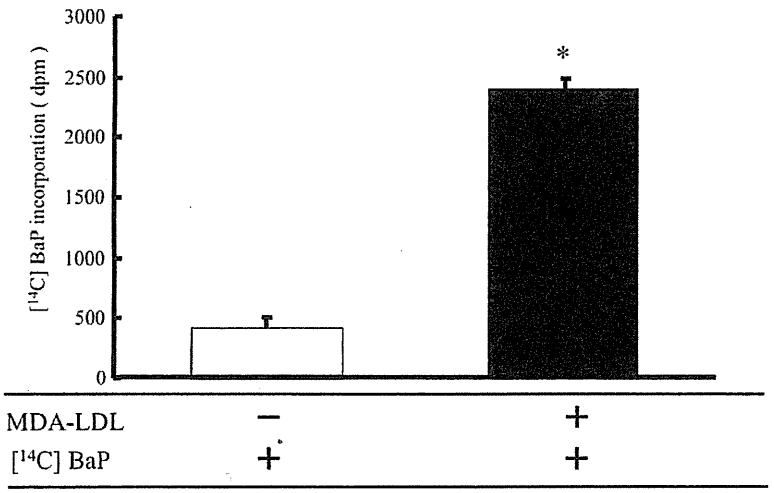

Fig. 4. Effect of MDA-LDL on [^{14}C]BaP incorporation into THP-1 cells. THP-1 cells were incubated with either [^{14}C]BaP or BaP and MDA-LDL for 24 hr. Incorporation of BaP was assessed by a [^{14}C]BaP incorporation assay. Concentrations of [^{14}C]BaP and MDA-LDL were 0.1 μCi/ml and 0.4 mg/ml, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.001$ compared with [^{14}C]BaP alone.

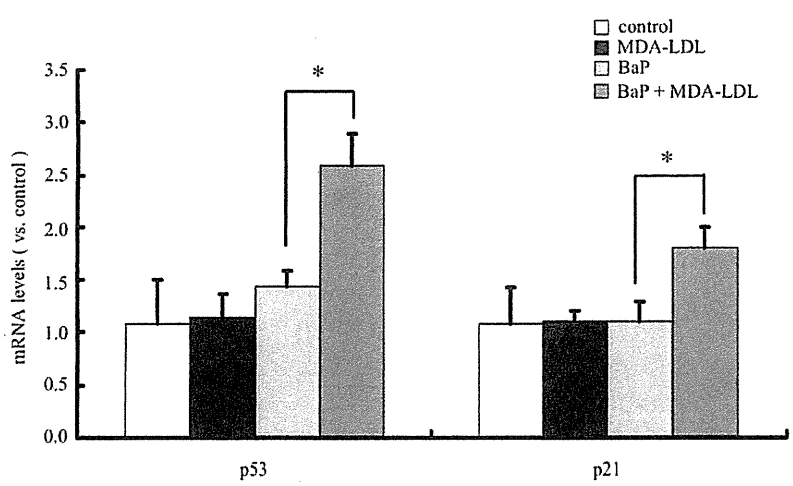

Fig. 5. Expression of p53 and p21 mRNAs in THP-1 cells. THP-1 cells were incubated with DMSO, BaP, MDA-LDL, or MDA-LDL along with BaP for 12 hr. Total RNA was prepared 12 hr later as described in the experimental procedures. The mRNA expression of the target genes was analyzed by real-time RT-PCR, and the mRNA levels of the target gene were normalized to that of GAPDH. Concentrations of MDA-LDL and BaP were 0.4 mg/ml and 0.1 μM, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.001$.

BaP and MDA-LDL than after treatment with BaP alone (Fig. 3). These findings suggest that the influence of PAHs on THP-1 cell growth is likely to be through AhR, and strong BaP activation is predicted to be due to the acceleration of BaP incorporation into cells.

BaP, as well as other PAHs, is poorly soluble in water.

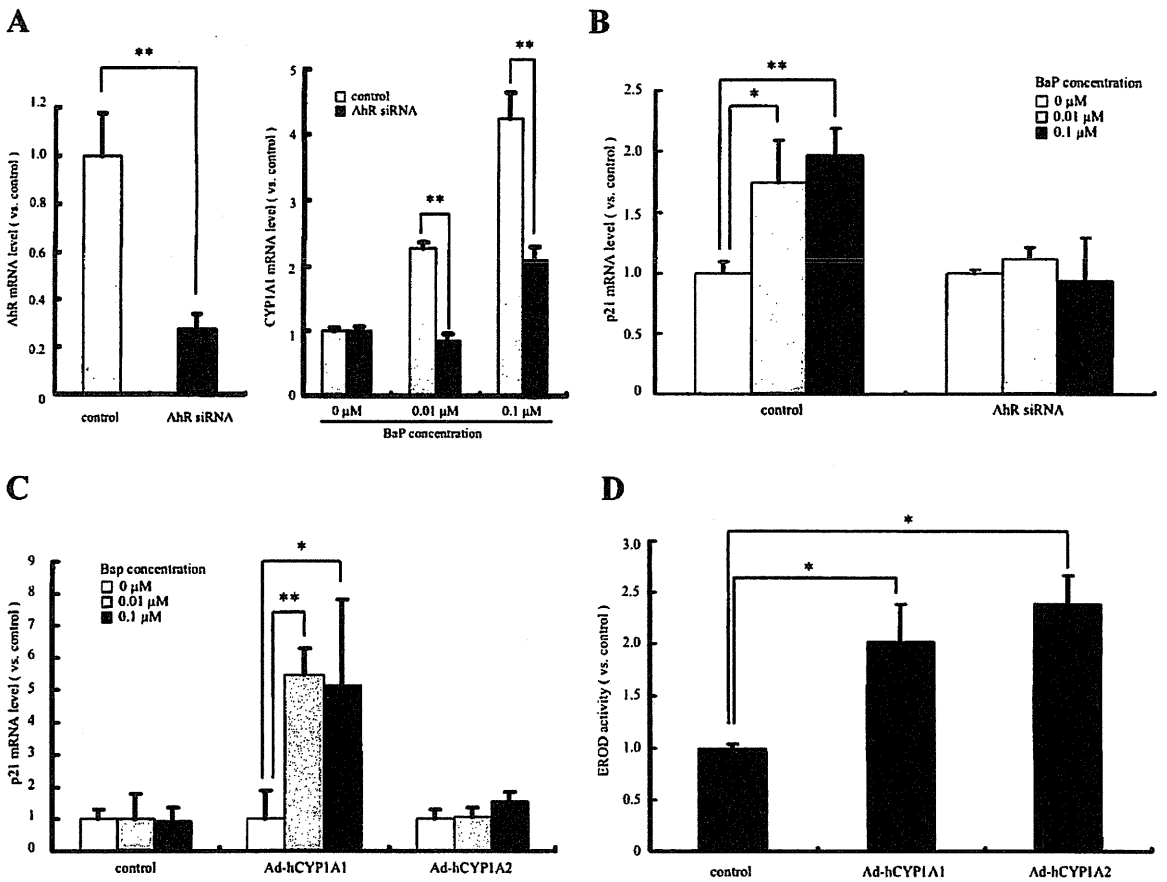

Fig. 6. (A) Effects of AhR siRNA on the expression levels of the AhR and CYP1A1 mRNA in HepG2 cells. Effects of AhR siRNA (B) and overexpression of hCYP1A1 (C) on the expression levels of p21 mRNA in HepG2 cells. (D) EROD activity of hCYP1A1 and hCYP1A2 in HepG2 cells. The mRNA expression of target genes was analyzed by real-time RT-PCR, and the mRNA levels of the target gene were normalized to that of GAPDH. EROD activity compared to the control is shown. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.05$; $^{**}p < 0.001$.

In plasma, BaP partitions readily incorporate into plasma lipoproteins (Avigan, 1959; Shu and Nichols, 1979). The entry of BaP into cells from plasma lipoproteins has been shown to be via diffusion and to be nonmediated by a transfer process (Remsen and Shireman, 1981; Plant *et al.*, 1985). Therefore, we investigated the uptake of BaP into THP-1 cell using [^{14}C]BaP. Interestingly, the uptake rate of [^{14}C]BaP was increased by cotreatment with MDA-LDL compared to treatment with [^{14}C]BaP alone (Fig. 4). The relatively slow rate of BaP uptake into cells suggests that the lipophilic properties of PAHs would likely result in extremely slow permeation into the tissues. It is important to understand the mechanism by which PAHs enter cells.

In a recent study, Iseki *et al.* (2005) suggested that the AhR in Leydig cells mediated growth inhibition through p21, which is a major transcriptional target of the tumor suppressor p53. Furthermore, p21 is known to play an important role in cell cycle control by interacting with the cyclin-dependent kinase complexes. Finally, the cell cycle may be arrested after the p53/p21 pathway is induced by DNA damage (Iwano *et al.*, 2006). Therefore, in the next experiment, we measured the expression levels of p53 and p21 mRNAs and found that expression of p53 and p21 mRNAs was significantly increased after cotreatment with BaP and MDA-LDL (Fig. 5). Our findings also indi-

cated that suppression of MDA-LDL-induced THP-1 cells growth by BaP may be attributable to apoptosis (Fig. 5). However, this finding is not compatible with the results of flow cytometry (data not shown). Furthermore, as mentioned previously, β-NF is a typical hCYP1A1 inducer that does not contribute to the suppression of MDA-LDL-induced THP-1 cell growth. These findings suggest that metabolites of BaP by hCYP1A1 induced through AhR activation may cause DNA damage. The cell cycle arrest was caused by p53/p21 activation through DNA damage. These results were strongly supported by the AhR siRNA experiment. AhR siRNA restored p21 mRNA expression level as shown in Fig. 6-B. The metabolic activation of BaP by hCYP1A1 could be a necessary step for suppression of MDA-LDL-induced THP-1 cell growth. Recently, knockout experiments of CYP1A1 have shown that CYP1A1 is not involved in BaP toxicity and was reported to be a detoxication enzyme (Uno *et al.*, 2006; Endo *et al.*, 2008). However, our overexpression experiment of hCYP1A1 using an hCYP1A1-expressing adenovirus further supported our findings. The reason for the discrepancy is not clear at this time but could be attributed to differences in the experimental conditions.

In conclusion, suppression of MDA-LDL-induced THP-1 cell growth by BaP may be due to accelerated incorporation of BaP. Thereafter, the hCYP1A1 gene is strongly induced by activation of the AhR by incorporated BaP. It seems that MDA-LDL may play an important role, at least in part, in that incorporation. In addition, BaP was metabolized to the activated form by strongly induced hCYP1A1. Subsequently, DNA damage was caused by the activated BaP, and the p53/p21 pathway was strongly activated. It is, therefore, possible that MDA-LDL-induced THP-1 cell growth was suppressed by PAHs.

Our findings suggest that compounds involved in arteriosclerosis are easily incorporated into cell via MDA-LDL, and further investigation is needed.

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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[1]**

*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.

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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

[1]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.

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

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[a]	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 diplotype	
									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
						Liver dysfunction			Allopurinol	–/>5 years		
									Etizolam	–/>5 years		
									Sodium pravastatin	–/>5 years		
2 (5)[b]	SJS	F/6	At least 1[c]	>37.0	<10%	GI tract disturbance	Not tested	9	None		4006/5101	B61/B51
3 (6)[b]	SJS	F/52	At least 1[c]	Unknown	<10%	Neutropenia	Not tested	14	Zonisamide	Not tested/346 days	4601/5901	B46/B59
						Liver dysfunction						
4	SJS	M/52	0	38	1	GI tract disturbance	Not tested	51	Tegafur/gimeracil/oteracil potassium	Not tested/38 days	0702/5201	B7/B52
						Neutropenia						
						Liver dysfunction						
						Renal dysfunction						
5	SJS	M/32	1	39	5	Liver dysfunction	Not tested	42	None		4002/5401	B60/B54
6 (2)	SJS	F/42	3	>39	5	GI tract disturbance	–	Shorter than 34	Sodium diclofenac	–/1 year	4001/5201	B60/B52
									L-carbocysteine	–/1 year		
									Cefteram pivoxil	–/4 days		
									Olopatadine hydrochloride	Not tested/unknown		
7	SJS	F/64	At least 1[c]	>37.0	10	Liver dysfunction	+	13	Mecobalamin	Not tested/13 days	**1511**/4002	**B75**/B60
8 (3)	SJS	M/45	3	>37.0	5	Liver dysfunction	Not tested	49	None		4801/5601	B48/B56
9 (4)	SJS	M/54	0	<37.0	0.5	None	+	34	None		1501/3501	B62/B35
10	TEN	M/38	3	40.3	40	Liver dysfunction	+	15	Troxipide	–/8 days	1302/4403	B13/B44
									Levofloxacin hydrate	–/15 days		
									Mecobalamin	–/9 days		
									Acyclovir	–/9 days		
11 (7)	TEN	M/17	3	39.7	20	Respiratory involvement	+	5	Zonisamide	+/33 days	4601/5601	B46/B56
						Neutropenia			Amoxicillin hydrate	+/1 day		
						Liver dysfunction			Promethazine methylenedisalicylate	Not tested/1 day		
12[d]	SJS	M/6	1	Unknown	<10%	Liver dysfunction	–	145	Zonisamide	+/24 days	**1511**/4006	**B75**/B61
13	Probable SJS	F/54	Unknown	<37.0	>10%	Liver dysfunction	Not tested	22	Sodium pravastatin	Not tested/unknown	4006/4403	B61/B44
									Nifedipine	Not tested/81 days		
									Etizolam	Not tested/15 days		
									Lansoprazole	Not tested/46 days		
									Sodium risedronate hydrate	Not tested/46 days		
14	Probable SJS	F/36	At least 1[c]	Unknown	5	None	+	15	Timiperone	Not tested/1 day	1301/**1511**	B13/**B75**
15	Atypical SJS	F/65	1	37.4	0.1	None	+	9	None		**1511**/3501	**B75**/B35

BT, body temperature; DLST, drug lymphocyte stimulation test; CBZ, carbamazepine.
[a]Number in parentheses is ID # from our previous study (Kaniwa et al., 2008).
[b]These patients were also included in Ikeda et al. (2010)
[c]Ophthalmic complications were observed, but severity was unknown.
[d]This patient was excluded from statistical analyses due to likely zonisamide-induced SJS.

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

	Population allele frequencies reported in allelefrequencies.net website[a]				
Ethnic group	*HLA-B*1502*	*HLA-B*1515*	*HLA-B*1521*	*HLA-B*1508*	*HLA-B*1511*
Japanese	0.001	Data unavailable	Data unavailable	Data unavailable	**0.004–0.008**[b,c]
Koreans	0.002	0.000	0.000	0.000	0.020
Han Chinese	**0.019–0.124**[b]	0.010	0.000–0.002	0.005–0.015	0.000–0.017[d]
Thai	**0.061–0.085**[b]	Data unavailable	**0.007–0.010**[b]	0.010	**0.010**[b]
Indians	**0.000–0.060**[b]	Data unavailable	Data unavailable	**0.005–0.033**[b]	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

[a]New Allele Frequency Database: http://www.allelefrequencies.net/ (Middleton et al., 2003).
[b]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.
[c]The frequency of 0.1 was reported by Tanaka et al. (1996).
[d]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; Locharernkul 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; Locharernkul 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).

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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.

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Review

Prediction of Severe Adverse Drug Reactions Using Pharmacogenetic Biomarkers

Masahiro TOHKIN[1,*], Akihiro ISHIGURO[1,2], Nahoko KANIWA[1], Yoshiro SAITO[1], Kouichi KUROSE[1] and Ryuichi HASEGAWA[1]

[1]*Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan*
[2]*Office of Safety II, Pharmaceuticals and Medical Devices Agency, Tokyo, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Severe adverse drug reactions (ADRs) are a major issue for drug therapy because they can cause serious disorders and be life-threatening. Many severe ADRs appear to be idiosyncratic and unpredictable. Genetic factors may underlie susceptibility to severe ADRs, and identification of predisposing genotypes may improve drug therapy by facilitating prescreening of carriers for specific genetic biomarkers. In this review, we clarify the current status of ADRs in Japan from open ADR data sources. Then, we introduce recent progress in the field of pharmacogenetic biomarkers for severe cutaneous ADRs, liver injury, and statin-induced myopathy. Key challenges for discovery of predictable risk alleles for these severe ADRs are also discussed.

Keywords: drug-induced liver injury; drug-induced myopathy; human lymphocyte antigen; Stevens-Johnson syndrome; toxic epidermal necrolysis

Introduction

Severe adverse drug reaction (ADR) is a major reason for failure of new drug development and withdrawal of approved drugs from the market. The classical pharmacological classification of ADRs by Rawlins and Thompson distinguished two types of severe ADRs.[1)] Type A reactions are dose-dependent and predictable on the basis of the drug's known pharmacological actions. Type A reactions are relatively common and include hypoglycemia induced by diabetic drugs and bleeding induced by warfarin, an oral anti-coagulant. By contrast, type B reactions are idiosyncratic, unpredictable from the pharmacological action of the drug, and are not necessarily dose-dependent. These type B reactions make up approximately 10–15% of all ADRs and include severe cutaneous disorders, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and drug-induced liver injury (DILI) caused by various drugs.

The aim of this review is to provide an update on the current understanding of pharmacogenetic analysis related to severe ADRs, especially severe cutaneous ADRs, DILI, and statin-induced myopathy; great progress has been recently observed in pharmacogenetic biomarkers of these ADRs, and this should facilitate early-stage detection of severe ADRs. Therefore, pharmacogenetic biomarkers of ADRs hold promise for reducing severe ADRs and pave the way for creating more affordable pharmaceuticals.

Domestic case reports for severe adverse drug reactions in Japan

Domestic cases of severe ADRs are reported to the Pharmaceuticals and Medical Devices Agency (PMDA) by pharmaceutical companies based on the Pharmaceutical Affairs Law in Japan. The ADR report in Japan includes information on suspicious drugs; ADR diagnoses, which are expressed using the Medical Dictionary for Regulatory Activities preferred terms (MedDRA-PT); and patient background such as gender, age, and concomitant use of other drugs. Because the quotation frequency of MedDRA-PTs in ADR reports reflects the number of ADR events, the quotation frequency provides basic information for estimating the event frequency of each ADR. Ac-

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**To whom correspondence should be addressed:* Masahiro TOHKIN, Ph.D., *Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-3700-9789, Fax. +81-3-3700-9788, E-mail: tohkin@nihs.go.jp*
The URL of the Drug Safety Information website of the Pharmaceuticals and Medical Devices Agency is "http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html" and the URL of material from the Committee on Safety of Drugs of the Ministry of Health, Labour and Welfare is "http://www.mhlw.go.jp/shingi/yakuji.html#anzen".

Table 1. Accumulated number of each adverse drug reaction term in cases reported from April 2004 to February 2009

Classification of accumulated frequency of each ADR term	Number of ADR terms in each classification	Representative ADR terms concerning SJS/TEN, hepatotoxicity, and rhabdomyolysis*
>1,000	30	Hepatic function abnormal (4,866), Liver disorder (3,936), Rhabdomyolysis (1,648), Stevens-Johnson syndrome (1,202)
500–1,000	26	Toxic epidermal necrolysis (751), Jaundice (715)
100–499	221	Hepatitis fulminant (406), Hepatitis acute (388), Oculomucocutaneous syndrome (324), Hepatitis (310), Cholestasis (184)
<100	4,491	
Total	4,768	

Based on the open data source MHLW website: http://www.mhlw.go.jp/shingi/yakuji.html#anzen
*Accumulated frequency is listed in parentheses.

Table 2. Number of case reports concerning SJS/TEN for fiscal years 2005 to 2008

Year (fiscal) Molecular entities	2005			2006			2007			2008		
	SJS	TEN	Sum	SJS	TEN	Sum	SJS	TEN	Sum	SJS	TEN	Sum
Allopurinol	14	12	26	22	8	30	15	11	27	33	18	51
Carbamazepine	17	5	22	17	4	21	24	6	30	26	10	36
Diclofenac	9	5	14	8	2	10	6	1	7	7	9	16
Loxoprofen	9	2	11	11	5	16	7	7	14	12	9	21
Phenobarbital	5	5	10	2	4	6	6	0	6	8	4	12
Non-pyrines	6	4	10	2	4	6	1	9	10	2	4	6
Zonisamide	9	1	10	3	2	5	1	4	5	9	6	15
Acetaminophen	1	4	5	1	5	6	1	12	13	3	10	13
Mortality (rate, %)	13 (6)	41 (27)		14 (5)	41 (30)		17 (7)	36 (23)		12 (4)	50 (26)	
Total	223	151	374	271	136	407	260	156	416	289	189	478

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

cording to material from the Committee on Safety of Drugs of the Ministry of Health, Labour and Welfare (MHLW, see footnote for URL address), the total number of MedDRA-PTs was 4,768 terms cited among 168,045 ADR events from domestic cases reported to PMDA from April 2004 to February 2009. As shown in **Table 1**, the number of MedDRA-PTs cited more than 100 times among 168,045 ADR events was 277. SJS/TEN, hepatotoxicity, and rhabdomyolysis are considered to be major ADRs in Japan because the frequently cited top 277 MedDRA-PTs included most ADR terms concerning SJS/TEN, hepatotoxicity, and rhabdomyolysis. In particular, accumulated quotation frequency of abnormal hepatic function (4,866) and liver disorder (3,936) ranked second and third, followed by interstitial pneumonia (5,190).

Major severe cutaneous ADRs, SJS and TEN, are life-threatening skin disorders which are often accompanied by high fever and systemic complications.[2,3] SJS/TEN incidence is generally very low and more than 100 different causative drugs have been reported.[4–6] We counted the event number for major suspected drugs in domestic cases concerning SJS and TEN from April 2005 to March 2009 based on the open data source of PMDA, and the results are shown in **Table 2**. Allopurinol, an anti-hyperuricemia drug, is the most frequently reported drug for SJS and TEN (**Table 2**). Many cases were reported with anticonvulsant drugs including carbamazepine (CBZ), phenobarbital, and zonisamide and non-steroidal anti-inflammation drugs (NSAID), which include diclofenac, loxoprofen, non-pyrines, and acetaminophen (**Table 2**). Although it is difficult to calculate the exact incidence of SJS and TEN in Japan, the Japanese mortality rates of SJS and TEN based on domestic cases were about 4–7% and 23–30%, respectively, which were in accordance with rates reported in other populations[7,8] (**Table 2**). Therefore, the mortality rate calculated by ADR reports can be a useful reference to estimate the mortality rate of severe ADRs such as SJS and TEN.

The liver is a common target for drug toxicity due to its pivotal role in drug metabolism. Moreover, any drug has the potential to cause liver injury.[9] Therefore, hepatotoxicity is the most common ADR, causing drug withdrawals and post-marketing regulatory decisions and

Table 3. Case reports concerning hepatotoxicity in fiscal year 2008

Molecular entities	Hepatic function abnormal	Liver disorder	Jaundice	Hepatitis fulminant	Hepatitis acute	Hepatitis	Cholestasis
Terbinafine HCl	52	47	3	0	5	11	2
Fluvastatin Na	33	21	0	0	0	1	0
Itraconazole	17	10	0	0	0	0	0
Loxoprofen Na	12	15	0	0	0	2	0
Carbamazepine	11	13	0	1	1	2	0
Rosuvastatin Ca	15	7	0	1	0	3	0
Tegafur/Uracil	12	10	1	3	1	0	0
Ticlopidine HCl	9	12	4	0	0	1	15
Gefitinib	16	2	0	1	0	0	0
Cyclosporine	11	3	3	0	0	0	1
Atorvastatin Ca	7	8	0	0	0	0	1
Fenofibrate	7	5	0	0	0	4	0
Acarbose	2	8	0	0	0	0	0
Tranilast	6	4	1	0	0	1	1
Aspirin	1	8	1	0	0	1	0
Non-pyrines (4)	3	5	0	0	0	0	0
Voglibose	3	4	1	0	1	3	0
Temozolomide	6	1	0	0	0	0	0
Cefcapene pivoxil HCl	3	3	2	2	0	0	0
Total	1,063	771	90	82	68	58	55

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

is associated with significant mortality.[10] We counted the event number of major suspected drugs in domestic cases concerning various hepatotoxicities based on the open data source from PMDA from April 2008 to March 2009, and the results are shown in **Table 3**. Highly reported hepatotoxicities were abnormal hepatic function, liver disorder, jaundice, fulminant hepatitis, acute hepatitis, hepatitis, and cholestasis (**Table 3**). Although the difference between abnormal hepatic function and hepatic disorder is unclear, these MedDRA-PTs are exclusively used. The total number of case reports for hepatotoxicity was 2,509. Other hepatotoxicity related reports (more than 10 cases) not in cluded in **Table 3** were hepatic failure (44), hepatocellular injury (41), cholelithiasis (21), acute hepatic failure (19), autoimmune hepatitis (19), hyperbilirubinemia (17), veno-occlusive liver disease (15), cholecystitis (14), mixed liver injury (12), cholecystitis acute (11), jaundice cholestatic (10), and cholangitis (10). There were 89 other hepatotoxicity-related reports (less than 9 cases) not included in **Table 3**. The estimated frequency of reported hepatotoxicity ranges from 1 per 10,000 to 1 per 10 million patient-years of exposure[11] (**Table 3**).

Rhabdomyolysis, one of the most serious myopathies, is characterized by the leakage of muscle cell content, including electrolytes, myoglobin, and other sarcoplasmic proteins [*e.g.*, creatine kinase, aldolase, lactate dehydrogenase, alanine aminotransferase (AST), and aspartate aminotransferase (AST)] into the circulation.[12] Lipid-lowering drugs (*e.g.*, statins and fibrates) are well known causes of rhabdomyolysis, and reports produced by the US Food and Drug Administration (FDA) showed that the rate of fatal rhabdomyolysis was 0.15 per 1 million statin

Table 4. Case reports concerning rhabdomyolysis for fiscal years 2004 to 2008

Molecular entities	Fiscal year				
	2004	2005	2006	2007	2008
Atorvastatin	51	41	48	31	28
Bezafibrate	16	22	17	16	11
Pravastatin	21	24	19	9	11
Simvastatin	21	15	5	8	2
Levofloxacin	9	10	10	9	4
Fluvastatin	13	8	7	4	6
Omeprazole	10	8	10	3	4
Propofol	8	14	2	5	8
Rosuvastatin	–	0	4	15	14
Risperidone	7	5	5	5	5
Fenofibrate	10	9	3	1	5
Pitavastatin	4	3	10	3	9
Total	389	351	359	291	332

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

prescriptions dispensed.[13,14] **Table 4** shows suspicious drugs in Japan, which are similar to US data, and the reported range of case numbers is 300 to 400 per year for the last 5 years (April 2004–March 2009).

Genomic analysis of severe adverse drug reactions

Severe ADRs affect only a minority of patients taking drugs. However, hereditary forms of severe ADRs and cases occurring in identical twins have been reported, implying involvement of certain genetic factors in predisposing individuals to such severe ADRs.[15,16] The genetic basis of ADRs can be categorized into two broad groups. The first group involves genes that drive pharmacological mechanisms (drug targets, drug metabolizing enzymes, and drug transporters).[17] Common mechanisms underlying these severe ADRs are unusual drug accumulation in the target organ due to polymorphisms in drug metabolizing enzyme and drug transporter genes, and unusual sensitivity in the target organ due to changes in drug target genes.[18] The second category involves the immune system in a drug-induced allergic reaction. One important molecule for ADRs associated with immune reactions is the human lymphocyte antigen (HLA), which plays a key role in initiation of immune responses and killing target cells by presenting antigens to the T-cell receptor.[19] The *HLA* gene region codes for three classical class I (HLA-A, HLA-B, and HLA-C) and three class II (HLA-DR, HLA-DP, and HLA-DQ) antigens. Class I antigens are recognized by cytotoxic $CD8^+$ T cells and class IIs by $CD4^+$ T cells. Of the highly polymorphic *HLA* genes, *HLA-B* is the most polymorphic with over 800 variants reported in the human genome.[20] *HLA* genes within each class encode structurally similar but distinct HLA proteins that bind and present HLA-type-specific antigenic peptides to T-cell receptors.[19,20] HLA disease associations that are related to genes with immunological and inflammatory functions have been identified in many autoimmune and inflammatory conditions.

In addition to these susceptible genes, recent advances in molecular biology have led to analysis of the association of whole genome polymorphisms with ADRs.[21,22] For example, recent high-density DNA microarrays can analyze more than one million genomic biomarkers at the same time. Therefore, association analysis has been conducted by both candidate gene and genome-wide analysis.

Severe cutaneous adverse drug reactions

Carbamazepine (CBZ) is one of the most widely used aromatic anticonvulsants and is often used as a pain-relief drug for prosopalgia. CBZ is metabolized by mainly hepatic CYP3A4, CYP2B6, and CYP2C8, which generate various potentially reactive metabolites, such as CBZ-10,11-epoxide; 3-hydroxy-CBZ; 2-hydroxy-CBZ; and CBZ-2,3-epoxide.[23,24] CBZ is generally well tolerated but also is associated with idiosyncratic adverse reactions such as SJS/TEN. A high frequency of CBZ-induced SJS/TEN was reported in Han Chinese (0.25% in new exposures to CBZ) compared to Caucasians (0.014% in new exposures to CBZ).[25–29] Furthermore, CBZ-induced SJS/TEN has been reported in identical twins.[15] These studies suggest that susceptibility to such reactions may be genetically determined. Since most reactive CBZ metabolites are detoxified to non-toxic dihydrodiols by liver microsomal epoxide hydrolase 1 (EPHX1) or to glutathione conjugates by glutathione S-transferase μ 1 (GSTM1),[30,31] some researchers have attempted to find the defective alleles of *EPHX* and *GST* genes in patients with SJS/TEN; however, these attempts have failed to find associations, indicating that reactive metabolite generation from CBZ is not sufficient to cause SJS/TEN.[32,33] Recently, Chung *et al.* reported a tight association between CBZ-induced SJS/TEN and *HLA-B*1502* allele in Han Chinese[25] (**Table 5**). They showed that all 44 Han Chinese patients with CBZ-induced SJS/TEN carried the *HLA-B*1502* allele and its odds ratio was 2,505 (95% confidence interval, 195 to 27,483, $Pc = 2.02 \times 10^{-32}$). The finding was further confirmed by the same group in

Table 5. Association between severe cutaneous adverse drug reaction and *HLA* type

Drug	HLA	Population	OR or (N)[a]	Pc[b]	Ref
Carbamazepine	B*1502	Han Chinese	2505	2.0×10^{-32}	25)
Carbamazepine	B*1502	Asian ancestry	(4/4)	–	37)
Carbamazepine	B*1502	European	(0/8)	–	37)
Carbamazepine	B*1502	Thai	25.5	0.0005	106)
Carbanazepine	B*1502	Japanese	(0/7)	–	36)
Carbamazepine	B*1502	Han Chinese	1357	1.6×10^{-41}	34)
Phenytoin	B*1502	Thai	18.5	0.005	106)
Antiepileptic	B*1502	Han Chinese	17.6	0.001	35)
Allopurinol	B*5801	Han Chinese	580	4.7×10^{-24}	44)
Allopurinol	B*5801	European	80	$<10^{-6}$	5)
Allopurinol	B*5801	Japanese	40.8	$<10^{-4}$	36)
Abacavir	B*5701	Australian	117	$<10^{-4}$	48)
Abacavir	B*5701	British	24	$<10^{-4}$	47)
Abacavir	B*5701	Australian	960	$<10^{-4}$	50)
Abacavir	B*5701	White	(36/65)	–	49)
		Black	(0/9)	–	
		Other	(1/10)	–	
Abacavir	B*5701	Japanese	(0/7)	–	40)
Nevirapine	DRB1*0101 and high CD4	Caucasian Australian	18	0.0006	59)
Nevirapine	Cw*0802-B*1402	Sardinian	15	0.05	60)
Nevirapine	B*3505	Thai	18.96	4.6×10^{-6}	62)
Nevirapine	Cw8	Japanese	6.2	0.03	61)

[a] OR is odds ratio and (N) is sensitivity (carrier cases/all cases).
[b] Pc indicates corrected P value.

an additional study that included patients who were Han Chinese or Chinese descendants from Taiwan, Hong Kong, China, and the USA[34] (**Table 5**). The involvement of *HLA-B*1502* was also detected in SJS/TEN caused by other aromatic anti-epileptic drugs, such as phenytoin in Han Chinese[35] (**Table 5**). However, such strong association between *HLA-B*1502* and CBZ-induced SJS/TEN has not been detected in Caucasian and Japanese SJS/TEN patients[36,37] (**Table 5**). *HLA-B*1502* is present at a higher allele frequency in South-east Asian populations than in Caucasian and Japanese populations.[38] *HLA-B*1502* was not detected in 486 healthy Japanese subjects[39] and in 935 USA Caucasians (http://www.allelefrequencies.net/), while the allele frequency in Han Chinese is 8.6%.[25] The low frequency of *HLA-B*1502* in Caucasian and Japanese populations may account for the fact that no association between *HLA-B*1502* and CBZ-induced SJS/TEN was observed in Caucasians and Japanese.[36,40] Alternatively, these results suggest that *HLA-B*1502* is involved in the mechanism, but is not sufficient for CBZ-induced SJS/TEN. There could be other co-factors, such as virus infection or other variants of genes, for example, *CYP3A4*, *CYP2B6*, *CYP2C8*, *EPHX1*, and *GSTM1*; T cell receptors; genes related to apoptosis; or genes for co-stimulatory molecules involved in the interaction between antigen-presenting cells and T cells.

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid and is commonly used for hyperuricemia and gout.[41] Allopurinol is metabolized by xanthine oxidase to oxipurinol, which forms ribonucleotide adduct and ribonucleoside adduct.[42] Allopurinol has been reported to be a causative drug of a variety of delayed cutaneous adverse reactions, such as SJS/TEN.[43] Recently, a strong association of *HLA-B*5801* with allopurinol-induced severe cutaneous adverse reactions (drug-induced hypersensitivity syndrome and SJS/TEN) was found in Han Chinese in Taiwan[44] (**Table 5**). They showed that the *HLA-B*5801* allele was present in all patients (51/51) with allopurinol-induced severe cutaneous adverse reactions, but only in 15% of tolerant patients (20/135). The odds ratio was 580 (95% confidence interval, 34 to 9781, $Pc = 4.7 \times 10^{-24}$). Although the association was confirmed in Caucasians[5] and Japanese,[36] the odds ratio in Han Chinese (580) was much higher than that in Caucasians (80) and Japanese (40) (**Table 5**). Approximately 9 to 11% of Han Chinese are carriers of the allele, and its prevalence is generally lower in Caucasian (1 to 6%), Japanese (0.68%), and African (2 to 4%) populations.[43,44] These reports suggest that *HLA-B*5801* might be a genetic biomarker for allopurinol-induced severe cutaneous adverse reactions; however, the extent of the association showed ethnic differences.

Abacavir is a potent nucleoside analog reverse transcriptase inhibitor that is used in combination with other drugs to treat human immunodeficiency virus infection. The most serious adverse reaction of abacavir that limits its use in therapy is a hypersensitivity reaction which includes the combination of fever, skin rash, constitutional symptoms, gastrointestinal tract symptoms, and respiratory symptoms.[45] Hypersensitivity to abacavir occurs in approximately 5 to 8% of patients treated with abacavir, typically within 1 to 6 weeks of the initial dose.[45] Abacavir is metabolized by type 1 alcohol dehydrogenase to an aldehyde-reactive metabolite.[46] The initial association between *HLA-B*5701* and abacavir-induced hypersensitivity reaction was elucidated by two independent research groups in 2002[47,48] (**Table 5**). The association was reported only in Caucasians and not in Africans or Japanese[40,49] (**Table 5**) because the allelic frequency of *HLA-B*5701* in Caucasians is approximately 8%, but low in individuals of African or Asian descent.[47,48] Fine recombinant genetic mapping has identified a significant linkage disequilibrium of the haplotypic M493T polymorphism of heat shock protein-Hom (Hsp70-Hom M493T) and *HLA-B*5701* in abacavir-induced hypersensitivity reaction cases in Western Australians, which enhanced the discrimination of hypersensitive subjects from the tolerant control (odds ratio, 3,893; $Pc < 0.00001$) when compared to *HLA-B*5701* only (odds ratio, 960; $Pc < 0.00001$).[50] The Hsp70-Hom M493T polymorphism may facilitate the loading of abacavir- or its metabolite-hapten endogenous peptide onto *HLA-B*5701*.[51] A large randomized, controlled clinical trial assessing the clinical effectiveness of *HLA-B*5701* screening in Caucasians (PREDICT-1 study),[52] and a case-control study of *HLA-B*5701* in both Caucasians and African Americans (SHAPE study),[53] were highly supportive of the use of *HLA-B*5701* screening in clinical practice to exclude *HLA-B*5701* carriers from patients treated with abacavir. However, 7 Japanese patients, who were all *HLA-B*5701*-negative, had abacavir-induced hypersensitivity reactions.[40] Thus, the genetic screening of *HLA-B*5701* is unlikely to be effective for the Japanese.

Nevirapine is a non-nucleoside reverse transcriptase inhibitor that is used in combination with antiretroviral therapy.[54] The major treatment-limiting toxicity associated with nevirapine use is skin rash and hypersensitivity, which emerge in 5% of patients who have initiated nevirapine therapy.[55] Nevirapine is metabolized by CYP3A4 predominantly, and to a lesser degree by CYP2B6 and other CYP isoforms, to several hydroxylated metabolites: 12-hydroxynevirapine has been implicated as a putative nevirapine metabolite causing hypersensitivity reactions.[56] $CD4^+$ T cells have been shown to be involved in the nevirapine-induced hypersensitivity reaction.[57,58] Thus, a high level of $CD4^+$ T cells (more than 25% above normal) is one risk factor for nevirapine-induced hypersensitivity reaction.[55] In addition to $CD4^+$ T cell levels, associations between several types of HLA and

nevirapine-induced hypersensitivity reactions have been reported in different countries or populations. For example, *HLA-DRB1*0101* (Western Australian),[59] *HLA-Cw* 0802-B*1402* haplotype (Sardinian patients, people in Italian autonomous regions),[60] *HLA-Cw8* (Japanese),[61] and *HLA-B*3505* (Thai)[62] have been reported (**Table 5**). These results imply that the primary determining *HLA* allele may be different among populations in the nevirapine-induced hypersensitivity reaction.

Drug-induced liver injury

Several patterns of DILI exist, with the most useful classification being hepatocellular, cholestatic, or a combination of both.[63] Hepatocellular injury involves marked elevation of serum ALT and AST levels, usually preceding an increase in total bilirubin and no increase or only modest increases in alkaline phosphatase (ALP) levels.[9] In cholestatic injury, increases in ALP levels predominate and precede increases in ALT and AST.[9] The patterns in which a drug causes liver injury are regarded as being either predictable (dose-dependent) or unpredictable (idiosyncratic).[64] Acetaminophen-induced hepatotoxicity has been considered the classic example of a dose-related hepatotoxin,[65] although few other drugs fit this pattern.[64] Rather, the majority of drugs that are capable of producing liver injury do so in an unpredictable fashion with variable latency periods.[9,66]

Single nucleotide polymorphisms (SNPs) in drug metabolizing enzymes and drug transporters, which regulate the metabolism and disposition of drugs, represent the best studied set of pharmaceutically important genetic markers of DILI. *N*-Acetyltransferase (NAT) functions by acetylating drugs, therefore causing active drug metabolites to be detoxified. Deficient alleles of *NAT* (such as *NAT1*14* and **15* and *NAT2*5*, **6*, and **7*), which reduce detoxification activity, increase the toxicity of drugs including isoniazid, sulfonamides, and procainamides.[67,68] The allele distribution of the Caucasian population differs from that reported in the Japanese population.[67,69] Another enzyme pathway of importance is that of glutathione in the detoxification of reactive metabolites. Genetically determined deficiencies in glutathione synthetase and GST have been associated with increased hepatotoxicity of certain drugs, including acetaminophen, metronidazole, and nitrofurantoin.[70,71] Frequencies of GST-deficient alleles show ethnic differences. For example, the homozygous deletion genotype frequency in *GSTM1* ranges from 0.38 to 0.67 in Caucasians, from 0.33 to 0.63 in East Asians, and from 0.22 to 0.35 in Africans and African-Americans.[72] Pacific Islanders have the highest reported frequency of homozygous deletion genotypes (0.64–1.0) of any group studied.[73,74] Troglitazone is a 2,4-thiazolidinedione anti-diabetic drug with insulin-sensitizing activities.[75,76] Troglitazone-associated idiosyncratic hepatic dysfunction and hepatic failure were reported after introduction of the drug into the market.[77–79] Yamamoto *et al.* reported that CYP3A4 catalyzed troglitazone into an epoxide of a quinone metabolite which may be eliminated by GSTs and EPHX.[80] To address the susceptible genetic factors responsible for the hepatotoxicity associated with the drug, Watanabe *et al.* performed a genetic polymorphic analysis by a target gene approach in troglitazone-treated Japanese patients with type 2 diabetes mellitus[81] (**Table 6**). They observed a correlation between hepatic failure and both *GSTT1* and *GSTM1* null genotypes. They reported that the odds ratio was 3.692 and its 95% confidence interval was 1.354 to 10.066 (Pc = 0.008). A more recent example of drug hepatotoxicity resulting from genetic polymorphisms of drug metabolizing enzymes and drug transporters is that of diclofenac, a non-steroidal anti-inflammatory drug that is among the most common drugs to cause idiosyncratic hepatotoxicity. Diclofenac-induced hepatotoxicity occurs at a rate of 6 per 100,000 users, and 8 to 20% of the patients who develop jaundice die of liver failure. It has been concluded that diclofenac hepatotoxicity is associated with the possession of variant UDP-glucuronosyltransferase 2B7 (*UGT2B7*2*), ATP-binding cassette transporter C2 (*ABCC2*, −24C>T), and *CYP2C8* haplotypes[82] (**Table 6**).

Table 6. Association between drug-induced liver injury and genetic polymorphisms

Drug	Gene variant	Population	OR[a]	Pc[b]	Ref
Troglitazone	GSTM1/T1	Japanese	3.69	0.008	81)
Ticlopidine	HLA-A*3303	Japanese	36.5	7.32×10^{-7}	86)
Diclofenac	UGT2B7*2	Unknown	8.5	0.03	82)
	ABCC2(C-24T)	Unknown	5.0	0.005	
	CYP2C8 haplotypes	Unknown	–	0.04	
Flucloxacillin	HLA-B*5701	European	45.0	8.7×10^{-33}	21)

[a] OR is the odds ratio.
[b] Pc indicates corrected P value.

Immune-mediated mechanisms via the reactive metabolite binding to macromolecules are believed to be associated with idiosyncratic DILI. *HLA* has been considered to be involved in T-cell mediated cytotoxic reactions and drug-induced allergic reactions. Therefore, *HLA* might be another type of candidate genetic biomarker of DILI.[64,66] Ticlopidine, an anti-platelet agent, which has been widely used for the secondary prevention of atherothrombosis,[83] has shown severe hepatotoxicity, mainly of the cholestatic type,[84] and there appears to be an increased rate of hepatic adverse reactions in Japanese compared with Caucasian patients.[85] Hirata *et al.* explored genetic risk factors for ticlopidine-induced hepatotoxicity using 22 Japanese patients with ticlopidine-induced hepatotoxicity and 85 Japanese patients who tolerated ticlopidine therapy without ex-

periencing adverse reactions and they found a significant correlation between ticlopidine-induced hepatotoxicity and *HLA-A*3303*[86] (**Table 6**). Allelic frequency of *HLA-A*3303* is 7.54% in Japanese, 0.6% in Caucasians, and 4.5% in African-Americans (http://www.allelefrequencies.net/). They reported that 12 patients (86%) among 14 patients who showed ticlopidine-induced cholestatic hepatotoxicity had *HLA-A*3303* and the odds ratio was 36.50 (95% confidence interval, 7.25 to 183.82). Another example of the correlation between *HLA* and DILI is that of flucloxacillin, which is widely used in many European countries and Australia for treatment of staphylococcal infection. Its use has been associated with a characteristic cholestatic hepatitis that is more common in females, the elderly, and patients with prolonged treatment courses.[87-89] In the United Kingdom, the incidence of flucloxacillin-related DILI has been estimated at 8.5 in every 100,000 new users in days 1 to 45 after starting treatment.[89] Daly *et al.* conducted a genome-wide association study using 51 cases (white European ancestry) of flucloxacillin-related DILI and 282 controls matched for sex and ancestry and found the strongest correlation between flucloxacillin-related DILI and a genetic marker (rs2395029) in complete linkage disequilibrium with *HLA-B*5701*[21] (**Table 6**). They reported that the odds ratio was 45 (95% confidence interval, 19.4 to 105). Among 51 cases, 43 patients (84%) carried the risk allele (G), which has a frequency of approximately 5% in the population controls and in European populations generally.

Statin-induced myopathy

In rare cases, statins can cause muscle pain or weakness in association with elevated creatine kinase levels (*i.e.*, myopathy), and occasionally this leads to muscle breakdown and myoglobin release (*i.e.*, rhabdomyolysis), with a risk of kidney failure and death.[90] The mechanisms by which statins cause myopathy remain unknown but appear to be related to statin concentrations in blood and muscle.[90]

Morimoto *et al.* studied genetic factors contributing to the risk of stain-induced myopathy and showed that the frequencies of *OATP-C*15* [tagged by 388A>G (N130D) and 521T>C, V174A], a mutant allele of *OATP-C* (OATP1B1, *SLC21A6/SLCO1B1*) was significantly higher in Japanese patients with myopathy who were receiving pravastatin or atorvastatin than in patients without myopathy[91] (**Table 7**). They also found another *OATP-C* mutant allele, 1628T>G (L543W), which is located in exon 12 of *SLC21A6/SLCO1B1* in a Japanese patient with pravastatin-induced myopathy[92] (**Table 7**). They examined the transporting activity for pravastatin and other substrates and found that the activity decreased significantly in HEK293 cells expressing mutant proteins with V174A and L543W compared to those in cells expressing *OATP-C*1a*, the reference allele of *OATC-C*.[93,94] OATP-C has been shown to mediate the hepatic uptake of statins.[95] From these results, they speculated that patients who are carrying these defective *OATP-C* mutant alleles have increased plasma concentrations of these statins and are thus more susceptible to the myotoxic effects of these statins compared to non-carrier patients treated with pravastatin and atorvastatin.[92] In fact, Ide *et al.* recently reported that *OATP-C*15* significantly influenced the relative bioavailability [F(rel)] of pravastatin; F(rel) was increased 1.50- and 1.95-fold in heterozygous and homozygous participants, respectively, for the *OATP-C*15* allele in comparison with participants without the allele from a covariate analysis of population pharmacokinetic analysis.[96]

Table 7. Candidates of *OATP-C* genomic biomarkers for statin-induced myopathy

Variant	*In vitro* activity	Statin	Ref
521T>C	Decrease	Pravastatin or atorvastatin	91)
521T>C	Decrease	Simvastatin	22)
1628T>G	Decrease	Pravastatin	91, 94)

The SEARCH Collaborative Group, which aims to determine whether a daily dose of 80 mg of simvastatin safely produces greater benefit than a daily dose of 20 mg, found 98 definite or incipient cases of myopathy among 6,031 participants who were assigned to receive 80 mg of simvastatin.[22] All participants were from the United Kingdom, but their ethnicity was not specified. They performed a genome-wide association study using approximately 300,000 genomic markers in 85 subjects with definite or incipient myopathy and 90 controls, and found a single strong association of myopathy with the rs4149056 (521T>C) SNP located within *SLCO1B1* (**Table 7**). They reported that the odds ratio for myopathy was 4.5 (95% confidence interval, 2.6 to 7.7) per copy of the C allele, and 16.9 (95% confidence interval, 4.7 to 61.1) in CC as compared with TT homozygotes. They concluded that this variant of *SLCO1B1* is strongly associated with an increased risk of statin-induced myopathy.

These studies suggest that variant *OATP-C* decreased the hepatic uptake of statin and increased blood and muscle concentrations of statin. The increase of the blood and muscle concentrations of statin may cause myopathy or rhabdomyolysis. Genotyping these *SLCO1B1* variants might help to achieve the benefits of statin therapy more safely.

Use of pharmacogenetic biomarkers in clinical practice

In principle, identifying genetic risk factors for severe ADRs, particularly type B reactions, could significantly

decrease the incidence rate of ADRs and improve the process of drug development.[97] Among these type B ADRs which we consider in this review, the usefulness of abacavir *HLA*-genetic biomarker (*HLA-B*5701*) has been confirmed in Caucasians from several prospective studies, such as the PREDICT-1 study.[52] The association of CBZ-induced SJS/TEN and an *HLA*-genetic biomarker (*HLA-B*1502*) in Han Chinese is extremely high compared with other drugs.[25] Therefore, *HLA-B*1502* screening is recommended for CBZ in clinical practice by the US FDA, and *HLA-B*5701* screening is recommended for abacavir by the US FDA and European Medical Agency. Before treatment with CBZ or abacavir, *HLA* analysis should be performed to exclude *HLA-B*1502* or *HLA-B*5701* unless the patient is from a population who shows extremely low frequency of these *HLA* types. Such exclusion of patients from treatment with causative drugs would markedly reduce the possibility of severe ADRs and prevent overestimation of severe cutaneous ADRs that could otherwise result in excessive discontinuation of treatment.[34,98,99] In Japan, package inserts of CBZ and abacavir describe these research results.

Perspective on pharmacogenetic biomarkers

An unresolved issue for genetic biomarkers is ethnic differences, since these *HLA* markers show ethnic specificity. For example, an *HLA* marker of abacavir (*B*5701*) or CBZ (*B*1502*) is present only in Caucasians or Han Chinese (and South East Asians), respectively, and its usefulness has not been shown in other populations such as Japanese.[37,40] On the other hand, the association between allopurinol treatment and *HLA-B*5801* was observed not only in Han Chinese but also in Caucasians and Japanese, although the odds ratios were lower than that of CBZ.[5,36,44] Therefore, it is absolutely necessary to explore the *HLA* marker for each population against each drug and also to find the universal genetic biomarker (if one exists) of severe ADRs for clinical practice. As shown for nevirapine, the association between the rash with hepatitis and *HLA-DRB1*0101* was observed in Western Australian patients with $CD4^+$ T-cell levels greater than 25% above normal levels.[59] This case suggests that the combination of *HLA* genetic biomarkers and other biomarkers might be useful to predict ADRs for some drugs. A prospective study or comparative study with other populations is necessary for *HLA* biomarkers of ticlopidine- and flucloxacillin-related DILI and *SLCO1B1* biomarkers of statin-induced myopathy.

In most cases of allergic reactions, such as SJS/TEN and DILI, *HLA*-drug toxicity associations are thought to arise as a result of the interaction of a specific *HLA* allele with the drug or its metabolite, causing an immune reaction to be triggered.[38,100,101] As shown in the abacavir-induced hypersensitivity reaction, the drug metabolite may play an important role in the allergic reaction process,[102] suggesting that sequential reactions from drug metabolism to the immune mechanism can exist in the allergic process. Thus, drug toxicities that are driven primarily by the immune response may require bioactivation of the drug to a specific metabolite to evoke the specific immune response that will lead to the generation of an adverse reaction.[38,100,101] These complex mechanisms may be involved in most cases of allergic reactions because reactive metabolites have been detected not only in abacavir but also in nevirapine, CBZ, and allopurinol.

Most of the currently available genetic biomarkers are limited in relation to *HLA*, drug metabolizing enzymes, and drug transporters.[17] Considering that the technology to identify genetic variants across the whole genome is advancing rapidly, many more significant genetic factors for ADRs are likely to be identified in the future. In such whole-genome case-control analysis, there might be critical points to resolve. The first problem is the size of case and control groups. Accrual of large numbers of cases is necessary for genome-wide association study of genetic factors underlying severe ADRs, even though the number of patients with specific types of ADRs is small.[103] Control subjects for such studies should be matched for drug exposure, concomitant use of other drugs that could affect the pharmacokinetics and pharmacodynamics of the drug in question, and subject background such as age, gender, and ethnicity.[103] The second problem is objective diagnosis of ADRs. Because one drug could induce many ADR phenotypes in which the mechanism may be different, standardization of diagnosis is necessary.[40,103] These critical points affect the sufficient statistical power to detect the genetic biomarker. In order to resolve these problems, several regional networks to study severe ADRs have been established, including our research group in Japan (for SJS/TEN, DILI, and myopathy in Japanese),[36] European collaboration for studying the genetic basis of adverse drug reactions (EUDRAGENE for six severe ADRs in multiple European populations),[104] the United States Drug Induced Liver Injury Network (DILIN, for DILI),[105] and the International Serious Adverse Event Consortium (SAEC, for SJS/TEN and DILI in global populations).[21] These networks involve scientists in regulatory agencies, healthcare systems, and pharmaceutical industries as well as academia. Moreover, for the goal of standardizing phenotypes and comparing ethnicity regarding genetic risk factors for severe ADRs, these networks may form a global consortium together with new networks from other communities in the future.

Conclusions

Specific types of *HLA* which showed strong association with severe cutaneous ADRs and DILI have been found as candidate pharmacogenetic biomarkers for each ADR. The *HLA* type was different for different causative drugs,

and the allelic frequency of *HLA* genetic polymorphisms showed ethnic differences. The genetic polymorphism of drug transporter gene *SLCO1B1* has been shown to be associated with statin-induced myopathy. It is necessary to conduct prospective studies to establish valid pharmacogenetic biomarkers for severe ADRs. A large research network for the collection of DNA samples from patients with ADRs is also necessary to explore a variety of pharmacogenetic biomarkers for ADRs.

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