polymorphism, the authors performed polymorphism analysis.

Methods. In 76 Japanese SJS/TEN patients with ocular surface complications and 160 healthy controls, the authors analyzed polymorphisms of the promoter −590C/T in the *IL-4* gene and of the promoter −1111C/T and Arg110Gln in the *IL-13* gene and assessed Gln551Arg in the *IL-4R* gene. Because Arg110Gln affects serum IL-13, plasma IL-13 levels were also examined.

Results. In the SJS/TEN patients, the Arg110Gln SNP of *IL-13* was significantly associated with the disease, and the frequency of Arg110 alleles was significantly higher than that in the controls. Plasma IL-13 tended to be lower in SJS/TEN patients than in the controls. Analysis of the genotype pattern of *IL-4R* SNP Gln551Arg and *IL-13* SNP Arg110Gln showed that the Gln551Gln(A/A)-Arg110Arg(G/G) genotype pattern was also associated with SJS/TEN.

Conclusions. *IL-13* gene polymorphisms might be associated with SJS/TEN with ocular surface complications. The present findings suggest that SJS/TEN is different from allergic diseases such as atopy and asthma because the ratio of each allele in the *IL-13* SNP Arg110Gln was the opposite of the ratio in those diseases. They also reveal that combined polymorphisms in the IL-13/IL-4R signaling pathway were associated with SJS/TEN with ocular surface complications. (*Invest Ophthalmol Vis Sci.* 2008;49:1809–1813) DOI:10.1167/iovs.07-1401

Stevens-Johnson syndrome (SJS), an acute inflammatory vesiculobullous reaction of the skin and mucous membranes first described in 1922,[1] is commonly associated with infectious agents and inciting drugs.[2,3] When there is extensive skin detachment and a poor prognosis, the condition is called toxic epidermal necrolysis (TEN).[4] In the acute stage, SJS/TEN patients manifest vesiculobullous skin lesions, severe conjunctivitis, and persistent corneal epithelial defects because of ocular surface inflammation. In the chronic stage, ocular surface complications, such as conjunctival invasion into the cornea caused by corneal epithelial stem cell deficiency, symblepharon, ankyloblepharon, and, in some instances, keratinization of the ocular surface, persist despite healing of the skin lesions.[5] SJS/TEN is one of the most devastating ocular surface diseases, and it leads to corneal damage and loss of vision. The reported incidence of ocular surface complications in SJS/TEN is 50% to 68%.[3,6]

We previously reported that not only environmental but also genetic factors may play important roles in an integrated etiology of SJS/TEN and that in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN with ocular surface complications.[7] We also documented that in Japanese patients with SJS/TEN, there was an association with toll-like receptor 3 (*TLR3*) polymorphisms.[2] Furthermore, we found that in Japanese patients with SJS/TEN, there is an association with polymorphisms in the allergy-related *IL-4R* gene and that the ratio of each allele in the polymorphism was the opposite of the ratio reported in atopy and asthma.[8]

IL-4Rα is a component of not only the IL-4 but also the IL-13 receptor and is essential for both IL-4 and IL-13 signaling. The type 1 IL-4 receptor is composed of 2 subunits, an α subunit (IL-4Rα), which binds IL-4 and transduces its growth-promoting and transcription-activating functions, and a γ c subunit, common to several cytokine receptors, that amplifies signaling of IL-4Rα. The IL-13 receptor (IL-13R) is composed of the IL-4Rα chain (IL-4Rα) and the IL-13Rα1 chain (IL-13Rα1).[9] Given that IL-4 is able to bind to this receptor, it is also called type 2 IL-4R.[9] There exists another IL-13 binding unit, the IL-13Rα2 chain (IL-13Rα2), which acts as a decoy receptor.[9]

Because there is an association between SJS/TEN and *IL-4Rα* polymorphism, we speculated that there might be an association between IL-4 or IL-13 signaling and SJS/TEN. Therefore, we examined IL-4 and IL-13 gene polymorphisms and the combination of these polymorphisms with *IL-4R* polymorphism.

With respect to *IL-4* gene polymorphisms, a variant of the promoter region of the *IL-4* gene, −590C/T, has been shown to be related to asthma.[10–12] Regarding *IL-13* gene polymorphisms, a variant of the promoter region of the *IL-13* gene, −1111C/T,[13,14] and a variant of Arg110Gln were reportedly associated with asthma.[15] Gln551Arg of the *IL-4R* gene was associated with atopy[16,17] and asthma.[18]

Here we examined polymorphisms of the promoter −590C/T (rs.2243250) in the *IL-4* gene, of −1111C/T (rs.1800925) and Arg110Gln (rs.20,541) in the *IL-13* gene, and of Gln551Arg (rs.1801275) in the *IL-4R* gene in Japanese SJS/TEN patients with ocular surface complications and healthy volunteers. We also examined their plasma IL-13 level because

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TABLE 1. Genotype Frequencies for Various SNPs and SJS/TEN Susceptibility

	Control (%) n = 160	SJS/TEN (%) n = 76	Allele 1 vs. Allele 2 P OR (95% CI)	Genotype 11 vs. 12 + 22 P OR (95% CI)	Genotype 11 + 12 vs. 22 P OR (95% CI)
IL-4 gene					
Promoter-590 (rs. 2243250)					
11 TT	82 (51.3)	39 (51.3)	0.54	0.99	0.08
12 TC	72 (45.0)	30 (39.5)	—	—	—
22 CC	6 (3.8)	7 (9.2)			
IL-13 gene					
Promoter-1111 (rs. 1800925)					
11 CC	101 (63.1)	57 (75.0)	0.049	0.07	0.23
12 CT	52 (32.5)	18 (23.7)	1.7	—	—
22 TT	7 (4.4)	1 (1.3)	(1.0–3.0)		
Arg(G) 110Gln(A) (rs. 20541)					
11 GG	77 (48.1)	47 (61.8)	0.014	0.049	0.035
12 GA	66 (41.2)	27 (35.5)	1.8	1.8	4.4
22 AA	17 (10.6)	2 (2.6)	(1.1–2.8)	(1.0–3.0)	(1.0–19.6)
IL-4R gene					
Gln(A)551Arg(G) (rs.1801275)					
11 AA	115 (71.9)	69 (90.8)	0.0008	0.0011	—
12 AG	41 (25.6)	7 (9.2)	3.7	3.9	—
22 GG	4 (2.5)	0 (0)	(1.7–8.5)	(1.6–9.0)	

P values were determined by χ^2 testing.

Arg110Gln in the *IL-13* gene has an effect on the serum level of IL-13.[19]

METHODS

Patients

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

For single-nucleotide polymorphism (SNP) analysis, we enrolled 76 patients with SJS/TEN in the chronic or subacute phase; all presented with ocular surface complications. The diagnosis of SJS/TEN was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites including the ocular surface. The controls were 160 healthy volunteers without allergic diseases such as atopic dermatitis or asthma. All participants and volunteers were Japanese residing in Japan. The average age of the patients and controls was 46.1 ± 17.3 (SD) and 36.2 ± 11.5 (SD) years, respectively. The male/female ratios in the patient and control groups were 33:43 and 57:103, respectively.

SNP Analysis

SNP analysis was performed by direct sequencing. PCR and sequence primers for SNPs of *IL-4* were 5′-CTTGAGCCCAGGAATTTGAG-3′ (sense) and 5′-ACAGGTGGCATCTTGGAAAC-3′ (antisense) for the −590 promoter (rs.2243250). For SNPs of *IL-13*, they were 5′-CCACATCTGTACAGTAGAGG-3′ (sense) and 5′-GGCTGAGGTCTAAGCTAAGG-3′ (antisense) for Arg110Gln (rs.20,541), and 5′-ATGCCTTGTGAGGAGGGTCAC-3′ (sense) and 5′-CCAGTCTCTGCAGGATCAACC-3′ (antisense) for promoter-1111 (rs.1800925). For Gln551Arg (rs.1801275) of *IL-4R* SNPs, they were 5′-AGCTTCAGCAACTCCCTGAG-3′ (sense) and 5′-CCCAAACCCACATTTCTCTG-3′ (antisense). Genomic DNA was isolated from human peripheral blood at SRL Inc. (Tokyo, Japan). PCR amplification was with DNA polymerase (Takara, Shiga, Japan) for 35 cycles at 94°C for 1 minute, annealing at 60°C for 1 minute, and 72°C for 1 minute on a commercial PCR machine (GeneAmp; Applied Biosystems, Foster City, CA) The PCR products were reacted (BigDye Terminator v3.1; Applied Biosystems), and sequence reactions were resolved on a genetic analyzer (ABI PRISM 3100; Applied Biosystems).

Statistical Methods Used for SNP Analysis

Alleles were counted manually. Each allele was assessed as an independent variable, and separate *P* values were calculated for each polymorphism. $P < 0.05$ was regarded as significant. In addition, *P* was corrected for the number of alleles tested in each gene (Bonferroni method).

Measurement of Plasma IL-13 Levels

For the measurement of plasma IL-13 levels, we enrolled 29 patients with SJS/TEN in the chronic or subacute phase; all presented with ocular surface complications (Arg110/Arg110, $n = 14$; Arg110/Gln110, $n = 14$; Gln110/Gln110, $n = 1$). Controls were 160 healthy volunteers (Arg110/Arg110, $n = 77$; Arg110/Gln110, $n = 66$; Gln110/Gln110, $n = 17$). Plasma, obtained at the time of genomic DNA isolation from peripheral blood, was used for analysis. Plasma IL-13 levels were immunoassayed with an ELISA kit (Biotrak Easy ELISA; Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. The minimum detectable level was 1.56 pg/mL.

RESULTS

A summary of our case-control association study with the four genotyped SNPs is shown in Table 1. All four SNPs were in Hardy-Weinberg equilibrium in the SJS/TEN patients and the healthy controls ($P > 0.01$). In the promoter −590C/T SNP of the *IL-4* gene related to higher IgE levels,[10] there was no significant association. In the promoter −1111C/T SNP of the *IL-13* gene related to asthma,[13,14] there was a weak association with allele frequency (C vs. T, raw $P = 0.049$, corrected $P = 0.099$; odds ratio = 1.7); correction of the *P* value for the number of alleles detected ($n = 2$) rendered the result not significant. Gln110Arg SNPs of *IL-13* exhibited a significant association with allele frequency (G vs. A, raw $P = 0.014$,

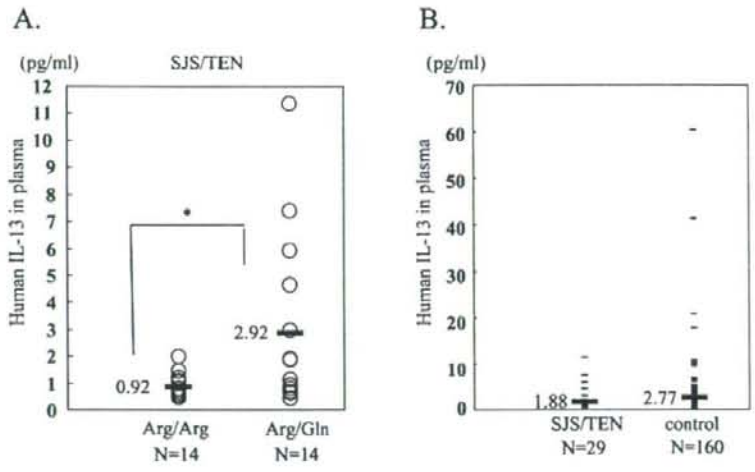

FIGURE 1. Plasma IL-13 levels in SJS/TEN patients. (**A**) Comparison of the plasma IL-13 levels in 28 SJS/TEN patients with Arg110Arg (n = 14) or Arg110Gln (n = 14) showed that levels were significantly higher in patients with Arg110Gln (*P < 0.05). Evaluation was with Student's t-test using a spreadsheet program. (**B**) Comparison of the plasma IL-13 level in 29 SJS/TEN patients (Arg110/Arg110, n = 14; Arg110/Gln110, n = 14; Gln110/Gln110, n = 1) and 160 controls (Arg110/Arg110, n = 77; Arg110/Gln110, n = 66; Gln110/Gln110, n = 17) showed that it tended to be lower in patients than in controls. However, the difference was not statistically significant. Evaluation was with Student's t-test using a spreadsheet program.

corrected P = 0.028; odds ratio = 1.8) even when we corrected P for the number of alleles detected of *IL13* SNPs (n = 2). They also exhibited a weak association with the dominant model (G/G vs. G/A + A/A, raw P = 0.049, corrected P = 0.097; odds ratio = 1.8) and the recessive model (G/G +G/A vs. A/A, raw P = 0.035, corrected P = 0.07; odds ratio = 4.4); correction of the P value for the number of alleles detected (n = 2) rendered the result not significant. These findings contrast with those of Heinzmann et al.,[15] who reported that Gln110 was significantly increased in human asthma. We detected a significant increase in Arg110 in our SJS/TEN patients.

The Gln551Arg SNP of the *IL-4R* gene showed a significant association with allele frequency (A vs. G, raw P = 0.0008; odds ratio = 3.7) and the dominant model (A/A vs. A/G + G/G, raw P = 0.0011; odds ratio = 3.9); these findings coincide with those we reported previously.[8]

We also studied the plasma IL-13 levels in our SJS/TEN patients because these levels were reportedly higher in patients with Gln110.[19] We compared plasma IL-13 levels in Arg110Arg and Arg110Gln genotypes, and, though in the controls it tended to be higher in the Arg110Gln genotype (data not shown), it was significantly higher in SJS/TEN patients with the Arg110Gln than the Arg110Arg genotype (Fig. 1A). Plasma IL-13 levels tended to be lower in SJS/TEN patients than in the controls, but the difference was not statistically significant (Fig. 1B). Our results are in accordance with findings that SJS/TEN with ocular surface complications is associated with Arg110Gln, which affects the plasma IL-13 level; in SJS/TEN there is a significant increase in the Arg110 allele, which might lead to lower serum IL-13 levels than the Gln110 allele.

We also analyzed the genotype pattern of *IL-4R* SNP Arg551Gln and *IL-13* SNP Arg110Gln. We found that the Gln551Gln(A/A)-Arg110Arg(G/G) genotype pattern also associated with SJS/TEN in Japanese patients (χ^2 test; P = 0.0006, OR = 2.6, 95% CI, 1.5–4.6; Table 2). In more detail, 69 of 76 (90.8%) SJS/TEN patients and 115 of 160 (71.9%) controls had IL-4R Gln551Gln (Table 1), and 44 of 76 (57.9%) SJS/TEN patients and 55 of 160 (34.4%) controls had the genotype pattern Gln551Gln(A/A) of the *IL-4R*-Arg110Arg(G/G) of the *IL-13* (type 1 pattern; Table 2). Therefore, 44 of 69 SJS/TEN patients with *IL-4R* Gln551Gln (63.8%) had the type 1 pattern, whereas only 55 of 115 controls with *IL-4R* Gln551Gln (47.8%) had the type 1 pattern. This result shows that SJS/TEN patients with *IL-4R* Gln551Gln have *IL-13* Arg110Arg more frequently than controls with *IL-4R* Gln551Gln; there was a significant difference between SJS/TEN and controls (χ^2 test; P = 0.036, OR = 1.9; 95% CI, 1.0–3.5). Thus, we suggest a combined effect exists between *IL-4R* and *IL-13* polymorphisms.

DISCUSSION

Arg110Gln SNP of *IL-13* was significantly associated with SJS/TEN with ocular surface complications. Arg110Gln affects the

TABLE 2. Pattern Structures and Frequencies of *IL-13* SNP Arg110Gln and *IL-4R* SNP Arg551Gln

Pattern Type	*IL-4R* SNP Arg551 Gln	*IL-13* SNP Arg110 Gln	Control (%) n = 160	SJS/TE (%) n = 76	P	OR (95% CI)
1	A/A	G/G	55/160 (34.4)	44/76 (57.9)	0.0006	2.6 (1.5–4.6)
2	A/A	A/G	48/160 (30.0)	23/76 (30.3)	NS	—
3	A/G	G/G	20/160 (12.5)	3/76 (3.9)	NS	—
4	A/G	A/G	16/160 (10.0)	4/76 (5.3)	NS	—
5	A/A	A/A	12/160 (7.5)	2/76 (2.6)	NS	—
6	G/G	G/G	2/160 (1.3)	0/76 (0.0)	NS	—
7	G/G	A/G	2/160 (1.3)	0/76 (0.0)	NS	—
8	A/G	A/A	2/160 (1.3)	0/76 (0.0)	NS	—

P values were determined by χ^2 testing.

plasma IL-13 level.[19] In SJS/TEN, Arg110 alleles are significantly increased, but in atopy and asthma, Gln110 alleles are significantly increased.[15] Plasma IL-13 tended to be lower in our SJS/TEN patients with ocular surface complications than in the controls because plasma IL-13 was lower in the presence of Arg110Arg than in the presence of Gln110Arg.[19] Although our results suggest that in Japanese SJS/TEN patients with ocular surface complications there might be an association with polymorphisms in the allergy-related *IL-13* genes, SJS/TEN is different from allergic diseases such as atopy and asthma because the ratio of each allele of the *IL-13* SNP Arg110Gln was opposite the ratio in atopy and asthma. In SJS/TEN, Arg110 rather than Gln110 alleles (which are significantly increased in asthma)[15] showed a significant increase. Arima et al.[19] have reported that the Gln110 variant of Gln110Arg decreased the affinity with IL-13Rα2, a decoy receptor, and enhanced stability as a protein, causing upregulation of the IL-13 concentration in vivo. The results we obtained by polymorphism analysis were supported by our findings that the plasma IL-13 level tended to be lower in patients with SJS/TEN.

Given that there is an association with *IL-4R* gene polymorphisms in SJS/TEN,[8] we analyzed the genotype pattern of the *IL-4R* SNP Gln551Arg and the *IL-13* SNP Arg110Gln. We found that Gln551Gln(A/A) of the *IL-4R*-Arg110Arg(G/G) of the *IL-13* genotype pattern associated with SJS/TEN in Japanese patients.

These results reveal not only that *IL-13* and *IL-4R* gene polymorphisms but also that combined polymorphisms in the IL-13/IL-4R signaling pathway are associated with SJS/TEN with ocular surface complications.

We previously reported that SJS/TEN was associated with Gln551Arg of *IL-4R* polymorphisms, which had no effect on IgE synthesis.[8] In addition, in Gln551Arg polymorphisms, Gln551 but not Arg551 alleles were significantly increased in SJS/TEN, whereas Arg551 alleles were significantly increased in atopy and asthma. Our earlier study on the relationship between serum IgE and SJS/TEN also showed that there was no significant difference between SJS/TEN patients and controls with respect to the incidence of high total serum IgE.[8]

In Arg110Gln of *IL-13* polymorphisms and Gln551Arg of *IL-4R* polymorphisms, the ratio of each allele was the inverse of the ratio reported for atopy and asthma; therefore, SJS/TEN appears to be different from those allergic diseases. Moreover, combined polymorphisms in the IL-13/IL-4R signaling pathway are also associated with SJS/TEN patients; we document that Gln551Gln(A/A) of the *IL-4R* and Arg110Arg(G/G) of the *IL-13* genotype pattern were also associated with SJS/TEN with ocular surface complications.

In patients with acute-phase SJS/TEN, dermatologists have examined IL-13 levels in serum or skin lesions and reported that the expression level of IL-13 is upregulated in patients with Steven-Johnson syndrome. They also reported that normalization in serum IL-13 levels was demonstrated in all three patients with SJS/TEN, who were tested after the resolution of the cutaneous disease. In this study, we examined the serum IL-13 levels in SJS/TEN patients in the chronic phase or the subacute phase, in which the cutaneous disease was resolved. Thus, we suggest that the serum IL-13 levels of baseline such as in the chronic phase or the subacute phase, but not in the acute phase, tend to be lower in SJS/TEN patients with ocular surface complications than in the controls because the Arg110Arg genotype, in which the IL-13 level was reportedly lower than in Arg110Gln, was significantly increased in SJS/TEN patients with ocular surface complications.

It has been suggested that the pathogenesis of TEN is immunologically mediated and that it involves cytotoxic $CD8^+$ lymphocytes.[20,21] Given that $CD8^+$ T cells involve Th1 cytokine-driven inflammatory mechanisms, such mechanisms may be involved in the skin inflammation seen in the acute stage of SJS/TEN. In contrast, Th2 cytokine-driven inflammatory mechanisms may play a role in the inflammation seen in allergic diseases such as atopy and asthma.[22] Thus, genetic alterations in the IL-13/IL-4R signaling pathway may regulate Th1 or Th2 cytokine-driven inflammatory mechanisms.

We suggested elsewhere that IL-4R might be linked to innate immunity.[8] The innate immune system may constitute a link between the environment and the adaptive immune system. We are continuing to examine the pathophysiology of SJS/TEN with ocular surface complications.

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References

1. Stevens AM, Johnson FC. A new eruptive fever associated with stomatitis and ophthalmia: report of two cases in children. *Am J Dis Child*. 1922;24:526-533.
2. Ueta M, Sotozono C, Inatomi T, et al. Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens-Johnson syndrome. *Br J Ophthalmol*. 2007;91:962-965.
3. Yetiv JZ, Bianchine JR, Owen JA Jr. Etiologic factors of the Stevens-Johnson syndrome. *South Med J*. 1980;73:599-602.
4. Auquier-Dunant A, Mockenhaupt M, Naldi L, et al. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. *Arch Dermatol*. 2002;138:1019-1024.
5. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. *Ophthalmology*. 2007;114:1294-1302.
6. Power WJ, Ghoraishi M, Merayo-Lloves J, Neves RA, Foster CS. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. *Opbthalmology*. 1995;102:1669-1676.
7. Ueta M, Sotozono C, Tokunaga K, Yabe T, Kinoshita S. Strong association between HLA-A*0206 and Stevens-Johnson syndrome in the Japanese. *Am J Opbthalmol*. 2007;143:367-368.
8. Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S. Association of IL-4R polymorphisms with Stevens-Johnson syndrome. *J Allergy Clin Immunol*. 2007;120:1457-1459.
9. Izuhara K, Arima K. Signal transduction of IL-13 and its role in the pathogenesis of bronchial asthma. *Drug News Perspect*. 2004;17:91-98.
10. Basehore MJ, Howard TD, Lange LA, et al. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol*. 2004;114:80-87.
11. Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin-4 promoter gene in Japanese. *Clin Exp Allergy*. 1998;28:449-453.
12. Walley AJ, Cookson WO. Investigation of an interleukin-4 promoter polymorphism for associations with asthma and atopy. *J Med Genet*. 1996;33:689-692.
13. Howard TD, Koppelman GH, Xu J, et al. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *Am J Hum Genet*. 2002;70:230-236.
14. Kabesch M, Schedel M, Carr D, et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. *J Allergy Clin Immunol*. 2006;117:269-274.
15. Heinzmann A, Mao XQ, Akaiwa M, et al. Genetic variants of IL-13 signalling and human asthma and atopy. *Hum Mol Genet*. 2000;9:549-559.
16. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med*. 1997;337:1720-1725.

17. Oiso N, Fukai K, Ishii M. Interleukin 4 receptor alpha chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan. *Br J Dermatol*. 2000;142:1003–1006.
18. Rosa-Rosa L, Zimmermann N, Bernstein JA, Rothenberg ME, Khurana Hershey GK. The R576 IL-4 receptor alpha allele correlates with asthma severity. *J Allergy Clin Immunol*. 1999;104:1008–1014.
19. Arima K, Umeshita-Suyama R, Sakata Y, et al. Upregulation of IL-13 concentration in vivo by the IL13 variant associated with bronchial asthma. *J Allergy Clin Immunol*. 2002;109:980–987.
20. Correia O, Delgado L, Ramos JP, Resende C, Torrinha JA. Cutaneous T-cell recruitment in toxic epidermal necrolysis: further evidence of $CD8^+$ lymphocyte involvement. *Arch Dermatol*. 1993;129:466–468.
21. Miyauchi H, Hosokawa H, Akaeda T, Iba H, Asada Y. T-cell subsets in drug-induced toxic epidermal necrolysis: possible pathogenic mechanism induced by CD8-positive T cells. *Arch Dermatol*. 1991;127:851–855.
22. Del Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. *Allergy*. 1992;47:450–455.

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HLA class I and II gene polymorphisms in Stevens-Johnson syndrome with ocular complications in Japanese

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Purpose: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute-onset mucocutaneous diseases induced by infectious agents and/or inciting drugs. Although the pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established, the extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility. Our previous study of polymorphisms in the HLA-class I genes of 40 Japanese SJS/TEN patients with ocular surface complications showed that in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN. In this study, we investigated the association between HLA class II antigens in addition to HLA class I antigens and SJS/TEN.

Methods: We studied the histocompatibility antigen genes, HLA-A, B, C, DRB1, and DQB1, of 71 Japanese SJS/TEN patients with ocular complications. We also genotyped 113 healthy volunteers for HLA-A, B, C, DRB1, and DQB1. We performed polymerase chain reaction amplification followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial bead-based typing kits.

Results: HLA-A*0206 was strongly associated with SJS/TEN. HLA-A*1101 was inversely associated. HLA-B*5901 exhibited a high odds ratio for SJS/TEN with ocular complications. However, when we corrected the p-value for the number of alleles detected (n=29), the results ceased to be significant. There was no association between HLA-C and SJS/TEN. There was also no significant association between HLA-DRB1 and SJS/TEN. HLA-DQB1*0502 was negatively and weakly associated with SJS/TEN although correction of the p-value for the number of alleles detected rendered the result not significant.

Conclusions: Because our findings are completely different from data reported on Caucasian patients, they suggest strong ethnic differences in the HLA-SJS associations.

Stevens-Johnson syndrome (SJS), an acute inflammatory vesiculobullous reaction of the skin and mucous membranes first described in 1922 [1], is commonly associated with infectious agents and/or inciting drugs [2,3]. In patients with extensive skin detachment and a poor prognosis, the condition is called toxic epidermal necrolysis (TEN) [4]. Although erythema multiforme (EM), SJS, and TEN were formerly accepted as part of a single "EM spectrum," a retrospective analysis of the type and distribution of skin lesions and the extent of epidermal detachment identified EM major and SJS/TEN as two separate clinical entities that differed with respect to histopathologic changes and etiology [5]. The annual incidence of SJS and TEN has been estimated as 0.4–1 and 1–6 cases per million persons, respectively [3,6]; the mortality rate is 3% and 27%, respectively [7]. Although rare, these reactions carry high morbidity and mortality rates and often result in severe and definitive sequelae such as vision loss. The pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established although the involvement of immune mechanisms [8,9], especially altered drug metabolism [10] and infections such as *Mycoplasma pneumoniae* [11], has been suggested. The extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility.

In the acute stage, SJS/TEN patients manifest severe conjunctivitis and persistent corneal epithelial defects due to ocular surface inflammation with vesiculobullous skin lesions. In the chronic stage, ocular surface complications, such as conjunctival invasion into the cornea due to corneal epithelial stem cell deficiency, symblepharon, ankyloblepharon, and in some instances, keratinization of the ocular surface, persist despite the healing of the skin lesions [12]. Moreover, we observed that more than 95% of patients with SJS/TEN with ocular complications had lost their fingernails in the acute or sub-acute stage and that some continue to have transformed nails even after the healing of their skin lesions [2,13]. SJS/TEN is one of the most devastating ocular surface diseases, leading to corneal damage

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and loss of vision. The reported incidence of ocular complications in SJS/TEN is 50%–68% [3,7]. In this study, we focused on patients with SJS/TEN accompanied by ocular surface complications.

Our previous study of polymorphisms in the HLA-class I genes (HLA-A, B, C) of 40 Japanese SJS/TEN patients with ocular surface complications showed that in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN with ocular surface complications [13]. We also documented that in Japanese SJS/TEN patients with ocular surface complications there was an association with TLR3 polymorphisms [2] and with IL4R polymorphisms [14]. Thus, genetic factors play an important role in an integrated etiology of SJS/TEN. However, HLA class II gene polymorphisms of Japanese SJS/TEN patients have not yet been reported.

Under the hypothesis of an immunologic reaction in susceptible individuals, we studied HLA class II (DRB1 and DQB1) gene polymorphisms in addition to HLA class I (HLA-A, B, C) in 71 Japanese SJS/TEN patients with ocular surface complications.

Table 1. HLA-A alleles and SJS/TEN with ocular complications.

HLA-A alleles	Carrier frequency				Allele frequency			
	SJS (n=71)	Normal (n=113)	p-value (X2)	Odds ratio	SJS (n=142)	Normal (n=226)	p-value (X2)	Odds ratio
*0101	0 (0/71)	0.035 (4/113)	0.16	-	0 (0/142)	0.018 (4/226)	0.3	-
*0201	0.268 (19/71)	0.221 (25/113)	0.47	-	0.155 (22/142)	0.111 (25/226)	0.22	-
*0206	0.423 (30/71)	0.15 (17/113)	0.00004 *	4.1	0.225 (32/142)	0.084 (19/226)	0.0001 *	3.2
*0207	0.07 (5/71)	0.08 (9/113)	1	-	0.035 (5/142)	0.04 (9/226)	1	-
*0301	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*1101	0.056 (4/71)	0.204 (23/113)	0.005	0.23	0.028 (4/142)	0.115 (26/226)	0.003 *	0.22
*2402	0.521 (37/71)	0.566 (64/113)	0.55	-	0.296 (42/142)	0.332 (75/226)	0.47	-
*2601	0.099 (7/71)	0.115 (13/113)	0.81	-	0.049 (7/142)	0.062 (14/226)	0.65	-
*2602	0.056 (4/71)	0.035 (4/113)	0.49	-	0.028 (4/142)	0.018 (4/226)	0.49	-
*2603	0.014 (1/71)	0.027 (3/113)	1	-	0.007 (1/142)	0.013 (3/226)	1	-
*3001	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*3101	0.099 (7/71)	0.186 (21/113)	0.14	-	0.049 (7/142)	0.093 (21/226)	0.16	-
*3303	0.225 (16/71)	0.212 (24/113)	0.84	-	0.113 (16/142)	0.111 (25/226)	0.95	-

HLA-A*0206 was strongly associated with SJS/TEN with ocular complications (carrier frequency: p<0.00005, corrected p value (Pc)<0.0005, OR=4.1; allele frequency: p<0.0005, Pc<0.005, OR=3.2). HLA-A*1101 was inversely associated (carrier frequency: p<0.01, Pc=0.07, OR=0.23; allele frequency: p<0.005, Pc<0.05, OR=0.22). SJS/TEN patients in this study consisted of 40 previously analyzed patients and 31 new patients. These results validate the strong association between HLA-A*0206 and SJS/TEN that we reported previously [13].

METHODS

Patients: This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Helsinki Declaration. The purpose of the research and the experimental protocols were explained to all participants, and their written informed consent was obtained.

Table 2. HLA-B alleles and SJS/TEN with ocular complications.

HLA-B alleles	Carrier frequency				Allele frequency			
	SJS (n=71)	Normal (n=113)	p	Odds ratio	SJS (n=142)	Normal (n=226)	p	Odds ratio
*0702	0.113 (8/71)	0.106 (12/113)	1	-	0.056 (8/142)	0.053 (12/226)	1	-
*1301	0.07 (5/71)	0.027 (3/113)	0.26	-	0.035 (5/142)	0.013 (3/226)	0.27	-
*1302	0 (0/71)	0.009 (1/113)	1	-	0 (0/142)	0.004 (1/226)	1	-
*1501	0.042 (3/71)	0.115 (13/113)	0.11	-	0.028 (4/142)	0.058 (13/226)	0.21	-
*1511	0 (0/71)	0.009 (1/113)	1	-	0 (0/142)	0.004 (1/226)	1	-
*1518	0.014 (1/71)	0.035 (4/113)	0.65	-	0.007 (1/142)	0.018 (4/226)	0.65	-
*1527	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*2704	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*3501	0.211 (15/71)	0.15 (17/113)	0.29	-	0.106 (15/142)	0.084 (19/226)	0.49	-
*3701	0 (0/71)	0.027 (3/113)	0.29	-	0 (0/142)	0.013 (3/226)	0.29	-
*3802	0 (0/71)	0.009 (1/113)	1	-	0 (0/142)	0.004 (1/226)	1	-
*3901	0.085 (6/71)	0.071 (8/113)	0.78	-	0.042 (6/142)	0.035 (8/226)	0.78	-
*3902	0.014 (1/71)	0.018 (2/113)	1	-	0.007 (1/142)	0.009 (2/226)	1	-
*4001	0.169 (12/71)	0.124 (14/113)	0.39	-	0.092 (13/142)	0.062 (14/226)	0.29	-
*4002	0.127 (9/71)	0.133 (15/113)	1	-	0.07 (10/142)	0.066 (15/226)	0.88	-
*4003	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*4006	0.099 (7/71)	0.097 (11/113)	1	-	0.049 (7/142)	0.049 (11/226)	1	-
*4402	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*4403	0.225 (16/71)	0.204 (23/113)	0.72	-	0.12 (17/142)	0.106 (24/226)	0.69	-
*4601	0.085 (6/71)	0.115 (13/113)	0.62	-	0.042 (6/142)	0.062 (14/226)	0.49	-
*4801	0.028 (2/71)	0.088 (10/113)	0.13	-	0.014 (2/142)	0.044 (10/226)	0.14	-
*5101	0.197 (14/71)	0.124 (14/113)	0.18	-	0.113 (16/142)	0.066 (15/226)	0.12	-
*5102	0.028 (2/71)	0.009 (1/113)	0.56	-	0.014 (2/142)	0.004 (1/226)	0.56	-
*5201	0.127 (9/71)	0.212 (24/113)	0.17	-	0.07 (10/142)	0.115 (26/226)	0.16	-
*5401	0.042 (3/71)	0.133 (15/113)	0.07	-	0.021 (3/142)	0.066 (15/226)	0.08	-
*5502	0.028 (2/71)	0.044 (5/113)	0.71	-	0.014 (2/142)	0.022 (5/226)	0.71	-
*5601	0.028 (2/71)	0.027 (3/113)	1	-	0.014 (2/142)	0.013 (3/226)	1	-
*5901	0.113 (8/71)	0.018 (2/113)	0.01	?	0.056 (8/142)	0.009 (2/226)	0.02	6.7
*6701	0 (0/71)	0.035 (4/113)	0.16	-	0 (0/142)	0.018 (4/226)	0.3	-

HLA-B*5901 exhibited a high odds ratio for SJS/TEN with ocular complications (carrier frequency: p<0.05, Pc=0.42, OR=7.0; allele frequency: p<0.05, Pc=0.46, OR=6.7). However, when we corrected the p-value for the number of alleles detected (n=29), the results ceased to be significant.

For HLA genotyping, we enrolled 71 Japanese patients with SJS/TEN in the chronic or sub-acute phase; all presented with ocular surface complications. The diagnosis of SJS/TEN was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites including the ocular surface. The average patient age was 45.8 ± 17.4 years; the male:female ratio was 31:40.

Controls: The normal control group consisted of 113 healthy volunteer blood donors for HLA class I (A, B, C) and HLA class II (DRB1, DQB1) for genotyping. All volunteers were Japanese residing in Japan.

HLA genotyping: We studied the histocompatibility antigen genes HLA-A, B, C, DRB1, and DQB1 of 71 Japanese SJS/TEN patients with ocular complications. We also genotyped 113 healthy volunteers for HLA-A, B, C, DRB1, and DQB1. These alleles were detected by the polymerase chain reaction (PCR)-Luminex typing method using the WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan). First, the target DNA was amplified by polymerase chain reactions with biotinylated primers specifically designed for each HLA locus. Then, the PCR product was denatured and hybridized to complementary oligonucleotide probes immobilized on fluorescent coded microsphere beads. At the same time, biotinylated PCR product was labeled with phycoerythrin-conjugated streptavidin and immediately examined with the Luminex 100 system. Genotype determination and data analysis were performed automatically, using the WAKFlow typing software.

Table 3. HLA-C alleles and SJS/TEN with ocular complications.

HLA-C alleles	Carrier frequency				Allele frequency			
	SJS (n=71)	Normal (n=113)	p	Odds ratio	SJS (n=142)	Normal (n=226)	p	Odds ratio
*0102	0.268 (19/71)	0.327 (37/113)	0.4	-	0.141 (20/142)	0.168 (38/226)	0.48	-
*0303	0.239 (17/71)	0.168 (19/113)	0.24	-	0.12 (17/142)	0.093 (21/226)	0.41	-
*0304	0.366 (26/71)	0.23 (26/113)	0.046	1.9	0.197 (28/142)	0.124 (28/226)	0.057	-
*0401	0.07 (5/71)	0.115 (13/113)	0.45	-	0.035 (5/142)	0.058 (13/226)	0.46	-
*0501	0.014 (1/71)	0 (0/113)	0.39	-	0.007 (1/142)	0 (0/226)	0.39	-
*0602	0 (0/71)	0.035 (4/113)	0.16	-	0 (0/142)	0.018 (4/226)	0.3	-
*0702	0.211 (15/71)	0.212 (24/113)	0.99	-	0.12 (17/142)	0.119 (27/226)	0.99	-
*0704	0.014 (1/71)	0.018 (2/113)	1	-	0.007 (1/142)	0.009 (2/226)	1	-
*0801	0.099 (7/71)	0.168 (19/113)	0.28	-	0.049 (7/142)	0.088 (20/226)	0.22	-
*0803	0.028 (2/71)	0.053 (6/113)	0.71	-	0.014 (2/142)	0.027 (6/226)	0.71	-
*1202	0.127 (9/71)	0.212 (24/113)	0.17	-	0.07 (10/142)	0.115 (26/226)	0.16	-
*1402	0.141 (10/71)	0.097 (11/113)	0.37	-	0.078 (11/142)	0.053 (12/226)	0.35	-
*1403	0.225 (16/71)	0.204 (23/113)	0.72	-	0.113 (16/142)	0.106 (24/226)	0.85	-
*1502	0.099 (7/71)	0.044 (5/113)	0.22	-	0.049 (7/142)	0.022 (5/226)	0.23	-

There was no association between HLA-C and SJS/TEN with ocular complications.

Statistical methods: For statistical analysis to compare carrier frequency and gene frequency, we used the χ^2-test for statistical analysis when the sample number was 10 and more than 10 and used the Fischer's exact test when the sample number was less than 10. The odds ratio (OR) with 95% confidence intervals (95% CI) was calculated using Labo Server software (World Fusion, Tokyo, Japan). Each allele was assessed as an independent variable and separate p values were calculated. A p value of <0.05 was regarded as significant. In addition, the p values were corrected for the number of alleles tested.

RESULTS

As shown in Table 1, HLA-A*0206 was strongly associated with SJS/TEN with ocular complications (carrier frequency: p<0.00005, corrected p value (Pc)<0.0005, OR=4.1; allele

Table 4. HLA-DRB1 alleles and SJS/TEN with ocular complications.

HLA-DRB1 alleles	Carrier frequency				Allele frequency			
	SJS (n=71)	Normal (n=113)	p	Odds ratio	SJS (n=142)	Normal (n=226)	p	Odds ratio
*0101	0.113 (8/71)	0.097 (11/113)	0.81	-	0.056 (8/142)	0.049 (11/226)	0.81	-
*0401	0.028 (2/71)	0.018 (2/113)	0.64	-	0.014 (2/142)	0.009 (2/226)	0.64	-
*0403	0.028 (2/71)	0.053 (6/113)	0.71	-	0.014 (2/142)	0.031 (7/226)	0.49	-
*0404	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*0405	0.197 (14/71)	0.248 (28/113)	0.43	-	0.106 (15/142)	0.133 (30/226)	0.44	-
*0406	0.028 (2/71)	0.062 (7/113)	0.49	-	0.014 (2/142)	0.031 (7/226)	0.49	-
*0407	0 (0/71)	0.035 (4/113)	0.16	-	0 (0/142)	0.018 (4/226)	0.3	-
*0410	0.028 (2/71)	0.018 (2/113)	0.64	-	0.014 (2/142)	0.009 (2/226)	0.64	-
*0701	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*0802	0.113 (8/71)	0.062 (7/113)	0.27	-	0.056 (8/142)	0.031 (7/226)	0.28	-
*0803	0.239 (17/71)	0.133 (15/113)	0.06	-	0.12 (17/142)	0.071 (16/226)	0.11	-
*0901	0.301 (22/71)	0.31 (35/113)	1	-	0.176 (25/142)	0.168 (38/226)	0.84	-
*1001	0 (0/71)	0.009 (1/113)	1	-	0 (0/142)	0.004 (1/226)	1	-
*1101	0.07 (5/71)	0.027 (3/113)	0.26	-	0.035 (5/142)	0.013 (3/226)	0.27	-
*1201	0.028 (2/71)	0.062 (7/113)	0.49	-	0.014 (2/142)	0.031 (7/226)	0.49	-
*1202	0.085 (6/71)	0.035 (4/113)	0.19	-	0.042 (6/142)	0.018 (4/226)	0.19	-
*1301	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*1302	0.169 (12/71)	0.195 (22/113)	0.66	-	0.085 (12/142)	0.097 (22/226)	0.68	-
*1401	0.028 (2/71)	0.053 (6/113)	0.71	-	0.014 (2/142)	0.027 (6/226)	0.72	-
*1403	0.042 (3/71)	0.062 (7/113)	0.74	-	0.021 (3/142)	0.031 (7/226)	0.75	-
*1405	0.056 (4/71)	0.062 (7/113)	1	-	0.028 (4/142)	0.031 (7/226)	1	-
*1406	0.014 (1/71)	0.018 (2/113)	1	-	0.007 (1/142)	0.009 (2/226)	1	-
*1501	0.183 (13/71)	0.106 (12/113)	0.14	-	0.092 (13/142)	0.058 (13/226)	0.21	-
*1502	0.127 (9/71)	0.186 (21/113)	0.31	-	0.07 (10/142)	0.102 (23/226)	0.31	-
*1602	0 (0/71)	0.035 (4/113)	0.16	-	0 (0/142)	0.018 (4/226)	0.3	-

There was no significant association between HLA-DRB1 and SJS/TEN with ocular complications.

TABLE 5. HLA-DQB1 ALLELES AND SJS/TEN WITH OCULAR COMPLICATIONS.

HLA-DQB1 alleles	Carrier frequency				Allele frequency			
	SJS (n=71)	Normal (n=113)	p	Odds ratio	SJS (n=142)	Normal (n=226)	p	Odds ratio
*0201	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*0301	0.254 (18/71)	0.212 (24/113)	0.52	-	0.141 (20/142)	0.106 (24/226)	0.32	-
*0302	0.169 (12/71)	0.177 (20/113)	0.89	-	0.085 (12/142)	0.093 (21/226)	0.78	-
*0303	0.296 (21/71)	0.354 (40/113)	0.41	-	0.169 (24/142)	0.195 (44/226)	0.54	-
*0401	0.197 (14/71)	0.239 (27/113)	0.51	-	0.106 (15/142)	0.128 (29/226)	0.51	-
*0402	0.056 (4/71)	0.053 (6/113)	1	-	0.028 (4/142)	0.027 (6/226)	1	-
*0501	0.113 (8/71)	0.097 (11/113)	0.81	-	0.056 (8/142)	0.053 (12/226)	1	-
*0502	0 (0/71)	0.08 (9/113)	0.01	0	0 (0/142)	0.04 (9/226)	0.01	0
*0503	0.085 (6/71)	0.062 (7/113)	0.57	-	0.042 (6/142)	0.031 (7/226)	0.57	-
*0601	0.352 (25/71)	0.274 (31/113)	0.26	-	0.19 (26/142)	0.164 (37/226)	0.63	-
*0602	0.155 (11/71)	0.106 (12/113)	0.33	-	0.078 (11/142)	0.058 (13/226)	0.45	-
*0603	0.014 (1/71)	0.009 (1/113)	1	-	0.007 (1/142)	0.004 (1/226)	1	-
*0604	0.169 (12/71)	0.195 (22/113)	0.66	-	0.085 (12/142)	0.097 (22/226)	0.68	-

HLA-DQB1*0502 showed a tendency of negative association with SJS/TEN with ocular complications (carrier frequency: p<0.05, Pc=0.17, OR=0; allele frequency: p<0.05, Pc=0.19, OR=0). Although none of the 71 SJS/TEN patients and 10 of the 117 healthy volunteers (8.5%) had the HLA-DQB1*0502 allele, the correction of the p-value for the number of alleles detected (n=14) rendered the result not significant.

frequency: p<0.0005, Pc<0.005, OR=3.2). HLA-A*1101 was inversely associated (carrier frequency: p<0.01, Pc=0.07, OR=0.23; allele frequency: p<0.005, Pc<0.05, OR=0.22). SJS/TEN patients in this study consisted of 40 previously analyzed patients and 31 new patients. These results validate the strong association between HLA-A*0206 and SJS/TEN that we reported previously [13].

Table 2 shows the results on HLA-B alleles. HLA-B*5901 exhibited a high odds ratio for SJS/TEN with ocular complications (carrier frequency: p<0.05, Pc=0.42, OR=7.0; allele frequency: p<0.05, Pc=0.46, OR=6.7). However, when we corrected the p-value for the number of alleles detected (n=29), the results ceased to be significant.

There was no association between HLA-C and SJS/TEN with ocular complications (Table 3). There was also no significant association between HLA-DRB1 and SJS/TEN with ocular complications (Table 4).

HLA-DQB1*0502 showed a tendency of negative association with SJS/TEN with ocular complications (carrier frequency: p<0.05, Pc=0.17, OR=0; allele frequency: p<0.05, Pc=0.19, OR=0); Table 5). Although none of the 71 SJS/TEN patients and 10 of the 117 healthy volunteers (8.5%) had the HLA-DQB1*0502 allele, the correction of the p-value for the number of alleles detected (n=14) rendered the result not significant.

We used the χ^2-test for statistical analysis when the sample number was 10 and more than 10 and used the Fischer's exact test when the sample number was less than 10. Carrier frequency is "frequency of the person with the allele

TABLE 6. ETHNIC DIFFERENCES IN THE ASSOCIATION OF SJS/TEN WITH HLA.

Allele	Japanese		Caucasian [19]		Taiwanese [18]	
	SJS	Control	SJS	Control	Carbamazepine induced SJS	Control
A*0206	0.423	0.15	-	(0 ~1.4%)	-	(3.1 ~24%)
B*1502	0	0		(0 ~0.2%)	1	0.086
B*4402	0.014	0	-	(6.7 ~26.5%)	-	0
B*4403	0.225	0.204	-	(6.6 ~20.0%)	-	(0 ~2%)
DQB1*0601	0.352	0.274	0.17	0.03	-	-

Carrier frequency of SJS-associated alleles in Japanese and Caucasian. Data in parentheses are from "Allele Frequency in Worldwide Populations."

at the population level," and allele frequency is "frequency of alleles at the population level" [15].

DISCUSSION

Analysis of our 71 Japanese patients showed that HLA-A*0206 was strongly associated with SJS/TEN with ocular complications. On the other hand, HLA-A*1101 was negatively associated. We postulate that the decreased B*5401 frequency in the patients is attributable to its linkage disequilibrium with A*1101. We also found that HLA-B*5901 was weakly associated with SJS/TEN with ocular complications although when we corrected the p-value for the number of alleles detected, the result ceased to be significant. We postulate that B*5901 could be a risk factor independent of A*0206 because only one patient had both alleles. Interestingly, none of the 71 SJS/TEN patients but 10 of the 117 volunteers (8.5%) had the HLA-DQB1*0502 allele. HLA-DQB1*0502 was also weakly associated with SJS/TEN with ocular complications although correction of the p-value for the number of alleles detected rendered the result not significant.

Regarding HLA-class I, previous reports from the United States [16] and France [17] showed that the HLA-B12 (HLA-Bw44) antigen was significantly increased in Caucasian SJS patients. In our study population, we did not find an association with HLA-B12 probably because in Caucasians, the HLA-B12 antigen is primarily coded by HLA-B*4402 whereas in the Japanese, it is almost exclusively coded by HLA-B*4403 [15]. HLA-A*0206, strongly associated with SJS/TEN with ocular complications in the Japanese, is absent in Caucasians. While we were unable to identify the causative drug(s) unequivocally, we suspect that antibiotics, cold remedies, or non-steroid anti-inflammatory drugs were involved in some of our patients. Limiting carbamazepine-induced SJS, the HLA-B*1502 allele was documented to show a very strong association [18].

With respect to HLA-class II, Power et al. [19] reported that HLA-DQB1*0601 was associated with Caucasian patients with ocular complications of SJS. In French SJS/TEN patients, HLA-DR antigens (DR) was not associated at all [16]. Different from the findings of others, we found that in our Japanese patients, there is no significant association between SJS/TEN and HLA-DQB1*0601.

Thus, our findings suggest strong ethnic differences in the association of SJS/TEN with HLA (Table 6). Because SJS/TEN is a rare condition probably with a complex genetic inheritance background, specific combinations of genes and certain environmental factors may be required for the manifestation of this rare phenotype. Since the strong association of specific HLA antigens with SJS with ocular complications may be a clue to understanding its basic pathobiology, we are attempting to develop a reliable test for identifying individuals susceptible to SJS with ocular complications.

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REFERENCES

1. Stevens AM, Johnson FC. A new eruptive fever associated with stomatitis and ophthalmia: Report of two cases in children. Am J Dis Child 1922; 24:526-33.
2. Ueta M, Sotozono C, Inatomi T, Kojima K, Tashiro K, Hamuro J, Kinoshita S. Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens-Johnson syndrome. Br J Ophthalmol 2007; 91:962-5. [PMID: 17314152]
3. Yetiv JZ, Bianchine JR, Owen JA Jr. Etiologic factors of the Stevens-Johnson syndrome. South Med J 1980; 73:599-602. [PMID: 7375977]
4. Roujeau JC, Kelly JP, Naldi L, Rzany B, Stern RS, Anderson T, Auquier A, Bastuji-Garin S, Correia O, Locati F, Mockenhaupt M, Paoletti C, Shapiro S, Shear N, Schöpf E, Kaufman DW. Medication use and the risk of Stevens-

Johnson syndrome or toxic epidermal necrolysis. N Engl J Med 1995; 333:1600-7. [PMID: 7477195]

5. Auquier-Dunant A, Mockenhaupt M, Naldi L, Correia O, Schroder W, Roujeau JC. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: Results of an international prospective study. Arch Dermatol 2002; 138:1019-24. [PMID: 12164739]
6. Chan HL, Stern RS, Arndt KA, Langlois J, Jick SS, Jick H, Walker AM. The incidence of erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis. A population-based study with particular reference to reactions caused by drugs among outpatients. Arch Dermatol 1990; 126:43-7. [PMID: 2404462]
7. Power WJ, Ghoraishi M, Merayo-Lloves J, Neves RA, Foster CS. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. Ophthalmology 1995; 102:1669-76. [PMID: 9098260]
8. Correia O, Delgado L, Ramos JP, Resende C, Torrinha JA. Cutaneous T-cell recruitment in toxic epidermal necrolysis. Further evidence of CD8+ lymphocyte involvement. Arch Dermatol 1993; 129:466-8. [PMID: 8466217]
9. Correia O, Delgado L, Roujeau JC, Le Cleach L, Fleming-Torrinha JA. Soluble interleukin-2 receptor and interleukin-1 alpha in toxic epidermal necrolysis: A comparative analysis of serum and blister fluid samples. Arch Dermatol 2002; 138:29-32. [PMID: 11790164]
10. Wolkenstein P, Charue D, Laurent P, Revuz J, Roujeau JC, Bagot M. Metabolic predisposition to cutaneous adverse drug reactions. Role in toxic epidermal necrolysis caused by sulfonamides and anticonvulsants. Arch Dermatol 1995; 131:544-51. [PMID: 7741540]
11. Leaute-Labreze C, Lamireau T, Chawki D, Maleville J, Taieb A. Diagnosis, classification, and management of erythema multiforme and Stevens-Johnson syndrome. Arch Dis Child 2000; 83:347-52. [PMID: 10999875]
12. Sotozono C, Ang LP, Koizumi N, Higashihara H, Ueta M, Inatomi T, Yokoi N, Kaido M, Dogru M, Shimazaki J, Tsubota K, Yamada M, Kinoshita S. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology 2007; 114:1294-302. [PMID: 17475335]
13. Ueta M, Sotozono C, Tokunaga K, Yabe T, Kinoshita S. Strong association between HLA-A*0206 and Stevens-Johnson syndrome in the Japanese. Am J Ophthalmol 2007; 143:367-8. [PMID: 17258541]
14. Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S. Association of IL4R polymorphisms with Stevens-Johnson syndrome. J Allergy Clin Immunol 2007; 120:1457-9. [PMID: 17900677]
15. Tokunaga K, Ishikawa Y, Ogawa A, Wang H, Mitsunaga S, Moriyama S, Lin L, Bannai M, Watanabe Y, Kashiwase K, Tanaka H, Akaza T, Tadokoro K, Juji T. Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. Immunogenetics 1997; 46:199-205. [PMID: 9211745]
16. Mondino BJ, Brown SI, Biglan AW. HLA antigens in Stevens-Johnson syndrome with ocular involvement. Arch Ophthalmol 1982; 100:1453-4. [PMID: 7115173]
17. Roujeau JC, Huynh TN, Bracq C, Guillaume JC, Revuz J, Touraine R. Genetic susceptibility to toxic epidermal necrolysis. Arch Dermatol 1987; 123:1171-3. [PMID: 3477129]
18. Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, Wu JY, Chen YT. Medical genetics: A marker for Stevens-Johnson syndrome. Nature 2004; 428:486. [PMID: 15057820]
19. Power WJ, Saidman SL, Zhang DS, Vamvakas EC, Merayo-Lloves JM, Kaufman AH, Foster CS. HLA typing in patients with ocular manifestations of Stevens-Johnson syndrome. Ophthalmology 1996; 103:1406-9. [PMID: 8841298]

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Association of Fas Ligand gene polymorphism with Stevens Johnson syndrome

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Association of Fas Ligand gene polymorphism with Stevens–Johnson syndrome

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ABSTRACT

Background: Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute severe blistering diseases of the skin and also two of the most devastating ocular surface diseases leading to corneal damage and loss of vision. The extreme rarity of cutaneous and ocular surface reactions to drug therapies led us to suspect individual susceptibility. SJS/TEN patients in the acute stage were reported to manifest increased serum levels of Fas Ligand (FasL). Thus, we performed SNP association analysis of the FasL gene.

Methods: In 76 Japanese SJS/TEN patients with ocular surface complications and 160 Japanese healthy controls, we examined four SNPs of FasL reported in the Japanese Single Nucleotide Polymorphisms (JSNP) database by sequencing.

Results: The SNP rs.3830150 A/G showed a significant strong inverse association with SJS/TEN. Analysis of the genotype pattern of SNPs rs.3830150 and rs.2639614 (rs.3830150 A/A–rs.2639614 G/G) also manifested a strong inverse association with SJS/TEN.

Conclusion: FasL gene polymorphisms might be associated with SJS/TEN.

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), considered variants of a single disease, are acute-onset mucocutaneous diseases induced by infectious agents and/or inciting drugs.[1 2] SJS/TEN are acute severe blistering diseases of the skin which carry high mortalities.[3–4] In addition, SJS and TEN are also two of the most devastating ocular surface diseases leading to corneal damage and loss of vision. The reported incidence of ocular complications in SJS/TEN is 50–68%.[2 4] From the ophthalmological standpoint, in the acute stage, SJS/TEN patients manifest severe conjunctivitis, and persistent corneal epithelial defects due to ocular surface inflammation. In the chronic stage, ocular surface complications such as conjunctival invasion into the cornea due to corneal epithelial stem-cell deficiency, symblepharon and, in some instances, keratinisation of the ocular surface persist despite the healing of the skin lesions.[5] We observed that more than 95% of SJS/TEN patients with ocular surface complications had lost their fingernails in the acute or subacute stage and that some continue to have transformed nails even after healing of the skin lesions.[1 6]

Although the pathobiological mechanisms underlying the onset of SJS/TEN have not been fully established, the extreme rarity of cutaneous- and ocular surface reactions to drug therapies led us to suspect individual susceptibility. We have documented the association with TLR3[7] and IL4R polymorphisms in Japanese SJS/TEN patients with ocular complications.[6] We also reported that in the Japanese, HLA-A*0206 was strongly associated with SJS/TEN with ocular surface complications.[7] Thus, genetic and environmental factors may play an important role in an integrated aetiology of SJS/TEN.

It has been reported that the skin lesion of SJS/TEN in the acute stage is histologically characterised by marked keratinocyte apoptosis in the epidermis with dermo-epidermal separation, resulting in bullae.[8] Moreover, SJS/TEN patients in the acute stage manifested increased serum levels of Fas Ligand (FasL),[9 10] and the activation of Fas through FasL was reported to be an initial important step leading to the diffused apoptotic cell death of epidermal cells in SJS/TEN.[9 10] Thus, we performed SNP association analysis of the FasL gene. We examined four SNPs of FasL reported in the Japanese Single Nucleotide Polymorphisms (JSNP) database and found that rs.3830150 A/G (intron) were significantly associated with SJS/TEN. Analysis of the genotype pattern of SNPs rs.3830150 and rs.2639614 (rs.3830150 A/A–rs.2639614 G/G) also manifested a strong inverse association with SJS/TEN in Japanese patients.

MATERIALS AND METHODS

Patients

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the principles set forth in the Declaration of Helsinki.

For SNPs analysis, we enrolled 76 patients with SJS/TEN in the chronic or subacute phase; all presented the symptom of ocular surface complications. The diagnosis of SJS/TEN was based on a confirmed history of the acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least two mucosal sites, including the ocular surface. The controls were 160 healthy volunteers. All participants and volunteers were Japanese residing in Japan. The average age of the patients and controls was 46.1 (SD 17.3) and 36.2 (11.5) years, respectively. The male:female ratio in the patient- and control groups was 33:43 and 57:103, respectively.

SNPs analysis

FasL SNP analysis was performed by sequencing from both sides, forward and reverse, in order to carefully confirm the results. For SNPs of FasL, the PCR- and sequence primers were 5'-TTTGGGAAACCCTCTCAAGC-3' (sense) and 5'-CGTGGCTGAGTGCCAGATTAG-3' (antisense)

Table 1 Genotype frequencies of FasL SNPs among Japanese Stevens–Johnson syndrome/toxic epidermal necrolysis patients and healthy controls

	Control (%) (n = 160)	Stevens–Johnson syndrome/toxic epidermal necrolysis (%) (n = 76)	Allele 1 vs Allele 2		Genotype 11 vs 12+22		Genotype 11+12 vs 22	
			p Value (χ^2)	OR (95% CI)	p Value (χ^2)	OR (95% CI)	p Value (χ^2)	OR (95% CI)
rs.929087								
11 TT	49 (30.6)	18 (23.7)	0.667	–	0.269	–	0.645	–
12 TC	73 (45.6)	42 (55.3)						
22 CC	38 (23.8)	16 (21.1)						
rs.3830150								
AA	118 (73.8)	40 (52.6)	0.004	0.496 (0.3 to 0.8)	0.001	0.395 (0.2 to 0.7)	0.966	–
AG	40 (25.0)	35 (46.1)						
GG	2 (1.3)	1 (1.3)						
rs.2639614								
GG	131 (81.9)	52 (68.4)	0.025	0.526 (0.3 to 0.9)	0.021	0.480 (0.3 to 0.9)	0.589	–
GA	28 (17.5)	23 (30.3)						
AA	1 (0.6)	1 (1.3)						
rs.2859247								
GG	93 (58.1)	37 (48.7)	0.447	–	0.173	–	0.427	–
GA	54 (33.8)	35 (46.1)						
AA	13 (8.1)	4 (5.3)						

OR, odds ratio.

for rs. 929087, 5'-TGTATGCAGCGTTGTCGAA-3' (sense) and 5'-TTTTGTTGAGGCTACACAGAGG-3' (antisense) for rs. 3830150, 5'-ATCATTCAGGCCCACTCACC-3' (sense) and 5'-TTGCTAGTCTCATCCCTTGCAC-3' (antisense) for rs. 2639614 and rs. 2859247. All primers except rs. 3830150 were those recommended in the JSNP database. Genomic DNA was isolated from human peripheral blood at SRL (Tokyo). PCR amplification was with DNA polymerase (Takara, Shiga, Japan) for 35 cycles at 94°C for 1 min, annealing at 60°C for 1 min, and at 72°C for 1 min on a commercial PCR machine (GeneAmp; Perkin-Elmer Applied Biosystems, Waltham, MA). The PCR products were reacted with BigDye Terminator v3.1 (Perkin-Elmer Applied Biosystems), and sequence reactions were resolved on an ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems).

Statistical methods

Alleles were counted manually. Genotype patterns were also counted manually. The χ^2 test was used for Hardy–Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions. The odds ratio (OR) with 95% CI was calculated using Labo Server software (World Fusion, Tokyo). Each allele and genotype pattern was assessed as an independent variable, and separate p values were calculated for each polymorphism. A p value of <0.05 was regarded as significant. In addition, the p values were corrected for the number of alleles tested (Bonferroni method).

RESULTS

A summary of the case-control association study with the four genotyped SNPs is shown in table 1. All four SNPs examined were in Hardy–Weinberg equilibrium in our SJS/TEN patients and controls ($p>0.01$). SNP rs.3830150 A/G showed a significant strong inverse association with allele frequency (A versus G, raw and corrected p value 0.004 and 0.017, respectively; odds ratio (OR) 0.496) and with the dominant model (A/A versus A/G + G/G, raw and corrected p value 0.001 and 0.005, respectively; OR 0.395). SNP rs.2639614 G/A exhibited a significant inverse association with allele frequency (G versus A, raw p value 0.025; OR 0.526) and with the dominant model (G/G versus G/A + A/A, raw p value 0.021; OR 0.48) (table 1), although the results ceased to be significant when we corrected the p value for the number of alleles tested (n = 4).

We also analysed the genotype pattern of SNPs rs.3830150 and rs.2639614. Analysis of the genotype pattern of SNPs rs.3830150 and rs.2639614 (rs.3830150 A/A–rs.2639614 G/G) manifested a strong inverse association with SJS/TEN in Japanese patients (χ^2 test, p = 0.001, OR = 0.391, 95% CI 0.2 to 0.7) (table 2).

DISCUSSION

Our results suggest that polymorphisms in the FasL gene may be associated with SJS/TEN in the Japanese population. SNP rs.3830150 A/G showed a significant strong inverse association with allele frequency and with the dominant model. Analysis of

Table 2 Pattern structures and frequency of rs.3830150 and rs.2639614 SNPs of FasL Gene

Pattern type	rs.3830150	rs.2639614	Control (%) (n = 160)	Stevens–Johnson syndrome/ toxic epidermal necrolysis (%) (n = 76)	p Value (χ^2)	OR (95% CI)
1	A/A	G/G	115/160 (72.5)	38/76 (50.0)	0.001	0.39 (0.2 to 0.7)
2	A/G	A/G	25/160 (15.6)	21/76 (27.6)	0.03	2.1 (1.1 to 4.0)
3	A/G	G/G	14/160 (8.8)	14/76 (18.4)	0.03	2.4 (1.1 to 5.2)
4	A/A	A/G	3/160 (1.9)	2/76 (2.6)	NS	–
5	G/G	G/G	2/160 (1.3)	0/76 (0.0)	NS	–
6	A/G	A/A	1/160 (0.6)	0/76 (0.0)	NS	–
7	G/G	A/A	0/160 (0.0)	1/76 (1.3)	NS	–

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the genotype pattern of SNPs rs.3830150 and rs.2639614 (rs.3830150 A/A–rs.2639614 G/G) also manifested a strong inverse association with SJS/TEN in Japanese patients. We have confirmed that the age–gender differences do not skew the data. All the controls and the patients are viable.

According to the International HapMap project, the rs.3830150 and rs.2639614 SNP, which showed a significant association with SJS/TEN, exists not only in Japanese-, but also in Han Chinese- and Caucasian populations, indicating that it is important to examine FasL SNPs in non-Japanese populations.

Previously, we have also documented the association with TLR3[1] and IL4R polymorphisms in Japanese SJS/TEN patients with ocular complications.[6] Because SJS/TEN is a rare condition, probably with a complex genetic-inheritance background, specific combinations of genes and certain environmental factors may be required for the manifestation of this rare phenotype.

It has been reported that the skin lesion of SJS/TEN in the acute stage is histologically characterised by marked keratinocyte apoptosis in the epidermis with dermo-epidermal separation, resulting in bullae.[8] Moreover, SJS/TEN patients in the acute stage manifested increased serum levels of FasL,[9 10] and the activation of Fas through FasL was reported to be an initial important step leading to the diffused apoptotic cell death of epidermal cells in SJS/TEN.[9 10]

Regarding eyes, it is reported that in the mouse eye, FasL is expressed by the corneal epithelium and endothelium,[11] and to maintain the cornea as a transparent barrier, the Fas-FasL pathway has a special significance in corneal immune privilege and in limiting inflammation.[12] However, no report has described the role of Fas/FasL-induced apoptosis in the pathogenesis of the ocular surface complications of SJS/TEN patients. The role of Fas/FasL signalling in chronic ocular surface inflammation remains elusive.

Drugs are probably the most widely accepted aetiological factor in SJS/TEN.[13] In addition, it is noteworthy that SJS/TEN patients often had the prodromata, including non-specific fever, coryza, and sore throat, that closely mimic upper-respiratory-tract infections commonly treated with antibiotics. These prodromata were evident from the clinical records of our SJS/TEN patients. Mycoplasma pneumoniae was responsible in five of 17 cases of childhood SJS,[14] and a viral aetiology involving herpes simplex-, Epstein–Barr-, cytomegalo-, and varicella zoster virus has been reported.[15 16]

On the other hand, apoptosis can reportedly be regulated by innate immunity.[17] Given the association between the onset of SJS/TEN and infections,[1 2] and the opportunistic infection of ocular surfaces by bacteria such as MRSA or MRSE,[18] we considered the possibility that there is an association between SJS/TEN and a disordered innate immune response. We postulated that drugs and/or viral infection may trigger a disorder in the host's innate immune response and that this event is followed by aggravated inflammation of the mucous membranes, ocular surface, and skin. The innate immune system may constitute a link between the environment and the adaptive immune system. We are continuing to examine the pathophysiology of SJS/TEN with ocular surface complications. A large international case-control study, called the Severe Cutaneous Adverse Reaction study, prospectively evaluated and supported the hypothesis that SJS and TEN are severity variants of a single entity.[19] The classification was based on the clinical appearance and pathology of skin lesions present in the acute stage. Although we were unable to accurately classify our patients into either SJS or TEN due to the fact that many of our patients were in the chronic stage, analysis of each condition independently may be worth pursuing in future studies.

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Competing interests: None.

Ethics approval: Ethics approval was obtained.

Patient consent: Prior written informed consent was obtained from all participants after the purpose of the research and the experimental protocols were thoroughly explained.

REFERENCES

1. **Ueta M,** Sotozono C, Inatomi T, *et al*. Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens–Johnson syndrome. *Br J Ophthalmol* 2007;**91**:962–5.
2. **Yetiv JZ,** Bianchine JR, Owen JA Jr. Etiologic factors of the Stevens–Johnson syndrome. *South Med J* 1980;**73**:599–602.
3. **Chan HL,** Stern RS, Arndt KA, *et al*. The incidence of erythema multiforme, Stevens–Johnson syndrome, and toxic epidermal necrolysis. A population-based study with particular reference to reactions caused by drugs among outpatients. *Arch Dermatol* 1990;**126**:43–7.
4. **Power WJ,** Ghoraishi M, Merayo-Lloves J, *et al*. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens–Johnson syndrome/toxic epidermal necrolysis disease spectrum. *Ophthalmology* 1995;**102**:1669–76.
5. **Sotozono C,** Ang LP, Koizumi N, *et al*. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens–Johnson syndrome. *Ophthalmology* 2007;**114**:1294–302.
6. **Ueta M,** Sotozono C, Inatomi T, *et al*. Association of IL4R polymorphisms with Stevens–Johnson syndrome. *J Allergy Clin Immunol* 2007;**120**:1457–9.
7. **Ueta M,** Sotozono C, Tokunaga K, *et al*. Strong association between HLA-A*0206 and Stevens–Johnson Syndrome in the Japanese. *Am J Ophthalmol* 2007;**143**:367–8.
8. **Paul C,** Wolkenstein P, Adle H, *et al*. Apoptosis as a mechanism of keratinocyte death in toxic epidermal necrolysis. *Br J Dermatol* 1996;**134**:710–14.
9. **Abe R,** Shimizu T, Shibaki A, *et al*. Toxic epidermal necrolysis and Stevens–Johnson syndrome are induced by soluble Fas ligand. *Am J Pathol* 2003;**162**:1515–20.
10. **Viard I,** Wehrli P, Bullani R, *et al*. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;**282**:490–3.
11. **Griffith TS,** Brunner T, Fletcher SM, *et al*. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 1995;**270**:1189–92.
12. **Niederkorn JY.** See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat Immunol* 2006;**7**:354–9.
13. **Roujeau JC,** Kelly JP, Naldi L, *et al*. Medication use and the risk of Stevens–Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med* 1995;**33**:1600–7.
14. **Leaute-Labreze C,** Lamireau T, Chawki D, *et al*. Diagnosis, classification, and management of erythema multiforme and Stevens–Johnson syndrome. *Arch Dis Child* 2000;**83**:347–52.
15. **Choy AC,** Yarnold PR, Brown JE, *et al*. Virus induced erythema multiforme and Stevens–Johnson syndrome. *Allergy Proc* 1995;**16**:157–61.
16. **Forman R,** Koren G, Shear NH. Erythema multiforme, Stevens–Johnson syndrome and toxic epidermal necrolysis in children: a review of 10 years' experience. *Drug Saf* 2002;**25**:965–72.
17. **Jiang D,** Liang J, Fan J, *et al*. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005;**11**:1173–9.
18. **Sotozono C,** Inagaki K, Fujita A, *et al*. Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* infections in the cornea. *Cornea* 2002;**21**:S94–101.
19. **Auquier-Dunant A,** Mockenhaupt M, Naldi L, *et al*. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens–Johnson syndrome, and toxic epidermal necrolysis: results of an international prospective study. *Arch Dermatol* 2002;**138**:1019–24.

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Diagnosis and Treatment of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis with Ocular Complications

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Purpose: To present a detailed clarification of the symptoms at disease onset of Stevens-Johnson syndrome (SJS) and its more severe variant, toxic epidermal necrolysis (TEN), with ocular complications and to clarify the relationship between topical steroid use and visual prognosis.

Design: Cross-sectional study.

Participants: Ninety-four patients with SJS and TEN with ocular complications.

Methods: A structured interview, examination of the patient medical records, or both addressing clinical manifestations at disease onset were conducted for 94 patients seen at Kyoto Prefectural University of Medicine. Any topical steroid use during the first week at the acute stage also was investigated.

Main Outcome Measures: The incidence and the details of prodromal symptoms and the mucosal involvements and the relationship between topical steroid use and visual outcomes.

Results: Common cold-like symptoms (general malaise, fever, sore throat, etc.) preceded skin eruptions in 75 cases, and extremely high fever accompanied disease onset in 86 cases. Acute conjunctivitis and oral and nail involvements were reported in all patients who remembered the details. Acute conjunctivitis occurred before the skin eruptions in 42 patients and simultaneously in 21 patients, whereas only 1 patient reported posteruption conjunctivitis. Visual outcomes were significantly better in the group receiving topical steroids compared with those of the no-treatment group ($P<0.00001$).

Conclusions: Acute conjunctivitis occurring before or simultaneously with skin eruptions accompanied by extremely high fever and oral and nail involvement indicate the initiation of SJS or TEN. Topical steroid treatment from disease onset seems to be important for the improvement of visual prognosis.

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Stevens-Johnson syndrome (SJS) and its more severe variant, toxic epidermal necrolysis (TEN), are acute inflammatory disorders that affect the skin and mucous membranes.[1–4] Although the incidence of SJS and TEN is very low, approximately 0.4 to 1 case per 1 million persons and 1 to 6 cases per 1 million persons, respectively, both can affect anybody at any age, usually as a consequence of adverse drug reactions.[5–7] A variety of drugs including antibiotics, nonsteroidal anti-inflammatory drugs, and antiepileptic medications, that is, any of the popularly used drugs, have been reported to cause severe drug reactions and to induce SJS or TEN.

The mortality rates for SJS and TEN are high: 1% to 5% and 25% to 35%, respectively.[8,9] Ocular complications occur in more than 50% of the patients, and ocular surface inflammation develops rapidly at the acute stage.[10,11] Extensive inflammation of the ocular surface often is accompanied by pseudomembranous formation and corneal or conjunctival epithelial defects, or both. The common pathway after the acute stage includes persistent epithelial defects, ulceration, and perforation, finally developing into corneal cicatricial changes such as neovascularization, opacification, keratinization, and symblepharon.[12,13] Even after the acute-stage impairments subside, permanent visual impairment or blindness remains and conjunctival inflammation prolongs at the chronic stage.[14] Patients with SJS or TEN require life-long management for ocular discomfort and morbidity. Stevens-Johnson syndrome or TEN accompanied by ocular complications, at both the acute and chronic stage, are 2 of the most devastating ocular surface diseases, and both are extremely difficult to treat.

The loss of corneal epithelial stem cells, which are located in the limbal region,[15–18] evidenced by the loss of palisades of Vogt, is the most common ocular feature of SJS.[13] As soon as the corneal epithelial stem cells are lost at the acute stage of SJS or TEN, the corneal epithelium does not regenerate, thus resulting in conjunctival epithelial invasion into the cornea (conjunctivalization) and cicatricial changes of the ocular surface. In contrast, the regeneration of the epidermis develops rather smoothly at the remission of the diseases.

Penetrating keratoplasty (PK) generally is contraindicated for eyes with SJS or TEN because PK does not supply the limbal region of the eye with corneal epithelial stem

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cells. Moreover, PK-initiated, immunologically driven ocular surface inflammation may induce persistent epithelial defects and corneal melting, perforation, or both, ultimately resulting in blindness.[12] Allograft transplantation of healthy limbal tissue is useful for the reconstruction of the ocular surface. However, long-term outcomes are poor in eyes with SJS or TEN.[19] Groundbreaking surgical procedures have been developed over the past 12 years. The authors first reported the usefulness of cultivated corneal epithelial transplantation for SJS with persistent epithelial defects after the acute stage.[20–23] In another report, they clarified the efficacy of ex vivo expanded autologous oral mucosal epithelial cells to the ocular surface.[24] Cultivated oral mucosal epithelial transplantation and the 2-step surgical combination of cultivated oral mucosal epithelial transplantation and PK have provided the patients with SJS or TEN with a surgical pathway toward restoration of their visual function.[25–27] However, it is impossible for the ocular surface of those patients to be restored to its previously normal state.

Diagnosis of SJS or TEN at disease onset is complex, often confusing, and very difficult. Moreover, the use of steroids for treatment remains controversial.[10,28–30] The authors' recent reports and those of others indicated the influence of genetic endowment in SJS and TEN.[31–40] For instance, there are statistically significant differences in single nucleotide polymorphisms of toll-like receptor 3, interleukin (IL)-4R/IL-13, and Fas ligand in SJS and TEN; thus, genetic screening may help to deliver a more rapid diagnosis in the future. At present, however, the understanding of the typical clinical picture of SJS and TEN is still a vital aspect of early diagnosis and the initiation of treatment. Therefore, this study investigated the clinical manifestation at disease onset of SJS and TEN with ocular complications and evaluated the relationship between ophthalmic management at the acute stage and the visual outcomes.

AQ: 1

Patients and Methods

From November 2005 through May 2008, extensive interviews were conducted with 94 patients (45 males and 49 females) with SJS or TEN with ocular complications seen at the SJS outpatient service at Kyoto Prefectural University Hospital. Of those patients, 88 cases were referral patients from the greater Japan area who had come to the SJS service at the acute stage (n = 14) or at the chronic stage (n = 74). Their ages ranged from 1 to 83 years (mean age±standard deviation, 41.6±18.5 years). At disease onset, the patients' ages ranged from 0 to 77 years (mean age±standard deviation, 26.2±18.8 years), and the duration of the illness ranged from 1 to 48 years (mean±standard deviation, 16.1±15.2 years). The questionnaires used in this study were structured as follows: (1) age of the patient at disease onset; (2) causative drugs; (3) the presence of prodromal symptoms; and (4) the episodes of high fever, conjunctivitis, skin eruptions, fingernail loss, and associated mucous membrane involvements. Medical records also were examined or the patients were asked directly regarding any ophthalmic management, especially the use of topical steroids, during the first week from disease onset. Then, the Mann–Whitney *U* test was used to analyze the correlation between the use of topical steroids and the visual outcomes. This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan.

The diagnosis of SJS or TEN at the acute stage was based on the acute onset of high fever, serious mucocutaneous illness with skin eruptions, involvement of at least 2 mucosal sites, and the pathologic findings of a skin biopsy that demonstrated necrotic changes of the dermis. The diagnosis of SJS or TEN at the chronic stage was based on ocular cicatricial findings such as symblepharon, severe dry eye, corneal neovascularization, opacification, and conjunctivalization, and a confirmed history of the 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 patients where disease onset occurred before age 10 years or in those who had lost consciousness at the acute stage because the illness, specific details were obtained by directly interviewing members of the immediate family.

Results

Of the 94 patients, drugs were the most commonly associated etiologic factor in 84 patients (89.4%). The causative drugs were cold remedies in 30 patients, antibiotics in 23 patients, nonsteroidal anti-inflammatory drugs in 19 patients, anticonvulsants in 5 patients, and others (anticancer agents, antirheumatic drugs, antimalarial, Chinese medicine, etc.).

Best-corrected visual acuity obtained at the chronic stage was 20/20 or better in 34 eyes (18.3%; Fig 1A), worse than 20/20 and up to and including 20/200 in 55 eyes (29.6%; Fig 1B), worse than 20/200 and up to and including 20/2000 in 53 eyes (28.5%; Fig 1C), and worse than 20/2000 in 44 eyes (23.7%; Fig 1D). Two eyes of 1 boy who was 1 year or age were excluded from the results because his visual acuity could not be assessed. F1

Characteristics of Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis with Ocular Complications

Of the 94 patients, common coldlike symptoms (general malaise, fever, sore throat, etc.) preceded skin eruptions in 75 patients. Extremely high fever (more than 39° C) was reported by 86 patients, whereas 1 patient reported no fever and the remaining 7 patients could not remember the extent of the fever. Acute conjunctivitis and oral involvements (blisters, erosions, and bleeding of the mouth and lips) occurred in all patients who could recollect their symptoms in detail. Fingernail loss at the acute stage or deformation at present existed in all patients (Table 1; Fig 2). Other mucous membrane involvements included those of the pharynx, respiratory tract, or ear canal. T1,F2

Forty-two patients reported episodes of acute conjunctivitis several hours to 4 days before the skin eruptions, and 21 patients reported that skin eruptions and conjunctivitis occurred simultaneously. Only 1 patient reported posteruption conjunctivitis (Table 2). T2

Topical Steroid Instillation and Visual Outcomes

Thirty-three patients (13 males and 20 females; mean age±standard deviation at disease onset, 31.5±18.6 years) began topical steroid treatment during the first week from disease onset, whereas 31 patients (14 males and 17 females; mean age±standard deviation, 27.9±19.5 years) received no topical steroid treatment or any other treatment for their eyes. The remaining 30 patients could not recall the details of ocular management during the first week from disease onset. Visual outcomes were significantly better in the group that received topical steroids at the acute stage compared with those of the no-treatment group ($P<0.00001$; Fig 3). F3

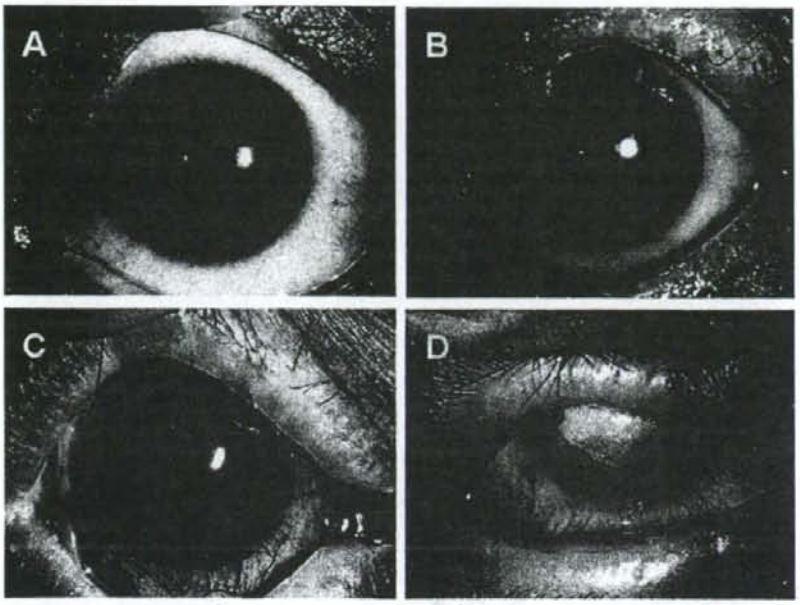

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Figure 1. Photographs showing representative ocular manifestations at the chronic stage, with corresponding visual acuity. **A**, Clear cornea and best-corrected visual acuity of 20/20 or better: 34 eyes (18.3%). **B**, Moderate conjunctivalization and visual acuity worse than 20/20 and up to and including 20/200: 55 eyes (29.6%). **C**, Severe conjunctivalization and neovascularization and visual acuity worse than 20/200 and up to and including 20/2000: 53 eyes (28.5%). **D**, Keratinization, severe opacification, and visual acuity worse than 20/2000: 44 eyes (23.7%).

Diagnosis at the Acute Stage

Eleven patients were diagnosed with acute conjunctivitis by ophthalmologists before the development of systemic eruptions. An additional 12 patients were misdiagnosed as having measles (n = 4), chickenpox (n = 2), herpetic infection (n = 2), rubella (n = 1), or other diseases by physicians in other fields.

Among 94 patients, only 37 patients were diagnosed as having SJS or TEN at disease onset. Seven patients were diagnosed properly at several weeks (range, 2–8 weeks) after the onset, and surprisingly, 6 patients obtained the diagnosis at 2 to 45 years after the onset. For the remaining patients, when they received a proper diagnosis could not be ascertained.

Table 1. Symptoms and Mucosal Involvements of the 94 Patients at the Acute Stage

Symptoms	Experienced	Did Not Experience	Unknown
Prodromal common cold-like symptoms	75	17	2
Extremely high fever (>39° C)	86	1	7
Ocular involvement	94	0	0
Oral involvement	82	0	12
Genital involvement	46	18	30
Fingernail loss or deformation	94	0	0

Discussion

Stevens-Johnson syndrome and TEN are rare but potentially fatal skin disorders. Ocular involvement is common and often results in long-term complications such as serious visual impairment with ocular discomforts.[13,28] Although much has been learned over the past 50 years about the management of SJS and TEN, the following 3 important problems still remain: (1) the difficulty of obtaining a prompt and accurate diagnosis of SJS or TEN at disease onset, (2) ocular involvement often is overlooked easily because of the serious general symptoms and high lethality of these 2 diseases, and (3) a universally accepted treatment regimen for SJS and TEN has yet to be adopted and treatment with corticosteroids remains controversial.[10,28–30] There is also no standardized ophthalmologic treatment for the prevention of ocular complications.

In this study, 12 patients were misdiagnosed as having chickenpox, measles, herpetic infection, or other diseases. For early diagnosis, the clinical pictures of SJS and TEN need to be well understood, and to that end, the results of this study provided new and important data. Common cold-like symptoms (general malaise, slight fever, sore throat, etc.) preceded skin eruptions in 82% of the cases, and in all but 1 patient, the disease was accompanied by very high fever (more than 39° C) at the onset. It should be emphasized that acute conjunctivitis occurred before or simulta-