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55 Biomarkers associated with severe cutaneous adverse reactions

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INTRODUCTION

Although skin rash is a frequently experienced adverse drug reaction, Stevens–Johnson syndrome (SJS), toxic epidermal necrosis (TEN), and drug-induced hypersensitivity syndrome (DIHS) are life-threatening severe cutaneous adverse reactions accompanied by fever and systemic complications. SJS and TEN, with characteristic mucosal disorders, are considered to be variations of the same disease expressed with different levels of severity (1), although this designation is controversial. The most widely accepted classification is based on the level of skin detachment area as follows; a skin detachment level less than 10% of the body surface, SJS; a skin detachment level from 10% to less than 30% of the body surface, SJS-TEN overlap; and a skin detachment level not less than 30%, TEN (1), while in Japan TEN is defined as a severity of skin detachment more than 10% of the body surface (2). DIHS, also called DRESS or HSS as acronyms for drug reaction with eosinophilia and systemic symptoms or hypersensitivity syndrome (3,4), is a severe disease with multiorgan failure and has been proposed to associate with reactivation of herpes virus-6 (5).

SJS/TEN and DIHS are idiosyncratic adverse reactions and the events are not usually dependent on the dose or plasma level of the causative drug. Moreover, these diseases are generally not identified during the drug development process due to their extremely low incidence but are initially recognized after the broad use of a drug in the post-approval period. These factors complicate the ability of physicians to administer drugs safely. Recently, several types of human leukocyte antigens (HLAs) have been reported to be associated with particular drug-induced severe cutaneous adverse reactions, which establish them as promising predictors for such reactions.

HLA AND OTHER GENOMIC BIOMARKERS RELATED TO SEVERE CUTANEOUS ADVERSE REACTIONS

HLAs are proteins involved in immune reactions. HLA-A, HLA-B, and HLA-C are categorized as class I molecules, which are ubiquitously expressed on the surface of cells including keratinocytes; and HLA-DR, HLA-DQ, and HLA-DP are categorized as class II molecules, which are mainly expressed on the surface of antigen presenting cells such as B-cells, macrophages or dendritic cells. Coding genes of all HLAs are on the short arm of chromosome 6 and are known to be diversely polymorphic. For example, more than 1000 alleles of *HLA-A*, *HLA-B*, and *HLA-C* have been identified to date (6).

Carbamazepine-Induced Severe Cutaneous Adverse Reactions

Chung et al. found for the first time a strong association between carbamazepine-induced SJS/TEN and *HLA-B*1502* in Han Chinese living in Taiwan (7). They reported that the carrier frequency of *HLA-B*1502* in these cases (44/44, 100%) was significantly higher than in carbamazepine-tolerant patients (3/101, 3%) ($p = 3.13E-27$), and that the odds ratio was 2504 (95% confidence interval (CI); 126–49,522). As shown in Table 55.1, this strong association has been confirmed by their continuing follow-up study (8), and studies involving Han Chinese patients in Hong Kong (9), Asian-originating patients living in Europe (10), Indian patients (11), and Thai patients (12–14). *HLA-B*1502* was also found to be a risk factor for carbamazepine-induced SJS/TEN in mainland Chinese similar to its being a risk factor for Southeastern Asian patients (15,16). However, the association of carbamazepine-induced SJS/TEN with *HLA-B*1502* has not been found in European (10), Japanese (17,18), or Korean patients (19). Moreover, no associations of *HLA-B*1502* with other carbamazepine-induced cutaneous adverse reactions such as maculopapular exanthema (MPE) or HSS were identified even with Thai patients (10) or Han Chinese patients in Taiwan (8) in addition to there being no correlations with Japanese (20), Korean (19), or European patients (21).

The incidence of carbamazepine-induced SJS/TEN in some Southeastern Asian countries such as Taiwan, Thailand, Malaysia, and the Philippines is 10-fold higher than in European countries, the United States, and Japan (22,23). Population allele frequencies of *HLA-B*1502* in Southeastern Asian countries are much higher (2–12%) than in Caucasians (rare) and in East Asian countries (Japan and Korea, 0–0.4%) (24), and this HLA type may be causative for the higher incidence of carbamazepine-induced SJS/TEN observed in Southeastern Asian countries.

Although carriers of *HLA-B*1502* have not been detected, Kaniwa et al. found four carriers of *HLA-B*1511* from 14 Japanese carbamazepine-induced SJS/TEN patients (18). *HLA-B*1511* and *HLA-B*1502* belong to the same serotype HLA-B75. Other major members of HLA-B75 are *HLA-B*1508* and *HLA-B*1521*. To date carbamazepine-induced SJS/TEN patients in Asia who carry *HLA-B*1511* (13,18,19), *HLA-B*1508* (11), and *HLA-B*1521* (13) have been reported, and particularly in East Asian countries such as Japan (18) and Korea (19), allele frequencies of *HLA-B*1511* in carbamazepine-induced SJS patients are much higher than carbamazepine-tolerant or healthy control subjects (Table 55.1). Although the alleles *HLA-B*1508,*

TABLE 55.1
Associations Between HLA Alleles and Carbamazepine-Induced Severe Cutaneous Adverse Reactions

Biomarker (HLA Allele)	Disease Phenotype	Ethnic Group	Carrier Frequency in Cases	Allele Frequency in Cases	Carrier Frequency in Controls	Allele Frequency in Controls	p-value	Odds Ratio (95% CI)	Reference
*B*1502*	SJS/TEN	Han Chinese in Taiwan	59/60		6/144		2.6E-41	1357 (193.4–8838.3)	(8)
		Asians living in Europe	4/4						(10)
		Han Chinese in Hong Kong	4/4						(9)
		Indians	6/8		0/10		0.0014	71.40 (3.0–1698)	(11)
		Thai	6/6		8/42		0.0005	25.5 (2.68–242.61)	(12)
		Thai	37/42		5/42		2.89E-12	54.76 (14.62–205.13)	(13)
		Han Chinese in mainland	9/9		11/80		<0.001	114.8 (6.3–2111.0)	(15)
		Chinese in mainland	16/17		2/21		<0.0001	152 (12–1835)	(16)
		Europeans	0/8						(10)
		Japanese	0/14						(18)
		Koreans	1/7		1/50		N.S.		(19)
	Hypersensitivity	Caucasians	0/56		0/43				(20)
	MPE/HSS	Han Chinese in Taiwan	1/31		6/144		N.S.		(8)
	MPE	Thai	2/9		8/42		N.S.		(12)
	MPE/HSS	Han Chinese in mainland	10/39		11/80		N.S.		(15)
*B*1508*	SJS/TEN	Indians	1/8						(11)
*B*1511*	SJS/TEN	Thai	1/42						(13)
		Japanese	4/14	4/28		10/986[b]	0.0004[c]	16.3 (4.76–55.6)	(18)
		Koreans	3/7		250		0.011	18.0 (2.3–141.2)	(19)
*B*1521*	SJS/TEN	Thai	2/42						(13)
*A*3101*	SJS	Europeans	5/12		10/257		8.0E-5	25.93 (4.93–116.18)	(26)
	SJS/TEN	Japanese	5/6		54/420		2.35E-4	33.9 (3.9–295.6)	(28)
	SCARs[a]	Japanese		11/44		53/742[b]	0.0004[c]	4.33 (2.07–9.06)	(27)
	SCARs[a]	Koreans	13/24		7/50		0.001	7.3 (2.3-22.5)	(19)
	MPE/HSS	Han Chinese in Taiwan	8/31		4/144		0.0021	12.17 (3.6–41.2)	(8)
	DIHS	Japanese	21/36		54/420		2.06E-9	9.5 (4.6–19.5)	(28)
	Others than SJS/TEN/DIES	Japanese	19/35		54/420		4.74E-8	8.0 (3.9–16.6)	(28)
	HSS	Europeans	10/27		10/257		0.03	12.41 (1.27–121.0)	(26)
	MPE	Europeans	23/106		10/257		8.0E-7	8.33 (3.59–19.36)	(26)

[a]Severe cutaneous adverse reactions.
[b]Healthy control.
[c]Allele frequencies between cases and controls were compared.
Abbreviations: DIHS, drug-induced hypersensitivity syndrome; HSS, hypersensitivity syndrome; MPE, maculopapular exanthema; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrosis.

*HLA-B*1511*, and *HLA-B*1521* are not as common as the allele *HLA-B*1502* in Southeastern Asians, the allele frequencies in Asians (0–3%) are higher than those in Caucasians (24). A recent *in vitro* study suggested the potential involvement of HLA-B*1511, HLA-B*1508, and HLA-B*1521 expressed in KERTrs in binding to CBZ and CTL activation (25). Thus, some members of the HLA-B75 serotype as well as *HLA-B*1502* are also risk factors for the development of carbamazepine-induced SJS/TEN in Asian countries.

In addition to *HLA-B*1502* or *HLA-B75* serotype, *HLA-A*3101* which was previously reported as a risk factor for carbamazepine-induced HSS and MPE in Han Chinese (8) has been reported to be a biomarker for carbamazepine-induced cutaneous adverse reactions in Europeans (26), Japanese (27,28), and Koreans (19) as shown in Table 55.1. In these three populations, *HLA-A*3101* is a risk factor for various carbamazepine-induced cutaneous adverse reactions ranging from mild skin rash such as MPE to sever cutaneous adverse reactions including SJS/TEN, while it is a risk factor for MPE and HSS but not for SJS/TEN in Taiwanese (8). Genome-wide association studies have detected SNPs (single nucleotide polymorphisms) on chromosome 6 close to the region of *HLA-A* that are in strong linkage disequilibrium with *HLA-A*3101* (26,28).

Thus, genetic biomarkers for carbamazepine-induced cutaneous adverse reactions are ethnic specific as well as probably phenotype specific.

Other Aromatic Antiepileptics-Induced Severe Cutaneous Adverse Reactions

Other aromatic antiepileptics such as phenytoin, lamotrigine, and phenobarbital also often cause cutaneous adverse reactions. Biomarkers related to these aromatic antiepileptics-induced cutaneous adverse reactions are summarized in Table 55.2, although the associations are typically weaker or less well established than the carbamazepine cases.

TABLE 55.2
Associations Between HLA Alleles and Aromatic Anti-Epileptics-Induced Severe Cutaneous Adverse Reactions

Causative Drug	Biomarker (HLA Allele)	Disease Phenotype	Ethnic Group	Carrier Frequency in Cases	Allele Frequency in Cases	Carrier Frequency in Controls	Allele Frequency in Controls	P-value	Odds Ratio (95% CI)	Reference
Phenytoin	*B*1502*	SJS/TEN	Taiwanese and Chinese	8/26		9/113		0.0041	5.1 (1.8–15.1)	(29)
		SJS/TEN	Thai	4/4		8/45		0.005	15.8 (1.82–188.4)	(12)
		MPE	Thai	3/4		8/45		N.S.		(12)
	*B*1301*	SJS/TEN	Taiwanese and Chinese	9/26		14/113		0.0154	3.7 (1.4–10.0)	(29)
Lamotrigine	*B*1502*	SJS/TEN	Taiwanese and Chinese	2/6						(29)
		TEN	Han Chinese in Hong Kong	1/1						(9)
		SJS/TEN	Han Chinese	1/3		1/21		N.S.		(32)
		MPE	Han Chinese	2/22		1/21		N.S.		(32)
		SJS/TEN	Han Chinese		0/4		4/56	N.S.		(33)
		MPE	Han Chinese		1/22		4/56	N.S.		(33)
	*B*5801*	SJS/TEN HSS	Mainly Europeans		3/44		0/86	0.037		(34)
Phenytoin, phenobarbital, or carbamazepine										
	*B*1301*	DIHS	Japanese	4/13						(20)
Oxycarbazepine										
	*B*1502*	SJS/TEN	Taiwanese and Chinese	3/3						(29)

Abbreviations: DIHS, drug-induced hypersensitivity syndrome; HSS, hypersensitivity syndrome; MPE, maculopapular exanthema; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrosis.

For phenytoin-induced Han Chinese SJS/TEN patients of Taiwan, Hung et al. reported that the carrier frequency of *HLA-B*1502* (8/26, 30.8%) was significantly higher than for tolerant controls (9/113, 8.0%), with an odds ratio of 5.1 (95% CI; 1.8–15.1, p = 0.0041) (29). For patients from Thailand, all of the four patients with phenytoin-induced SJS/TEN carried *HLA-B*1502*, and the association was significant in comparison to the carrier frequency of phenytoin-tolerant patients (1/9), while no associations were observed between *HLA-B*1502* and phenytoin-induced MPE (12). Man et al. detected a phenytoin-induced SJS/TEN case with *HLA-B*1502* and five phenytoin-induced MPE cases without *HLA-B*1502* (9). There are two case reports from mainland China on phenytoin-induced SJS/TEN patients; one report of two patients with the negative *HLA-B*1502* allele (30) and another report of one patient out of two with a positive *HLA-B*1502* allele (31). These results indicate that *HLA-B*1502* seems to be at least one of the risk factors for development of SJS/TEN caused by phenytoin in Southeastern Asian countries, although the association is not as strong as in the case of carbamazepine-induced SJS/TEN. Hung et al. also found a moderate association between *HLA-B*1301* and phenytoin-induced SJS/TEN (29).

For lamotrigine-induced SJS/TEN, two patients out of six from Taiwan (29) and one patient from Hong-Kong (9) carried *HLA-B*1502*. However, two recently performed case control studies using Han Chinese lamotrigine-induced patients including SJS/TEN and MPE failed to show an association with *HLA-B*1502*. A weak association of lamotrigine-induced severe cutaneous adverse reactions with *HLA-B*5801*, a known risk factor for allopurinol-induced severe cutaneous adverse reactions, discussed later, was detected in Europeans (34). It is interesting that two out of six Chinese lamotrigine-induced SJS patients also carried *HLA-B*5801* (29). Further large scale studies are required to elucidate genetic associations with lamotrigine-induced severe cutaneous adverse reactions.

Kano et al. pointed out a possible association between *HLA-B*1301* and a particular virus reactivation based on the observation that three out of four Japanese patients carrying *HLA-B*1301* had cytomegalovirus reactivation during the course of DIHS/DRESS caused by phenytoin, carbamazepine or Phenobarbital (20).

Allopurinol-Induced Severe Cutaneous Adverse Reactions

Allopurinol, one of the most often used drugs for hyperuricemia, is the most common cause of SJS/TEN in Europe (35). For the first time, a strong association between allopurinol-induced severe cutaneous adverse reactions and *HLA-B*5801* for Han Chinese in Taiwan was found by Hung et al. (Table 55.3) (36). This association has been confirmed in patients for Thailand (37), Japan (38,17,39), Europe (40), and Korea (41), although the strength of the association is dependent on the ethnic groups. Interestingly, the biomarker *HLA-B*5801* is a predictor for a wide variety of cutaneous reactions including SJS/TEN, HSS, and MPE unlike in the case of *HLA-B*1502*, which is a risk factor only for SJS/TEN. *HLA-B*5801* is a rather common allele (population allele frequencies are greater than 5%) in ethnic groups in which high sensitivity was observed in case–control studies, while population allele frequencies of *HLA-B*5801* are very low in Japanese and Europeans (less than 1%) (24). SNPs on chromosome 6 are completely linked with *HLA-B*5801* as detected by a genome-wide association in Japanese (39), and as such, they could be used as surrogate biomarkers in screening tests for *HLA-B*5801* prior to the initiation of allopurinol therapy.

TABLE 55.3
Associations Between HLA Alleles and Severe Cutaneous Adverse Reactions Caused by Various Drugs

Causative Drug	Biomarker (HLA Allele)	Disease Phenotype	Ethnic Group	Carrier Frequency in Cases	Allele Frequency in Cases	Carrier Frequency in Controls	Allele Frequency in Controls	P-value	Odds Ratio (95% CI)	Reference
Allopurinol	*B*5801*	SJS/TEN/HSS	Han Chinese in Taiwan	51/51		20/135		4.7E-24	580.3 (34.4–9780.9)	(36)
		SJS/TEN	Thai	27/27		7/54		1.61E-13	348.3 (19.2–6336.9)	(37)
		SJS/TEN/DIHS	Japanese	3/3						(38)
		SJS/TEN	Japanese		10/36		6/986[a]	5.39E-12[b]	62.8 (21.2–185.8)	(39)
		SJS/TEN	Europeans	15/27		28/1822[a]		<E-8	80	(40)
		SJS/TEN/DIHS	Koreans	24/26		6/57		2.45E-11	97.8 (18.3–521.5)	(41)
Abacavir	*B*5701*	HSS	Western Australian	14/18		4/167		<0.0001	117 (29–481)	(42)
	*B*5701 _HLA-DR7_ HLA-DQ3*	HSS	Western Australian	13/18		0/167			822 (43–15,675)	(42)
	*B*5701*	HSS	Western Australian	17/18		4/230		<0.0001	960	(43)
	*B*5701_ Hsp70-Hom M493T*	HSS	Western Australian	17/18		1/230		<0.00001	3893	(43)
	*B*5701*	HSS	Caucasians	Not indicated		Not indicated		8.4E-23	21.4 (9.5–48.1)	(45)
	*B*5701*	HSS	Hispanics	Not indicated		Not indicated		2.1E-4	30.4 (1.74–530.9)	(45)
	*B*5701*	HSS	Africans	Not indicated		Not indicated		0.27		(45)
	*B*5701*	HSS	Caucasians	57/129		8/202			19 (8–48)	(46)
	*B*5701*	HSS	Caucasians (cases with positive skin patch test result)	42/42		8/202			1945 110–34,352	(46)
	*B*5701*	HSS	Africans	10/69		2/206			17 (4–164)	(46)
	*B*5701*	HSS	Africans (cases with positive skin patch test result)	5/5		2/206			900 (38–21,045)	(46)
Methazolamide	*B59*	SJS	Japanese	3/3						(53)
		SJS	Koreans	6/6						(55)
		SJS/TEN	Koreans	2/2						(56)
	*B*5901*	SJS/TEN	Koreans	5/5		20/485[a]		<0.001	249.8 (13.4–4813.5)	(57)
Acetazolamide	*B59*	SJS	Koreans	2/2						(58,59)
Nevirapine	*B*3505*	Skin rash	Thai	25/143		2/181		4.9E-8	18.96 (4.87–73.44)	(60)

[a]Healthy control.
[b]Allele frequencies between cases and controls were compared.
Abbreviations: DIHS, drug-induced hypersensitivity syndrome; HSS, hypersensitivity syndrome; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrosis.

Abacavir-Induced Hypersensitivity Syndrome

Abacavir is a commonly used nucleotide analog with antiviral activity against HIV-1. Approximately 5–9% of patients develop HSS within six weeks after initial exposure to abacavir (42). Mallal et al. first reported the association between abacavir-induced HSS and *HLA-B*5701* in Western Australian HIV patients as shown in Table 55.3 (42). In Western Australian patients groups, *HLA-B*5701* is highly linked with *HLA-DR7* and *HLA-DQ3* (serotypes of HLA class II molecules) (42), or with a nonsynonymous SNP, *Hsp70-Hom M493T* (Heat shock 70 kDa protein 1L) (43), and the odds ratios for combinations of these genomic markers (822 for *B*5701_HLA-DR7_HLA-DQ3*, and 3893 for *B*5701_ Hsp70-Hom M493T*) were much higher than the odds ratio (100) with *HLA-B*5701* alone (42). Initially, an association between *HLA-B*5701* and abacavir-induced HSS was ambiguous in African patients (44,45). Hughes et al. examined the association between *HLA-B*5701* and abacavir-induced HSS in various ethnic groups with a cohort study in which patients from 12 countries participated. They confirmed an association between *HLA-B*5701*

and abacavir-induced HSS in Caucasian males and females and Hispanic, but failed to detect a significant association in African (45). As shown in Table 55.3, the carrier frequency of *HLA-B*5701* in abacavir-induced HSS in African patients seemed to be low (14%) compared with similar Caucasian patients (44%) in a study performed in the United States by Saag et al. (46) However, for patients immunologically confirmed by skin patch test using 1% and 10% abacavir solutions, the carrier frequencies of *HLA-B*5701* were 100% both in Caucasian (42/42) and African patients (5/5) (46). Thus, *HLA-B*5701* is now regarded as being a risk factor for abacavir-induced HSS both for Caucasians and Black people.

*HLA-B*5701* is a common allele in Caucasians that has recently been found to also be a risk factor for flucloxacillin-induced liver injury (47). However, *HLA-B*5701* is a rare allele in Asian countries such as Taiwan (48), Japan (49), and Korea (50). Only one out of 320 patients with HIV carried *HLA-B*5701* in Taiwan (48), and no carriers were found in 534 Korean patients with HIV (50). The availability of a screening test for *HLA-B*5701* did not affect the incidence of abacavir-induced HSS in Koreans (screening unavailable (3/57) versus screening available (4/93)) (50). Abacavir-induced HSS is encountered less frequently in Japan (1.3%) (51) and Taiwan (0.9%) (48) compared with Western countries.

Methazolamide-Induced SJS/TEN

Methazolamide, a sulfonamide derivative, is a carbonic anhydrase inhibitors used to lower the intraocular pressure in glaucoma. Most reports on methazolamide-induced SJS/TEN have been related to Japanese (52,53) and Koreans patients (54–56), and some of these reports (53,36,37) have suggested a strong association between *HLA-B59* serotype and SJS/TEN (Table 55.3). A recent case–control study performed in Korea using five cases and 485 control subjects from the general population revealed that *HLA-B*5901* is a risk factor for methazolamide-induced SJS/TEN (57). Case reports (58,59) on Korean acetazolamide-induced SJS patients positive for *HLA-B59* suggest that *HLA-B59* (*HLA-B*5901*) is also a risk factor for SJS/TEN caused by acetazolamide, another sulfonamide derivative carbonic anhydrase inhibitor. The allele frequency of *HLA-B*5901* is 1–2% in Japanese and Korean, but is very rare in Caucasians and Blacks (24).

Nevirapine-Induced Cutaneous Adverse Reactions

Nevirapine is a potent non-nucleoside reverse transcriptase inhibitor used for the treatment of HIV-1 infection and is known to often cause various types of skin rash. The *HLA-B*3505* allele was observed for 17.5% of Thai patients with nevirapine-induced skin rash compared with only 1.1% of nevirapine-tolerant Thai patients (OR =18.96; 95% CI: 4.87–73.44, $Pc = 4.6 \times 10^{-6}$) and 0.7% of the general Thai population (OR = 29.87; 95% CI = 5.04–175.86, $Pc = 2.6 \times 10^{-5}$) (60). Thus, *HLA-B*3505* allele is a strong predictor for nevirapine-induced skin adverse reactions in HIV-infected Thai patients. A genome-wide association study identified variations in *CCHCR1* associated with *HLA-B*3505* (61), which could be used as surrogate predictors for screening patients prior to the initiation of nevirapine treatment.

SJS/TEN with Ophthalmic Sequelae

Mucocutaneous damage caused by SJS/TEN often inflicts severe, lifelong visual dysfunction. The carrier frequency of *HLA-A*0206* in Japanese patients with visual dysfunction after SJS/TEN (30/71, 42.3%) was significantly higher than in Japanese healthy volunteers (17/113, 15.0%) (62,63). In addition, the Toll-like receptor 3 gene (TLR3) polymorphisms, rs293248 and rs299698, have been suggested to be associated with ocular surface complications in Japanese SJS/TEN patients (64).

Genome-Wide Association Studies

Currently high-throughput technologies such as genome-wide SNP analysis are available for exploring biomarkers associated with severe adverse reactions; for example, biomarkers related to drug-induced liver injury caused by flucloxacillin (47) and myopathy caused by simvastatin (65) have been successfully detected by genome-wide association studies. For severe cutaneous adverse reactions, SNPs strongly linked with known biomarkers such as *HLA-B*5801* (39), *HLA-A*3101* (26,28) and *HLA-B*3505* (61) have been additionally found by GWAS of allopurinol-, carbamazepine- and nevirapine-induced severe cutaneous adverse reactions, respectively, as mentioned earlier. However, two recently performed genome-wide association studies on skin rash with large sample sizes and multiple causative drugs that include 424 European cases and 1881 control subjects (66), and 96 cases and more than 4000 control subjects (67), failed to detect new biomarkers, although SNPs associated with *HLA-B*5801* were detected by the former study performed by the RegiScar group. This result was presumably due to the involvement of a considerable number of patients with allopurinol-induced skin rash. As we have mentioned, biomarkers for severe cutaneous adverse reactions are usually causative drug-specific and have generally been identified by case–control studies on each causative drug with the exception of studies on patients with complicating visual dysfunction (62–64). Therefore, if a genome-wide association study with a large sample size is applied to a particular causative drug-induced cutaneous adverse reaction, new genomic biomarkers other than *HLA*-related genes might be found.

OTHER BIOMARKERS

Several biomarkers related to the pathophysiology of SJS/TEN have been reported. Epidermal skin detachment is a characteristic of SJS/TEN, and several cytotoxic proteins released from immune cells are known to be involved in epidermal keratinocyte apoptosis/necrolysis including Fas–Fas ligand (FasL) interaction and soluble cytotoxic factors such as granulysin (68).

The soluble form of FasL (sFasL) was reported to be released from peripheral blood mononuclear cells (PBMCs) by stimulation of causal drugs and to induce keratinocyte apoptosis in SJS/TEN patients (69). Furthermore, in a pilot study using four previously identified SJS/TEN patients induced by carbamazepine, their PBMC secreted approximately twice the amount of sFasL as in two healthy, age-matched control subjects, and sFasL release increased in a culprit drug concentration-dependent manner (70). Thus, sFasL was thought to be a potential biomarker for SJS/TEN. Indeed, two to four days before the onset of SJS/TEN, serum sFasL levels increased to more than 100 pg/mL in five out of seven patients, where the disease onset was defined as erosion/ulceration of mucocutaneous regions or first development of ocular lesion (71). These increases rapidly diminished within five days of disease onset, and were not observed throughout the same time period in 33 patients with ordinary types of drug-induced skin reactions as well as in 32 healthy control subjects. Thus, serum

TABLE 55.4

Performance Characteristics of Screening for *HLA-B*5701* in the Control Group

	HLA-B*5701			Performance Characteristics[a]
	Positive	Negative	Total	
Clinical diagnosis				
HSS	30	36	66	Sensitivity 45.5%
No reaction	19	762	781	Specificity 97.6%
				PPV 61.2%, NPV 96.5%
Immunologically diagnosis				
HSS	23	0	23	Sensitivity 100%
No reaction	25	794	819	Specificity 96.9%
				PPV 47.9%, NPV 100%

Abbreviations: HSS, hypersensitivity syndrome; NPV, negative predictive value; PPV, positive predictive value.

sFasL levels could be a predictive biomarker for the onset of SJS/TEN. However, serum levels of sFasL were too low to use in a rapid diagnostic device.

Blister cells from skin lesions of SJS/TEN predominantly consist of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells (72). These blister fluids were cytotoxic against keratinocytes and were found to contain cytotoxic proteins including granulysin, which were released from CTLs and NK cells. The cationic protein granulysin was detected at ~7 μg/mL in the blister fluids, which was two to four orders of magnitude higher than other cytotoxic proteins, perforin (~30 ng/mL), granzyme B (~5 ng/mL), and sFasL (~0.4 ng/mL) in the blister fluids from the afflicted skin regions of the SJS/TEN patients. Furthermore, the increase in granulysin levels was specific to SJS/TEN and depending on the severity (the levels were TEN > overlapping SJS-TEN>SJS), and not found in similar skin lesions from burn injury or bullous pemphigoid. Thus, the skin detachments in SJS/TEN could be attributed primarily to granulysin. Granulysin is also detected in the sera of SJS/TEN patients on the order of ng/mL from two to four days before onset of the disease (day 1 was defined the same as the above sFasL case) (73). If the cut-off value was set at 10 ng/mL (100-fold higher than sFasL level), four out of the five SJS/TEN patients were positive, but only one of 24 ordinary types of drug-induced skin reaction patients and none of the 31 healthy control subjects were positive. Serum granulysin levels decreased rapidly within five days after disease onset. Although this study used a small sample size and the results need to be confirmed, granulysin appears to be a promising biomarker for predicting the onset of SJS/TEN.

The levels of other biomarkers including serum lactate dehydrogenase and serum creatine kinase were also increased in SJS/TEN patients, but they are non-specific for the disease and only increase after the disease onset.

Not a biomarker, but the prognostic score for fatality is SCORTEN, a severity of illness score for TEN, which is now also applying to SJS (74). The SCORTEN evaluates seven independent factors within 24 h of admission: age ≥40 years old, malignancy, initial percentage of epidermal detachment ≥10%, tachycardia ≥120/min, serum urea >10 mM, serum glucose >14 mM, and serum bicarbonate <20 mM. These seven factors are allotted equal weighting in the score, and thus SCOTEN ranges from 0 (no factor present) to 7 (all factors present). Discriminating between death and recovery was shown to be excellent by several studies with a receiver operating characteristic (ROC) of approximately 80% (74–77). Recently, some alterations of the calculation methods have been introduced. The estimation three days after admission was more accurately predictive than the original first day calculation (78). In addition, an auxiliary method using only age, malignancy, and percentage of epidermal detachment ≥30% was proposed (76). This score does not depend on vital or laboratory parameters, and thus can easily apply to patients, but is poorer in discrimination by ROC curve analysis than the SCORTEN.

USEFULNESS OF GENOMIC BIOMARKERS FOR PREVENTION OF SEVERE CUTANEOUS ADVERSE REACTIONS AND CLINICAL IMPLEMENTATION

Biomarkers for severe cutaneous adverse reactions have largely been found through retrospective studies. Prospective randomized clinical studies could more efficiently evaluate the utility of screening for biomarkers to reduce drug-induced severe cutaneous reactions.

For this purpose, a prospective, randomized, multicenter, double-blind study called PREDICT-I was conducted to evaluate screening for *HLA-B*5701*, in which 1956 abacavir-naïve HIV-infected patients in Europe and Australia participated (79,80). In this study, patients were randomly divided into two groups; in one group, prospective screening for *HLA*5701* was applied to exclude *HLA-B*5701*-positive patients from abacavir treatment, while in the second group (control group), abacavir was given to all patients. The prevalence of *HLA-B*5701* in this study was 5.6%. The incidence of HSS in the group receiving prospective screening was significantly lower (3.4%) than in the control group (7.8%). Moreover, the screening completely eliminated HSS, immunologically confirmed with the epicutaneous patch test using abacavir solution in the prospective screening group (incidence 0%), while the incidence of immunologically confirmed HSS was 2.7% in the control group. The performance characteristics of *HLA-B*5701* screening in the control group were shown in Table 55.4. These results suggest that screening for *HLA-B*5701* prior to initiation of abacavir treatment can reduce the risk of hypersensitive reaction. Another large scale study performed using a racially diverse North American population (n = 725) also showed that prospective *HLA-B*5701* screening could reduce the risk of immunologically confirmed HSS to less than 1% among *HLA-B*5701*-negative individuals (81). A study conducted in France also indicated that prospective screening for *HLA-B*5701* reduced the incidence of suspected hypersensitivity from 22.5% to less than 1% and definite hypersensitivity from 12% to 0% (82). Moreover the rate of unwarranted interruption of abacavir therapy could be decreased from 10.2% to 0.73% possibly due to lowering the rate of false-positive diagnosis of hypersensitivity (82). Thus, all of these studies indicated the usefulness of prospective genetic-screening in lowering the incidence of abacavir-induced hypersensitivity. Currently, prospective screening for *HLA-B*5701* is required in Europe and the United States before the initiation of treatment with abacavir.

In Taiwan, a warning for *HLA-B*1502* as a risk factor for carbamazepine-induced SJS/TEN was introduced for the first time to the package inserts of carbamazepine products in December 2007, followed shortly by an announcement by the FDA. From 2002 to 2004, carbamazepine was newly prescribed to around 50,000 patients

each year, and the average incidence of carbamazepine-induced SJS/TEN during this period was estimated to be 0.22%, which corresponded to around 115 patients a year (83). A prospective study was performed in Taiwan in which 4877 subjects from 23 hospitals were recruited, and carbamazepine was administered to only *HLA-B*1502* negative patients (83). None of the 4120 patients who took carbamazepine and were followed for two months after the initiation of carbamazepine therapy developed SJS/TEN within two months. According to the above-mentioned historical data of the disease incidence, 10 patients would have been expected to develop SJS/TEN in this prospective study, and this difference was statistically significant ($p < 0.001$).

Prospective screening for *HLA-B*1502* and *HLA-B*5801* are now required in Taiwan before the initiation of therapy with carbamazepine and allopurinol, respectively. FDA has also approved a revision of product labels containing carbamazepine to clearly state that patients at genetically high risk should be screened for the *HLA-B*1502* allele before starting carbamazepine treatment.

CONCLUSIONS

Genomic biomarkers associated with the risk of severe cutaneous adverse reactions caused by several particular drugs have been accumulating, and some of these biomarkers are currently used in prospective screening before commencing drug therapy. However, some of the biomarkers are ethnic group-specific, and the strength of the association between biomarkers and adverse reactions is dependent on ethnic group. Even after excluding patients at high risk from treatment with a causative drug, clinical vigilance is still necessary for patients without relevant biomarkers during drug therapy. Biomarkers other than genomic risk factors could be useful for discriminating severe cutaneous adverse reactions from mild or moderate reactions, and they may be useful for determining the therapeutic management of patients.

Further *in vivo* and *in vitro* investigations are necessary to explore biomarkers for various drugs for which risk factors are unknown and to clarify the pathogenesis of severe cutaneous adverse reactions. In addition, the establishment of screening methods for use in the drug development process to identify drug candidates with a high risk for severe cutaneous reactions is also required.

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Note

Development of a Rapid and Inexpensive Assay for Detecting a Surrogate Genetic Polymorphism of *HLA-B*58:01*: A Partially Predictive but Useful Biomarker for Allopurinol-related Stevens-Johnson Syndrome/toxic Epidermal Necrolysis in Japanese

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Summary: Allopurinol-induced Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) is strongly associated with *HLA-B*58:01* in various populations including Japanese. We demonstrated that several single nucleotide polymorphisms (SNPs) around the *HLA* region on chromosome 6 were strongly linked with *HLA-B*58:01* in a previous study using Japanese allopurinol-related SJS/TEN patients. Their very strong linkage suggests that these SNPs could be used as surrogate biomarkers to find carriers of *HLA-B*58:01* to avoid these serious adverse effects. In the present study, to expedite the application of this pharmacogenomic information to the proper usage of allopurinol in a clinical situation, we developed a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay for the genotyping of rs9263726 in the *psoriasis susceptibility 1 candidate 1* (*PSORS1C1*) gene, which is in absolute linkage disequilibrium ($r^2 = 1$, $D' = 1$) with *HLA-B*58:01*. The developed PCR-RFLP assay using FokI restriction enzyme was able to detect three different genotypes, GG, GA, and AA of rs9263726 robustly, and thus to find *HLA-B*58:01* carriers. This robust and inexpensive assay would be useful for pre-screening the subjects with *HLA-B*58:01*, a genetically high risk factor for allopurinol-induced SJS/TEN.

Keywords: allopurinol; PCR-RFLP; screening test; Stevens-Johnson syndrome; toxic epidermal necrolysis

Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse reactions (SCARs).[1)] SJS and TEN, considered variants of the same skin disorder, are characterized by the development of limited (in SJS) or widespread (in TEN) detachment and blistering of the skin epidermis and mucous epithelium, often with organ involvement.[1,2)] The incidence of SJS/TEN is very rare, estimated to occur at about 2 patients per million individuals per year in Caucasians,[3)] but these SCARs require intensive care due to the high mortality rates (1–5% for SJS and 20–30% for TEN) and long-term treatments for subsequent complications, especially ocular pathologies.[1)] SJS/TEN are idiosyncratic SCARs that have been considered, for a long time, to be difficult to predict, but human lymphocyte antigen (HLA) types have recently been reported to be associated with the onset of SJS/TEN in a drug-specific manner.[1,4)]

Allopurinol is a widely-prescribed urate-lowering drug and has known to be the most common causative drug for SJS/TEN in Japan.[4,5)] In 2005, Hung *et al.* reported that an *HLA* allele *B* variant, *HLA-B*58:01*, is strongly associated with allopurinol-induced SCARs consisting of SJS, TEN and

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hypersensitivity syndrome in a Han Chinese population.[6] They found that 100% (51/51 patients) of the case patients had this *HLA* type, while only 15% (20/135 patients) of the tolerant control had, giving an odds ratio (OR) of 580.3 (sensitivity = 100%, specificity = 85%). This association was later confirmed in Thai (SJS/TEN patients, OR = 348.3, sensitivity = 100%, specificity = 87%),[7] Korean (SJS/TEN/drug-induced hypersensitivity syndrome patients, OR = 97.8, sensitivity = 92%, specificity = 89%),[8] European (SJS/TEN patients, OR = 80, sensitivity = 56%)[9] and Japanese (SJS/TEN patients, OR = 62.8, sensitivity = 56%)[10,11] populations. Although the associations have been partial, especially in Europeans and Japanese, *HLA-B*58:01* is thought to be a useful biomarker for allopurinol-induced SJS/TEN.

A recent report showed that based on the very strong association of the *HLA-B*15:02* allele with SJS/TEN in the Han Chinese population (sensitivity = 98%, specificity = 96%),[12] prospective testing for *HLA-B*15:02* and subsequent avoidance of carbamazepine therapy resulted in zero occurrence of SJS/TEN in Taiwan.[13] Based on this result and the severity of these adverse reactions, a pre-screening test is now mandatory and covered by the National Health Insurance in Taiwan, although its positive predictive value could be estimated at around 3% using the values of their study. Thus, examining *HLA-B*58:01* prior to allopurinol administration may be also valuable to avoid allopurinol-induced SJS/TEN. However, testing *HLA* types is relatively laborious, time-consuming and expensive. Very recently, we found that several single nucleotide polymorphisms (SNPs) around the *HLA* region on chromosome 6 were strongly linked with *HLA-B*58:01* in a group of SJS/TEN patients.[11] In general, a single SNP can be easily genotyped and inexpensively compared to the *HLA* type. Thus, the linked SNPs could be used as alternatives to testing for *HLA-B*58:01* when deciding on the application of drug therapies involving allopurinol. To expedite the application of this pharmacogenomic information for the proper usage of allopurinol in clinical settings, we developed a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method that can genotype SNPs easily without high skills and inexpensively.

Materials and Methods

Patients: Japanese SJS/TEN patients from unrelated families were recruited from July 2006 through April 2010 at participating institutes of the Japan Severe Adverse Reactions (JSAR) research group.[11] In addition, SJS/TEN patients were recruited through a nationwide blood-sampling network system in Japan for severe drug adverse reactions operated by the National Institute of Health Sciences under the auspices of the Ministry of Wealth, Labour and Welfare and the Federation of Pharmaceutical Manufacturers' Associations of Japan. Genomic DNA was extracted from blood leukocytes as described previously.[10] DNA samples extracted from the cord blood of healthy Chinese-Americans were purchased from AllCells (Emeryville, CA, USA). The ethics committees of the National Institute of Health Sciences and each participating institute of the JSAR research group approved this study. Written informed consent was obtained from all cases and healthy Chinese-American subjects.

Genotyping of single nucleotide polymorphism by TaqMan assay and *HLA* types: *HLA-B* types were determined by the sequencing-based method as reported in a previous paper.[11] Of the several SNPs linked with *HLA-B*58:01*, we selected rs9263726 (110G>A, Arg37His) in *psoriasis susceptibility 1 candidate 1* (*PSORS1C1*) as a surrogate marker for *HLA-B*58:01*, because this SNP was in absolute linkage disequilibrium ($r^2 = 1$, $D' = 1$) with *HLA-B*58:01* and associated with SJS/TEN with an odds ratio of 61.2 ($p = 3.64 \times 10^{-8}$) in the dominant genotype mode.[11] This variation was located *ca.* 215 kb away from the *HLA-B* gene, detected at a minor allele frequency of 0.006 (12/1982 alleles), which was the same as that of the reported Japanese frequency of *HLA-B*58:01* (0.006),[14] and in Hardy-Weinberg equilibrium ($p = 0.847$).[11] In allopurinol-related SJS/TEN patients, the minor allele frequency of *HLA-B*58:01* and rs9263726 was 0.278.[11] rs9263726 was genotyped using TaqMan SNP Genotyping Assays (C_30352071_10, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions using 5 ng of genomic DNA from Japanese SJS/TEN patients or healthy Chinese-Americans. Hardy-Weinberg equilibrium was analyzed by Fisher's exact test using SNPAlyze ver. 3.1 software (Dynacom, Chiba, Japan).

Genotyping of rs9263726 by PCR-RFLP: PCR primers (forward: 5′-AAGCTCCATCCACCCCTGGT-3′ and reverse: 5′-ACACATTGGGTGGGGGACAT-3′) were designed to amplify a *PSORS1C1* genomic fragment containing the rs9263726 SNP locus. PCR was performed using *Ex*-Taq (0.625 units) (Takara Bio Inc., Shiga, Japan) with a pair of primers (0.2 μM) and genomic DNA (50 ng). The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 7 min. Aliquots of PCR products (5 μl) were then digested by the addition of 0.4 units of FokI restriction endonuclease (New England Biolabs, Beverly, MA, U.S.A.) in the presence of 1 × Buffer 4 (New England Biolabs) at 37°C for 2 h. Restriction mixtures were incubated at 65°C for 20 min to inactivate FokI, and then electrophoresed through a 15–25% gradient acrylamide gel (MULTIGEL II Mini, Cosmo Bio Co., Ltd., Tokyo, Japan). Following electrophoresis, the gels were stained with ethidium bromide, and DNA was visualized by placing the gel on a UV transilluminator.

Results and Discussion

First, we compared the results of genotyping rs9263726 in *PSORS1C1* with the PCR-RFLP and TaqMan SNP Geno-

typing assays (C_30352071_10). DNA from Chinese-Americans was used since the frequency of *HLA-B*58:01* in this population is reportedly higher than in the Japanese population.[6,10] Preliminary, experiments using the TaqMan assay showed that the 200 DNA samples from Chinese-Americans contained 161 homozygotes of the major allele (GG), 36 heterozygotes (GA), and 3 homozygotes of the minor allele (AA) of rs9263726 (data not shown), which distribution was in Hardy-Weinberg equilibrium ($p = 0.550$). In addition, we confirmed that the 3 subjects with homozygous AA surely had homozygous *HLA-B*58:01* (data not shown). From the DNA from Chinese-Americans genotyped by TaqMan assay, 5 samples with GG, 4 with GA, and 2 with AA of rs9263726 were selected to establish the PCR-RFLP method. In the developed assay, the 260 bp PCR products derived from the A allele of rs9263726 were digested with Fok I produced two bands (141 bp and 119 bp), while those derived from the G allele remained as the parent single band (260 bp) (**Supplementary Fig. 1A**). Genotypes of these samples by PCR-RFLP assay were 100% in concordance with those from the TaqMan SNP assay, indicating that this is a robust method of genotyping rs9263726.

Next, in order to validate this PCR-RFLP assay, the rs9263726 locus was genotyped for the DNA samples with or without *HLA-B*58:01* of 27 Japanese SJS/TEN patients for whom *HLA-B* types had been previously determined.[10,11] The following SJS/TEN samples were selected: 5 *HLA-B*58:01* heterozygous carriers and 22 other *HLA-B* allele carriers. The other *HLA* types were selected based on an allele frequency ≥ 0.01 in Japanese control populations,[14,15] although a *HLA-B*44:02* sample (allele frequency = 0.01) was not available. As shown in **Table 1** and **Supplementary Fig. 1B**, the 5 patients with heterozygous *HLA-B*58:01* were also heterozygotes for rs9263726 (GA), and the remaining 22 patients with the other *HLA-B* types were major homozygotes for this SNP (GG). Thus, our developed PCR-RFLP assay can robustly predict the *HLA-B*58:01* status of SJS/TEN patients.

Very recently, Kostenko *et al.* generated a monoclonal antibody to recombinant HLA-B*57:01 protein and developed a flow cytometric assay for the detection of HLA-B57-positive peripheral blood mononuclear cells.[16] This antibody can cross-react with HLA-B58 proteins and thus could be used to pre-screen for *HLA-B*58:01* carriers. However, this assay method cannot discriminate *HLA-B*57:01* from *B*58:01* and uses blood cells, making it laborious and expensive (*i.e.*, a flow cytometer is necessary). In contrast, our PCR-RFLP method does not require a high skill set, and can be done at a low cost without use of specific machines, although a DNA extraction step is necessary.

Although the testing of rs9263726 or *HLA-B*58:01* cannot perfectly predict allopurinol-induced SJS/TEN, it may be better for the *HLA-B*58:01*-positive patients to avoid the administration of allopurinol, as do the *HLA-B*15:02*-positive patients for carbamazepine in Taiwan. Because allopuirol is a xantine oxidase inhibitor, febuxostat, having the same pharmacological effect through a different structure, might be an alternative drug for the *HLA-B*58:01*-positive patients, although further studies are clearly necessary to prove that SJS/TEN induced by febuxostat is surely not to be associated with *HLA-B*58:01*.

Table 1. *HLA* and rs9263726 genotypes in Japanese SJS/TEN patients

ID	*HLA-B**		rs9263726
1	**58:01**	46:01	**G/A**
2	**58:01**	51:01	**G/A**
3	**58:01**	51:01	**G/A**
4	**58:01**	38:02	**G/A**
5	**58:01**	07:02	**G/A**
6	07:02	51:01	G/G
7	13:01	35:01	G/G
8	15:01	40:02	G/G
9	15:11	40:02	G/G
10	15:18	38:02	G/G
11	35:01	40:02	G/G
12	37:01	40:06	G/G
13	39:01	51:01	G/G
14	40:01	40:01	G/G
15	48:01	51:02	G/G
16	40:02	40:06	G/G
17	40:06	52:01	G/G
18	44:03	44:03	G/G
19	46:01	35:01	G/G
20	51:01	35:01	G/G
21	52:01	52:01	G/G
22	54:01	54:01	G/G
23	55:02	51:01	G/G
24	56:01	46:01	G/G
25	59:01	35:01	G/G
26	67:01	39:01	G/G
27	40:03	54:01	G/G

In conclusion, we have developed a robust PCR-RFLP genotyping assay for rs9263726 in *PSORS1C1*, which is in absolute linkage disequilibrium with *HLA-B*58:01*, a partially predictive but useful biomarker for allopurinol-related SJS/TEN in Japanese. The genotyping of rs9263726 by this easy and inexpensive method makes it useful for the prospective screening of patients with *HLA-B*58:01* in the future.

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日本人を対象にしたゲノム・メタボローム解析によるバイオマーカー探索

Biomarker exploration by genomic and metabolomic analyses in Japanese

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1 はじめに

バイオマーカーは医薬品の開発効率の改善や，安全性の向上等に役立つことが期待されている。このため米国では，バイオマーカーを利用した臨床試験が，この10年間で実に約30倍に増加している。さらに，本邦でも市販後に添付文書の改訂が行われ，バイオマーカーに関する記載が追加される例が増加しており，特にゲノムバイオマーカーに関しては，順調に増えている[1]。

内閣府・総合科学技術会議の平成24年度科学技術重要施策アクションプランには，「科学的根拠に基づいたバイオマーカーを開発，利用することで，客観的，確度の高い診断と予測，治療の実現を目指すことが可能となる。そのため，「先制医療（早期医療介入）の実現による発症率の低下」を課題として選択した」と記載されている。バイオマーカーを早期の臨床指標として用いる取組，また医薬品の安全対策に用いる取組は，今後も加速すると考えられている。

本稿では，日本人を対象にした医薬品の薬効・副作用を予測するためのゲノム・メタボローム解析によるバイオマーカー探索に関し，筆者らの成果を中心に述べる。

2 薬効・副作用に関するゲノムマーカー探索

2.1 薬効

(1) ワルファリン

経口抗凝固剤ワルファリンは，安定投薬量に至るまでに時間がかかること，また投薬量に個人差が大きいことが知られている。ワルファリンは薬物代謝酵素CYP2C9で主として代謝されるが，本酵素には活性低下をもたらす遺伝子多型*CYP2C9*3*（I359L，日本人での染色体別頻度：約3%）が知られている。また標的酵素であるビタミンKエポキシド還元酵素複合体（サブユニット1，*VKORC1*）にも発現量の低下と関連する多型（−1639G＞A，頻度：約90%）が知られている。

国際ワルファリン薬理遺伝学コンソーシアム（IWPC）は，上記の遺伝子多型情報に加えて，患者背景情報（年齢，身長，体重，人種，併用薬等）を考慮した投薬量計算式を発表した[2]。日本人についても従来の投薬量算出法より，IWPCの方法が適切に投薬量を予測できることが報告されている[3]。

(2) タモキシフェン

抗乳癌剤タモキシフェンは，エストロゲン受容体拮抗作用により薬効を示すとされる。原薬のCYP2D6およびCYP3A4による代謝で生成する4-ヒドロキシ体やエ

ンドキシフェンは，原薬に比して強い薬効を有する。

CYP2D6では，日本人で頻度の高い（頻度：約38%）酵素活性低下をもたらす遺伝子多型 *10*（P34S，他）および酵素活性の消失をもたらす *5*（頻度：約6%）が主として知られている。多型群を有する日本人患者では，無再発生存期間が有意に短いことが報告されている[4]。

2.2 副作用

(1) 好中球減少症

抗がん剤イリノテカンは，多くの消化器癌等に，また抗がん剤ゲムシタビンは膵臓癌等に用いられる。これらの抗がん剤では，副作用として骨髄抑制が知られているが，その発症に遺伝子多型の関与が報告されている。イリノテカンに関しては，活性代謝物SN-38を解毒代謝するグルクロン酸転移酵素UGT1A1の活性低下型遺伝子多型である *6*（G71R，頻度：約16%）と *28*（−54_−39A(TA)$_6$TAA ＞ A(TA)$_7$TAA，頻度：約11%）が，重篤な好中球減少症の発現に関連しており，添付文書での注意喚起に加えて，多型診断用の対外診断薬も販売されている[5]。またゲムシタビンに関しては，解毒代謝酵素であるシチジンデアミナーゼ（CDA）の活性低下型遺伝子多型である *3*（A70T，頻度：約4%）が，重篤な好中球減少症の発現に関連している[6]。

(2) 重症薬疹

重症薬疹は医薬品による健康被害救済制度において常に上位を占めている。中でも重篤なのが，スティーブンス・ジョンソン症候群（SJS）及び中毒性表皮壊死症（TEN）である。SJSとTENは，多くの医薬品が発症原因となり，皮膚・粘膜部の発疹・びらん，発熱等を主症状とし，表皮の水疱・剥離面積等によりSJSとTENに分類される。致死率も比較的高く，重い後遺症が残ることがある。近年，その発症とヒト白血球抗原（HLA）遺伝子の特定のタイプとの間の強い関連が明らかとなっている。

抗てんかん薬カルバマゼピン誘因性SJS/TEN発症に関し，漢民族・タイ人等で，*HLA-B*1502* との非常に強い関連が報告されているが，白人や日本人では認められない。一方で，韓国人と日本人では，*HLA-B*1502* と同じ血清型B75に属する *HLA-B*1511*（日本人での頻度：約1%）との関連が報告されている[7]。さらに最近，白人，日本人において，*HLA-A*3101*（日本人での頻度：約9%）との相関が認められている[8]。本邦では，*HLA-B*1502* および *HLA-A*3101* に関する注意喚起が添付文書でなされている。

また，高尿酸血症薬アロプリノールによるSJS/TEN等の発症と *HLA-B*5801*（日本人での頻度：約1%）との関連が，台湾の漢民族でまず報告され，日本人を含めた諸民族（韓国人，白人等）でも，関連が報告されている[9]。日本では添付文書における注意喚起がなされている。

(3) 薬物性肝障害

薬物性肝障害は中毒性と特異体質性に大別され，さらに特異体質性はアレルギー性と代謝性に分類される。

抗血小板薬チクロピジンは，主に胆汁うっ滞型肝障害を誘因することが知られている。日本人を対象にした遺伝子解析の結果，*HLA-A*3303* との間に強い関連が認められた[10]。その発症頻度は白人よりも日本人において高いことが知られているが，日本人母集団における *HLA-A*3303* の染色体別頻度（約7%）が，白人（約0.7%）より高いことが一因と考えられる。

トログリタゾンは，肝障害により市場撤退した経口糖尿病薬である。日本人につき遺伝子解析が行われた結果，グルタチオン *S*-転移酵素である *GSTM1* および *GSTT1* 両遺伝子の欠損との関連が示された[11]。日本人における *GSTM1/T1* 両欠損型の頻度は約25%である。

3 薬効・副作用に関するメタボロームマーカーの探索

メタボロームは，アミノ酸，糖，脂質等の内在性代謝物を網羅的に測定する手法である。日本人を対象にした薬効に関するマーカーの報告例はないが，副作用に関しては散見される。薬物性肝障害に関して，電荷を有する内在性代謝物の解析を行った結果，ALT＋γ-グルタミルシトルリンがマーカーとなりうることが報告されている[12]。筆者らは，6カ所のナショナルセンターおよび慶応大学と，腎がん，肥満症，大動脈瘤，脊柱管狭窄症

等の13疾患に関し，日本人患者を対象としたメタボローム解析を行っており，その成果である疾患バイオマーカーは薬効の指標となりうると考えている。また別途，バイオマーカー探索・検証用試料の品質要件に関する研究を開始した。

4 おわりに

最近では，予測が困難であった重篤副作用に関しても，次々とバイオマーカーが発見されている。しかし現段階での報告の多くは，市販後に見出されたものである。有用なバイオマーカーが，本邦にて創薬段階で見出され，市販後にも継続して適正使用に用いられることを祈念したい。

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Epistatic Interactions Associated with Stevens–Johnson Syndrome

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Abstract: Stevens–Johnson syndrome (SJS) is an acute inflammatory vesiculobullous reaction of the skin and mucosa, often including the ocular surface, and sometimes progresses to toxic epidermal necrolysis (TEN). SJS/TEN may be initiated by certain types of medication coupled with possible infection; however, few susceptibility genes have been identified as indicators for risk prediction, except for certain human leukocyte antigen alleles, although several candidate susceptibility genes were identified by candidate gene and genome-wide approaches. As an alternative genetic model, gene–gene interactions between candidate genes were investigated using high-dimensional variable selection methods such as iterative sure independence screening. Linkage disequilibria around the toll-like receptor (*TLR*)*3* and prostaglandin E receptor (*PTGER*)*3* genes were also investigated using additional single nucleotide polymorphisms in an extended additional sample set. A murine experimental allergic conjunctivitis (EAC) model was used to examine the functional interactions. Iterative sure independence screening analyses of Japanese SJS/TEN patients with ocular surface complications revealed 2 interactions that exerted SJS/TEN susceptibility effects, which were locus pairs of *TLR3–PTGER3* and *HLA-A–IL1α*. Furthermore, functional interactions between TLR3 and EP3 (the protein of *PTGER3*) were demonstrated using the EAC model. Identification of functional interactions between TLR3 and EP3 supports an epistatic interaction conferring an increased risk for SJS with ocular complications.

Key Words: prostaglandin E receptor 3, Stevens–Johnson syndrome, susceptibility genes, toll-like receptor 3, toxic epidermal necrolysis

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Stevens–Johnson syndrome (SJS), an acute inflammatory vesiculobullous reaction of the skin and mucous membranes, was first described in 1922.[1] Stevens and Johnson, both pediatricians, encountered 2 boys aged 7 and 8 years who manifested an extraordinary generalized skin eruption, inflamed buccal mucosa, persistent fever, and severe purulent conjunctivitis resulting in visual disturbance.[1] Subsequently, some pediatricians have reported that SJS is associated with infectious agents such as *Mycoplasma pneumoniae*[2] and may have a viral etiology involving herpes simplex virus, varicella zoster virus, cytomegalovirus, and Epstein–Barr virus.[3] However, dermatologists have claimed that more than 100 different drugs are implicated in eliciting SJS and its severe form, toxic epidermal necrolysis (TEN).[4] There have been a number of reports of life-threatening severe adverse drug reactions characterized by rapidly developing blistering exanthema macules, high fever, and target-like lesions accompanied by mucosal involvement and skin detachment.[4,5]

The annual incidence of SJS and TEN is estimated to be 0.4–1 and 1–6 cases per million persons, respectively,[6,7] with respective mortality rates of 3% and 27%.[8] Although rare, these reactions have high mortality and morbidity rates and often result in definitive and severe sequelae such as vision loss.[9] The reported incidence of ocular complications in SJS/TEN is 50% to 68%.[7,8] During the acute stage, patients with severe ocular surface complications manifest vesiculobullous lesions of the mucosa (especially the eyes and mouth) and skin, persistent corneal epithelial defects as a result of ocular surface inflammation, and severe conjunctivitis. Oral involvement, including erosions, blisters, and bleeding of the mouth and lips, has been observed in all SJS/TEN patients with severe ocular surface complications. Furthermore, almost all such patients lose their fingernails in the acute or subacute stage because of paronychia.[10–12] During the chronic stage, severe ocular surface complications including conjunctival invasion into the cornea, dry eyes, symblepharon, and in some instances, keratinization of the ocular surface, persist, despite healing of the skin lesions.[10] Ophthalmologists see SJS/TEN patients in the acute and chronic stages, and because vesiculobullous skin lesions present during the acute stage may have healed in the chronic stage, a differential diagnosis of SJS or TEN may be difficult. Thus, ophthalmologists usually diagnose both SJS and TEN as SJS in a broad sense.

It was previously reported that the HLA-Bw44 antigen, a subgroup of HLA-B12, was significantly increased in 15 Caucasian SJS patients with ocular involvement relative to a control Caucasian population of 411 individuals[13] and that the onset of SJS with ocular involvement was associated with putative viral syndromes or administration of drugs.[13] Another report indicated that 45 French SJS/TEN patients, whose disorder was clearly drug induced, showed a significantly increased

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frequency of the HLA-B12 antigen in comparison with a French control population comprising 66 individuals.[14] The causative agents were nonsteroidal antiinflammatory drugs (NSAIDs) in 25 of the 45 patients (56%).[14]

An association between HLA molecules and drug-induced severe cutaneous adverse reactions including SJS and TEN has also been reported. The *HLA-B*1502* allele shows a very strong association with carbamazepine-induced SJS/TEN in the Han Chinese of Taiwan.[15] In contrast, no such association between *HLA-B*1502* and carbamazepine-induced SJS/TEN has been found in Caucasian patients.[16,17] Another Taiwanese study showed that *HLA-B* 5801* was present in all Han Chinese with allopurinol-induced SJS/TEN and drug-induced hypersensitivity.[18]

Recently, dermatologists have reported that allopurinol (a uric acid–lowering drug) and anticonvulsants such as carbamazepine are frequently the main causative drugs of SJS/TEN.[19] However, among patients we examined with severe ocular surface complications, 89 of 116 patients (76.7%) presented with SJS after receiving antibiotics, cold remedies, and/or NSAIDs for treatment of the common cold. In addition, only 6 of 116 patients (5.2%) developed SJS after receiving drug treatment for the prevention of convulsions, and only 2 of 116 patients (1.7%) developed SJS after taking allopurinol. Therefore, we recognized that our patients constituted a subgroup among the overall population of those with SJS/TEN (Fig. 1).

We previously reported that the frequency of carriers of the *HLA-A*0206* antigen is significantly higher among Japanese patients with severe ocular surface complications than in controls.[20] We also performed single nucleotide polymorphism (SNP) association analysis of candidate genes and observed the associated polymorphisms of several immune-related genes including toll-like receptor (*TLR*)*3*,[12] interleukin (*IL*)*4R*,[21,22] *FasL*[23], and *IL13*[22] in Japanese SJS/TEN patients with severe ocular surface complications. Furthermore, we performed a genome-wide association study of SJS/TEN patients to elucidate the detailed pathophysiology of SJS/TEN and found associations between 6 SNPs in the prostaglandin E receptor 3 (EP3) gene (*PTGER3*) and SJS/TEN accompanied by severe ocular surface complications.[24] We suggested that these *PTGER3* polymorphisms might be associated with NSAID-related susceptibility to SJS/TEN because 76 of 100 (76%) SJS/TEN patients were found to have used cold medications, possibly including NSAIDs, before the onset of disease.[24] Drugs are probably the most widely accepted etiologic factor for SJS/TEN.[5,25] Many patients who develop SJS/TEN with severe ocular surface complications do so after taking remedies for the common cold or NSAIDs, which inhibit the production of the EP3 ligand prostaglandin E_2 (PGE_2), lending support to the idea that EP3 is involved in the development of SJS/TEN.[24]

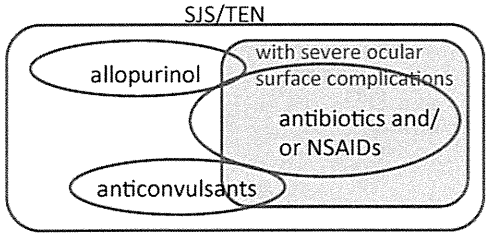

FIGURE 1. SJS/TEN patients with severe ocular surface complications. SJS/TEN patients with severe ocular surface complications constituted a subgroup among the overall population of those with SJS/TEN.

For the past decade, SNPs have been widely used as genetic markers for identifying human disease susceptibility genes. However, it has become apparent that gene–gene interactions should also be considered in addition to major single-locus effects.[26] In particular, some nonadditive (epistatic) models for some complex diseases fit naturally to actual observations, suggesting interactions involving multiple loci.[27]

Therefore, to reveal other genetic components of SJS/TEN associated with severe ocular surface complications, a statistical search for interactions between all possible pairs of loci was performed. We applied high-dimensional variable selection methods such as sure independence screening (SIS) to the comprehensive data set obtained from our previous studies, for a total of 14 immune-related genes, including *PTGER3, TLR3*, and *HLA-A* (Table 1).[28]

STATISTICALLY SUSCEPTIBLE INTERACTIONS

After filtering with the standard SNP quality control filter, 36 SNPs were used for SIS to scan a total of 5778 ($3 \times 36 + 9 \times 36 \times (36-1)/2$) dummy variables.[28] Iterative SIS analyses identified 2 variables with susceptible effects on SJS, which were involved in the locus pairs of *PTGER3–TLR3*, and *HLA-A–IL1α* (Table 2).[28]

ADDITIONAL ANALYSIS OF *PTGER3* AND *TLR3* SNPs AND LINKAGE DISEQUILIBRIA AROUND *TLR3* AND *PTGER3* LOCI

Next, we focused on the epistatic interactions between *PTGER3* and *TLR3* and analyzed an additional 32 SNPs of *PTGER3* and 10 SNPs of *TLR3*, resulting in 38 SNPs of *PTGER3* and 17 SNPs of *TLR3* in total. Both the case and control samples were in Hardy–Weinberg equilibrium ($P > 0.01$). The results showed that 14 additional SNPs of *PTGER3* and 5 additional SNPs of *TLR3* were associated with SJS/TEN with ocular complications, besides the previously reported 6 SNPs of *PTGER3* and 2 SNPs of *TLR3*.[28]

Based on the squared correlation coefficient, R^2, we investigated linkage disequilibria around the *TLR3* and *PTGER3* loci using additional SNPs in all samples (original and additional: 116 for patients and 221 for controls). One SNP (rs3775293), for which the minor allele frequency in both cases and controls was <5%, was excluded. We found 3 solid-spine linkage disequilibrium blocks in each locus.[28] Iterative SIS reported 14 variables with nonzero regression coefficients that were localized between *TLR3* and *PTGER3*, as if connecting the 5′ region of *TLR3* in block 1 and the 3′ region of *PTGER3* in block 1 (Fig. 2).[28]