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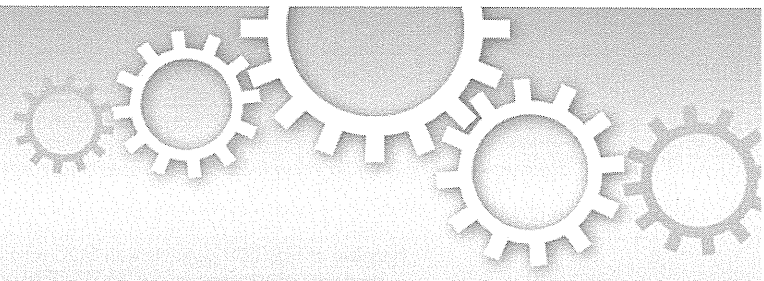

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Correspondence and requests for materials should be addressed to M.U. (mueta@koto.kpu-m.ac.jp)

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

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

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

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

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

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

Table 1 | Demographic and background data of patients and controls

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

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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

Methods

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

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

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

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

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

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

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

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

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Author contributions

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

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Original Investigation

Genetic Variants Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions

Wen-Hung Chung, MD, PhD; Wan-Chun Chang, MS; Yun-Shien Lee, PhD; Ying-Ying Wu, MS; Chih-Hsun Yang, MD; Hsin-Chun Ho, MD; Ming-Jing Chen, MD; Jing-Yi Lin, MD; Rosaline Chung-Yee Hui, MD, PhD; Ji-Chen Ho, MD; Wei-Ming Wu, MD, PhD; Ting-Jui Chen, MD; Tony Wu, MD, PhD; Yih-Ru Wu, MD, PhD; Mo-Song Hsih, MD; Po-Hsun Tu, MD; Chen-Nen Chang, MD, PhD; Chien-Ning Hsu, PhD; Tsu-Lan Wu, PhD; Siew-Eng Choon, MD; Chao-Kai Hsu, MD, PhD; Der-Yuan Chen, MD, PhD; Chin-San Liu, MD, PhD; Ching-Yuang Lin, MD, PhD; Nahoko Kaniwa, PhD; Yoshiro Saito, PhD; Yukitoshi Takahashi, MD, PhD; Ryosuke Nakamura, PhD; Hiroaki Azukizawa, MD, PhD; Yongyong Shi, PhD; Tzu-Hao Wang, MD, PhD; Shiow-Shuh Chuang, MD, PhD; Shih-Feng Tsai, MD, PhD; Chee-Jen Chang, PhD; Yu-Sun Chang, PhD; Shuen-Iu Hung, PhD; for the Taiwan Severe Cutaneous Adverse Reaction Consortium and the Japan Pharmacogenomics Data Science Consortium

IMPORTANCE The antiepileptic drug phenytoin can cause cutaneous adverse reactions, ranging from maculopapular exanthema to severe cutaneous adverse reactions, which include drug reactions with eosinophilia and systemic symptoms, Stevens-Johnson syndrome, and toxic epidermal necrolysis. The pharmacogenomic basis of phenytoin-related severe cutaneous adverse reactions remains unknown.

OBJECTIVE To investigate the genetic factors associated with phenytoin-related severe cutaneous adverse reactions.

DESIGN, SETTING, AND PARTICIPANTS Case-control study conducted in 2002-2014 among 105 cases with phenytoin-related severe cutaneous adverse reactions (n=61 Stevens-Johnson syndrome/toxic epidermal necrolysis and n=44 drug reactions with eosinophilia and systemic symptoms), 78 cases with maculopapular exanthema, 130 phenytoin-tolerant control participants, and 3655 population controls from Taiwan, Japan, and Malaysia. A genome-wide association study (GWAS), direct sequencing of the associated loci, and replication analysis were conducted using the samples from Taiwan. The initial GWAS included samples of 60 cases with phenytoin-related severe cutaneous adverse reactions and 412 population controls from Taiwan. The results were validated in (1) 30 cases with severe cutaneous adverse reactions and 130 phenytoin-tolerant controls from Taiwan, (2) 9 patients with Stevens-Johnson syndrome/toxic epidermal necrolysis and 2869 population controls from Japan, and (3) 6 cases and 374 population controls from Malaysia.

MAIN OUTCOMES AND MEASURES Specific genetic factors associated with phenytoin-related severe cutaneous adverse reactions.

RESULTS The GWAS discovered a cluster of 16 single-nucleotide polymorphisms in *CYP2C* genes at 10q23.33 that reached genome-wide significance. Direct sequencing of *CYP2C* identified missense variant rs1057910 (*CYP2C9*3*) that showed significant association with phenytoin-related severe cutaneous adverse reactions (odds ratio, 12; 95% CI, 6.6-20; $P=1.1 \times 10^{-17}$). The statistically significant association between *CYP2C9*3* and phenytoin-related severe cutaneous adverse reactions was observed in additional samples from Taiwan, Japan, and Malaysia. A meta-analysis using the data from the 3 populations showed an overall odds ratio of 11 (95% CI, 6.2-18; $z=8.58$; $P < .00001$) for *CYP2C9*3* association with phenytoin-related severe cutaneous adverse reactions. Delayed clearance of plasma phenytoin was detected in patients with severe cutaneous adverse reactions, especially *CYP2C9*3* carriers, providing a functional link of the associated variants to the disease.

CONCLUSIONS AND RELEVANCE This study identified *CYP2C* variants, including *CYP2C9*3*, known to reduce drug clearance, as important genetic factors associated with phenytoin-related severe cutaneous adverse reactions.

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Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The Taiwan Severe Cutaneous Adverse Reactions Consortium comprises the physicians, pharmacists, and other investigators from Taipei Medical University Hospital, Wan Fang Hospital, Chung Shan University Hospital, Taichung Veterans General Hospital, Changhua Christian Hospital, China Medicine University Hospital, National Cheng Kung University Hospital, National Taiwan University Hospital, and Chang Gung Memorial Hospitals.

Corresponding Authors: Wen-Hung Chung, MD, PhD, Department of Dermatology, Chang Gung Memorial Hospital, No. 5, Fusing St, Taoyuan, 333 Taiwan (chung1@cgmh.org.tw); Shuen-Iu Hung, PhD, Institute of Pharmacology, School of Medicine, National Yang-Ming University, No. 155, Section 2, Linong St, Taipei, 112 Taiwan (sihung@ym.edu.tw).

Phenytoin (diphenylhydantoin) is a widely prescribed antiepileptic drug and remains the most frequently used first-line antiepileptic drug in hospitalized patients.[1,2] Although effective for treating neurological diseases, phenytoin can cause cutaneous adverse reactions ranging from mild rash (maculopapular exanthema) to life-threatening severe cutaneous adverse reactions.[3-6] Phenytoin-related severe cutaneous adverse reactions include drug reactions with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN).[4-6] Stevens-Johnson syndrome and TEN are variants of the same mucocutaneous blistering reaction disease and carry high morbidity and mortality (10%-50%).[4-6] DRESS, also known as drug-induced hypersensitivity syndrome, is characterized by generalized maculopapular eruptions, high fever, eosinophilia, atypical lymphocytes, and visceral involvement and has a mortality rate of approximately 10%.[6,7] Phenytoin-related severe cutaneous adverse reactions frequently impair the internal organs, leading to the highest mortality among the different antiepileptic drug-related cutaneous reactions.[5]

ALDEN *algorithm of drug causality for epidermal necrolysis*

DRESS *drug reaction with eosinophilia and systemic symptoms*

GWAS *genome-wide association study*

SJS *Stevens-Johnson syndrome*

SNP *single-nucleotide polymorphism*

TEN *toxic epidermal necrolysis*

The pharmacogenomic basis of phenytoin-related severe cutaneous adverse reactions remains unknown. By candidate gene approach, *HLA-B*15:02* was identified as associated with phenytoin-related severe cutaneous adverse reactions in Asians; however, the strength of the association is much weaker than that found for carbamazepine-related SJS-TEN.[8-12] The US Food and Drug Administration has suggested that physicians should avoid prescribing phenytoin or fosphenytoin as an alternative to carbamazepine in patients who carry *HLA-B*15:02*.[13] To investigate the genetic factors associated with phenytoin-related severe cutaneous adverse reactions, we carried out a genome-wide association study (GWAS) followed by direct sequencing of the associated genes and replication analyses using samples from Taiwan, Japan, and Malaysia.

Methods

Recruitment of Cases and Drug-Tolerant Controls

Patients with phenytoin-related cutaneous adverse reactions were recruited from the Chang Gung Memorial Hospital (CGMH) health system and Taiwan Severe Cutaneous Adverse Reactions Consortium in Taiwan, Hospital Sultanah Aminah Johor Bahru in Malaysia, and centers collaborating with the National Institute of Health Sciences and Osaka University in Japan between 2002 and 2014. All cases were evaluated by at least 2 dermatologists, who reviewed all available photographs, histological data, and clinical information, including type of cutaneous reactions, date of onset, and drug history, dosage, and duration. Phenotypes of severe cutaneous adverse reactions were clinically assessed using the diagnostic criteria established by the Registry of Severe Cutaneous Adverse Reactions Consortium, which maintains a multinational registry of severe cutaneous adverse reactions cases reported by physicians from many countries (Austria, France, Germany, Israel, Italy, the Netherlands, Spain, South Africa, Taiwan, the United Kingdom, and Vietnam, etc).[4,7] Stevens-Johnson syndrome and TEN are characterized by a rapidly developing blistering exanthema of purpuric macules and target-like lesions accompanied by mucosal involvement and skin detachment. Stevens-Johnson syndrome was defined as skin detachment less than 10% of the body surface area, SJS-TEN overlap as skin detachment from 10% to 29%, and TEN as skin detachment greater than 30%.[4,5] The criteria and scoring system of DRESS include cutaneous involvement with typical rash (eg, exfoliative dermatitis, diffuse maculopapular exanthema), fever, eosinophilia, lymph node enlargement, atypical lymphocytes, internal organ involvement (liver, kidney, central nervous system, lung, heart, muscle), and time of resolution.[7] The maculopapular exanthema phenotype is characterized by generalized cutaneous erythematous macules and papules and is self-limited without systemic involvement. We used 2 methods, the Naranjo score[14] and ALDEN (algorithm of drug causality for epidermal necrolysis),[15] to determine the drug causality as phenytoin. Drug-tolerant patients who had received phenytoin for more than 3 months without evidence of adverse reactions were enrolled as controls from the departments of neurology or neurosurgery of the CGMH health system in Taiwan in 2002-2014. Written informed consent was obtained from each participant. This study was approved by the institutional review board of the ethical standards committee of each study site/institute.

GWAS, Direct Sequencing, and Linkage Disequilibrium Analysis

GWAS was performed using the Affymetrix SNP Array 6.0 platform, which is composed of 909 622 single-nucleotide polymorphisms (SNPs). The genotype calls were generated using the Birdseed method (Birdseed version 2) with Affymetrix Power Tools (version apt-1.10.2). The mean call rate of each array is 98.7% (SD, 0.95%). We excluded SNPs with a call rate of less than 0.90 and $P<5.5\times10^{-8}$ (0.05/909 622) ($P<.05$ with Bonferroni correction for multiple comparisons) in a Hardy-Weinberg equilibrium test of data from participants from the general population of Taiwan. We performed GWAS analysis and principal component analysis and constructed a quantile-quantile plot using MATLAB version 8.1 and Bioinformatics Toolbox version 4.3 (MathWorks). After quality control measures and principal component analysis implementation, a total of 854 035 SNPs were used in the GWAS discovery. To investigate functional SNPs, we designed polymerase chain reaction primers (listed in eTable 1 in the Supplement) for direct sequencing (Sanger method) of the exons of associated genes in severe cutaneous adverse reactions cases. Then, the genotypes of missense/nonsense SNPs identified from direct sequencing were further examined in the samples of severe cutaneous adverse reactions cases, phenytoin-tolerant controls, and population controls by TaqMan assays (Life Technologies). Haploview software (version 4.1) was used to draw the linkage disequilibrium maps of chromosome 10: 96.0-97.5 Mb

containing the *CYP2C* region. We calculated the D′ and r^2 values to estimate the independence of the SNPs in the samples.

Analysis of Concentrations of Plasma Phenytoin

We obtained convenience plasma samples from phenytoin-tolerant controls (including those with continuous use of phenytoin and those who were able to discontinue phenytoin therapy and provided their serial blood samples before or after drug withdrawal) and severe cutaneous adverse reactions cases. Plasma samples of controls who received the maintenance dosage were collected within 24 hours after the last dose of phenytoin. Available samples from phenytoin-tolerant controls and patients with severe cutaneous adverse reactions were obtained before or after withdrawal of phenytoin. The date of drug withdrawal in patients with severe cutaneous adverse reactions was usually the same day or near the onset of severe cutaneous adverse reactions when phenytoin was recognized as the associated drug. The plasma concentration of total phenytoin in samples was determined by fluorescence polarization immunoassay using Axsym Phenytoin Assay (Abbott) in the Department of Laboratory Medicine of the CGMH (College of American Pathologists number 3291201-02). Standard calibrators (0.0, 2.5, 5.0, 10.0, 20.0, and 40.0 μg/mL) were used to generate the standard curve. The assay system has a sensitivity of 0.5 μg/mL. This sensitivity is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. Interday and intraday variability in precision were determined using human serum with 6.9, 14.0, and 24.0 μg/mL of phenytoin added, which yielded a coefficient of variation of less than 2.9%. Accuracy by recovery was determined by adding phenytoin to human serum and to buffer at concentrations of 2.5, 4.0, 8.0, 12.0, 16.0, 20.0, 30.0, and 36.0 μg/mL, and the mean recovery was 101.5% (SD, 3.9%).

Statistical Analysis

We conducted the statistical analysis for the association by comparing the allele or genotype frequencies between cases and controls in modes of inheritance (additive model, recessive model, or dominant models). The associations were examined by Fisher exact tests and rank-ordered according to the lowest *P* value in these models. All *P* values were 2-tailed. A Bonferroni correction was applied for the multiple comparisons and adjusted the *P* values using the numbers of tests (n=854 035 SNPs for GWAS, n=17 for HLA-A genotypes, and n=36 for HLA-B genotypes). A corrected $P<.05$ was considered to be statistically significant, and significant *P* values were $P=.0029$ for HLA-A (0.05/17), $P=.0014$ for HLA-B (0.05/36), and $P=5.9 \times 10^{-8}$ for GWAS (0.05/854 035). Odds ratios (ORs) were calculated using a Haldane modification, which added 0.5 to all cells to accommodate possible zero counts. Fisher exact tests, Bonferroni correction, and OR calculation were performed by MATLAB version 8.1 and Statistics Toolbox version 8.2 (MathWorks), and a meta-analysis was conducted using Review Manager (RevMan) version 5.2. Pooled ORs using a random-effects model were calculated from studies with phenytoin-related severe cutaneous adverse reactions or population controls and *CYP2C9*3* allele analysis. Study heterogeneity was investigated by calculating τ^2 and I^2. The statistical significance was defined as $P < .05$. The concentrations of plasma phenytoin in the different groups were compared by nonparametric tests.

Additional information regarding methods to determine drug causality, estimates of the prevalence of phenytoin-related cutaneous adverse reactions, and HLA genotyping methods is provided in the eAppendix in the Supplement.

Results

For the initial GWAS, direct sequencing of the associated loci, and replication analysis, we enrolled a total of 168 cases with phenytoin-related cutaneous reactions (n=90 severe cutaneous adverse reactions [n=48 SJS-TEN and n=42 DRESS] and n=78 maculopapular exanthemas) and 130 tolerant controls from Taiwan (**Table 1**). Of the 90 cases with severe cutaneous adverse reactions, 13 patients died as a result of the episode (Table 1). The average daily dose of phenytoin showed no significant difference between the 90 severe cutaneous adverse reactions cases (mean, 314 mg/d; 95% CI, 292-330 mg/d) and 130 phenytoin-tolerant controls (mean, 323 mg/d; 95% CI, 309-337 mg/d; $P=.42$) (Table 1). Based on the data from the Taiwan National Health Insurance and CGMH databases, the estimated prevalence was 0.24% for phenytoin-related SJS-TEN, 0.21% for phenytoin-related DRESS, and 3.6% for phenytoin-related maculopapular exanthema in Taiwan.

As control participants in the GWAS, we randomly selected 412 healthy individuals from a Taiwan biobank under a nationwide population study, which comprises 9980 Han Chinese descendants.[16] There was no self-report of adverse drug events by any of these 412 participants from Taiwan, where 98% of the population is made up of Han Chinese. We performed the GWAS using samples from 60 cases of phenytoin-related severe cutaneous adverse reactions (n=38 SJS-TEN and n=22 DRESS) initially enrolled from a referral center (CGMH) and the 412 controls from Taiwan. The principal component analysis plots (eFigure 1 in the Supplement) could not separate the 60 severe cutaneous adverse reactions cases from 412 general controls, suggesting that there is no population stratification between cases and controls. The principal component analysis located most of the Taiwanese severe cutaneous adverse reactions cases as among southern, central, and northern Han Chinese of mainland China (eFigure 2 in the Supplement).

The GWAS discovered a cluster of 16 SNPs on chromosome 10q23.33 (96.4-97.0 Mb) that reached the genome-wide significance threshold ($P < 5.9 \times 10^{-8}$) for association with phenytoin-related severe cutaneous adverse reactions (**Figure 1**). Eight SNPs with the lowest *P* values were located on *CYP2C* genes, comprising *CYP2C18* (NCBI Entrez gene 1562), *CYP2C19* (NCBI Entrez gene 1557), *CYP2C9* (NCBI Entrez gene 1559), and *CYP2C8* (NCBI Entrez gene 1558) (**Table 2**). The quantile-quantile plot confirmed a marked excess of significantly associated SNPs on chromosome 10 (eFigure 3 in the Supplement). Direct sequencing of the *CYP2C* genes of patients identified 2 missense variants, rs1057910 (*CYP2C9*3*; p.I359L) and rs3758581 (*CYP2C19*1C*; p.V331I), showing statistically significant association with phenytoin-related severe cutaneous adverse reactions (Table 2 and

Table 1. Demographic Data and Clinical Features of Patients With Phenytoin-Related Cutaneous Adverse Reactions and Phenytoin-Tolerant Controls Enrolled in Taiwan

Characteristics	Cases of Phenytoin-Related Cutaneous Adverse Reactions (n = 168)						
	Severe Cutaneous Adverse Reactions (n = 90)						
	SJS (n = 39)	SJS-TEN Overlapping (n = 3)	TEN (n = 6)	DRESS (n = 42)	Total (n = 90)	MPE (n = 78)	Phenytoin-Tolerant Controls (n = 130)
Age, y							
Mean (SD)	56 (17)	63 (3.5)	64 (16)	57 (21)	57 (17)	52 (21)	41 (14)
Median (range)	59 (19-77)	61 (61-67)	68 (37-77)	59 (13-91)	61 (13-91)	54 (1-88)	39 (15-79)
Sex, No. (%)							
Male	15 (39)	1 (33)	3 (50)	18 (43)	37 (41)	39 (50)	82 (63)
Female	24 (61)	2 (67)	3 (50)	24 (57)	53 (59)	39 (50)	48 (37)
Phenytoin exposure							
Dosage, mean (95% CI) [range], mg/d	309 (268-343) [100-750]	300 (300-300) [300-300]	325 (245-405) [300-400]	317 (289-339) [300-750]	314 (292-330) [100-750]	312 (287-338) [100-800]	323 (309-337) [100-600]
Duration, mean (range), d	23 (2-58)[a]	21 (10-31)	24 (12-63)	30 (7-59)	27 (2-63)[a]	23 (2-58)	89 mo (3-303 mo)
Deceased cases, No. (%)	2 (5.1)	2 (67)	5 (83)	4 (9.5)	13 (14)	0	0
Internal organ involvement							
Hepatitis, GPT, IU/L, No. (%)[b]							
<100	18 (46)	3 (100)	3 (50)	16 (38)	40 (44)	NA	NA
100-500	15 (39)	0	3 (50)	18 (43)	36 (40)	NA	NA
501-1000	5 (13)	0	0	4 (9.5)	9 (10)	NA	NA
>1000	1 (3.1)	0	0	4 (9.5)	5 (5.5)	NA	NA
Acute renal failure[c]	2 (6.3)	2 (67)	1 (17)	6 (14)	11 (12)	NA	NA
Hematologic abnormalities							
Eosinophilia, absolute eosinophil counts/μL, No. (%)							
<700	20 (51)	2 (67)	4 (67)	13 (31)	39 (43)	NA	NA
700-1499	15 (39)	1 (33)	1 (17)	17 (41)	34 (38)	NA	NA
≥1500	4 (10)	0	1 (17)	12 (29)	17 (19)	NA	NA
Atypical lymphocytosis	12 (31)	1(33)	4 (67)	22 (52)	39 (43)	NA	NA
Mucosal involvement, No. (%)							
Oral	39 (100)	3 (100)	6 (100)	16 (38)	64 (71)	NA	NA
Eyes	19 (49)	2 (67)	5 (83)	3 (7.1)	29 (32)	NA	NA
Genital	12 (31)	2 (67)	4 (67)	1 (2.4)	19 (21)	NA	NA

Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; GPT, glutamic pyruvic transaminase; MPE, maculopapular exanthema; NA, not applicable; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

[a] A duration of 2 days was observed in a patient who received intravenous phenytoin infusion.

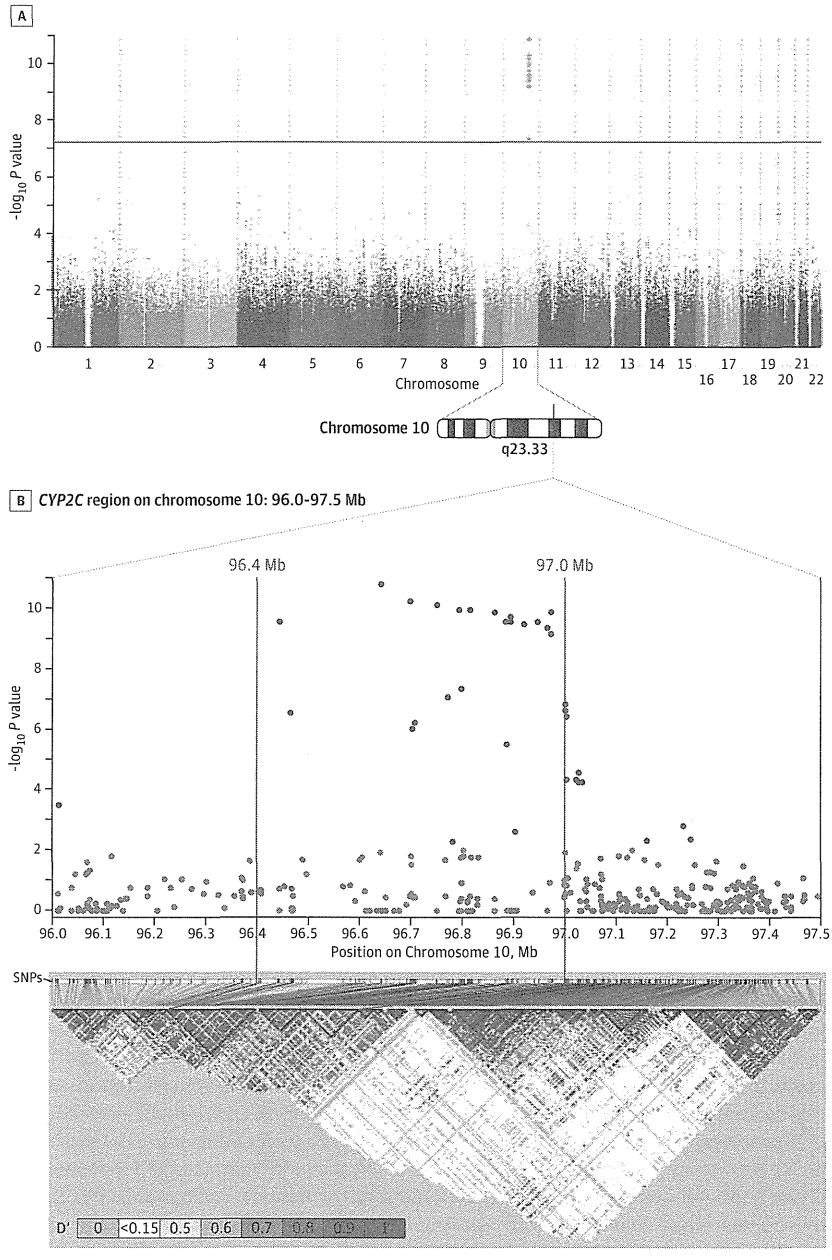
[b] Values of glutamic-pyruvic transaminase were 2 times greater than normal.

[c] The creatinine value was 1.5-fold higher than the normal value range (0.4-1.5 mg/dL) after drug intake.

eTable 2 in the Supplement). The association between the 10 variants and phenytoin-related severe cutaneous adverse reactions was replicated in an independent set of 30 cases of phenytoin-related severe cutaneous adverse reaction (n=10 SJS-TEN and n=20 DRESS) recruited from the Taiwan Severe Cutaneous Adverse Reactions Consortium and 130 phenytoin-tolerant controls (Table 2). All 10 SNPs in the 412 general controls were in Hardy-Weinberg equilibrium (Table 2).

The 10 SNPs are common (minor allele frequencies ≥0.19) in severe cutaneous adverse reactions cases, yet rare (minor allele frequencies 0.017-0.063) in the population controls from Taiwan (n = 412) and the southern (n = 500), central (n = 500), and northern (n = 500) Han Chinese samples (eTable 3 in the Supplement). The 10 SNPs showed strong linkage disequilibrium in the data sets of 412 controls and 90 severe cutaneous adverse reactions cases but a smaller linkage disequilibrium block in 130 phenytoin-tolerant controls (**Figure 2** and eTables 4-6 and eFigure 4 in the Supplement). Among the 7 haplotypes inferred from 3 SNPs (rs3758581, rs1057910, and rs6583967), the risk haplotype (haplotype 2) was absent in 130 phenytoin-tolerant controls and showed significant association with phenytoin-related severe cutaneous adverse reactions (eFigure 5 in the Supplement). Because the estimated prevalence of phenytoin-related severe cutaneous adverse reactions is very low in Taiwan, the genotyping data of 130 phenytoin-tolerant controls and 412 population controls was combined. All 10 SNPs exhibited significant association with phenytoin-related severe cutaneous adverse reactions in the combined-samples analysis (90 cases and 542 controls) and showed $P>.05$ for heterogeneity between studies (Table 2). Data from the GWAS, replication, and combined-samples analysis all revealed that *CYP2C9**3* showed significant association with

Figure 1. Genome-Wide Association Scan and Linkage Disequilibrium Map for the *CYP2C* Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions



A, Manhattan plot showing associations between *CYP2C* single-nucleotide polymorphisms (SNPs) and phenytoin-related severe cutaneous adverse reactions. Each dot represents a $-\log_{10} P$ value calculated by Fisher exact test for the allele frequency in 60 severe cutaneous adverse reaction cases and 412 population controls. The red horizontal line represents $P = 5.9 \times 10^{-8}$, indicating $P = .05$ by Bonferroni correction for the multiple comparisons (0.05/854 035). B, The $-\log_{10} P$ values of SNPs on the chromosome 10q23.33 (physical position: 96.0-97.5 Mb) and the linkage disequilibrium heat map based on pairwise D' values from genome-wide association study data from 412 controls. The genomic coordinates are based on the NCBI Human Genome build 37.5, and the standard ideogram of chromosome 10 was taken from the NCBI Human Genome resource site. Top, Single-nucleotide polymorphisms with *P*#.01 are indicated by blue and *P*>.01 by cyan. The physical position between chromosome 10 (96.4-97.0 Mb), which spans *CYP2C18*, *CYP2C19*, *CYP2C9*, *CYP2C8*, and *C10orf129* genes, is indicated by pink lines. Bottom, Black triangles mark the linkage disequilibrium blocks. A D' value of 1 indicates that the examined loci exhibit complete dependency while a value of 0 demonstrates the independence of one another. The colors represent the D' values: red (0.5#D' values #1), white to pink (0<D' values <0.5), purple (D' = 0).

phenytoin-related severe cutaneous adverse reactions (OR, 12; 95% CI, 6.6-20; $P = 1.1 \times 10^{-17}$ in the combined-samples analysis) (Table 2).

We compared the SNP data of the subgroups of 168 patients with phenytoin-related cutaneous adverse reactions and 130 phenytoin-tolerant controls and found that *CYP2C9*3* exhibited significant association with phenytoin-related SJS-TEN (OR, 30; 95% CI, 8.4-109; $P = 1.2 \times 10^{-10}$), DRESS (OR, 19; 95% CI, 5.1-71; $P = 7.0 \times 10^{-7}$), and maculopapular exanthema (OR, 5.5; 95% CI, 1.5-21; $P = .01$) (eTable 7 in the Supplement). The significant association between *CYP2C9*3* and phenytoin-related cutaneous adverse reactions was also noted when comparing data from cases with that of the 412 population controls (eTable 7).

We examined the association between *CYP2C9*3* and phenytoin-related severe cutaneous adverse reactions using

Table 2. Ten Significant SNPs Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions in the GWAS Discovery, Direct Sequencing, Replication, and Combined Samples

SNP	Position on Chromosome 10 (bp)[a]	Nearby Gene (Location)[a]	Minor allele	GWAS Discovery[b] MAF Cases	MAF Controls	P Value[e]	OR (95% CI)	Replication Analysis[c] MAF Cases	MAF Controls	P Value[e]	OR (95% CI)	Combination[d] P Value[e]	OR (95% CI)	P Value HET	P Value HWE
rs17110192	10q23.33 (96441927)	*CYP2C18* (5'UTR)	C	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.019	9.0×10^{-6}	11.5 (3.9-34)	5.5×10^{-16}	9.8 (5.8-17)	.78	.15
rs3758581 (*CYP2C19*1C*)	10q23.33 (96602623)	*CYP2C19* (exon 7)	A	0.21	0.03	3.4×10^{-11}	8.4 (4.6-15)	0.18	0.023	2.2×10^{-5}	9.5 (3.4-27)	3.5×10^{-15}	8.5 (5.1-14)	.79	.30
rs17110321	10q23.33 (96639896)	*CYP2C9*, *CYP2C19*	G	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.012	1.0×10^{-6}	19.2 (5.2-71)	1.3×10^{-16}	11 (6.2-18)	.34	.15
rs9332093	10q23.33 (96696555)	*CYP2C9* (5'UTR)	G	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.015	3.3×10^{-6}	14.4 (4.4-47)	2.7×10^{-16}	10 (6.0-18)	.55	.15
rs1057910 (*CYP2C9*3*)	10q23.33 (96741053)	*CYP2C9* (exon 7)	C	0.21	0.024	1.5×10^{-12}	11 (5.7-20)	0.18	0.012	1.0×10^{-6}	19.2 (5.2-71)	1.1×10^{-17}	12 (6.6-20)	.42	.61
rs9332245	10q23.33 (96749181)	*CYP2C9* (3'UTR)	A	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.019	9.0×10^{-6}	11.5 (3.9-34)	5.5×10^{-16}	9.8 (5.8-17)	.78	.15
rs1592037	10q23.33 (96792328)	*CYP2C8* (3'UTR)	A	0.21	0.033	1.0×10^{-10}	7.8 (4.3-14)	0.18	0.035	1.8×10^{-4}	6.3 (2.5-16)	6.9×10^{-14}	7.3 (4.4-12)	.70	.39
rs6583967	10q23.33 (96814475)	*CYP2C8* (Intron)	C	0.21	0.033	1.0×10^{-10}	7.8 (4.3-14)	0.2	0.027	1.2×10^{-5}	9.0 (3.4-24)	5.1×10^{-15}	8.0 (4.9-13)	.79	.39
rs10882551	10q23.33 (96905783)	*CYP2C8* (5'UTR)	T	0.2	0.033	4.6×10^{-10}	7.4 (4.1-13)	0.2	0.019	2.0×10^{-6}	12.8 (4.3-38)	6.5×10^{-15}	8.2 (5.0-14)	.38	.39
rs12262878	10q23.33 (96971504)	*C10orf129* (Intron)	C	0.2	0.03	1.6×10^{-10}	8.0 (4.4-15)	0.18	0.027	4.8×10^{-5}	8.1 (3.0-22)	2.9×10^{-14}	7.9 (4.8-13)	.98	.30

Abbreviations: GWAS, genome-wide association study; HET, *P* value of the heterogeneity test between studies; HWE, Hardy-Weinberg equilibrium *P* values for 412 controls from the general population; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslational region.

[a] The genomic coordinates are based on NCBI Human Genome Build 37.5. Gene ID: *CYP2C18*: NCBI Entrez gene 1562; *CYP2C19*: NCBI Entrez gene 1557; *CYP2C9*: NCBI Entrez gene 1559; *CYP2C8*: NCBI Entrez gene 1558; and *C10orf129*: NCBI Entrez gene 142827.

[b] Sixty cases of severe cutaneous adverse reactions vs 412 controls from the general population.

[c] Thirty cases of severe cutaneous adverse reactions vs 130 phenytoin-tolerant controls.

[d] Ninety cases of severe cutaneous adverse reactions vs 542 controls.

[e] *P* values were calculated by Fisher exact test for the risk allele. All SNPs were discovered by the GWAS except rs3758581 and rs1057910, which were identified by direct sequencing on the associated genes.

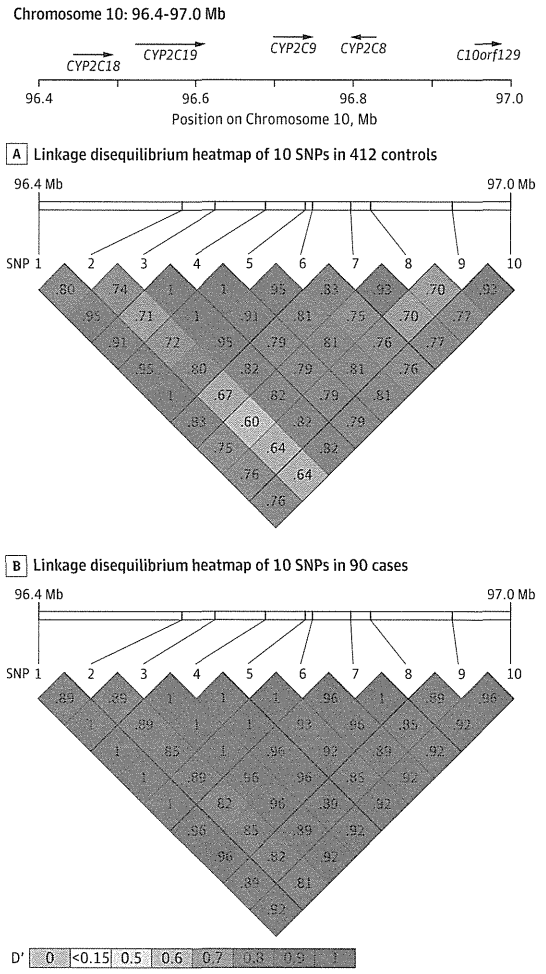
samples from 9 patients with SJS-TEN and 2869 population controls from Japan and 6 severe cutaneous adverse reactions cases and 374 population controls from Malaysia.[17] Demographic and clinical data for patients with phenytoin-related severe cutaneous adverse reactions from Japan and Malaysia are shown in eTable 8 in the Supplement. Allele frequencies of *CYP2C9*3* were 17% to 22% in samples from patients with phenytoin-related severe cutaneous adverse reactions but only 2.7% to 2.8% in samples from the population controls of Japan and Malaysia (eTable 9 in the Supplement). *CYP2C9*3* showed statistically significant association with phenytoin-related severe cutaneous adverse reactions in both Japanese (OR, 10; 95% CI, 3.4-32; $P = 1.2 \times 10^{-3}$) and Malaysians (OR, 6.9; 95% CI, 1.4-34; $P = .048$) (eTable 9). We further analyzed the association between *CYP2C9*3* and phenytoin-related severe cutaneous adverse reactions by meta-analysis using a random-effects model and classified cases and controls according to their phenotype (SJS-TEN or DRESS) and ethnicity (Taiwanese, Japanese, or Malaysian) (**Figure 3**). The results of the meta-analysis showed a pooled OR of 12 (95% CI, 6.4-22; $z = 7.82$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related SJS-TEN, a pooled OR of 9.2 (95% CI, 4.3-20; $z = 5.70$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related DRESS, and an overall OR of 11 (95% CI, 6.2-18; $z = 8.58$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related severe cutaneous adverse reactions in Asians (Figure 3).

Although no SNPs on HLA region reached genome-wide significance, we examined the HLA association because of the immunological characteristics of phenytoin-related severe cutaneous adverse reactions.[3-6,18] Phenytoin-related severe cutaneous adverse reactions showed no link with *HLA-A* and a very weak association with *HLA-B*13:01*, *HLA-B*15:02*, and *HLA-B*51:01*, in which their *P* values become nonsignificant after Bonferroni correction (eTable 10 in the Supplement). In the subgroup analysis, only phenytoin-related SJS-TEN showed significant association with *HLA-B*15:02* (OR, 5.0; 95% CI, 2.0-13; $P = 7.0 \times 10^{-4}$; $P = .025$ after Bonferroni correction) (eTable 10). Adding *HLA-B*1502* to *CYP2C9*3* genetic screening improved the sensitivity to 62.5% for phenytoin-related SJS-TEN but decreased the specificity (eTable 11 in the Supplement).

The concentrations of plasma phenytoin were determined in the samples of participants, including (1) 90 phenytoin-tolerant controls with continuous use of phenytoin; (2) 11 phenytoin-tolerant controls who were able to discontinue phenytoin therapy; (3) 14 patients with SJS-TEN; and (4) 26 patients with DRESS (**Table 3** and eFigure 6 in the Supplement). The average concentration of plasma phenytoin in the 90 phenytoin-tolerant controls was 11.8 μg/mL (95% CI, 11.0-12.6 μg/mL) (Table 3). The day of drug withdrawal in the 11 phenytoin-tolerant controls and 40 patients with severe cutaneous adverse reactions was labeled as day 0 (eFigure 6, B-D). Nine samples from patients with severe cutaneous adverse reac-

tions were obtained before drug withdrawal because these patients were hospitalized and received phenytoin for seizure prophylaxis. Before drug withdrawal, plasma concentrations of phenytoin were significantly higher in patients with SJS-TEN (mean, 34 μg/mL; 95% CI, 1.8-66 μg/mL) compared with the phenytoin-tolerant controls (mean, 11 μg/mL; 95% CI, 9.1-13 μg/mL; $P = .015$) (Table 3). After drug withdrawal for 1 to 5 days, concentrations of plasma phenytoin rapidly decreased in phenytoin-tolerant controls (mean, 2.5 μg/mL; 95% CI, 1.5-3.5 μg/mL) but remained significantly high in patients with SJS-TEN (mean, 12 μg/mL; 95% CI, 4.6-19 μg/mL; $P = .0004$) and patients with DRESS (mean, 5.5 μg/mL; 95% CI, 2.8-8.3 μg/mL; $P = .029$) (Table 3). Furthermore, significantly delayed clearance of plasma phenytoin was observed in patients with severe cutaneous adverse reactions with *CYP2C9*3* (mean, 17 μg/mL; 95% CI, 5.9-27 μg/mL; $P = .0002$) and in noncarriers (mean, 4.9 μg/mL; 95% CI, 3.1-6.7 μg/mL; $P = .015$) (Table 3). The *CYP2C9*3* carriers with severe cutaneous adverse reactions had significantly higher levels of plasma phenytoin than patients without the risk allele ($P = .022$). However, the average daily dose showed no difference between patients with severe cutaneous adverse reactions carrying *CYP2C9*3* (n = 12; mean, 300 mg/d; 95% CI, 300-300 mg/d) and noncarriers (n = 28; mean, 304 mg/d; 95% CI, 291-316 mg/d). These data suggest that rs1057910 (*CYP2C9*3*) contributes to phenytoin-related severe cutaneous adverse reactions.

Figure 2. Linkage Disequilibrium Heat Maps for the *CYP2C* Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions



The linkage disequilibrium heat maps are drawn based on pairwise D' values of the 10 risk single-nucleotide polymorphisms using the data of 412 controls (A) and 90 cases of phenytoin-related severe cutaneous adverse reactions (B).

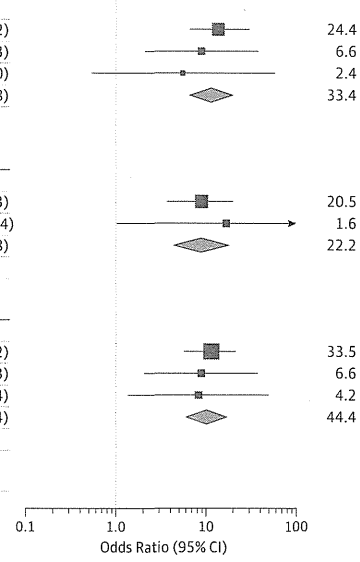
Discussion

Phenytoin has a narrow therapeutic range (10-20 μg/mL) and nonlinear pharmacokinetics and is metabolized to inactive hydroxyphenytoin, 5-(4'-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH), primarily (90%) by the cytochrome P450 (CYP) 2C9 enzyme.[19] Formation of *p*-HPPH is thought to proceed via a reactive arene oxide intermediate, which has been proposed for the induction of phenytoin hypersensitivity.[19,20] In this study, we report *CYP2C* variants, including *CYP2C9*3*, known to cause 93% to 95% reduction in phenytoin clearance,[21-24] as important genetic factors for phenytoin-related severe cutaneous adverse reactions. We detected accumulated phenytoin in patients with severe cutaneous adverse reactions, particularly *CYP2C9*3* carriers. Patients with SJS-TEN exhibited slower metabolism and a stronger strength of association with the *CYP2C* SNPs than patients with DRESS. Delayed clearance was also noted in patients with severe cutaneous adverse reactions without *CYP2C9*3*, suggesting that nongenetic factors such as renal insufficiency, hepatic dysfunction, and concurrent use of substances that compete or inhibit the enzymes may also affect phenytoin metabolism and contribute to severe cutaneous adverse reactions. Such characteristics share the features of the drug-accumulation hypothesis of allopurinol-related severe cutaneous adverse reactions, in which the risk factors include high-dose regimen, renal failure, concomitant diuretic, and high concentration of oxypurinol.[25-27] Further studies are needed to investigate how the *CYP2C* variants and the accumulated reactive metabolites affect cutaneous adverse reactions.

Among the 10 risk alleles, the missense rs1057910 is the only one with known function associated with reduced CYP2C9 enzyme activity and phenytoin-related neurological toxicity.[22,23] The SNP rs1057910 forms *CYP2C9*3* and part of *CYP2C9*18*. Another risk SNP, rs3758581, present on the *CYP2C19*1B* and *CYP2C19*1C* normal haplotypes, is a missense mutation yet has no obvious effects on CYP2C19 activity or drug metabolism.[24,28,29] The SNP rs3758581 may be a surrogate marker for rs1057910 because of the strong linkage disequilibrium between the 2 SNPs. In our 90 samples from patients with severe cutaneous adverse reactions, we did not detect *CYP2C9*2* (rs1799853). The frequencies of *CYP2C9*3* vary in ethnic groups (0.8%-10%).[28-30] *CYP2C9*3* was reported to be associated with phenytoin maculopapular exanthema ($P = .007$) in Koreans.[31] A GWAS using samples from 40 cases

Figure 3. Distribution of the *CYP2C9*3* Variant in Cases With Phenytoin-Related Severe Cutaneous Adverse Reactions and Population Controls

Subgroup	Cases of Phenytoin-Related Severe Cutaneous Adverse Reactions, No.		Population Controls, No.		Odds Ratio (95% CI)	Weight, %
	*CYP2C9*3* Carriers	Total Participants	*CYP2C9*3* Carriers	Total Participants		
SJS/TEN						
Taiwan	20	48	20	412	14.00 (6.75-29.02)	24.4
Japan	3	9	153	2869	8.88 (2.20-35.83)	6.6
Malaysia	1	4	21	374	5.60 (0.56-56.20)	2.4
Subtotal		61		3655	11.96 (6.42-22.28)	33.4
Total No. of *CYP2C9*3* carriers	24		194			
Heterogeneity: $\tau^2=0.00$; $\chi^2_3=0.77$; $P=.68$; $I^2=0\%$						
Test for overall effect: $z=7.82$; $P<.00001$						
DRESS						
Taiwan	13	42	20	412	8.79 (3.97-19.43)	20.5
Malaysia	1	2	21	374	16.81(1.02-278.24)	1.6
Subtotal		44		786	9.22 (4.30-19.78)	22.2
Total No. of *CYP2C9*3* carriers	14		41			
Heterogeneity: $\tau^2=0.00$; $\chi^2_1=0.19$; $P=.66$; $I^2=0\%$						
Test for overall effect: $z=5.70$; $P<.00001$						
All severe cutaneous adverse reactions (SJS/TEN and DRESS)						
Taiwan	33	90	20	412	11.35 (6.10-21.12)	33.5
Japan	3	9	153	2869	8.88 (2.20-35.83)	6.6
Malaysia	2	6	21	374	8.40 (1.46-48.54)	4.2
Subtotal		105		3655	10.63 (6.20-18.24)	44.4
Total No. of *CYP2C9*3* carriers	38		194			
Heterogeneity: $\tau^2=0.00$; $\chi^2_2=0.18$, $P=.91$; $I^2=0\%$						
Test for overall effect: $z=8.58$; $P<.00001$						



Patients with phenytoin-related severe cutaneous adverse reactions were recruited at the Chang Gung Memorial Hospital health system and the Taiwan Severe Cutaneous Adverse Reaction Consortium in Taiwan, Hospital Sultanah Aminah Johor Bahru in Malaysia, and centers collaborating with the National Institute of Health Sciences and Osaka University in Japan. Study weighting (indicated by size of data markers) refers to the proportion of participants who were recruited from each study. The τ^2 and I^2 represent measures of heterogeneity. Diamonds represent pooled odds ratios (Mantel-Haenszel method, random effects) and error bars indicate 95% CIs. DRESS indicates drug reaction with eosinophilia and systemic symptoms; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

with maculopapular exanthema and 4 cases with severe cutaneous adverse reactions caused by phenytoin and 1296 controls from a British population[32] failed to discover genome-wide significant variants; this may be explained by the limited sample size and maculopapular exanthema phenotype of most of the cases.

This study has several limitations. The sample sizes of severe cutaneous adverse reaction cases and phenytoin-tolerant controls were small, and we did not have samples from other population groups to replicate the genetic association. For the pharmacokinetic analysis, we had only a few available plasma samples, and most of the severe cutaneous adverse reaction samples were collected after drug withdrawal. Additionally, drug-tolerant participants were younger and more likely to be male than patients with severe cutaneous adverse reactions, which may account for some of the observed differences in drug metabolism.

This study highlights that genetic variants of metabolizing enzymes contribute to severe cutaneous adverse reactions, which is different from the previous HLA studies.[33-38] Although the clinical manifestations and prognosis are quite different between SJS-TEN and DRESS, our data suggest some shared genetic factors. We propose that delayed clearance and accumulation of reactive metabolites caused by genetic variants of drug-metabolizing enzymes may be the primary factor, and that immunogenicity, such as the presence of risk HLA alleles and specific T-cell receptor clonotypes in susceptible individuals, may facilitate the development and guide the different types of cutaneous adverse reactions.[39,40] Further investigation is required to determine how a complex interplay of impaired drug metabolism, accumulation of reactive drug compounds, HLA presentation of the drug/peptide antigens, T-cell receptor recognition, and historical immune memory triggers drug hypersensitivity.

Conclusions

This study identified *CYP2C* variants, including *CYP2C9*3*, known to reduce drug clearance, as important genetic factors associated with phenytoin-related severe cutaneous adverse reactions. These findings may have potential to improve the safety profile of phenytoin in clinical practice and offer the possibility of prospective testing for preventing phenytoin-related severe cutaneous adverse reactions. More research is required to replicate the genetic association in different populations and to determine the test characteristics and clinical utility.

Table 3. Comparison of Mean Concentrations of Plasma Phenytoin in Phenytoin-Tolerant Controls and Patients With Phenytoin-Related Severe Cutaneous Adverse Reactions

	Phenytoin-Tolerant Controls		Cases			
					Severe Cutaneous Adverse Reactions	
Time Period	Continued Phenytoin Use (n = 90)	Discontinued Phenytoin Use (n = 11)	SJS-TEN (n = 14)	DRESS (= 26)	Without *CYP2C9*3* (n = 28)	With *CYP2C9*3* (n = 12)
During continuous use of phenytoin						
Phenytoin concentration, mean (95% CI) [range], μg/mL	11.8 (11.0-12.6) [5.6-20]					
Before phenytoin withdrawal						
No. of plasma samples[a]		23	4	5	2	7
Phenytoin concentration, mean (95% CI) [range], μg/mL		11 (9.1-13) [4.8-19]	34 (1.8-66) [12-52]	11 (7.0-14) [7.2-15]	11 (2.1-21) [11-12]	24 (5.7-42) [7.2-52]
P value[b]			.02	.86	.96	.12
1 to 5 d after phenytoin withdrawal						
No. of plasma samples[a]		32	17	14	20	11
Time since drug withdrawal, mean (95% CI), h[c]		62 (50-73)	65 (48-82)	79 (58-100)	72 (55-89)	70 (48-92)
Phenytoin concentration, mean (95% CI) [range], μg/mL		2.5 (1.5-3.5) [0-12]	12 (4.6-19) [0.8-46]	5.5 (2.8-8.3) [0.5-14]	4.9 (3.1-6.7) [0.5-14]	17 (5.9-27) [1.3-46]
P value[b]			4.0×10^{-4}	.029	.015	2.0×10^{-4}
>5 d after phenytoin withdrawal						
No. of plasma samples[a]		4	25	37	44	18
Time since drug withdrawal, mean (95% CI), h		168 (137-199)	278 (229-328)	255 (223-288)	267 (234-301)	259 (209-300)
Phenytoin concentration, mean (95% CI) [range], μg/mL		0.3 (-0.7-1.3) [0-1.2]	3.3 (0.5-6.2) [0-21]	0.7 (0.3-1.1) [0-6.3]	0.6 (0.3-0.9) [0-6.3]	4.7 (0.9-8.6) [0-21]
P value[b]			.33	.44	.54	.16

Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

[a] The plasma samples for the pharmacokinetic analysis were based on sample availability; more than 1 sample could be obtained from a particular participant at different time points.

[b] *P* values were calculated by nonparametric tests for the comparison between the difference of plasma phenytoin concentrations in the cases and 11 phenytoin-tolerant controls.

[c] A nonparametric test was used to examine the differences in the time interval after drug withdrawal among the samples obtained from the subgroups. No significant difference in time intervals was found between the subgroups of cases and 11 phenytoin-tolerant controls.

ARTICLE INFORMATION

Author Affiliations: Department of Dermatology, Chang Gung Memorial Hospital, Keelung, Taiwan (Chung, H.-C. Ho, J.-Y. Lin); Department of Dermatology, Drug Hypersensitivity Clinical and Research Center, Chang Gung Memorial Hospital, Linkou, Taiwan (Chung, Yang, M.-J. Chen, Hui); College of Medicine, Chang Gung University, Taoyuan, Taiwan (Chung, Yang, H.-C. Ho, M.-J. Chen, J.-Y. Lin, Hui, J.-C. Ho, W.-M. Wu, T. Wu, Y.-R. Wu, Hsih, Tu, Chen-Nen Chang, Chien-Ning Hsu, T.-L. Wu, Wang, Chuang, C.-J. Chang); Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan (W.-C. Chang, Y.-Y. Wu, T.-J. Chen, Hung); Department of Biotechnology, Ming Chuan University, Taoyuan, Taiwan (Lee); Department of Dermatology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan (J.-C. Ho, W.-M. Wu); Department of Dermatology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan (T.-J. Chen); Department of Neurology, Chang Gung Memorial Hospital, Linkou, Taiwan (T. Wu, Y.-R. Wu, Hsih); Department of Neurosurgery, Chang Gung Memorial Hospital, Linkou, Taiwan (Tu, Chen-Nen Chang); Department of Pharmacy, Chang Gung Memorial Hospital, Kaohsiung, Taiwan (Chien-Ning Hsu); Department of Laboratory Medicine, Chang Gung Memorial Hospital, Linkou, Taiwan (T.-L. Wu); Department of Dermatology, Hospital Sultanah Aminah Johor Bahru, Johor, Malaysia (Choon); Department of Dermatology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan (C.-K. Hsu); Department of Rheumatology, Taichung Veterans General Hospital, Taichung, Taiwan (D.-Y. Chen); Department of Neurology, Changhua Christian Hospital, Changhua, Taiwan (Liu); Department of Pediatrics, China Medicine University, Taichung, Taiwan (C.-Y. Lin); Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan (Kaniwa, Saito, Nakamura); Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan (Takahashi); Department of Dermatology, Course of Integrated Medicine, Osaka University Graduate School of Medicine, Suita, Japan (Azukizawa); Shanghai Genome Pilot Institutes for Genomics and Human Health, Shanghai, China (Shi); Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Linkou, Taiwan (Wang); Genomic Medicine Research Core Laboratory, Chang Gung Memorial Hospital, Linkou, Taiwan (Wang); Department of Plastic Surgery and Burn Center, Chang Gung Memorial Hospital, Linkou, Taiwan (Chuang); Institute of Molecular and Genomic Medicine, National Health Research Institutes, Zhunan, Miaoli, Taiwan (Tsai); Biostatistical Center for Clinical Research, Chang Gung Memorial Hospital, Linkou, Taiwan (C.-J. Chang); Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan (Yu-Sun Chang).

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Study concept and design: Chung, Hung.
Acquisition, analysis, or interpretation of data: All authors.
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症　例

ラモトリギンによる重症薬疹の4例

久田　恭子[1)]，松倉　節子[1)]，大野真梨恵[1)]，磯田　祐士[1)]
渡邉　裕子[1)]，守田亜希子[1)]，相原　道子[2)]，蒲原　毅[1)]

要　旨

抗てんかん薬であるラモトリギン（以下LTG）は，既存の薬剤とは異なる作用機序を有し，わが国では2008年に承認された比較的新しい薬剤である。ラモトリギンによるStevens-Johnson症候群（以下SJS）3例と薬剤性過敏症症候群（以下DIHS）1例を経験した。全例ともパルス療法を含むステロイド全身投与で改善し，眼症状等の後遺症は認めなかった。わが国では2009年から2012年までにラモトリギンの薬疹は58例の報告があり，DIHSが22例と最多でSJSと中毒性表皮壊死症（以下TEN）は合わせて13例であった。1985年から2012年の海外報告では33例中TENが18例と最多で，つぎにSJSが10例であった。ラモトリギンは2011年より双極性障害にも保険適応となった。今後も使用頻度の増加が見込まれる薬剤であり，それに伴う重症薬疹の発症に注意が必要である。

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キーワード：ラモトリギン，Stevens-Johnson症候群，薬剤性過敏症症候群，薬剤添加リンパ球刺激試験

はじめに

ラモトリギンは，既存の抗てんかん薬と異なる作用機序をもった比較的新しい薬剤である。ヨーロッパでは1991年から，米国では1994年から使用されており[1)]，わが国では2008年10月に承認され，他の抗てんかん薬で効果が得られないてんかん発作に対する併用療法に用いられている。また，双極性障害の気分安定剤としての使用も2011年に承認された。バルプロ酸ナトリウム（デパケン®）（Sodium Valproate；以下VPA）との併用ではラモトリギンの血中濃度が上昇するため，少量から投与開始し，維持療法も単剤投与時の半量投与が推奨されている。

今回われわれは，ラモトリギンによるStevens-Johnson症候群（以下SJS）3例，薬剤性過敏症症候群（Drug-induced hypersensitivity syndrome；以下DIHS）1例を経験した。今後使用頻度が増えることが予想される薬剤であることから，重症薬疹の発症には注意が必要である。薬剤添加リンパ球刺激試験（以下DLST）の結果も含め若干の文献的考察を加えて報告する。

症例報告

症例1：38歳，女性。

既往歴：双極性障害。

現病歴：双極性障害により数年来VPA，ミルナシプラン，クロサキゾラム，アビリプラゾールを内服していた。2011年10月よりラモトリギン50 mg/日が追加投与された。投与16日目より結膜の瘙痒，紅斑が出現。19日目より全身に拡大し，流涙，眼脂，37度台の微熱が出現したため当科紹介受診した。

現　症：体温37.2℃。口唇に出血性びらん，口

[1)] 横浜市立大学附属市民総合医療センター皮膚科
〒232-0024　神奈川県横浜市南区浦舟町4-57
[2)] 横浜市立大学附属病院皮膚科
連絡先：久田　恭子
掲載決定日：2013年12月18日

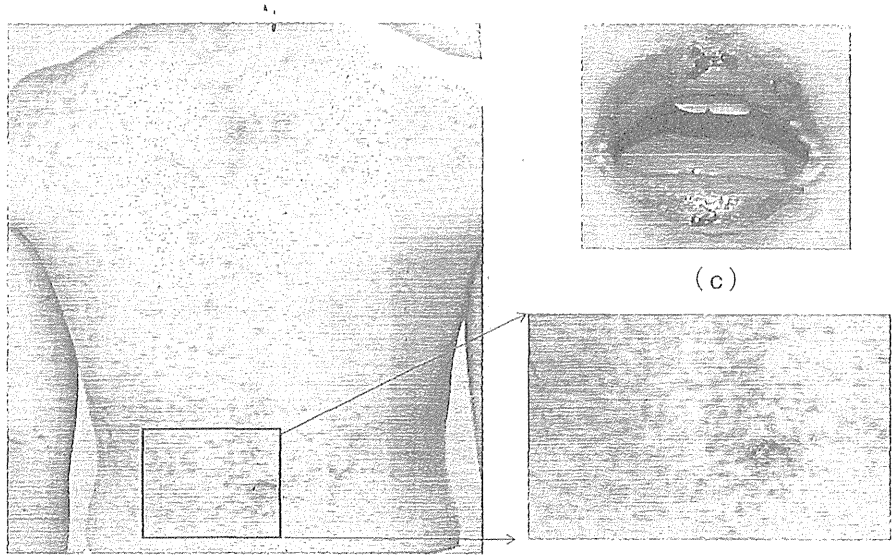

Fig. 1 : Case 1 ; Clinical examination revealed erythema on the back (a), conjunctivitis (b), and bleeding erosive lesions on the lips (c).

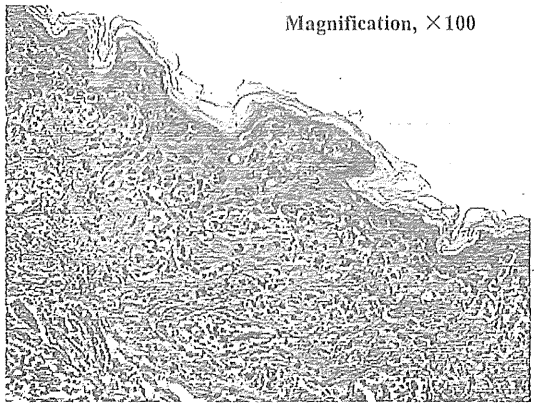

Fig. 2 : Histopathology revealed apoptosis in the epidermis and liquefaction of the basal cell layer with infiltration of lymphocytes in the upper dermis.

腔内，陰部にびらんを認めた。体幹に浸潤を触れる多形紅斑，Target lesion を認めた（Fig. 1）。

病理組織学的所見：表皮に好酸性壊死と基底層の液状変性を認めた。真皮上層の血管周囲リンパ球浸潤とリンパ球の表皮向性を認めた（Fig. 2）。

一般検査：WBC 4750/μl（neutrophil 67.7%，lymphocyte 19.6%，eosinophil 19.6%），AST 35 mU/l，ALT 36 mU/l，CRP 2.031 mg/dl，そのほか生化学的検査は正常だった。

治療および経過：粘膜および皮膚症状と病理組織所見より SJS と診断し，ベタメタゾン 8 mg/日（体重 58.2 kg）の投与を開始した。皮疹はすみやかに改善したため，以後ステロイドを漸減，中止した。入院 18 日目で退院した。

パッチテスト方法および判定：被疑薬剤を白色ワセリンにて 10%希釈し，鳥居パッチテスターにて無疹の背部に 48 時間閉鎖貼付した。判定は，ユニット除去 30 分後に 1 回目の判定を行い，その 24 時間後に 2 回目の判定（貼付 72 時間後判定）貼付 1 週間後に 3 回目の判定（貼付 1 週間後判定）を行った。判定には International Contact Dermatitis Research Group（以下 ICDRG）基準を用い，72 時間または 1 週間後に＋以上を陽性とした。

パッチテスト結果：発症 22 日目（ステロイド中止 4 日目）に行ったパッチテストでは両者とも陰性だった。

DLST：発症 19 日目（ステロイド中止 1 日目）と 27 日目（ステロイド中止 9 日目）では陰性であったが，発症 46 日目（ステロイド中止 21 日目）では VPA，ラモトリギンともに陽性となった。

診　断：DLST でラモトリギン，VPA ともに陽性となり，両者によるアレルギーも否定はできなかったが，VPA は数年来内服されており，発症から 15 日前に追加されたラモトリギンによる SJS である可能性が高いと判断した。

症例 2：38 歳，女性。

既往歴：脳性麻痺，てんかん。

現病歴：幼少時よりてんかんに対し VPA を内服しており，2011 年 7 月よりラモトリギン 25 mg/日が追加された。投与 21 日目より感冒症状が出現し，

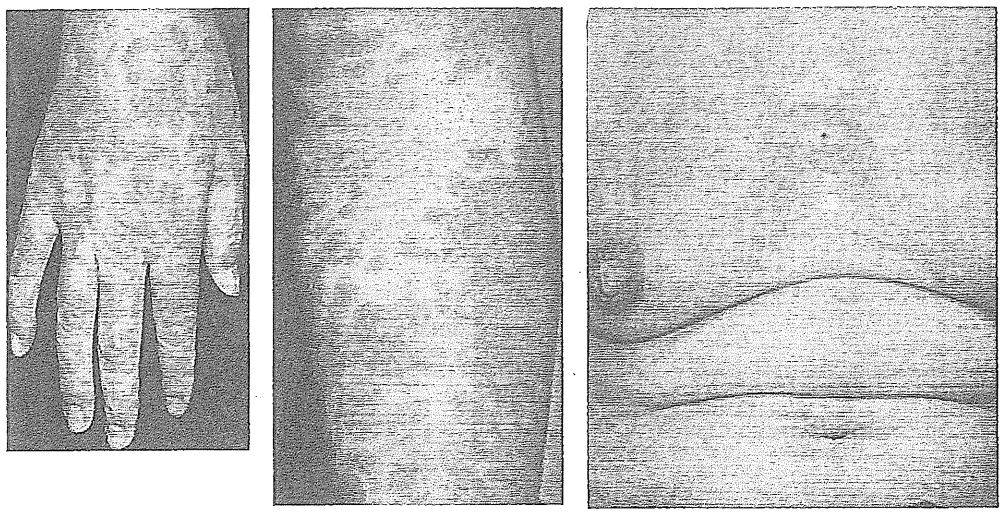

Fig. 3：Case 2；Presence of erythema on the extremities and trunk.

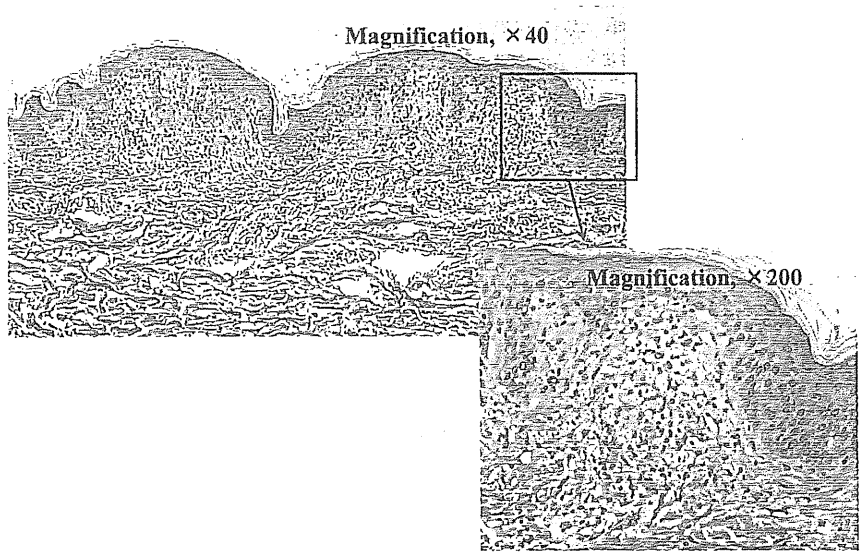

Fig. 4：Case 2；Histopathology showed liquefaction and lymphocyte infiltration in the epidermal basement membrane.

アモキシシリンを内服した。25日目より38度台の熱発とともに全身に紅斑が出現したため，投与より35日目に当科紹介受診し，薬疹の疑いで入院となった。

現　症：体温38.3℃。体幹，四肢に多形紅斑を認め，体幹は癒合して紅皮症となっていた（Fig. 3）。顔面の腫脹と頸部に有痛性のリンパ節腫脹を認めた。粘膜疹はみられなかった。

病理組織所見：表皮基底層の軽度の液状変性と，真皮のリンパ球を主体とする炎症細胞浸潤がみられた（Fig. 4）。

一般検査：WBC 7,440/μl（neutrophil 51.5%，lymphocyte 14.5%，eosinophil 22.5%），AST 84 mU/l，ALT 136 mU/l，CRP 2.089 mg/dl その他の生化学検査は正常だった。

治療と経過：ラモトリギンによるDIHSを疑い，VPA以外の薬剤を中止し補液を開始した。入院3日目に異型リンパ球0.5%が出現した。皮疹の改善なく，38度台の発熱が持続したため，ベタメタゾン8 mg/日（体重60.1 kg）より開始し，症状はすみやかに改善した。プレドニゾロン（以下PSL）20 mg内服で入院14日後に退院となった。退院から14日後，PSL 5 mg/日内服中に紅斑が再燃し，好酸球数増多を認めた。ステロイドを減量せずに経過をみたところ，数日で改善したため，その後緩徐に漸減中止した。

パッチテスト：症例1と同様の方法でラモトリギン，アモキシシリンのパッチテストを施行した。発症136日目（ステロイド中止後70日後）にラモトリギンのみ陽性となった。

DLST：発症12日目（ベタメタゾン静注中）にラモトリギン，アモキシシリン陰性。発症41日目（PSL 5 mg/日内服中）でも両者陰性。発症136日後のDLST（ステロイド中止後70日後）でラモトリギンのみ陽性となった。

ウイルス学的検査：13病日に測定し，ヒトヘルペスウイルス（以下HHV）6型IgM 10倍未満，HHV 6型IgG 80倍だった。24病日に再度測定し，6型IgM 10倍未満，HHV 6型IgG 40倍と再活性化はなかった。

診　断：約2週間遷延する紅斑と発熱，リンパ節腫脹，好酸球増多，肝機能障害があり，HHV-6の再活性化がなかったことから，診断基準よりラモトリギンによるDIHS非典型例と診断した。

症例3：38歳，女性。

既往歴：てんかん。

現病歴：てんかん発作に対し，VPA，アモキサピンを数年来内服していた。2010年9月にアモキサピンを中止し，ラモトリギン100 mg/日に変更された。内服9日目から発熱があり，11日目に紅斑と結膜充血，口唇びらんが出現した。内服開始2週間後にラモトリギンを中止し，翌日当院紹介受診した。

現　症：体温37.8℃。体幹，四肢に多形紅斑を認めた。結膜充血と口唇には出血性のびらんを認めた（Fig. 5）。

病理組織学的所見：表皮に好酸性個細胞壊死と，表皮内のリンパ球浸潤を認めた（Fig. 6）。

一般検査：WBC 6,020/μl（neutrophil 70.0%，lymphocyte 16.6%，eosinophil 5.5%），AST 42 mU/l，ALT 27 mU/l CRP 5.814 mg/dl その他の生化学検査は正常だった。

治療と経過：入院のうえ，ベタメタゾン7 mg/日（体重52.5 kg）を開始し，皮疹は改善した。以後漸減中止し，入院15日後に退院となった。

パッチテスト：症例1と同様の方法でラモトリギンのパッチテストを施行した。発症78日目（ステロイド中止後19日目）のパッチテストは陰性だった。

DLST：発症10日目（ベタメタゾン静注中）のDLSTは陰性だった。

診　断：臨床および病理所見と経過よりラモトリギンによるSJSと診断した。

症例4：32歳，女性。

既往歴：脳性麻痺，てんかん。

現病歴：2002年よりVPA，フェニトイン，クロナゼパム，クロバザム，アセタゾラミドを内服していた。2011年5月よりラモトリギン25 mg隔日投与を開始し，投与2週間後に25 mg/日に増量された。投与開始から28日後に結膜充血，流涙が出現した。内服33日後には口唇のびらん，四肢に皮疹が出現し，37度台の発熱も認めたため，同日当科紹介受診となった。

現　症：体温36.6℃。両眼瞼結膜に充血，眼脂があり，上下口唇に出血性びらんを認めた。口腔内にもびらんを認めた。体幹，四肢に皮疹はみられなかった。眼科にて，結膜炎，角膜炎が確認された。偽膜，結膜びらんは認めなかった（Fig. 7）。

病理組織学的所見：皮膚生検は施行しえなかった。

一般検査：末梢血でWBC 9,820/μl（neutrophil 82.0%，lymphocyte 10.0%，eosinophil 6.0%）AST 23 mU/l，ALT 35 mU/l，γ-GTP 76 U/l，CRP 0.418 mg/dl そのほかの生化学的検査は正常だった。

治療と経過：経過よりラモトリギンによるSJSを疑い，入院のうえ，VPAをのぞくすべての薬剤を中止した。入院後もてんかん発作の頻発があったため，神経内科と相談のうえクロバザムを再開した。ベタメタゾン4 mg/日（体重27.3 kg）の全身投与を開始し，症状は軽快した。以後漸減中止し，入院18日後に退院となった。退院時，眼症状も改善し，その後も後遺症はなかった。

パッチテスト：症例1と同様の方法でラモトリギンのパッチテストを施行した。発症59日後（ステロイド中止33日目）にフェニトイン，アセタゾラミド，クロバザムについてパッチテストを行いすべて陰性であった。

DLST：経過と重症薬疹の発症頻度よりラモトリギン，フェニトインを被疑薬と考え，2剤についてDLSTを施行した。発症23日目（PSL 10 mg/日内服中）および発症59日目（ステロイド中止後33日目）に施行されいずれも陰性だった。

診　断：ラモトリギン以外の薬剤は約10年間継続使用されており，発症前の追加薬剤はラモトリギンのみであったため，パッチテスト，DLSTは陰性であったものの経過よりラモトリギンによるSJSと診断した。