

## BRIEF COMMUNICATION

# HLA-B\*1511 is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients

\*Nahoko Kaniwa, \*Yoshiro Saito, †Michiko Aihara, ‡Kayoko Matsunaga, \*Masahiro Tohkin, \*Kouichi Kurose, §Hirokazu Furuya, ¶Yukitoshi Takahashi, #Masaaki Muramatsu, \*\*Shigeru Kinoshita, ‡Masamichi Abe, ¶Hiroko Ikeda, #Mariko Kashiwagi, #Yixuan Song, \*\*Mayumi Ueta, \*\*Chie Sotozono, †Zenro Ikezawa, and \*Ryuichi Hasegawa, for the JSAR research group<sup>1</sup>

\*Division of Medicinal Safety Sciences, National Institute of Health Sciences, Tokyo, Japan; †Department of Environmental Immunodermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan; ‡Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan; §Department of Neurology, Neuro-Muscular Center, National Oomuta Hospital Oomuta, Japan; ¶Shizuoka Institute of Epilepsy and Neurological Disorders, National Epilepsy Center, Shizuoka, Japan; #Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; and \*\*Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

### SUMMARY

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but life-threatening severe cutaneous adverse reactions. Recently, strong associations of HLA-B\*1502 with carbamazepine-induced SJS/TEN have been found in Han Chinese patients. These associations have been confirmed in several Asian populations, excluding Japanese. SJS patients carrying HLA-B\*1508, HLA-B\*1511, or HLA-B\*1521, which are members of the HLA-B75 type

along with HLA-B\*1502, were detected in studies in India and Thailand. In the current study, we genotyped the HLA-B locus from 14 Japanese typical and atypical SJS/TEN patients in whom carbamazepine was considered to be involved in the onset of adverse reactions. Although there were no HLA-B\*1502 carriers, four patients had HLA-B\*1511. Our data suggest that HLA-B\*1511, a member of HLA-B75, is a risk factor for carbamazepine-induced SJS/TEN in Japanese.

**KEY WORDS:** HLA-B\*1502, HLA-B75, Serotype.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe adverse drug reactions (ADRs) with mucosal and cutaneous disorders, and often are accompanied by high fever and systemic complications. Although incidence is low, SJS and TEN are life-threatening and their mortalities are estimated at 5% and 30%, respectively. On the basis of summarized spontaneous reports of severe ADRs to the Ministry of Health, Labor and Welfare (MHLW) from 2006 to 2008, the incidence of SJS/TEN in Japan can be calculated as 3.4 patients per million per year (approximately 430 cases annually), and major causative drugs are allopurinol and carbamazepine.

As for carbamazepine-induced SJS/TEN, involvement of HLA-B\*1502 in Han Chinese SJS/TEN patients has been reported (Chung et al., 2004), and has been confirmed in Asians in Hong Kong (Man et al., 2007), Europe (Lonjou et al., 2006), Thailand (Locharernkul et al., 2008), and India (Mehta et al., 2009). However, no association between HLA-B\*1502 and carbamazepine-related SJS/TEN was detected in our previous study with seven Japanese SJS/TEN patients (Kaniwa et al., 2008). Therefore, we extended the investigation to explore other biomarkers in Japanese SJS/TEN patients who were administered carbamazepine.

### METHODS

#### Patients

The ethics committee of each participating institute of the JSAR (Japan Severe Adverse Reactions) research group approved this study. Written informed consent was obtained from each patient. Fifteen unrelated Japanese patients who were prescribed carbamazepine before the onset of SJS/TEN were recruited from participating institutes or through

Accepted September 3, 2010; Early View publication November 3, 2010.

Address correspondence to Nahoko Kaniwa, PhD, Division of Medicinal Safety Sciences, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. E-mail: nkaniwa@nihs.go.jp

<sup>1</sup>The JSAR (Japan Severe Adverse Reactions) research group: the representative of the research group is Nahoko Kaniwa at National Institute of Health Sciences, and all authors are members of the research group.

Wiley Periodicals, Inc.

© 2010 International League Against Epilepsy

a nationwide blood sampling network in Japan operated by the National Institute of Health Sciences in cooperation with the MHLW and the Federation of Pharmaceutical Manufacturers' Association of Japan. Patient characteristics are summarized in Table 1. Seven patients were included in our previous report (Kaniwa et al., 2008), and two patients were in another study (Ikeda et al., 2009). Twelve patients were diagnosed as definite SJS or TEN and three patients were diagnosed as probable SJS due to atypical or mild symptoms by the JSAR research group experts. This diagnosis was based on criteria proposed by Bastuji-Garin et al. (1993) using a standardized case report form including medicinal records, disease progress, and involvement of systemic complications as well as treatment. Severity of ocular complication was scored as follows: 0, no involvement; 1, only hyperemia of bulbar and palpebral conjunctiva; 2, pseudomembrane formation; 3, defect of conjunctival or corneal epithelia.

#### HLA-B typing

High-resolution *HLA-B* typing was performed by a sequence-based method using SeCore B Locus Sequencing kit (Invitrogen Corp., Brown Deer, WI, U.S.A.) and an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Genomic DNA (250 ng) was used for PCR amplification and sequencing exons 2, 3, and 4. *HLA-B* haplotype was estimated with the Assign SBT software (version 3.2.7b; Conexio Genomics, Applecross, WA, Australia).

#### Statistical analysis

*HLA-B\*1511* allele frequency reported by Tanaka et al. was used as the control frequency (Tanaka et al., 1996). Fisher's exact test was conducted using JMP ver. 7.0.1 (SAS Institute Japan, Co., Ltd., Tokyo, Japan) to calculate the odds ratio and its 95% confidence interval (CI).

## RESULTS

Demographics, symptomatic state, coadministered drugs with carbamazepine, and *HLA-B* diplotypes of 15 patients are summarized in Table 1. However, Patient 12 was excluded from the following statistical analyses because zonisamide was a more likely causative drug. Involvement of carbamazepine in the onset of SJS/TEN could not be excluded for the remaining 11 definite SJS/TEN patients and three probable SJS patients.

In contrast to data on Han Chinese (Chung et al., 2004) and Thai populations (Locharernkul et al., 2008), *HLA-B\*1502* was not detected in this work. However, two patients with definite SJS/TEN and two patients with probable SJS carried *HLA-B\*1511*. The allele frequencies of *HLA-B\*1511* in the SJS/TEN groups were compared with the allele frequency in a Japanese population reported by Tanaka et al. (1996) ( $n = 493$ ) instead of that in carbamazepine-tolerant patients, because the incidence of SJS/TEN in Japan is very low (three per million/year). Allele frequencies of *HLA-B\*1511* increased significantly in the SJS/TEN group regardless of the exclusion or inclusion of probable SJS patients [0.0909 (2 of 22) and 0.143 (4 of 28), respectively] than in the Japanese population (0.01), and the odds ratios were 9.76 ( $p = 0.0263$ , CI 2.01–47.5) and 16.3 ( $p = 0.0004$ , CI 4.76–55.6), respectively. No patients with *HLA-B\*1511* had severe ocular complications.

## DISCUSSION

Recently, *HLA-B\*1502* involvement has been reported in carbamazepine-induced SJS/TEN in Southern Asian patients (Chung et al., 2004; Man et al., 2007; Locharernkul et al., 2008; Mehta et al., 2009) and patients of Asian ancestry living in Europe (Lonjou et al., 2006). Although we did not detect SJS/TEN patients receiving carbamazepine who carried *HLA-B\*1502*, we did find four patients carrying *HLA-B\*1511*. *HLA-B\*1511* and *HLA-B\*1502* belong to the same *HLA-B75* serotype. Other major members of *HLA-B75* are *HLA-B\*1508*, *HLA-B\*1515*, and *HLA-B\*1521*. Mehta et al. (2009) have investigated the association between *HLA-B\*1502* and carbamazepine-induced SJS using eight Indian patients. Although in their study most patients (six of eight) did carry *HLA-B\*1502*, one patient was homozygous *HLA-B\*1508*. Tassaneeyakul et al. (2010) have also performed a case-control study using 42 CBZ-induced SJS/TEN patients and 42 carbamazepine-tolerant controls in a Thai population. In their study, 37 SJS/TEN patients carried *HLA-B\*1502* and the very strong association of *HLA-B\*1502* with SJS/TEN was again confirmed. Although the statistical significance was not examined, two patients carrying heterozygous *HLA-B\*1521* and one patient carrying heterozygous *HLA-B\*1511* were detected, suggesting that not only *HLA-B\*1502* but also some subfamilies of serotype *HLA-B75* are involved in the onset of carbamazepine-induced SJS/TEN.

Allele frequencies of individual *HLA* genotypes in worldwide populations obtained from various studies are shown at AlleleFrequencies.net (Middleton et al., 2003). Table 2 summarizes the population allele frequencies of representative types of *HLA-B75* in various ethnic groups. In Han Chinese, Thai and Indians, carriers of *HLA-B\*1502*, *HLA-B\*1521*, and *HLA-B\*1508* are at high risk of carbamazepine-induced SJS/TEN, although *HLA-B\*1502* is mainly involved. A comparable allele frequency of *HLA-B\*1511* (higher than 3.8%) to that of *HLA-B\*1502* in Han Chinese in Beijing has been reported recently by Yang et al. (Yang et al., 2010). Because the allele frequency of *HLA-B\*1511* is higher than that of *HLA-B\*1502* in Japanese and Koreans, carriers of the former may more easily be detected in association studies than carriers of the latter in northeast Asian populations. *HLA-B\*1521* can be a risk

Table 1. Backgrounds and HLA-B diplotypes of Japanese carbamazepine-related SJS/TEN patients

ID <sup>a</sup>	ADR type	Sex/Age	Severity score in ophthalmic disorders	Highest BT (°C)	Total area of blistering skin (%)	Systemic complications	Result of DLST to CBZ	Period of onset for CBZ (days)	Coadministered drugs		HLA-B diplotypes	
									Drug name	DLST result/period of onset	High resolution	Low resolution
1 (1)	TEN	M/73	1	>39	20	Neutropenia	-	14	Potassium citrate/sodium citrate hydrate	-/4 days	1511/4801	B75/B48
2 (5) <sup>b</sup>	SJS	F/6	At least 1 <sup>c</sup>	>37.0	<10%	GI tract disturbance	Not tested	9	Allopurinol	-/5 years	4006/5101	B61/B51
3 (6) <sup>b</sup>	SJS	F/52	At least 1 <sup>c</sup>	Unknown	<10%	Neutropenia	Not tested	14	Etizolam	-/5 years	4601/5901	B46/B59
4	SJS	M/52	0	38	1	Liver dysfunction	Not tested	51	Sodium pravastatin	Not tested/346 days	0702/5201	B7/B52
5	SJS	M/32	1	39	5	GI tract disturbance	Not tested	42	None	-/1 year	4002/5401	B60/B54
6 (2)	SJS	F/42	3	>39	5	Renal dysfunction	-	Shorter than 34	Sodium diclofenac	-/1 year	4001/5201	B60/B52
7	SJS	F/64	At least 1 <sup>c</sup>	>37.0	10	GI tract disturbance	+	13	Cefteram pivoxil	Not tested/	1511/4002	B75/B60
8 (3)	SJS	M/45	3	>37.0	5	Liver dysfunction	Not tested	49	Olopatadine hydrochloride	Not tested/	4801/5601	B48/B56
9 (4)	SJS	M/54	0	<37.0	0.5	None	+	34	Mecobalamin	Not tested/13 days	1501/3501	B62/B35
10	TEN	M/38	3	40.3	40	Liver dysfunction	+	15	None	-/8 days	1302/4403	B13/B44
11 (7)	TEN	M/17	3	39.7	20	Respiratory involvement	+	5	Levofloxacin hydrate	-/15 days	4601/5601	B46/B56
12 <sup>d</sup>	SJS	M/6	1	Unknown	<10%	Neutropenia	-	145	Mecobalamin	-/9 days	1511/4006	B75/B61
13	Probable SJS	F/54	Unknown	<37.0	>10%	Liver dysfunction	Not tested	22	Acyclovir	-/9 days	4006/4403	B61/B44
									Zonisamide	+/33 days	unknown	
									Amoxicillin hydrate	+/1 day	Not tested/81 days	
									Promethazine	Not tested/1 day	Not tested/15 days	
									methylenebisacrylate		Not tested/46 days	
									Zonisamide	+/24 days	Not tested/46 days	
									Sodium pravastatin	Not tested/	Not tested/46 days	
									Nifedipine	unknown	Not tested/46 days	
									Etizolam	Not tested/	Not tested/46 days	
									Lansoprazole	Not tested/	Not tested/46 days	
									Sodium risedronate hydrate	Not tested/	Not tested/46 days	
14	Probable SJS	F/36	At least 1 <sup>c</sup>	Unknown	5	None	+	15	Timperone	Not tested/1 day	1301/1511	B13/B75
15	Atypical SJS	F/65	1	37.4	0.1	None	+	9	None	Not tested/	1511/3501	B75/B35

BT, body temperature; DLST, drug lymphocyte stimulation test; CBZ, carbamazepine.

<sup>a</sup>Number in parentheses is ID # from our previous study (Kaniwa et al., 2008).<sup>b</sup>These patients were also included in Ikeda et al. (2010)<sup>c</sup>Ophthalmic complications were observed, but severity was unknown.<sup>d</sup>This patient was excluded from statistical analyses due to likely zonisamide-induced SJS.

**Table 2. Population allele frequencies of individual types of HLA-B75 in various ethnic groups**

Ethnic group	Population allele frequencies reported in allelefrequencies.net website <sup>a</sup>				
	<i>HLA-B*1502</i>	<i>HLA-B*1515</i>	<i>HLA-B*1521</i>	<i>HLA-B*1508</i>	<i>HLA-B*1511</i>
Japanese	0.001	Data unavailable	Data unavailable	Data unavailable	<b>0.004–0.008</b> <sup>b,c</sup>
Koreans	0.002	0.000	0.000	0.000	0.020
Han Chinese	<b>0.019–0.124</b> <sup>b</sup>	0.010	0.000–0.002	0.005–0.015	0.000–0.017 <sup>d</sup>
Thai	<b>0.061–0.085</b> <sup>b</sup>	Data unavailable	<b>0.007–0.010</b> <sup>b</sup>	0.010	<b>0.010</b> <sup>b</sup>
Indians	<b>0.000–0.060</b> <sup>b</sup>	Data unavailable	Data unavailable	<b>0.005–0.033</b> <sup>b</sup>	Data unavailable
Caucasians (West Europe)	0.000	0.000	0.000	0.000–0.004	0.000–0.003
Caucasians (East Europe)	0.000	0.000	0.000	0.000–0.009	0.000
Sub-Saharan Africans	0.000	0.000–0.008	Data unavailable	0.000	0.000
Hispanics	0.000	0.004–0.008	0.000	0.000–0.006	0.000
Arabians	0.000	0.000	0.000	0.000–0.007	0.000
Australian aborigine	0.000–0.007	Data unavailable	0.026–0.135	Data unavailable	Data unavailable

<sup>a</sup>New Allele Frequency Database: <http://www.allelefrequencies.net/> (Middleton et al., 2003).  
<sup>b</sup>SJS/TEN patients carrying the allele shown in the second row have been reported in the study using an ethnic group shown in the first column.  
<sup>c</sup>The frequency of 0.1 was reported by Tanaka et al. (1996).  
<sup>d</sup>Higher value than 0.038 in Han Chinese in Beijing was recently reported by Yang et al. (2010).

factor for carbamazepine-induced SJS/TEN for Thai and Australian aborigine. Interestingly, HLA-B75 has not been detected in carbamazepine-induced SJS/TEN Caucasian patients (Lonjou et al., 2006). This may be due to extremely low allele frequencies or no existence of HLA-B75 subfamilies.

*HLA-B\*1502* has been reported to have associations with SJS/TEN caused by other aromatic antiepileptic drugs such as phenytoin and lamotrigine in Han Chinese and Thai (Man et al., 2007; Lochareernkul et al., 2008). In this study we detected a patient carrying *HLA-B\*1511* whose causative drug was probably zonisamide, an aromatic antiepileptic drug. Therefore, *HLA-B\*1511* may be also involved in the onset of SJS/TEN induced by other aromatic antiepileptic drugs as well as *HLA-B\*1502*, although further investigation is needed.

The odds ratio of *HLA-B\*1511* for SJS/TEN obtained in this study was low in comparison with those observed in Thai, Indians, and Han Chinese in Taiwan (25.5, 71.4, and 25.04 respectively) (Chung et al., 2004; Lochareernkul et al., 2008; Mehta et al., 2009). One reason for this may be the low allele frequency (<0.01) of *HLA-B\*1511* among the Japanese. The administration of multiple drugs to Japanese patients may also contribute to the low odds ratio. Indeed, on average, more than three drugs were administered to the patients in this study. We concluded that patients receiving multiple drugs developed SJS/TEN due to carbamazepine by comparing the periods of latency of the individual drugs prior to SJS/TEN onset. However, we cannot completely exclude the possibility of other causative drugs. Another possibility is that *HLA-B\*1502* is more prone than *HLA-B\*1511* to cause carbamazepine-induced SJS/TEN. Carbamazepine or its metabolites may covalently (Weltzien et al., 1996) or noncovalently (Wu et al., 2007; Yang et al., 2007) bind more easily to the HLA-B\*1502 protein or its binding peptide.

There are no SJS/TEN patients carrying *HLA-B\*1511* who had severe ocular complications. This result coincides with the previous report that none of the 71 SJS/TEN patients with ocular surface complications had *HLA-B\*1511* (Ueta et al., 2008).

## ACKNOWLEDGMENTS

This study was supported in part by the Health and Labor Sciences Research Grant (Research on Advanced Medical Technology) from the Ministry of Health, Labor and Welfare. We deeply appreciate the Federation of Pharmaceutical Manufacturers' Association of Japan for their assistance in recruiting patients. We also thank all patients and medical doctors for their cooperation with our study. We thank Ms. Sachiko Tsutsumi, Ms. Hina Kato, Dr. Akiko Miyamoto, and Mr. Jun Nishikawa for their assistance.

## DISCLOSURE

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 has any conflict of interest to disclose.

## REFERENCES

- Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. (1993) Clinical classification of cases of toxic epidermal necrolysis, Stevens–Johnson syndrome, and erythema multiforme. *Arch Dermatol* 129:92–96. The diagnostic criteria are reflected in the currently used following Japanese guidance; Diagnostic criteria of Stevens–Johnson syndrome (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005) and Diagnostic criteria of Toxic Epidermal Necrolysis (Hashimoto K representing a Research Group on the Conquest of Intractable Diseases, Health and Labor Sciences Research Grant from the Ministry of Health, Labor and Welfare, 2005).
- Chung WH, Hung SI, Hong HS, Hsieh MS, Yang LC, Ho HC, Wu JY, Chen YT. (2004) Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 428:486.
- Ikeda H, Takahashi Y, Yamazaki E, Fujiwara T, Kaniwa N, Saito Y, Aihara M, Kashiwagi M, Muramatsu M. (2010) HLA Class I markers in

- Japanese patients with carbamazepine-induced cutaneous adverse reactions. *Epilepsia* 51:297–300.
- Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, Sawada J, Furuya H, Takahashi Y, Muramatsu M, Kinoshita S, Abe M, Ikeda H, Kashiwagi M, Song Y, Ueta M, Sotozono C, Ikezawa Z, Hasegawa R; JSAR research group. (2008) HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics* 9:1617–1622.
- Locharernkul C, Loplumlert J, Limotai C, Korkij W, Desudchit T, Tongkobpetch S, Kangwanshiratada O, Hirankarn N, Suphapeetiporn K, Shotelersuk V. (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, Graf E, Schumacher M, Hovnanian A, Mockenhaupt M, Roujeau JC; Regi-SCAR Group. (2006) 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.
- Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, Ng MH. (2007) Association between HLA-B\*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48:1015–1018.
- Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, Dave DM, Goyal RK. (2009) Association of HLA-B\*1502 allele and carbamazepine-induced Stevens–Johnson syndrome among Indians. *Indian J Dermatol Venereol Leprol* 75:579–582.
- Middleton D, Menchaca L, Rood H, Komerofsky R. (2003) Brief communication. New allele frequency database: <http://www.allelefrequencies.net>. *Tissue Antigens* 61:403–407.
- Tanaka H, Akaza T, Juji T. (1996) Report of the Japanese central bone marrow data center. *Clin Transpl* 1996:139–144.
- Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Chen P, Lin SY, Chen WH, Konyoung P, Khunarkornsiri U, Auvichayapat N, Pavakul K, Kulkantrakorn K, Choonhakarn C, Phonhiamhan S, Piyatrakul N, Aungaree T, Pongpakdee S, Yodnopaglaw P. (2010) Association between HLA-B\*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. *Epilepsia* 51:926–930.
- Ueta M, Sotozono C, Inatomi T, Kojima K, Hamuro J, Kinoshita S. (2008) HLA class I and II gene polymorphisms in Stevens–Johnson syndrome with ocular complications in Japanese. *Mol Vis* 14:550–555.
- Weltzien HU, Moulton C, Martin S, Padovan E, Hartmann U, Kohler J. (1996) T cell immune responses to haptens. Structural models for allergic and autoimmune reactions. *Toxicology* 107:141–151.
- Wu Y, Farrell J, Pirmohamed M, Park BK, Naisbitt DJ. (2007) Generation and characterization of antigen-specific CD4+, CD8+, and CD4+ CD8+ T-cell clones from patients with carbamazepine hypersensitivity. *J Allergy Clin Immunol* 119:973–981.
- Yang CW, Hung SI, Juo CG, Lin YP, Fang WH, Lu IH, Chen ST, Chen YT. (2007) HLA-B\*1502-bound peptides: implications for the pathogenesis of carbamazepine-induced Stevens–Johnson syndrome. *J Allergy Clin Immunol* 120:870–877.
- Yang G, Deng YJ, Qin H, Zhu BF, Chen F, Shen CM, Sun ZM, Chen LP, Wu J, Mu HF, Lucas R. (2010) HLA-B\*15 subtypes distribution in Han population in Beijing, China, as compared with those of other populations. *Int J Immunogenet* 37:205–212.