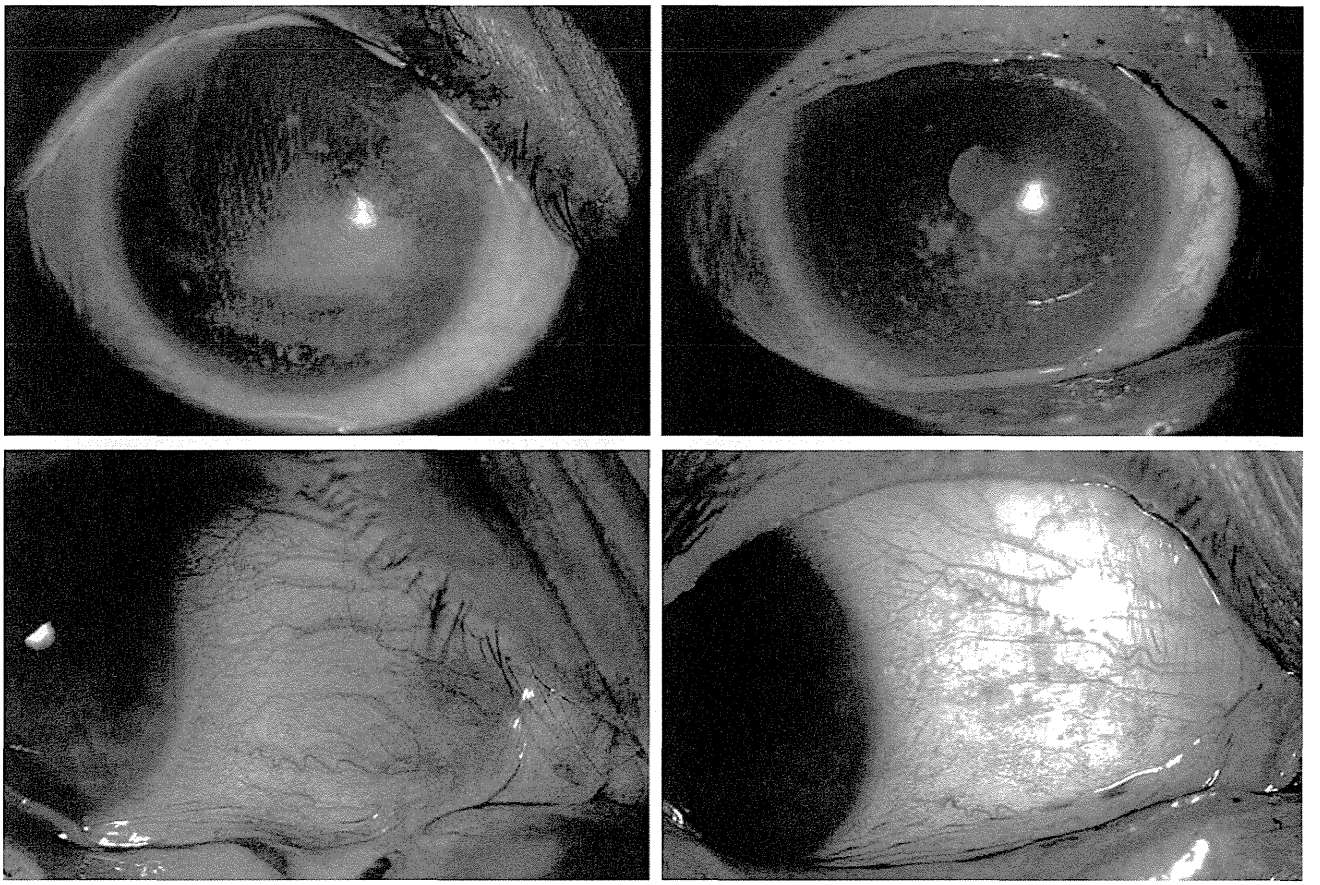


FIGURE 2. Photographs of (Top left) fluorescein corneal staining at baseline, (Top right) fluorescein corneal staining at 52 weeks, (Bottom left) lissamine green conjunctival staining at baseline, and (Bottom right) lissamine green conjunctival staining at 52 weeks, in which corneal and conjunctival disorders were improved in dry eye patients with 2% rebamipide (OPC-12759) ophthalmic suspension treatment over 52 weeks.

the same fluorescein corneal staining score, the eye with the higher lissamine green conjunctival staining score was used; (4) if both eyes had the same lissamine green conjunctival staining score, the right eye was used. In the analysis of dry eye–related ocular symptoms, patients with a dry eye–related ocular symptom score of 0 at baseline were excluded.

Analysis of change from baseline to each time point was calculated for the fluorescein corneal staining score, lissamine green conjunctival staining score, TBUT, and dry eye–related ocular symptoms. Scores at all visits were compared with baseline using the paired *t* test. With a target of 100 patients completing the 52-week study, the sample size was set at 153 patients.

RESULTS

• CHARACTERISTICS OF THE PARTICIPANTS: A total of 154 patients were treated, and 127 of those patients (82.5%) completed the study (Table 1). All 154 patients who received treatment were included in the efficacy and safety analyses. Demographic and other baseline characteristics of the patients are shown in Table 2. Of the 154 total patients, 15 patients (9.7%) were male and 139 patients (90.3%) were female. The mean age was 59.3 years (range: 24-86 years). Of the 154 patients, 26 patients (16.9%) had primary or secondary Sjögren syndrome and 5 patients (3.2%) had Stevens-Johnson syndrome as the underlying cause of dry eye. At baseline, the most frequent patient-reported symptom was dryness, followed by foreign body sensation.

• EFFICACY EVALUATION: *Objective signs.* At baseline, the mean fluorescein corneal staining score was 6.6. The mean fluorescein corneal staining score at week 2 (4.3) was significantly decreased compared with that at baseline ($P < .001$, paired *t* test), and further improvements were observed at almost every visit up to 52 weeks (1.9 at week 52) (Figure 1). The mean lissamine green conjunctival staining score at baseline was 9.4. At week 2, the mean lissamine green conjunctival staining score (6.8) was significantly decreased compared with that at baseline

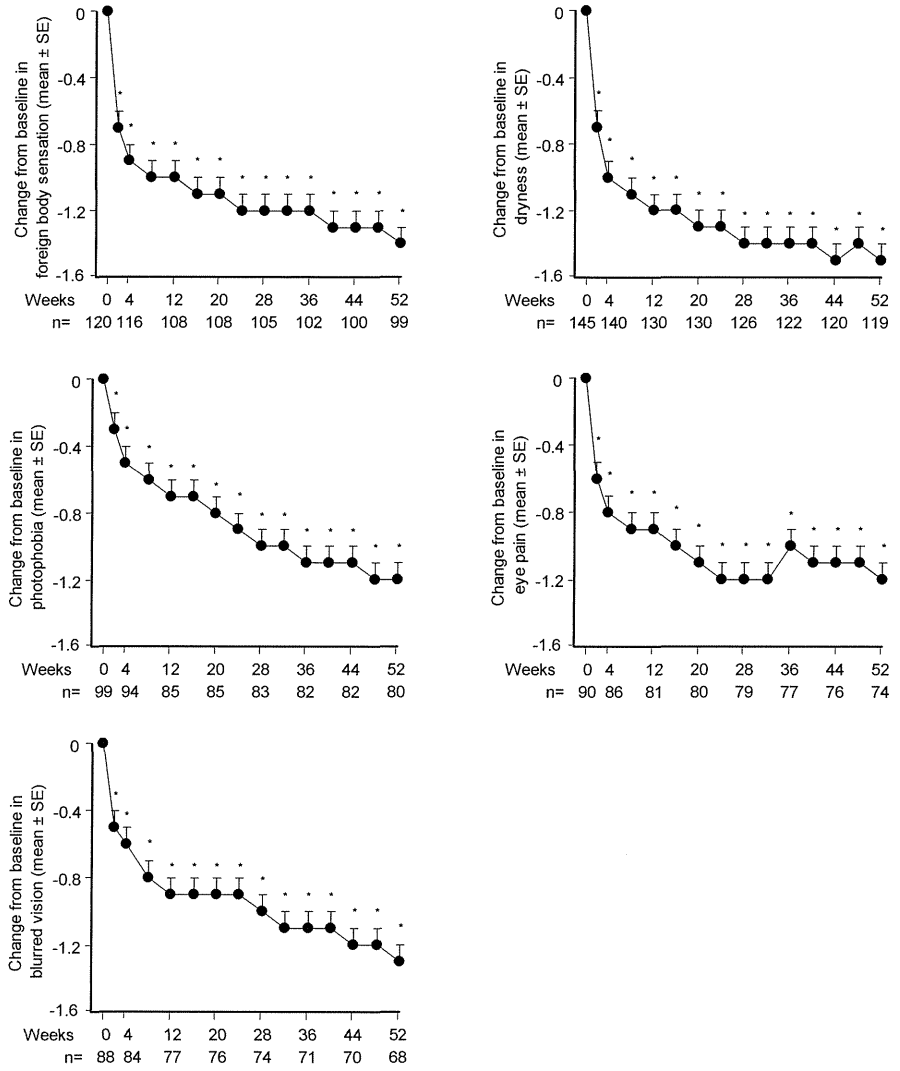


FIGURE 3. Change from baseline in dry eye–related ocular symptoms: (Top left) foreign body sensation, (Top right) dryness, (Middle left) photophobia, (Middle right) eye pain, and (Bottom) blurred vision, with 2% rebamipide (OPC-12759) ophthalmic suspension treatment in dry eye patients over 52 weeks. The scores of all 5 dry eye–related ocular symptoms significantly improved at week 2 compared with baseline, and further improvements were observed at almost every visit up to 52 weeks. *P < .001 compared with respective baseline, paired *t* test. SE = standard error.

($P < .001$, paired *t* test), and further improvements were observed at every visit up to 52 weeks (3.2 at week 52) (Figure 1). Figure 2 shows the fluorescein corneal staining and lissamine green conjunctival staining photographs at baseline and at week 52, in which corneal and conjunctival disorders were improved.

The mean TBUT gained significantly from baseline (2.41 seconds) to week 2 (2.96 seconds) ($P < .001$, paired *t* test), and further improvements were observed at every visit up to 52 weeks (3.74 at week 52) (Figure 1).

Subjective symptoms. At baseline, the mean subjective dry eye–related ocular symptom scores were 1.9, 2.2, 1.7, 1.6, and 1.8 for foreign body sensation, dryness, photophobia, eye pain, and blurred vision, respectively. The mean scores for those same subjective symptoms at week 2 (1.2, 1.5, 1.4, 1.1, and 1.3, respectively) were significantly decreased compared with those at baseline (all $P < .001$, paired *t* test; Figure 3). Further improvements were observed at almost every visit up to 52 weeks for all dry eye–related ocular symptoms (0.5, 0.8, 0.6, 0.4, and 0.4, respectively).

• SAFETY EVALUATION: Adverse events with an incidence of ≥3% are shown in Table 3. Adverse events were observed in 126 of the 154 patients (81.8%), and the most frequent adverse events were nasopharyngitis (22.1%) and dysgeusia (13.6%). Incidences of any of the adverse events did not markedly increase throughout the 52-week treatment period (Table 3).

TABLE 3. All Adverse Events With an Incidence of ≥3% in Dry Eye Patients Treated With 2% Rebamipide (OPC-12759) Ophthalmic Suspension in This Multicenter, Open-Label, 52-Week Study

		Time to Onset of Event			
	2% Rebamipide (N = 154) n (%)	Week 14 or Earlier	Week 14 to Week 28	Week 28 to Week 42	Week 42 or Later
Total number of patients	126 (81.8)	81 (52.6)	62 (40.3)	52 (33.8)	32 (20.8)
Ocular events					
Blepharitis	5 (3.2)	1 (0.6)	3 (1.9)	1 (0.6)	0 (0)
Conjunctival hemorrhage	8 (5.2)	5 (3.2)	0 (0)	3 (1.9)	0 (0)
Eye discharge	5 (3.2)	4 (2.6)	1 (0.6)	0 (0)	0 (0)
Eye irritation	6 (3.9)	2 (1.3)	3 (1.9)	0 (0)	1 (0.6)
Conjunctivitis	7 (4.5)	4 (2.6)	0 (0)	2 (1.3)	1 (0.6)
Allergic conjunctivitis	8 (5.2)	0 (0)	2 (1.3)	5 (3.2)	1 (0.6)
Trichiasis	8 (5.2)	3 (1.9)	4 (2.6)	1 (0.6)	0 (0)
Blurred vision	5 (3.2)	5 (3.2)	0 (0)	0 (0)	0 (0)
Conjunctival hyperemia	5 (3.2)	1 (0.6)	0 (0)	1 (0.6)	3 (1.9)
Nonocular events					
Abdominal discomfort	5 (3.2)	1 (0.6)	2 (1.3)	2 (1.3)	0 (0)
Nasopharyngitis (infectious)	34 (22.1)	12 (7.8)	11 (7.1)	9 (5.8)	2 (1.3)
Contusion	6 (3.9)	0 (0)	4 (2.6)	1 (0.6)	1 (0.6)
Blood urea increased	5 (3.2)	1 (0.6)	0 (0)	1 (0.6)	3 (1.9)
White blood cell count decreased	6 (3.9)	5 (3.2)	0 (0)	0 (0)	1 (0.6)
Back pain	6 (3.9)	2 (1.3)	1 (0.6)	2 (1.3)	1 (0.6)
Dysgeusia	21 (13.6)	20 (13.0)	1 (0.6)	0 (0)	0 (0)
Allergic rhinitis	6 (3.9)	2 (1.3)	0 (0)	3 (1.9)	1 (0.6)
Eczema	5 (3.2)	2 (1.3)	2 (1.3)	0 (0)	1 (0.6)

When the same event repeatedly occurred in the same subject, the event was tabulated according to the date when the event initially occurred.

Drug-related adverse events occurred in 36 of 154 patients (23.4%). Dysgeusia (21 patients, 13.6%), blurred vision (5 patients, 3.2%), and eye irritation (4 patients, 2.6%) were frequently observed as drug-related adverse events. All events resolved, and all were mild in severity except for 1 case of dysgeusia (moderate).

No deaths were reported during the study period. Serious adverse events were observed in 6 patients (3.9%) and severe adverse events were observed in 2 patients (1.3%); however, none of these events was judged to be drug related.

DISCUSSION

THE FINDINGS OF THIS OPEN-LABEL, SINGLE-ARM STUDY suggest the long-term efficacy and safety of 2% rebamipide ophthalmic suspension, administered 4 times daily for up to 52 weeks, for the treatment of patients with dry eye. For all objective signs (fluorescein corneal staining, lissamine green conjunctival staining, and TBUT) and subjective symptoms, the scores significantly improved at week 2 compared with baseline, and further improvements were observed at almost every visit up to 52 weeks. The rapid (2-week) onset of the effect of rebamipide demonstrated in this study supported the findings of previous double-masked, placebo-controlled phase II study, which verified the significant improvements of rebamipide in both objective signs and subjective symptoms over placebo.[13] The assessment of symptoms is particularly important in patients with dry eye, as objective signs do not necessarily represent the patient's subjective symptoms. Indeed, it has been reported that there is only a weak association between self-reported symptoms and objective measures of dry eye.[16] Improvements in subjective symptoms, as well as objective signs, are needed for an effective treatment of dry eye.

In this study, the 5 patients with Stevens-Johnson syndrome showed an improvement in their objective signs and subjective symptoms of dry eye up to 52 weeks, and all of those patients completed the study without discontinuation. Severe dry eye was often seen in patients with Stevens-Johnson syndrome owing to the destruction of the mucosal architecture that can result in a decrease in ocular goblet cells.[17,18] These findings suggested that 2% rebamipide could be an effective agent for treating severe dry eye caused by Stevens-Johnson syndrome.

The 52-week ocular instillation of 2% rebamipide was well tolerated, and no significant safety concerns were reported. As reported in the previous phase II and III trials of rebamipide,[13,14] the most common drug-related adverse

event was dysgeusia, which was probably attributable to the bitter taste associated with the active ingredient. Nasopharyngitis was also relatively common and may reflect the year-long duration of the study, which encompassed the annual cold and flu seasons.

Given that dry eye is a chronic condition, effective long-term treatment is needed.[3] Some artificial tear products contain preservatives that can be detrimental to eye health with long-term use. One such preservative, benzalkonium chloride, has been shown to destabilize the tear film, disrupt the corneal epithelium, decrease goblet cells, and cause conjunctival squamous metaplasia, apoptosis, and damage to deeper ocular tissues.[19,20] Such adverse effects are clearly a concern, and the use of preservative-free ocular products is thus recommended.[20,21] The data from this present study are promising, showing that the efficacy and safety of preservative-free 2% rebamipide instilled 4 times daily is maintained over 52 weeks. These findings suggested that 2% rebamipide may prove to be an excellent treatment option for patients with dry eye requiring long-term management.

In summary, the findings of this multicenter, open-label, single-arm study show that 2% rebamipide is effective in improving both the objective signs and subjective symptoms in patients with dry eye, and its efficacy was maintained for at least 52 weeks with no particular safety concerns. The ability of 2% rebamipide to reduce the corneal and conjunctival epithelial damage and symptoms associated with dry eye, together with its well-tolerated safety profile, make it a promising treatment option for patients with dry eye.

THE AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST. Shigeru Kinoshita and Norihiko Yokoi have signed an agreement as a medical specialist with Otsuka Pharmaceutical Co, Ltd, and receive a fee for their services; Saki Awamura, Norihiro Nakamichi, Hiroyuki Suzuki, and Kazuhide Oshiden are employees of Otsuka Pharmaceutical Co, Ltd. This study was sponsored and funded by Otsuka Pharmaceutical Company, Ltd, Tokyo, Japan. Contributions of authors: design of the study (S.K., S.A., N.N., H.S., K.O.); conduct of the study (N.Y., the Rebamipide Ophthalmic Suspension Long-term Study Group); collection, management, analysis, and interpretation of the data (S.A., N.N., H.S., K.O.); preparation, review and approval of the manuscript (S.K., S.A., N.N., H.S., K.O., N.Y.).

Collaborators: The Rebamipide Ophthalmic Suspension Long-term Study Group: Kazushige Sado (Sado Eye Clinic, Miyagi, Japan), Tamihiko Seimiya (Juntendo Tokyo Koto Geriatric Medical Center, Tokyo, Japan), Kenji Inoue (Inoue Eye Hospital, Tokyo, Japan), Tairo Kimura (Ueno Eye Clinic, Tokyo, Japan), Miki Uchino (Ryogoku Eye Clinic, Tokyo, Japan), Yoshinori Takahashi (Tachikawadori Eye Clinic, Tokyo, Japan), Junji Ono (Ono Ophthalmic Clinic, Shizuoka, Japan), Masako Matsuura (Matsuura Eye Clinic, Aichi, Japan), Chie Sotozono (Kyoto Prefectural University of Medicine, Kyoto, Japan), Keiko Ouchi (Ouchi Eye Clinic, Kyoto, Japan), Mitsuko Sugao (Sugao Eye Clinic, Osaka, Japan), Torao Sugiura (Sugiura Eye Institute, Osaka, Japan), Yutaka Sakiyama (Nara Hospital Kinki University Faculty of Medicine, Nara, Japan), Emiko Tani (Tani Eye Clinic, Hyogo, Japan), Kaoru Sasaki (Ideta Eye Hospital, Kumamoto, Japan), Masayoshi Migita (Migita Eye Institute, Oita, Japan), and Hidehito Kawabata (Kawabata Eye Clinic, Chiba, Japan).

REFERENCES

1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop. *Ocul Surf* 2007;5(2):75–92.
2. Lin PY, Tsai SY, Cheng CY, et al. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology* 2003;110(6):1096–1101.
3. Friedman NJ. Impact of dry eye disease and treatment on quality of life. *Curr Opin Ophthalmol* 2010;21(4):310–316.
4. de Pinho Tavares F, Fernandes RS, Bernardes TF, Bonfioli AA, Soares EJC. Dry eye disease. *Semin Ophthalmol* 2010;25(3):84–93.
5. Reddy P, Grad O, Rajagopalan K. The economic burden of dry eye: a conceptual framework and preliminary assessment. *Cornea* 2004;23(8):751–761.
6. Gipson IK. The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture. *Invest Ophthalmol Vis Sci* 2007;48(10):4391–4398.
7. Corfield AP, Carrington SD, Hicks SJ, Berry M, Ellingham R. Ocular mucins: purification, metabolism and functions. *Prog Retin Eye Res* 1997;16(4):627–656.
8. Danjo Y, Watanabe H, Tisdale AS, et al. Alteration of mucin in human conjunctival epithelia in dry eye. *Invest Ophthalmol Vis Sci* 1998;39(13):2602–2609.
9. Argueso P, Balaram M, Spurr-Michaud S, Keutman HT, Reza Dana M, Gipson IK. Decreased levels of the goblet cell mucin MUC5AC in tears of patients with Sjögren syndrome. *Invest Ophthalmol Vis Sci* 2002;43(4):1004–1011.
10. Urashima H, Okamoto T, Takeji Y, Shinohara H, Fujisawa S. Rebamipide increases the amount of mucin-like substances on the conjunctiva and cornea in the N-acetylcysteine-treated in vivo model. *Cornea* 2004;23(6):613–619.
11. Urashima H, Takeji Y, Okamoto T, Fujisawa S, Shinohara H. Rebamipide increases mucin-like substance contents and periodic acid Schiff reagent-positive cells density in normal rabbits. *J Ocul Pharmacol Ther* 2012;28(3):264–270.
12. Takeji Y, Urashima H, Aoki A, Shinohara H. Rebamipide increases the mucin-like glycoprotein production in corneal epithelial cells. *J Ocul Pharmacol Ther* 2012;28(3):259–263.
13. Kinoshita S, Awamura S, Oshiden K, et al. Rebamipide (OPC-12759) in the treatment of dry eye: a randomized, double-masked, multicenter, placebo-controlled Phase II study. *Ophthalmology* 2012;119(12):2471–2478.
14. Kinoshita S, Oshiden K, Awamura S, et al. A randomized, multicenter phase III study comparing 2% rebamipide (OPC-12759) with 0.1% sodium hyaluronate in the treatment of dry eye. *Ophthalmology* 2013;120(6):1158–1165.
15. Lemp MA. Report of the National Eye Institute/Industry workshop on clinical trials in dry eyes. *CLAO J* 1995;21(4):221–232.

16. Hay EM, Thomas E, Pal B, Hajeer A, Chambers H, Silman AJ. Weak association between subjective symptoms of and objective testing for dry eyes and dry mouth: results from a population based study. *Ann Rheum Dis* 1998;57(1):20–24.
17. Ralph RA. Conjunctival goblet cell density in normal subjects and in dry eye syndromes. *Invest Ophthalmol* 1975; 14(4):299–302.
18. Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease. *Arch Ophthalmol* 1984;102(7):1049–1051.
19. Burstein NL. The effects of topical drugs and preservatives on the tears and corneal epithelium in dry eye. *Trans Ophthalmol Soc U K* 1985;104(4):402–409.
20. Baudouin C, Labbé A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. *Prog Retin Eye Res* 2010;29(4):312–334.
21. Asbell PA, Spiegel S. Ophthalmologist perceptions regarding treatment of moderate-to-severe dry eye: results of a physician survey. *Eye Contact Lens* 2010;36(1):33–38.

Re: "Folliculitis in Clinically "Quiet" Chronic Stevens-Johnson Syndrome"

To the Editor:

Stevens-Johnson syndrome (SJS) is an acute inflammatory disorder that predominantly affects the skin and mucous membranes including the ocular surface.[1] In the acute phase, >50% of SJS patients experience ocular complications.[2] In the chronic phase, ocular complications and persistent conjunctival inflammation may develop.[3]

We previously reported the severity of eyelid complications that was significantly correlated with visual loss and the presence of ongoing histologic conjunctival inflammation in chronic SJS.[4] It reported that cicatrization of the eyelid margin and the tarsus might result in entropion and deficiency in eyelid closure and blinking, leading to the corneal complications.[5] Though the management of eyelid complications in chronic SJS is often difficult because of the recurrence of trichiasis and entropion, little is known about the histologic changes in the eyelid during the chronic phase of SJS. To the best of our knowledge, there have been no histopathologic studies of the ciliary follicles in SJS, and hence, we examined eyelid tissue from chronic SJS patients.

We examined specimens including ciliary follicles from 7 consecutive patients with SJS and 4 patients with no ocular surface disorders as controls (non-SJS) who underwent eyelid surgery for treatment of trichiasis with or without entropion between December 2008 and May 2012.

T1 Patients' information is shown in Table. Cases 1 to 7 were SJS patients. Case 1 had severe trichiasis and entropion with ongoing conjunctival inflammation and corneal erosions since her initial attack 2 years previously. Cases 2 to 7 were chronic SJS, and Cases 8 to 11 were non-SJS with no clinical evidence of ongoing inflammation. Resected tissues of eyelid margin including ciliary follicles for treatment of trichiasis were examined histopathologically (all cases) and immunohistochemically (4 of SJS and 2 of non-SJS). Patients with a confirmed history of SJS and chronic ocular complications that persisted for at least 1 year from the onset of SJS were included. Routine paraffin sections were prepared, and histopathologic and immunohistochemical examinations were performed.

Histopathologic examination showed severe diffuse inflammatory cell infiltration in Case 1, and moderate inflammation limited around the ciliary follicles in Cases 2, 5, and 6. Mild inflammation was seen in Cases 3, 4, and 7 to 11. Immunohistochemical staining was performed in Cases 1 to 4, 8, and 9. CD3, CD4, and CD45RO positive cells were strongly present in Case 1, moderately present limited around the ciliary follicles in Case 2, and weakly present in Cases 3, 4, 8, and 9 (Fig.). CD68 positive cells were strongly present in Case 1, moderately in Case 2, and mildly in Cases 3, 4, 8, and 9. CD8 positive cells were moderate in Case 1 and weakly present in the others. CD20 positive cells and mast cell tryptase were weakly positive in all cases. Neutrophil erastase positive cells were mildly present in Case 3 and 4.

It is interesting to note that recurrence of trichiasis requiring further surgery was seen not only in Case 1 who had ongoing conjunctival inflammation but also in Cases 2, 5, and 6 who had chronic SJS during 1 year after the first surgery. All recurrent cases had at least moderate histologic inflammation at the time of initial surgery. Additional surgery was not required in the other cases. This may suggest a correlation between recurrence of trichiasis and subclinical inflammation.

Our results indicate that significant inflammatory cells around the ciliary follicles can be found in some cases of clinically "quiet" chronic SJS. This ongoing subclinical inflammation in patients who may appear clinically quiescent may contribute to the propensity for recurrence of entropion and trichiasis in those with chronic SJS.

Akihide Watanabe, M.D.
Chie Sotozono, M.D., Ph.D.
Mayumi Ueta, M.D., Ph.D.
Katsuhiko Shinomiya, Ph.D.
Shigeru Kinoshita, M.D., Ph.D.
Hirohiko Kakizaki, M.D., Ph.D.
Dinesh Selva, F.R.A.C.S., F.R.A.N.Z.C.O.

Correspondence: Akihide Watanabe, M.D., Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Kamigyo-ku, Kyoto, Japan 602-0841 (awatanab@koto.kpu-m.ac.jp)

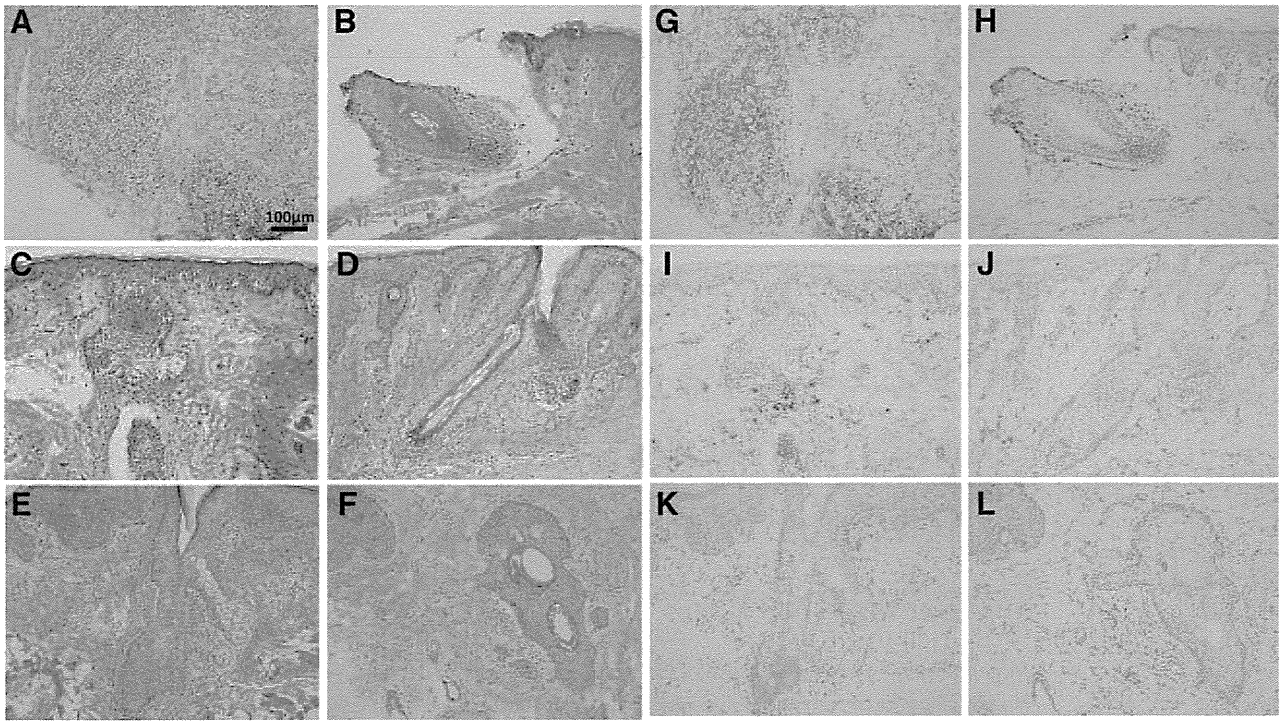
Patients' data

Case no.	Age	Sex	Disease	Recurrence	Clinical inflammation	Histologic inflammation	CD3	CD4	CD45RO	CD68	CD8	CD20	Mast cell tryptase	Neutrophil erastase
1	38	F	SJS	1	Severe	Severe	+++	+++	+++	+++	+	−	+	−
2	30	M	SJS	2	None	Moderate	++	++	++	++	−	−	+	−
3	38	M	SJS	0	None	Mild	−	−	−	+	−	−	+	+
4	70	F	SJS	0	None	Mild	−	−	−	+	−	−	+	+
5	1	M	SJS	2	None	Moderate								
6	38	F	SJS	1	None	Moderate								
7	38	M	SJS	0	None	Mild								
8	6	M	Non-SJS	0	None	Mild	−	−	−	+	−	−	+	−
9	79	M	Non-SJS	0	None	Mild	−	−	−	+	−	−	+	−
10	85	M	Non-SJS	0	None	Mild								
11	69	F	Non-SJS	0	None	Mild								
12	38	F	Non-SJS	0	None	Mild								

Age, age at surgery; Clinical inflammation, clinical inflammation at the time of initial surgery; F, Female; Histologic inflammation, inflammative cell infiltration; M, Male; Recurrence, recurrence of trichiasis requiring further surgery; SJS, Stevens-Johnson syndrome; +++, strong immunoreactive; ++, moderatate immunoreactive; +, mild immunoreactive; −, weakly immunoreactive.

<z10.1097/01.iop.0000440709.90516.87>

S. Anita	12/6/13	4 Color Fig: F1	14:19	Art:OPRS-D-13-00327



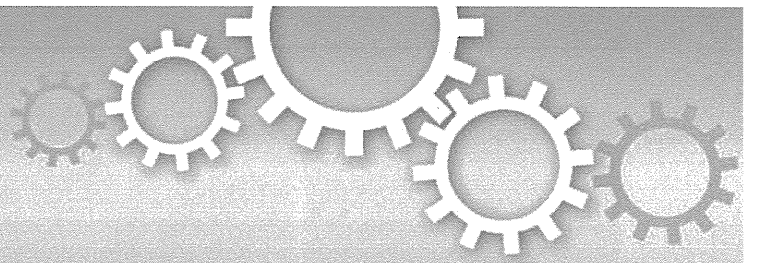
Hematoxylin-eosin staining (A–F) and immunohistochemical staining (anti-CD45RO) (G–L). (A–D, G–J) SJS patients, A and G: Case 1, B and H: Case 2, C and I: Case 3, D and J: Case 4. (E, F, K, L): non-SJS patients, **E** and **K**: Case 8, **F** and **L**: Case 9. **A,** Severe inflammation; **B,** Moderate inflammation limited around the follicle; **C–F,** Slight inflammation. **G,** Strong immunopositive; **H,** Moderate immunopositive limited around the follicle; **I–L,** Weakly immunopositive.

AQ1 The authors have no financial or conflicts of interest to disclose.

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. 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.
3. Kawasaki S, Nishida K, Sotozono C, et al. Conjunctival inflammation in the chronic phase of Stevens-Johnson syndrome. *Br J Ophthalmol* 2000;84:1191–3.
4. 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.
5. Di Pascuale MA, Espana EM, Liu DT, et al. Correlation of corneal complications with eyelid cicatricial pathologies in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis syndrome. *Ophthalmology* 2005;112:904–12.

SCIENTIFIC REPORTS



OPEN

SUBJECT AREAS:
DISEASES
BIOMARKER RESEARCH

Received
28 November 2013

Accepted
14 April 2014

Published
30 April 2014

Correspondence and requests for materials should be addressed to M.U. (mueta@koto.kpu-m.ac.jp)

Independent strong association of *HLA-A*02:06* and *HLA-B*44:03* with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement

Mayumi Ueta[1,2], Nahoko Kaniwa[3], Chie Sotozono[1], Katsushi Tokunaga[4], Yoshiro Saito[3], Hiromi Sawai[4], Hiroko Miyadera[4], Emiko Sugiyama[3], Keiko Maekawa[3], Ryosuke Nakamura[3], Masaki Nagato[5], Michiko Aihara[6], Kayoko Matsunaga[7], Yukitoshi Takahashi[8], Hirokazu Furuya[9], Masaaki Muramatsu[10], Zenrou Ikezawa[11] & Shigeru Kinoshita[1]

[1]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan, [3]Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan, [4]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [5]Molecular Diagnostics Division, Wakunaga Pharmaceutical Co., Hiroshima, Japan, [6]Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, Kanagawa, Japan, [7]Department of Dermatology, Fujita Health University School of Medicine, Aichi, Japan, [8]National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan, [9]Department of Neurology, Faculty of Medicine, Kochi University, Kochi, Japan, [10]Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan, [11]Department of Dermatology, International University of Health and Welfare (IUHW) Atami Hospital, Shizuoka, Japan.

Stevens-Johnson syndrome (SJS) and its severe variant, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes. Cold medicines including non-steroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient cold medications are reported to be important inciting drugs. We used two sample sets of Japanese patients to investigate the association between HLA genotypes and cold medicine-related SJS/TEN (CM-SJS/TEN), including acetaminophen-related SJS/TEN (AR-SJS/TEN) with severe mucosal involvement such as severe ocular surface complications (SOC). *HLA-A*02:06* was strongly associated with CM-SJS/TEN with SOC and AR-SJS/TEN with SOC. *HLA-B*44:03* was also detected as an independent risk allele for CM-, including AR-SJS/TEN with SOC. Analyses using data obtained from CM-SJS/TEN patients without SOC and patients with CM-unrelated SJS/TEN with SOC suggested that these two susceptibility alleles are involved in the development of only CM-SJS/TEN with SOC patients.

Stevens-Johnson syndrome (SJS) is an acute inflammatory vesiculobullous reaction of the skin and mucous membranes such as the ocular surface, oral cavity, and genitals. It is rare but often associated with inciting drugs and/or infectious agents[1–3]. In patients with extensive skin detachment and a poor prognosis the condition is called toxic epidermal necrolysis (TEN)[4]. The annual incidence of SJS and TEN has been reported as 1–6 and 0.4–1.0 cases per million persons, respectively[3,5] and the mortality rate as 3% and 27%, respectively[6].

The association between human leukocyte antigen (HLA) genotypes and drug-induced severe cutaneous adverse reactions (SCAR) including SJS/TEN has been reported. In Taiwanese Han Chinese patients the *HLA-B*15:02* allele exhibited a very strong association with carbamazepine-induced SJS/TEN[7]. Similarly, in Japanese-[8] and European individuals[9] the *HLA-A*31:01* allele was strongly associated with carbamazepine-induced SCAR including SJS/TEN and drug-induced hypersensitivity syndrome (DIHS). Allopurinol, a uric acid-lowering drug, often induced SCAR including SJS, TEN and DIHS, and allopurinol-induced SCARs were strongly associated with *HLA-B* 58:01* in Han Chinese-[10], Caucasian-[11], and Japanese patients[12], suggesting that different ethnic groups may share the same risk factor for allopurinol-induced SCARs. Mockenhaupt et al.[13] reported that

Table 1 | Demographic and background data of patients and controls

Explaination of subjects		Group 1 (KPUM)	Group 2 (NIHS)
a	Number of SJS/TEN patients with SOC who had taken cold medicines for treatment of common cold (CM-SJS/TEN with SOC group)	131	20
	Female/Male	80/51	14/6
	Age of onset (years, mean ± SD)	26.6 ± 17.5	54.0 ± 17.7
b (which are included in a)	Number of SJS/TEN patients with SOC who had taken acetaminophen for treatment of common cold (Acetaminophen-SJS/TEN with SOC group)	(59)	(14)
	Female/Male	37/22	9/5
	Age of onset (years, mean ± SD)	31.1 ± 15.8	35.2 ± 16.9
c	Patients with SJS/TEN without SOC who had taken cold medicines for treatment of common cold (CM-SJS/TEN without SOC group)		16
	Female/Male	-	9/7
	Age of onset (years, mean ± SD)		62.0 ± 25.0
d	Patients with SJS/TEN with SOC who had taken medicines not for treatment of common cold (CM unrelated-SJS/TEN with SOC group)	14	38
	Female/Male	11/3	19/19
	Age of onset (years, mean ± SD)	44.8 ± 19.3	57.4 ± 23.1
the samples excluded because of drug unrelated or detail unknown		17	-
total number of the SJS/TENpatients		162	74
Controls	Healthy volunteers	419	220
	Female/Male	350/69	131/89
	Age (years, mean ± SD)	-	35.5 ± 11.0

CM-SJS/TEN: Cold medicine-related SJS/TEN.
SOC: severe ocular surface complications.
KPUM: Kyoto Prefectural University of Medicine, NIHS: National Institute of Health Sciences.

allopurinol and anticonvulsants such as carbamazepine are the main inciting drugs for SJS/TEN; we[14] and others[2,4] found that cold medicines including non-steroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient cold medications are also major causative drugs for SJS/TEN. However, there have been no reports on the association between HLA genotypes and cold medicines in patients with SCAR.

Many SJS/TEN survivors suffer severe sequelae such as visual disturbance due to severe ocular surface complications (SOC) in the acute phase of the disease. In our earlier study of 71 Japanese SJS/TEN patients we reported the strong association between *HLA-A*02:06* and SJS/TEN with SOC[15]. We found that a considerable number of these patients used cold medicines to treat the common cold[14]. Therefore, in this study we focused on a possible association between HLA genotypes and cold medicine (NSAIDs and analgesics)-related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement including SOC.

Results

HLA-type associated with CM-SJS/TEN with SOC. First we compared the carrier frequencies of HLA alleles in the 131 CM-SJS/TEN with SOC patients and in 419 controls. The results are summarized in Table 2.

*HLA-A: HLA-A*02:06* was strongly associated with CM-SJS/TEN with SOC ($p = 2.8 \times 10^{-16}$, $Pc = 4.8 \times 10^{-15}$, odds ratio (OR) = 5.7). *HLA-A*24:02* was inversely associated with CM-SJS/TEN with SOC ($p = 3.9 \times 10^{-4}$, Pc = 0.0066, OR = 0.5). *HLA-A*03:01* was weakly associated with the risk for- and *HLA-A*11:01* was weakly associated with resistance to CM-SJS/TEN with SOC; the association was not significant after Bonferroni correction.

*HLA-B: HLA-B*13:01, HLA-B*44:02, HLA-B*44:03*, and *HLA-B*46:01* were weakly associated with CM-SJS/TEN with SOC; the association was not significant after correction. *HLA-B*15:01, HLA-B*52:01* and *HLA-B*54:01* were weakly inversely associated with CM-SJS/TEN with SOC; the association was not significant after correction.

*HLA-C: HLA-C*03:04* and *HLA-C*05:01* were weakly associated- and *HLA-C*12:02* was weakly and inversely associated with CM-SJS/TEN with SOC; the association was not significant after correction.

Next, to confirm these associations we compared the carrier frequency of HLA alleles with p values less than 0.05 before Bonferroni correction in the 131 CM-SJS/TEN with SOC of Group 1a, in another 20 CM-SJS/TEN with SOC patients (Group 2a) and 220 healthy controls of Group2.

In Group 2a (n = 20), *HLA-A*02:06* and *HLA-B*44:03* were significantly associated with CM-SJS/TEN with SOC (p = 0.0014, Pc = 0.0056, OR = 5.2 and p = 0.0058, Pc = 0.0406, OR = 4.22, respectively) (Table 3). However, the other HLA alleles examined were not significantly associated. Although the patient backgrounds were a little bit different in Groups 1a and 2a (1a: CM-SJS/TEN with SOC as sequelae, 2a: CM-SJS/TEN with SOC in the acute phase), we identified the same HLA types, *HLA-A*02:06* and *HLA-B*44:03*, as risk factors for CM-SJS/TEN with SOC.

As we observed the same tendency in Groups 1a and 2a, we combined the 151 CM-SJS/TEN with SOC patients (Group 1a, n = 131; Group 2a, n = 20) to compare the carrier frequencies of *HLA-A*02:06* and *HLA-B*44:03* with the frequencies in the 639 combined healthy controls. (Group 1, n = 419; Group 2, n = 220). The combined data revealed a strong association of *HLA-A*02:06* and *HLA-B*44:03* with CM-SJS/TEN with SOC (*HLA-A*02:06*, $p = 2.7 \times 10^{-20}$, OR = 5.6; *HLA-B*44:03*, $p = 1.25 \times 10^{-3}$, OR = 1.99) (Table 4a).

Comparison between CM-SJS/TEN with and without SOC. Among 16 CM-SJS/TEN without SOC patients (Group 2c), 2 carried *HLA-A*02:06* and none carried *HLA-B*44:03* (Table 4b). These carrier frequencies did not differ significantly from the Group 2 controls (p = 1.000 and p = 0.2324, respectively). These results suggest that *HLA-A*02:06* and *HLA-B*44:03* are not common risk factors for both CM-SJS/TEN with and without SOC, but were risk factors for only CM-SJS/TEN with SOC.

For further confirmation we compared the carrier frequency of both HLA alleles in the 151 combined CM-SJS/TEN with SOC patients (Group 1a, n = 131, Group 2a, n = 20) and in the 16 CM-SJS/TEN without SOC patients in Group 2c. The carrier frequencies of both alleles were significantly higher in the CM-SJS/TEN with SOC (Group 1a + Group 2a) than in the CM-SJS/TEN without

Table 2 | Results of association analysis for HLA types and CM-SJS/TEN with SOC in Group 1 (KPUM)

HLA genotype	Carrier frequency (%)		Dominant model analysis		
	Case (n = 131)	Control (n = 419)	P	Pc	Odds ratio (95% CI)
HLA-A					
*A*02:06*	62/131 (47.3%)	57/419 (13.60%)	**2.79.E-16**	**4.75E-15**	**5.71 (3.666-8.881)**
*A*03:01*	5/131 (3.82%)	4/419 (0.95%)	**0.0242**	0.412	4.12 (1.089-15.564)
*A*11:01*	10/131 (7.6%)	71/419 (16.95%)	**8.67.E-03**	0.147	0.405 (0.202-0.811)
*A*24:02*	57/131 (43.5%)	256/419 (61.10%)	**3.89.E-04**	**6.60.E-03**	**0.490 (0.330-0.730)**
HLA-B					
*B*13:01*	10/131 (7.6%)	13/419 (3.10%)	**0.0237**	0.807	2.58 (1.104-6.032)
*B*15:01*	11/131 (8.4%)	69/419 (16.47%)	**0.0222**	0.755	0.465 (0.238-0.908)
*B*44:02*	5/131 (3.82%)	5/419 (1.19%)	**0.0498**	1.69	3.29 (0.936-11.532)
*B*44:03*	31/131 (23.7%)	66/419 (15.75%)	**0.0381**	1.29	1.66 (1.024-2.682)
*B*46:01*	22/131 (16.8%)	38/419 (9.07%)	**0.0133**	0.453	2.02 (1.148-3.566)
*B*52:01*	12/131 (9.2%)	79/419 (18.85%)	**9.16.E-03**	0.311	0.434 (0.228-0.825)
*B*54:01*	10/131 (7.6%)	61/419 (14.56%)	**0.0391**	1.33	0.485 (0.241-0.976)
HLA-C					
*C*03:04*	42/131 (32.1%)	98/419 (23.39%)	**0.0467**	0.841	1.55 (1.00-2.38)
*C*05:01*	5/131 (3.82%)	5/419 (1.19%)	**0.0498**	0.897	3.29 (0.936-11.532)
*C*12:02*	13/131 (9.9%)	80/419 (19.09%)	**0.0145**	0.262	0.467 (0.251-0.870)

P: P values obtained with χ^2-tests.
Pc: P values corrected for the multiplicity of testing by the number of comparisons (17, 34, and 18 for *HLA-A*, *HLA-B* and *HLA-C*, respectively).
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: confidence interval.

SOC (Group 2c) (*HLA-A*02:06*, p = 0.00812, OR = 6.2; *HLA-B*44:03*, p = 0.02023, OR = 11.59) (Table 4b).

Analysis of CM unrelated-SJS/TEN with SOC. As shown in Table 1, Group 1d contained 14- and Group 2d contained 38 patients with CM unrelated (other medicine related) -SJS/TEN with SOC. Among the 14 CM unrelated-SJS/TEN with SOC patients from Group 1d, 3 carried *HLA-A*02:06* and 4 carried *HLA-B*44:03*. Among the 38 CM unrelated SJS/TEN with SOC patients from Group 2d, 4 manifested *HLA-A*02:06* and 2 had *HLA-B*44:03*. To obtain higher power, we combined the data from the 52 CM unrelated -SJS/TEN with SOC patients from Groups 1d (n = 14) and 2d (n = 38) and compared their carrier frequency with that of combined healthy volunteers (n = 639). As shown in Table 4c, the carrier frequencies of *HLA-A*02:06* and *HLA-B*44:03* were comparable in the 2 groups (52 CM unrelated -SJS/TEN with SOC patients and 639 controls) and the difference was not statistically significant.

Analysis of acetaminophen-SJS/TEN with SOC (AR-SJS/TEN with SOC). Acetaminophen is contained as an analgesic in most cold medicines. At least 59 patients in Group 1b and 14 in Group 2b were known to have taken acetaminophen for a few ~ several days before the onset of SJS/TEN. Therefore we examined the association of *HLA-A*02:06* and *HLA-B*44:03* with acetaminophen-related SJS/TEN (AR-SJS/TEN) with SOC using the combined data (73 AR-SJS/TEN with SOC from 59 in Group 1b and 14 in Group 2b). In all 73

Table 3 | Results of association analysis between HLA types and CM-SJS/TEN with SOC in Group 2 (NIHS)

HLA genotype	Carrier frequency (%)		Dominant model analysis		
	Case (n = 20)	Control (n = 220)	P	Pc	Odds ratio (95% CI)
HLA-A					
*A*02:06*	9/20 (45.0%)	30/220 (13.6%)	**0.0014**	**0.00560**	**5.18 (1.98–13.56)**
*A*03:01*	0/20 (0%)	19/220 (8.6%)	0.3804		
*A*11:01*	2/20 (10.0%)	39/220 (17.7%)	0.5408		
*A*24:02*	14/20 (70.0%)	132/220 (60.0%)	0.4770		
HLA-B					
*B*13:01*	2/20 (10%)	6/220 (2.7%)	0.1364		
*B*15:01*	2/20 (10%)	39/220 (17.7%)	0.5408		
*B*44:02*	0/20 (0%)	4/220 (1.8%)	1.0000		
*B*44:03*	8/20 (40.0%)	30/220 (13.6%)	**0.0058**	**0.0406**	**4.22 (1.59–11.19)**
*B*46:01*	2/20 (10%)	18/220 (8.2%)	0.6764		
*B*52:01*	1/20 (5.0%)	48/220 (21.8%)	0.0857		
*B*54:01*	5/20 (25%)	33/220 (15.0%)	0.3316		
HLA-C					
*C*03:04*	6/20 (30%)	43/220 (19.5%)	0.2573		
*C*05:01*	0/20 (0%)	4/220 (1.8%)	1.0000		
*C*12:02*	1/20 (5.0%)	47/220 (21.4%)	0.1388		

P: p-values obtained by Fisher's exact tests are shown.
Pc: p-values corrected for the mutiplicity of testing by the number of comparisons: (4, 7 and 3 for HLA-A, HLA-B and HLA-C, respectively).
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: Confidence interval.

Table 4 | Results of association analyses using combined SJS/TEN patients' data

a. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	CM-SJS/TEN with SOC (Group 1a and Group 2a)	Control (Combined healthy controls)	p	Odds ratio (95% CI)
*A*02:06*	71/151 (47.0%)	87/639 (13.6%)	2.72E-20	5.63 (3.81–8.33)
*B*44:03*	39/151 (25.8%)	95/639 (14.9%)	0.00125	1.99 (1.30–3.05)

b. Comparison between CM-SJS/TEN with SOC (Group 1a and Group 2a) and without SOC (Group 2c)

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	CM-SJS/TEN with SOC (Group 1a and Group 2a)	CM-SJS/TEN without SOC (Group 2c)	p	Odds ratio (95% CI)
*A*02:06*	71/151 (47%)	2/16 (12.5%)	0.00812	6.21 (1.36–28.28)
*B*44:03*	39/151 (25.8%)	0/16 (0%)	0.02023	11.59* (0.68–197.7)

c. Comparison of CM unrelated SJS/TEN with SOC and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis
HLA genotype	CM unrelated-SJS/TEN with SOC (Group 1d and Group 2d)	Control (Combined healthy controls)	p
*A*02:06*	7/52 (13.5%)	87/639 (13.6%)	0.975
*B*44:03*	6/52 (11.5%)	95/639 (14.9%)	0.514

d. Comparison between Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b) and combined healthy volunteers' data

	Carrier frequency (%)		Dominant model analysis	
HLA genotype	Acetaminophen-SJS/TEN with SOC (Group 1b and Group 2b)	Control (Combined healthy controls)	p	Odds ratio (95% CI)
*A*02:06*	37/73 (50.7%)	87/639 (13.6%)	2.54E-15	6.52 (3.91–10.88)
*B*44:03*	20/73 (27.4%)	95/639 (14.9%)	0.0059	2.16 (1.27–3.78)

*Woolf's correction.
P: P values obtained by χ^2-tests.
CM-SJS/TEN: cold medicine related SJS/TEN who had taken cold medicine.
SOC: severe ocular surface complications.
CI: Confidence interval.

patients with AR-SJS/TEN with SOC, we found a significant association with both alleles (*HLA-A*02:06*, p = 2.5 × 10^{-15}, OR = 6.5; *HLA-B*44:03*, p = 0.0059, OR = 2.2) (Table 4d).

Discussion

In this study we examined possible HLA risk factors for CM-SJS/TEN with SOC using two independently collected data sets of Japanese SJS/TEN patients.

The carrier frequency of *HLA-A*02:06*, which we reported to have a very strong association with causative drug-unspecified SJS/TEN with SOC[15,19], was significantly higher in CM-SJS/TEN with SOC patients than in the healthy controls. This significant association was maintained in AR-SJS/TEN with SOC.

On the other hand, the carrier frequency of *HLA-A*02:06* in the 16 CM-SJS/TEN without SOC patients of Group 2c and the 52 CM-unrelated SJS/TEN with SOC patients from Groups 1d and 2d did not significantly differ from that in our healthy controls. These results suggest that *HLA-A*02:06* is a risk factor for CM-SJS/TEN with SOC but not for CM-SJS/TEN without SOC or CM-unrelated SJS/TEN with SOC.

Moreover, *HLA-A*02:06* and *HLA-B*44:03* might not be primarily associated with only infection related SJS/TEN, because drug-unrelated SJS/TEN with SOC in KPUM, which seemed to be only infectious agents-related SJS/TEN, was not associated with *HLA-A*02:06* and *HLA-B*44:03* in our preliminary study (Supplemental Table 1).

The carrier frequeny of *HLA-A*02:06* in all of our healthy controls was 13.6% (Tables 2 and 3), indicating that *HLA-A*02:06* is a very common allele in the Japanese. However, as it is very rare in Caucasians and less frequent in Southern Han Chinese[20], in these populations, this allele might not be a major risk factor for CM-SJS/TEN with SOC. We also found a significant association between *HLA-B*44:03* and CM-SJS/TEN with SOC (including AR-SJS/TEN with SOC). This association was not detected in CM-SJS/TEN without SOC patients nor in CM-unrelated SJS/TEN with SOC patients. This again suggests *HLA-B*44:03* as a risk factor for CM-SJS/TEN with SOC. Data on our controls (Tables 2 and 3) indicate that *HLA-B*44:03* is a common *HLA-B* type in the Japanese population. Unlike *HLA-A*02:06*, *HLA-B*44:03* is observed in Asians, Caucasians and Africans[21]. Reports from the USA[22] and France[23,24] showed that the *HLA-B12* (*HLA-Bw44*) antigen was significantly increased in Caucasian SJS patients. The *HLA-B12* antigen is mainly coded by *HLA-B*44:02* or *HLA-B*44:03* (http://www.allelefrequencies.net/).

Cold medicines were reported to be major causative drugs in SJS/TEN in Europe[4] and in its drug safety communications, the U.S. Food and Drug Administration (http://www.fda.gov/Drugs/DrugSafety/ucm363041.htm) alerted to the possibility of serious skin reactions to acetaminophen. The significant association of HLA-B12 with SJS/TEN in European patients may be attributable to their genetic backgrounds. To determine whether *HLA-B*44:03* is a common risk

factor for CM-SJS/TEN with SOC in various populations, independent association studies in divergent ethnic groups are needed.

Because *HLA-A*02:06* is rarely a haplotype with *HLA-B*44:03* (http://www.allelefrequencies.net/), these two HLA alleles might be independent genetic risk factors that render the host susceptible to severe mucosal disorders and to severe sequelae such as visual disturbance when SJS/TEN develops after the administration of cold medicines including NSAIDs. In our study, 96 of 151 patients (63.6%) with CM-SJS/TEN with SOC (group 1, n = 131; group 2, n = 20) harbored either *HLA-A*02:06* or *HLA-B*44:03*. On the other hand, only 177 of our 639 controls (27.7%) had one of these *HLA* alleles.

Forman et al.[25] and Leaute-Labreze[26] reported other infectious agents as triggers of SJS/TEN. Elsewhere[27] we showed that rs3775296T/T, a SNP of *Toll-like receptor 3 (TLR3)*, was a risk factor for SJS/TEN with SOC and that the interaction between rs3775296T/T and *HLA-A*02:06* exerted more than additive effects. TLR3 is a pattern-recognizing receptor related to innate immunity after viral infections that often produce common cold symptoms. Moreover, cold medicines such as acetaminophen and NSAIDs, including ibuprofen and loxoprofen, commonly down-regulate the production of prostanoid including PGE_2. We also reported earlier that in our study population, EP3, which is one of the PGE_2 receptors, polymorphisms were strongly associated with SJS/TEN with SOC[14] and that the EP3 protein levels were much lower in the conjunctival epithelial cells of SJS/TEN patients than in the control subjects[14,28]. It is noteworthy that in our earlier study of SJS/TEN with SOC patients[14] about 80% had CM-SJS/TEN with SOC. It might be possible that not only cold medicine but cold medicine with infectious agent could cause CM-SJS/TEN with SOC, because the patients develop CM- SJS/TEN with SOC by taking cold medicines after having common cold induced by infectious agents. We believe that interactions between *HLA* risk factors detected in the current study and *TLR3*, and/or *EP3* might be keys in the pathogenesis of CM-SJS/TEN with SOC.

In summary, we reported the association between certain *HLA* types and CM-SJS/TEN with SOC. We propose that *HLA-A*02:06* and *HLA-B*44:03* be considered as strong risk factors for CM-SJS/TEN with SOC. Our findings may help to elucidate the pathogenesis of CM-SJS/TEN with SOC.

Methods

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

Patients and controls. Japanese SJS/TEN patients (n = 236) were independently recruited at Kyoto Prefectural University of Medicine (KPUM)(Group 1, n = 162) and by the Japan Severe Adverse Reactions Research Group, mainly conducted by the National Institute of Health Sciences (NIHS) (Group 2, n = 74).

Between October 2004 and May 2013, 162 SJS/TEN with SOC were treated at Kyoto Prefectural University of Medicine; of these, 71 were included in our previous study[15]. The diagnosis of SJS/TEN with SOC was based on a confirmed history of acute-onset high fever, serious mucocutaneous illness with skin eruptions, and the involvement of at least 2 mucosal sites including the oral cavity and ocular surface. Some of the patients had developed SJS/TEN many years before recruitment for this study. Of the 162 patients in Group 1, 131 patients had taken cold medicines such as NSAIDs and multi-ingredient cold medications for a few ~ several days before disease onset for common-cold symptoms; they were classified as CM-SJS/TEN with SOC (Group 1a). Although the specific drugs were not identified by all 131 CM-SJS/TEN with SOC patients, 59 of 131 CM-SJS/TEN with SOC patients (45%) reported taking medicines containing acetaminophen (AR-SJS/TEN with SOC, Group 1b). Among the 162 of SJS/TEN with SOC patients (Group 1), 14 patients (Group 1d) were classified as CM unrelated-SJS/TEN with SOC, because they manifested anticonvulsants-related SJS/TEN with SOC (n = 10) or SJS/TEN with SOC after being treated with antimalarial-, anticancer-, or anti-depressive agents or steroids n = 4). We also excluded 17 patients; in 9 SJS/TEN with SOC the drugs were unknown and in 8 SJS/TEN with SOC were not related to drugs.

Group 2 (n = 74) consisted of patients with newly-developed SJS/TEN; they were recruited between June 2006 and May 2013 by participating institutes or via a nationwide blood sampling network operated by the NIHS in cooperation with the Ministry of Health, Labour and Welfare, the Pharmaceutical and Medical Devices Agency, and the Federation of Pharmaceutical Manufacturers' Association of Japan. The criteria proposed by Bastuji-Garin et al.[16] were used for a diagnosis of SJS/TEN in this group.

Ocular surface complications were judged to be severe ocular complications (SOC) when pseudo-membrane formation and/or conjunctival or corneal epithelial defects were observed in the acute phase. As shown in Table 1, Group 2 (n = 74) consisted of 20 patients with CM-SJS/TEN with SOC (Group 2a), all but 6 of these presented with AR-SJS/TEN with SOC (Group 2b). Group 2 also included 16 patients with CM-SJS/TEN without SOC (Group 2c), and 38 patients with CM-unrelated-SJS/TEN with SOC (Group 2d). The background of the 236 patients with SJS/TEN in group1 and group2 is summarized in Table 1.

Healthy Japanese volunteers (n = 639) served as the controls. They were independently recruited by the University of Tokyo (n = 419)[17] and by Kyoto Prefectural University of Medicine (n = 220)[18] and served for comparison studies of patient groups 1 and 2, respectively. In this study we enrolled only mainland Japanese.

HLA genotyping. We analyzed *HLA-A, -B, and -C* of all 162 group 1 patients, which consist of 131 CM-SJS/TEN with SOC (group 1a), 14 CM-unrelated (other medicine related) SJS/TEN with SOC (group 1d), and 17 SJS/TEN with SOC excluded because of being drug-unrelated and detail unknown. We performed polymerase chain reaction (PCR) assays followed by hybridization with sequence-specific oligonucleotide probes (PCR-SSO) using commercial bead-based typing kits (Wakunaga, Hiroshima, Japan). In group 2 (n = 74) we performed high-resolution HLA typing with a sequence-based method using SeCoreA, -B, and -C, locus sequencing kits (Invitrogen Corp., Brown Deer, WI, USA) and ABI 3730 and 3130 DNA sequencers (Applied Biosystems, Foster City, CA, USA). HLA genotypes were assigned using Assign SBT- or Assign ATF software (versions 3.2.7b and 1.0.2.41; respectively, Conexio Genomics, Western Australia, Australia). We also genotyped all volunteers for *HLA-A, -B, and -C* using PCR-SSO and commercial bead-based typing kits (Wakunaga or One Lambda, CA, USA).

Statistical analysis. We compared the carrier frequency of individual HLA alleles between our patients and controls based on the dominant model using the χ^2-test (Labo Server software;World Fusion, Tokyo, Japan) or Fisher's exact test (JMP version 7.0.1 software; SAS Institute Japan Ltd., Tokyo, Japan). Significance levels were corrected with the Bonferroni correction for multiple comparisons.

1. Ueta, M. *et al.* Toll-like receptor 3 gene polymorphisms in Japanese patients with Stevens-Johnson syndrome. *Br J Ophthalmol* **91**, 962–965 (2007).
2. Yamane, Y., Aihara, M. & Ikezawa, Z. Analysis of Stevens-Johnson syndrome and toxic epidermal necrolysis in Japan from 2000 to 2006. *Allergol Int* **56**, 419–425 (2007).
3. Yetiv, J. Z., Bianchine, J. R. & Owen, J. A., Jr. Etiologic factors of the Stevens-Johnson syndrome. *South Med J* **73**, 599–602 (1980).
4. Roujeau, J. C. *et al.* Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N Engl J Med* **333**, 1600–1607 (1995).
5. Chan, H. L. *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* **126**, 43–47 (1990).
6. Power, W. J., Ghoraishi, M., Merayo-Lloves, J., Neves, R. A. & Foster, C. S. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectrum. *Ophthalmology* **102**, 1669–1676 (1995).
7. Chung, W. H. *et al.* Medical genetics: A marker for Stevens-Johnson syndrome. *Nature* **428**, 486 (2004).
8. Ozeki, T. *et al.* Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Hum Molec Genetics* **20**, 1034–1041, DOI:10.1093/hmg/ddq537 (2011).
9. McCormack, M. *et al.* HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N Engl J Med* **364**, 1134–1143, DOI:10.1056/NEJMoa1013297 (2011).
10. Hung, S. I. *et al.* HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci USA* **102**, 4134–4139 (2005).
11. Lonjou, C. *et al.* A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* **18**, 99–107 (2008).
12. Tohkin, M. *et al.* A whole-genome association study of major determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Pharmacogenomics J* **13**, 60–69, DOI:10.1038/tpj.2011.41 (2013).
13. Mockenhaupt, M. *et al.* Stevens-Johnson syndrome and toxic epidermal necrolysis: Assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. *J Invest Dermatol* **128**, 35–44, DOI:10.1038/sj.jid.5701033 (2008).

14. Ueta, M. *et al.* Association between prostaglandin E receptor 3 polymorphisms and Stevens-Johnson syndrome identified by means of a genome-wide association study. *J Allergy Clin Immunol* **126**, 1218–1225 e1210, DOI:10.1016/j.jaci.2010.08.007 (2010).
15. Ueta, M. *et al.* HLA class I and II gene polymorphisms in Stevens-Johnson syndrome with ocular complications in Japanese. *Mol Vis* **14**, 550–555 (2008).
16. Bastuji-Garin, S. *et al.* Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch Dermatol* **129**, 92–96 (1993).
17. Kawashima, M., Ohashi, J., Nishida, N. & Tokunaga, K. Evolutionary analysis of classical HLA class I and II genes suggests that recent positive selection acted on DPB1*04:01 in Japanese population. *PloS one* **7**, e46806, DOI:10.1371/journal.pone.0046806 (2012).
18. Nakaji, S., Ueta, M., Sotozono, C., Inatomi, T. & Kinoshita, S. [HLA-class I gene polymorphisms in Japanese Stevens-Johnson syndrome patients with ocular surface complications]. *Nippon Ganka Gakkai Zasshi* **116**, 581–587 (2012).
19. 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* **143**, 367–368 (2007).
20. Tokunaga, K. *et al.* Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. *Immunogenetics* **46**, 199–205 (1997).
21. Middleton, D., Menchaca, L., Rood, H. & Komerofsky, R. New allele frequency database: http://www.allelefrequencies.net. *Tissue Antigens* **61**, 403–407 (2003).
22. Mondino, B. J., Brown, S. I. & Biglan, A. W. HLA antigens in Stevens-Johnson syndrome with ocular involvement. *Arch Ophthalmol* **100**, 1453–1454 (1982).
23. Roujeau, J. C. *et al.* HLA phenotypes and bullous cutaneous reactions to drugs. *Tissue Antigens* **28**, 251–254 (1986).
24. Roujeau, J. C. *et al.* Genetic susceptibility to toxic epidermal necrolysis. *Arch Dermatol* **123**, 1171–1173 (1987).
25. Forman, R., Koren, G. & Shear, N. H. Erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis in children: A review of 10 years' experience. *Drug safety* **25**, 965–972 (2002).
26. Leaute-Labreze, C., Lamireau, T., Chawki, D., Maleville, J. & Taieb, A. Diagnosis, classification, and management of erythema multiforme and Stevens-Johnson syndrome. *Arch Dis Childhood* **83**, 347–352 (2000).
27. Ueta, M. *et al.* HLA-A*0206 with TLR3 polymorphisms exerts more than additive effects in Stevens-Johnson syndrome with severe ocular surface complications. *PloS one* **7**, e43650, DOI:10.1371/journal.pone.0043650 (2012).
28. Ueta, M., Sotozono, C., Yokoi, N., Inatomi, T. & Kinoshita, S. Prostaglandin E receptor subtype EP3 expression in human conjunctival epithelium and its changes in various ocular surface disorders. *PloS one* **6**, e25209, DOI:10.1371/journal.pone.0025209 (2011).

Acknowledgments

This work was conducted as a part of the BioBank Japan Project supported by the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government, and in part by grants-in-aid for scientific research from the Japanese Ministry of Health, Labour and Welfare, and a research grant from the Kyoto Foundation for the Promotion of Medical Science and the Intramural Research Fund of Kyoto Prefectural University of Medicine. The funding agencies had no role in the study design, data collection and -analysis, the decision to publish, or the preparation of this manuscript.

Author contributions

M.U., N.K. and K.T. wrote the main manuscript text and made Table, M.U., N.K., C.S., K.T., Y.S., H.S., H.M., E.S., K.M., R.N., M.N., M.A., K.M., Y.T., H.F., M.M., Z.I. and S.K. contributed to material of the research and reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Ueta, M. *et al.* Independent strong association of *HLA-A*02:06* and *HLA-B*44:03* with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement. *Sci. Rep.* **4**, 4862; DOI:10.1038/srep04862 (2014).

Ther Adv Drug Saf
(2013) 4(6) 246–253
DOI: 10.1177/2042098613499791

The risk of cutaneous adverse reactions among patients with the *HLA-A* 31:01* allele who are given carbamazepine, oxcarbazepine or eslicarbazepine: a perspective review

Nahoko Kaniwa and Yoshiro Saito

Abstract: Carbamazepine is a drug that is widely used for the treatment of epilepsy, trigeminal neuralgia and bipolar disorder. This drug is also known to cause cutaneous adverse drug reactions (cADRs) in up to 10% of patients. The recent progress in pharmacogenetics has revealed that human leukocyte antigen (HLA) genotypes are associated with a susceptibility to the cADRs caused by particular drugs. For carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis, very strong associations with *HLA-B*15:02* have been found mainly in patients of Southeastern Asian origin. In some countries, prescreening *HLA-B*15:02* allele has already been put to practical use as a biomarker to avoid the life-threatening adverse drug reactions. In this review, another risk factor for carbamazepine-induced cADRs is discussed, namely *HLA-A*31:01*. We compare the strength of the association between *HLA-A*31:01* and carbamazepine-induced cADRs based on reports for various ethnic populations; discuss the difference between the *HLA-A*31:01* and *HLA-B*15:02* biomarkers and the usefulness of prescreening *HLA-A*31:01* to detect patients at high risk for carbamazepine-induced cADRs; and refer to points that remain to be resolved.

Keywords: Biomarker, HLA genotype, hypersensitivity syndrome, Stevens–Johnson syndrome, toxic epidermal necrolysis

Introduction

Carbamazepine is one of most commonly prescribed drugs for the treatment of epilepsy, trigeminal neuralgia and bipolar disorder. It is also known to be the most common inducer of cutaneous adverse drug reactions (cADRs). The clinical manifestations of cADRs caused by carbamazepine vary widely, ranging from a mild skin rash, such as maculopapular eruption (MPE) and erythema exsudativum multiforme (EEM) minor, to severe rashes such as EEM major, Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). SJS and TEN, with their characteristic mucosal and cutaneous disorders, including blisters, are considered to represent different severities of the same disease [Bastuji-Garin *et al.* 1993]. The most widely accepted classification for these two disorders is based on the degree of skin detachment expressed in terms of the percentage of body surface area affected. SJS is defined as an area of skin detachment that involves less than 10% of the body surface. SJS–TEN overlap is defined as an area of skin detachment that affects from 10% to less than 30% of the body surface. TEN is defined as a level of skin detachment of no less than 30%. DIHS and MPE are categorized as nonbullous cADRs [Naisbitt *et al.* 2003]. DIHS is a severe adverse reaction that leads to multiorgan failure and is hypothesized to be associated with the reactivation of herpesvirus 6 [Hashimoto, 2006]. DIHS has also been referred to as a drug reaction with eosinophilia and systemic symptoms (DRESS)

Correspondence to:
Nahoko Kaniwa, PhD
Senior Researcher,
Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
nkaniwa@nihs.go.jp

Yoshiro Saito, PhD
Director, Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan

or hypersensitivity syndrome (HSS). In this review, either DIHS or HSS is used according to the disease name used in the original article. Whereas MPE is a mild skin rash, SJS/TEN and DIHS are life-threatening adverse reactions. The incidences of SJS/TEN and DIHS are very low (two to three cases per million per year), but their mortality is very high (5–30%). SJS/TEN is currently understood to be reactions that involve cytotoxic CD8+ T cells, and DIHS and MPE are also believed to have immune etiologies [Naisbitt *et al.* 2003].

The occurrence of cADRs is a very significant problem, both for physicians and patients, because it is unpredictable and often leads to a discontinuation of treatment. However, recent studies have revealed that human leukocyte antigen (HLA) genotypes are linked to a predisposition to the cADRs induced by particular drugs, including carbamazepine, and these genotypes are thus thought to be promising biomarkers. In this review, the associations between *HLA-A*31:01* and cADRs induced by carbamazepine and its analogs are discussed.

HLA proteins

HLAs are a family of proteins that are involved in immune reactions by presenting antigens to T cells. HLA-A, -B and -C are categorized as class I molecules that are ubiquitously expressed on the surface of cells, including keratinocytes. HLA-DR, -DQ, and -DP are categorized as class II molecules that are expressed mainly on the surface of antigen-presenting cells, such as B cells, macrophages and dendritic cells. The genes for all the HLAs are on the short arm of chromosome 6 and are known to be highly polymorphic. For example, more than 1000 alleles of *HLA-A*, *-B* and *-C* have been identified to date [Robinson *et al.* 2011].

A brief introduction of associations of carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis with HLA-B*15:02 and HLA-B75

Very strong associations between *HLA-B*15:02* and carbamazepine-induced SJS/TEN have been found among the Han Chinese in Taiwan [Hung *et al.* 2006; Chung *et al.* 2004], which were confirmed by various case–control studies of Southeastern Asian patients [Kulkantrakorn *et al.* 2011; Wang *et al.* 2011; Zhang *et al.* 2011; Tassaneeyakul *et al.* 2010; Mehta *et al.* 2009; Locharernkul *et al.* 2008; Man *et al.* 2007; Lonjou *et al.* 2006]. *HLA-B*15:02* is a member of the serotype HLA-B75. In addition to *HLA-B*15:02*, carriers of some HLA-B75 members, including *HLA-B*15:08, HLA-B15:11* and *HLA-B*15:21*, with carbamazepine-induced SJS/TEN have also been detected in Asian countries, including India, Thailand, Korea and Japan [Kaniwa *et al.* 2010; Tassaneeyakul *et al.* 2010; Mehta *et al.* 2009]. The involvement of HLA-B75 members in the development of SJS/TEN was suggested by an *in vitro* study using a cell line transfected with cDNAs of these alleles, which underwent lysis by cytotoxic T cells activated by carbamazepine through recognition by the T-cell receptor (TCR) [Wei *et al.* 2012]. Thus, HLA-B75 can be said to be a risk factor for carbamazepine-induced SJS/TEN in Asian individuals. It is noteworthy that *HLA-B*15:02* is a risk factor only for SJS/TEN but not for other phenotypes of cADRs, and is also restricted to patients of Asian origin.

Associations of carbamazepine-induced cADRs with HLA-A*31:01

As shown in Table 1, *HLA-A*31:01* was reported for the first time to have associations with carbamazepine-induced MPE/HSS, but not with SJS/TEN, in Han Chinese patients in Taiwan [p = 0.0022, odds ratio (OR) = 17.5, 95% confidence interval (CI) = 4.6–66.5] [Hung *et al.* 2006]. The sensitivity of *HLA-A*31:01* in Han Chinese patients with carbamazepine-induced MPE/HSS was 0.25. This was followed by a report by Kashiwagi and colleagues that allelic frequency of *HLA-A*31:01* in Japanese patients with carbamazepine-induced severe cADRs (n = 22 including four SJS cases) was significantly higher than in a general Japanese population (p = 0.0004, OR = 4.33 and sensitivity = 0.50) [Kashiwagi *et al.* 2008]. The following five studies listed in Table 1, including our unpublished data, also revealed the tendency of a high allelic or carrier frequency of *HLA-A*31:01* in both SJS/TEN and various other types of cADRs, including DIHS/HSS, EEM or MPE, compared with that in tolerant control patients or in general populations. It should be noted, however, that the p values are dependent on the sample sizes of the studies, and sometimes no significant differences were detected because of a small sample size. In a study with Korean patients, three of seven patients with SJS/TEN and 10 of 17 patients with HSS carried *HLA-A*31:01*, and the carrier frequency in the latter was significantly higher than in tolerant

Table 1. Association of *HLA-A*31:01* or A31 with carbamazepine-induced cutaneous adverse reactions.

cADR phenotypes	Ethnic groups	Carrier frequency		*p* value	Odds ratio	95% Confidence interval	Reference
		Case group	Tolerant control group				
SJS/TEN	Han Chinese in Taiwan	1/60	4/144	NS			Hung *et al.* [2006]
MPE/HSS		8/31	4/144	0.0021	12.17	3.6–41.2	
HSS		2/13	4/144	NS			
MPE		6/18	4/144	2.2E-03	17.5	4.6–66.5	
Severe cADRs*	Japanese	11/22	53/371[$]	0.0004[‡]	4.33	2.07–9.06	Kashiwagi *et al.* [2008]
All phenotypes	Japanese	45/77	54/420	1.1E-19	9.5	5.6–16.3	Ozeki *et al.* [2011]
DIHS		21/36	54/420	2.1E-09	9.5	4.6–19.5	
SJS/TEN		5/6	54/420	2.4E-04	33.9	3.9–295.6	
Others		19/35	54/420	4.7E-08	8	3.9–16.6	
All phenotypes	Japanese	10/15	5/33	< 0.001	11.2	2.668–47.105	Niihara *et al.* [2012]
SJS/TEN		1/3	5/33				
DIHS		8/9	5/33				
EEM/MPE		1/3	5/33				
SJS/TEN	Japanese	9/21	484/2878[$]	0.0047	3.7	1.55–8.86	Our data (unpublished)
SJS	Korean	3/7	7/50	NS			Kim *et al.* [2011]
HSS		10/17	7/50	0.001 (p_c = 0.013)	7.3	2.3–22.5	
SJS	European	5/12	10/257	8.0E-05	25.93	4.93–116.18	McCormack *et al.* [2011]
HSS		10/27	10/257	3.5E-08	12.41	1.27–121.03	
MPE		23/106	10/257	1.1E-06	8.33	3.59–19.36	
SJS	Children originating from various ethnicities and living in Canada	0/9	3/91	NS			Amstutz *et al.* [2013]
HSS		3/6	3/91	0.0025	26.36	2.53–307.89	
MPE		6/26	3/91	0.0037	8.57	1.67–57.50	

*Erythroderma maculopapular and nutiform, $n = 6$; erythroderma, $n = 3$; DIHS, $n = 4$; SJS, $n = 2$ and other drug eruptions, $n = 7$.
[$]General population
[‡]Allelic frequencies were compared.
cADR, cutaneous adverse drug reaction; DIHS, drug-induced hypersensitivity syndrome; EEM, erythema exsudativum multiforme; HSS, hypersensitivity syndrome; MPE, maculopapular eruption; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

controls or a general population [Kim *et al.* 2011]. The sensitivity of *HLA-A*31:01* in Korean patients with carbamazepine-induced SJS or HSS was 0.54 (13/24). In a study of Japanese patients conducted by Ozeki and colleagues, *HLA-A*31:01* was significantly associated with carbamazepine-induced DIHS, SJS/TEN and other types of skin rashes (sensitivity for all phenotypes = 0.58) [Ozeki *et al.* 2011]. In their study, an especially strong association was detected between SJS/TEN and *HLA-B*31:01*. In another Japanese study by Niihara and colleagues, eight of nine patients with carbamazepine-induced DIHS carried *HLA-A*31:01*, an association that was statistically significant (sensitivity for all phenotypes = 0.67) [Niihara *et al.* 2012]. We previously reported the involvement of *HLA-B*15:11* in the development of carbamazepine-induced SJS/TEN in Japanese patients [Kaniwa *et al.* 2010]. In our sample, 9 of 21 patients with carbamazepine-induced SJS/TEN carried *HLA-A*31:01*, and the association was statistically significant ($p = 0.0047$, OR = 3.7, 95% CI = 1.55–8.86; sensitivity = 0.43) (unpublished data). *HLA-A*31:01* was also reported to be a biomarker for various carbamazepine-induced cADRs in Europeans, ranging from a mild skin rash, such as MPE, to severe cADRs, including SJS/TEN, and the sensitivity of *HLA-A*31:01* for all phenotypes was 0.26 (38/145) [McCormack *et al.* 2011]. The situation that *HLA-A*31:01* is involved in various phenotypes of skin rash caused by carbamazepine in white patients was similar to those observed in Asian patients. A recently conducted case–control study including children living in Canada also detected significant correlations of *HLA-A*31:01* with carbamazepine-induced HSS and MPE, but there were no correlations with SJS [Amstutz *et al.* 2013]. In this study, three patients with SJS who were of Asian origin carried the *HLA-B*15:02* allele.

As mentioned above, the sensitivities of *HLA-A*31:01* observed in studies with Korean and Japanese patients ranged from 43% to 67%, and they were higher than those observed among Han Chinese in Taiwan and among Europeans (26% for both ethnic groups). However, the observed variation in association strengths of *HLA-A*31:01* with carbamazepine-induced cADRs among various ethnic groups was smaller than that in associations of *HLA-B*15:02* with carbamazepine-induced SJS/TEN. Yip and colleagues estimated a pooled OR of 9.5 (95% CI = 6.4–13.9) for the association of *HLA-A*31:01* with carbamazepine-induced cADRs among the studies with Korean, Japanese, Chinese and European patients [Yip *et al.* 2012].

Population allelic frequency of HLA-A*31:01 in various ethnic groups

The *HLA-A*31:01* allele in general populations varies among different ethnic groups [Kurose *et al.* 2012]. *HLA-A*31:01* is a common allele among Japanese individuals (allelic frequency 0.071–0.093). Its frequency is comparable among Korean individuals and white individuals (0.050, and 0.018–0.042 respectively) and is lower among Chinese in both mainland China and Taiwan (0.022 and 0.018 respectively). HLA-A*31:01 is a rare allele among African individuals, in whom its frequency is on average 0.01. There have been no reports on whether *HLA-A*31:01* is linked to carbamazepine-induced cADRs in African patients.

Comparison between HLA-B*15:02 and HLA-A*31:01 as risk factors for carbamazepine-induced cutaneous adverse drug reactions

Although, as mentioned above, the association between *HLA-B*15:02*/HLA-B75 and carbamazepine-induced SJS/TEN appears to be restricted to Asian patients, associations between *HLA-A*31:01* and carbamazepine-induced cADRs have been detected both in Asian and European patients. However, its associations with cADRs were rather weak compared with the associations between *HLA-B*15:02* and carbamazepine-induced SJS/TEN observed in Southeast Asian countries, for which the sensitivities were nearly 100%.

At first, the association of *HLA-A*31:01* was thought to be limited to carbamazepine-induced HSS or MPE, but not with SJS/TEN in Han Chinese populations. However, various case–control studies that were conducted independently in other Asian countries and in Europe showed significant correlations between *HLA-A*31:01* and the SJS/TEN caused by carbamazepine. Therefore, it can be concluded that *HLA-A*31:01* is involved in the onset of both SJS/TEN and nonbullous cADRs, such as HSS and MPE.

The mechanism by which small molecules such as drugs (<1000 Da) become antigenic and recognized by T cells has not been elucidated. Two major concepts have been proposed [Adam *et al.* 2011]. One is the hapten/prohapten concept, and

the other is the p-i concept (pharmacological interactions of drugs with immune receptors). β-Lactam antibiotics have been shown to bind covalently to lysine residues of serum albumin as a hapten, and peptides modified with a hapten, which are generated by intracellular processing, embedded in HLA molecules are considered to be presented by antigen-presenting cells to TCRs (hapten concept) [Monshi *et al.* 2013; Jenkins *et al.* 2009]. Using a cell line transfected with *HLA-B*15*, Wei and colleagues showed that HLA-B75 members, including *HLA-B*15:02* and *HLA-B*15:11* proteins, promoted cell lysis by cytotoxic T cells that had been activated by carbamazepine [Wei *et al.* 2012]. In contrast, members of other serotypes of *HLA-B*15*, such as HLA-B62 and HLA-B72, cannot promote cell lysis by cytotoxic T cells activated by carbamazepine. The 63rd amino acid (the next amino acid of putative carbamazepine binding site) of members of serotype HLA-B75 is asparagine, whereas that of serotypes HLA-B62 or HLA-B72 is glutamic acid. Thus, carbamazepine is bound noncovalently to the HLA-B75 molecules, and the TCR recognizes its complex for T-cell activation (p-i concept). In addition to the specific HLA allele, *HLA-B*15:02*, a skewed usage of specific repertoires of the third complementarity-determining region of the TCR, such as VB-11-ISGSY, is reported to be required to develop carbamazepine-induced SJS/TEN [Ko *et al.* 2011].

To date, no information has been available on the pathogenic mechanisms of *HLA-A*31:01* molecules inducing hypersensitive reactions to carbamazepine (or its metabolites), including mechanisms of antigen presentation and TCR recognition. The pathogenesis for the HLA-A*31:01 molecule may be different from that for HLA-B*15:02 molecules, because *HLA-A*31:01* is linked not only to SJS/TEN but also to various phenotypes of cADRs. The diverging points for such clinical manifestations should be clarified.

The alleles *HLA-A*31:01* and *HLA-B*15:11* were found exclusively in each case of our Japanese patients (our unpublished data) and of Korean patients [Kim *et al.* 2011]. Unlike other ethnic groups, either *HLA-A*31:01* or *HLA-B*15:11* can be said to be a risk factor for carbamazepine-induced SJS/TEN in Korean and Japanese individuals because more than half of the patients with carbamazepine-induced SJS/TEN in these countries carry either of the alleles (6/7 in Korean patients and 14/21 in Japanese patients) [Kim *et al.* 2011] (our unpublished data). Therefore, the combined biomarkers may be of use to detect patients at high risk of carbamazepine-induced SJS/TEN in Korean and Japanese individuals.

Cutaneous adverse drug reactions caused by oxcarbazepine and eslicarbazepine

Oxcarbazepine and eslicarbazepine, which are metabolized differently from carbamazepine, have been developed to avoid the severe adverse reactions caused by carbamazepine. Oxcarbazepine was approved in 2007 in the USA and eslicarbazepine was approved in 2009 in Europe. Although SJS/TEN cases caused by oxcarbazepine were fewer than those caused by carbamazepine [Buggy *et al.* 2010; Dogan *et al.* 2008; Le Louët *et al.* 2008], there have been many reports of oxcarbazepine-caused severe cADRs. To date, two studies with Chinese patients have pointed out the involvement of *HLA-B*15:02* in oxcarbazepine-induced SJS/TEN [Hu *et al.* 2011; Hung *et al.* 2010]; however, another study with Chinese patients detected no significant correlation between the disease and this allele [He *et al.* 2012]. There have been no reports concerning the involvement of *HLA-A*31:01* in oxcarbazepine-induced SJS/TEN. Since eslicarbazepine, which is the active metabolite of oxcarbazepine, was approved only quite recently, reports on severe cADRs have not been accumulating.

Usefulness of prescreening HLA-A*31:01

On the basis of the knowledge obtained from various retrospective case–control studies with Southeastern patients [Kulkantrakorn *et al.* 2011; Wang *et al.* 2011; Zhang *et al.* 2011; Tassaneeyakul *et al.* 2010; Mehta *et al.* 2009; Locharernkul *et al.* 2008; Man *et al.* 2007; Hung *et al.* 2006; Lonjou *et al.* 2006; Chung *et al.* 2004] and a positive result obtained from a prospective case–control study performed in Taiwan to examine the usefulness of prescreening the risk factor [Chen *et al.* 2011], the screening for *HLA-B*15:02* prior to the initiation of carbamazepine treatment is currently mandatory in Taiwan and Singapore, and for patients in the USA who have ancestry at high risk for carbamazepine-induced SJS/TEN.

For *HLA-A*31:01*, Yip and colleagues [Yip *et al.* 2012] examined the usefulness of prescreening by a meta-analysis, using data obtained from three studies [McCormack *et al.* 2011; Ozeki *et al.*

2011; Hung *et al.* 2006]. The performance characteristics of *HLA-A*31:01* for Han Chinese, Japanese and European patients estimated by Yip and colleagues are as follows: sensitivity 0.262–0.584; specificity 0.871–0.972; positive predictive value 0.119–0.427; and negative predictive value 0.921–0.986. In every ethnic group, the number of patients needing to be tested in order to prevent one case (NNT) was estimated at less than 100, which was much smaller than the NNT (461) for screening *HLA-B*15:02* for Taiwanese individuals [Yip *et al.* 2012]. This difference may be caused by the fact that *HLA-B*15:02* is linked only with the rarer, but more severe cADRs, SJS/TEN, whereas *HLA-A*31:01* is linked even with frequently occurring mild skin rashes, such as MPE, as well as with SJS/TEN. The usefulness of *HLA-A*31:01* prescreening should be further discussed taking several points into consideration: the clinical impact of avoiding mild skin reactions, alternative drugs for *HLA-A*31:01*-positive patients and cost-effectiveness of the prescreening test. The results of an ongoing prospective study on the effects of a *HLA-A*31:01* prescreening test for prevention of carbamazepine-induced cADRs conducted in Japan by a Riken group (M. Kubo, http://www.biobankjp.org/pgx/outline/cbz.html) would have much impact on this issue.

Because HLA genotyping methods currently being used in clinical laboratory testing are laborious, time consuming and expensive, a more inexpensive, simple and rapid genotyping method is required for prescreening *HLA-A*31:01*. A new, simple and rapid pharmacogenetic test for detecting *HLA-A*31:01* was developed, which uses the InvaderPlus (Hologic, Inc., Bedford, MA, USA) assay and surrogate single-nucleotide polymorphisms that were found by a genome-wide association study to be highly linked with *HLA-A*31:01* [Aoki *et al.* 2012]. Uchiyama and colleagues developed another simple and inexpensive method for the detection of *HLA-A*31:01*, using a nested *HLA-A* allele-specific primer polymerase chain reaction combined with restriction fragment length polymorphism analysis [Uchiyama *et al.* 2013].

Conclusion

Unlike the case of HLA-B**15:02*, *HLA-A*31:01* is a risk factor for various types of carbamazepine-induced cADRs, ranging from mild ones such as MPE to severe ones, including SJS/TEN and DIHS, in both Asian and white patients. The pathogenesis of *HLA-A*31:01* involvement in the development of cADRs remains to be elucidated, which could help discriminate rashes that are likely to progress from those that are likely to resolve.

Funding

This study was supported in part by a Health and Labor Sciences Research grant from the Ministry of Health, Labor and Welfare.

Conflict of interest statement

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors have any conflict of interest to disclose.

References

Adam, J., Pichler, W. and Yerly, D. (2011) Delayed drug hypersensitivity: models of T-cell stimulation. *Br J Clin Pharmacol* 71: 701–707.

Amstutz, U., Ross, C., Castro-Pastrana, L., Rieder, M., Shear, N., Hayden, M. *et al.* (2013) *HLA-A*31:01* and *HLA-B*15:02* as genetic markers for carbamazepine hypersensitivity in children. *Clin Pharmacol Ther* 94: 142–149.

Aoki, M., Hosono, N., Takata, S., Nakamura, Y., Kamatani, N. and Kubo, M. (2012) New pharmacogenetic test for detecting an *HLA-A*31:01* allele using the InvaderPlus assay. *Pharmacogenet Genomics* 22: 441–446.

Bastuji-Garin, S., Rzany, B., Stern, R., Shear, N., Naldi, L. and Roujeau, J. (1993) Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch Dermatol* 129: 92–96.

Buggy, Y., Layton, D., Fogg, C. and Shakir, S. (2010) Safety profile of oxcarbazepine: results from a prescription-event monitoring study. *Epilepsia* 51: 818–829.

Chen, P., Lin, J., Lu, C., Ong, C., Hsieh, P., Yang, C. *et al.* (2011) Carbamazepine-induced toxic effects and *HLA-B*1502* screening in Taiwan. *N Engl J Med* 364: 1126–1133.

Chung, W., Hung, S., Hong, H., Hsih, M., Yang, L., Ho, H. *et al.* (2004) Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 428: 486.

Dogan, E., Usta, B., Bilgen, R., Senol, Y. and Aktekin, B. (2008) Efficacy, tolerability, and side effects of oxcarbazepine monotherapy: a prospective study in adult and elderly patients with newly diagnosed partial epilepsy. *Epilepsy Behav* 13: 156–161.

Hashimoto, K. (2006) Drug induced hypersensitivity syndrome. In Shiohara, T., Miyaji, Y. and Takigawa, M. (eds), *Dermatology Practice 19, Insight into Skin Rash*. Tokyo: Bunkoudo.

He, N., Min, F., Shi, Y., Guo, J., Liu, X., Li, B. *et al.* (2012) Cutaneous reactions induced by oxcarbazepine in Southern Han Chinese: incidence, features, risk factors and relation to *HLA-B* alleles. *Seizure* 21: 614–618.

Hu, F., Wu, X., An, D., Yan, B., Stefan, H. and Zhou, D. (2011) Pilot association study of oxcarbazepine-induced mild cutaneous adverse reactions with *HLA-B*1502* allele in Chinese Han population. *Seizure* 20: 160–162.

Hung, S., Chung, W., Jee, S., Chen, W., Chang, Y., Lee, W. *et al.* (2006) Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet Genomics* 16: 297–306.

Hung, S., Chung, W., Liu, Z., Chen, C., Hsih, M., Hui, R. *et al.* (2010) Common risk allele in aromatic antiepileptic-drug induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Han Chinese. *Pharmacogenomics* 11: 349–356.

Jenkins, R., Meng, X., Elliott, V., Kitteringham, N., Pirmohamed, M. and Park, B. (2009) Characterisation of flucloxacillin and 5-hydroxymethyl flucloxacillin haptenated HSA in vitro and in vivo. *Proteomics Clin* 3: 720–729.

Kaniwa, N., Saito, Y., Aihara, M., Matsunaga, K., Tohkin, M., Kurose, K. *et al.* (2010) *HLA-B*1511* is a risk factor for carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Epilepsia* 51: 2461–2465.

Kashiwagi, M., Aihara, M., Takahashi, Y., Yamazaki, E., Yamane, Y., Song, Y. *et al.* (2008) Human leukocyte antigen genotypes in carbamazepine-induced severe cutaneous adverse drug response in Japanese patients. *J Dermatol* 35: 683–685.

Kim, S., Lee, K., Song, W., Kim, S., Jee, Y., Lee, S. *et al.* (2011) Carbamazepine-induced severe cutaneous adverse reactions and HLA genotypes in Koreans. *Epilepsy Res* 97: 190–197.

Ko, T., Chung, W., Wei, C., Shih, H., Chen, J., Lin, C. *et al.* (2011) Shared and restricted T-cell receptor use is crucial for carbamazepine-induced Stevens–Johnson syndrome. *J Allergy Clin Immunol* 128: 1266–1276.

Kulkantrakorn, K., Tassaneeyakul, W., Tiamkao, S., Jantararoungtong, T., Prabmechai, N., Vannaprasaht, S. *et al.* (2011) *HLA-B*1502* strongly predicts carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Thai patients with neuropathic pain. *Pain Pract* 12: 202–208.

Kurose, K., Sugiyama, E. and Saito, Y. (2012) Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in Eastern Asians and Europeans: implications in the clinical trials for novel drug development. *Drug Metab Pharmacokinet* 27: 9–54.

Le Louët, H., Thomas, L. and Babai, S. (2008) DRESS: is oxcarbazepine safer than carbamazepine? An analysis of the French Pharmacovigilance database. *Eur J Neurol* 15: e43.

Locharernkul, C., Loplumlert, J., Limotai, C., Korkij, W., Desudchit, T. and Tongkobpetch, S. (2008) Carbamazepine and phenytoin induced Stevens–Johnson syndrome is associated with *HLA-B*1502* allele in Thai population. *Epilepsia* 49: 2087–2091.

Lonjou, C., Thomas, L., Borot, N., Ledger, N., de Toma, C., LeLouet, H. *et al.* (2006) A marker for Stevens–Johnson syndrome ...: ethnicity matters. *Pharmacogenomics J* 6: 265–268.

Man, C., Kwan, P., Baum, L., Ledger, N., de Toma, C., LeLouet, H. *et al.* (2007) Association between *HLA-B*1502* allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48: 1015–1018.

McCormack, M., Alfirevic, A., Bourgeois, S., Farrell, J., Kasperavičiūtė, D., Carrington, M. *et al.* (2011) *HLA-A*3101* and carbamazepine-induced hypersensitivity reactions in Europeans. *N Eng J Med* 364: 1134–1143.

Mehta, T., Prajapati, L., Mittal, B., Joshi, C., Sheth, J., Patel, D. *et al.* (2009) Association of *HLA-B*1502* allele and carbamazepine-induced Stevens–Johnson syndrome among Indians. *Indian J Dermatol Venereol Leprol* 75: 579–582.

Monshi, M., Faulkner, L., Gibson, A., Jenkins, R., Farrell, J., Earnshaw, C. *et al.* (2013) Human leukocyte antigen *(HLA)-B*57:01*-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology* 57: 727–739.

Naisbitt, D., Britschgi, M., Wong, G., Farrell, J., Depta, J., Chadwick, D. *et al.* (2003) Hypersensitivity reactions to carbamazepine: characterization of the specificity, phenotype, and cytokine profile of drug-specific T cell clones. *Mol Pharmacol* 63: 732–741.

Niihara, H., Kakamu, T., Fujita, Y., Kaneko, S. and Morita, E. (2012) *HLA-A31* strongly associates with carbamazepine-induced adverse drug reactions but not with carbamazepine-induced lymphocyte proliferation in a Japanese population. *J Dermatol* 39: 594–601.

Ozeki, T., Mushiroda, T., Yowang, A., Takahashi, A., Kubo, M., Shirakata, Y. *et al.* (2011) Genome-wide association study identifies *HLA-A*3101* allele