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ORIGINAL ARTICLE

A whole-genome association study of major determinants for allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients

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Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) are severe, cutaneous adverse drug reactions that are rare but life threatening. Genetic biomarkers for allopurinol-related SJS/TEN in Japanese were examined in a genome-wide association study in which Japanese patients ($n=14$) were compared with ethnically matched healthy controls ($n=991$). Associations between 890 321 single nucleotide polymorphisms and allopurinol-related SJS/TEN were analyzed by the Fisher's exact test (dominant genotype mode). A total of 21 polymorphisms on chromosome 6 were significantly associated with allopurinol-related SJS/TEN. The strongest association was found at rs2734583 in *BAT1*, rs3094011 in *HCP5* and GA005234 in *MICC* ($P=2.44\times10^{-8}$; odds ratio = 66.8; 95% confidence interval, 19.8–225.0). rs9263726 in *PSORS1C1*, also significantly associated with allopurinol-related SJS/TEN, is in absolute linkage disequilibrium with *human leukocyte antigen-B*5801*, which is in strong association with allopurinol-induced SJS/TEN. The ease of typing rs9263726 makes it a useful biomarker for allopurinol-related SJS/TEN in Japanese.
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Introduction

Allopurinol is a xanthine oxidase inhibitor that prevents the production of uric acid to reduce plasma uric acid levels to a normal range. It is the most frequently used anti-hyperuricemic agent in the world due to its long-term pharmacological effect.[1] However, allopurinol is also one of the most frequent causes of a variety of delayed severe cutaneous adverse drug reactions (SCARs).[2] According to spontaneous reports of severe adverse drug reactions to the Ministry of Health, Labor, and Welfare of Japan, allopurinol-related SCARs accounted for about 11% of all reported SCAR cases in Japan in 2008.[3] Allopurinol-related SCARs include the drug-induced hypersensitivity syndrome, Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[4] SJS/TEN are characterized by high fever, malaise and rapid development of blistering exanthema, with macules and target-like lesions, accompanied by mucosal involvement.[5] Even though the incidence of SJS/TEN is extremely low, the mortality rate of TEN can be as high as 26%.[5] Therefore, SJS/TEN is a serious problem in allopurinol therapy, in spite of the ideal anti-hyperuricemic effect of allopurinol.

Although previous works have suggested that the development of SJS/TEN depends on an immune mechanism involving a drug-dependent cytotoxic cell response against epidermal cells,[5,6] the pathophysiology of SJS/TEN remains largely unknown. Susceptibility to such idiosyncratic reactions is thought to be genetically determined, and familial predisposition to allopurinol-induced SJS/TEN has been reported.[6] Therefore, the exploratory studies for genetic risk factors related to SJS/TEN are needed. A strong association has been observed between allopurinol-induced SCAR and the human lymphocyte antigen (*HLA*) allele B variant (*HLA-B*5801*) in the Han Chinese in Taiwan[7] and in the Thai population.[8] These studies showed that the *HLA-B*5801* allele is present in all patients with allopurinol-induced SCAR (51/51 of Han Chinese and 27/27 of Thai patients) and in only 12–15% of tolerant patients (20/135 and 7/54, respectively). The odds ratio (OR) was 580 (95% confidence interval , 34–9781; $P = 4.7 \times 10^{24}$) for the Han-Chinese data[7] and 348.3 (95% confidence interval, 19.2–6336.9; $P = 1.61 \times 10^{13}$) for the Thai study.[8] Although the association was confirmed in both Caucasian and Japanese subjects,[9,10] the OR in the Han-Chinese and Thai populations were much higher than those in the Caucasian (OR = 80) and Japanese (OR = 40) groups. These reports indicated that *HLA-B*5801* is the valid genetic biomarker for allopurinol-induced SJS/TEN in various ethnic groups, but the mechanisms by which *HLA-B*5801* is specifically involved in allopurinol-induced SJS/TEN progression and the strength of the association showed ethnic differences are unknown.

Currently, genotyping by high-density array scanning of the whole genome allows discovery of previously unsuspected genetic risk factors that influence the pathogenesis of serious adverse drug reactions.[11–13] Genome-wide association studies (GWASs) provide opportunities to uncover polymorphisms that influence susceptibility to allopurinol-induced SJS/TEN free of mechanistic hypotheses. Therefore, in addition to *HLA-B* typing as shown in our previous study,[10] we further conducted a retrospective pharmacogenetic case–control study using whole-genome single nucleotide polymorphism (SNP) data from high-density DNA microarrays in order to identify new and effective genetic biomarkers for allopurinol-related SJS/TEN in Japanese patients.

Materials and methods

Recruitment of study subjects

A total of 141 Japanese SJS/TEN patients from unrelated families were recruited from July 2006 to April 2010 from participating institutes of the Japan Severe Adverse Reactions (JSAR) research group and through a nationwide blood-sampling network system in Japan for SJS/TEN onset patients, operated by the National Institute of Health Sciences.[10] In all, 121 of these patients were diagnosed as defined SJS or TEN by JSAR research group's dermatological experts based on diagnostic criteria[4] that are currently used in Japan. Information was collected using a standardized case report form that includes medical records, co-administrated drug records, disease progress and involvement of systemic complications, as well as SJS/TEN treatment. Among the 141 SJS/TEN patients, 20 were diagnosed as probable SJS due to atypical or mild symptoms. TEN and SJS were defined as mucocutaneous disorders characterized by extensive erythema, blisters, epidermal detachment, erosions, enanthema and high fever. SJS was defined as skin detachment of 10% or less of the body surface area, and TEN as skin detachment of more than 10%, excluding staphylococcal scaled skin syndrome.[5] In all enrolled cases defined as SJS or TEN, allopurinol was regarded as the drug responsible for SJS or TEN if the onset of SJS/TEN symptoms occurred within the first 2 months of allopurinol exposure. For the retrospective pharmacogenetic case–control study, 991 healthy, ethnically matched subjects in the Tokyo metropolitan area were used as the control group. Healthy subjects were used as the control group instead of allopurinol-tolerant patients because the incidence of SJS/TEN is extremely low (0.4–6 per million per year).[3]

The ethics committees of the National Institute of Health Sciences, each participating institute of the JSAR research group and the Japan Pharmacogenomics Data Science Consortium (JPDSC) approved this study. Written informed consent was obtained from all cases and ethnically matched controls.

Whole-genome genotyping of SNPs

Genome-wide genotyping of the 14 allopurinol-related SJS/TEN patients and 991 ethnically matched controls was conducted using the Illumina Human 1M-Duo BeadChip (Illumina, San Diego, CA, USA), which contained 1 163 218 SNPs. SNPs were discarded from case–control association analysis if they exhibited a minor allele frequency <0.001 in the control group (237 890 SNPs), a call rate <0.95 for each SNP (32 640 SNPs) or a P-value <0.001 in the test of Hardy–Weinberg equilibrium among controls (2 368 SNPs). These quality control steps removed a total of 272 897 SNPs. All samples had a call rate for each microarray above 0.99. Sample duplicates and hidden relatedness were investigated on the basis of pairwise identity-by-state analysis via PLINK;[14] however, there was no duplicate or hidden relatedness in the samples. This quality-control procedure ensured reliable genotyping data.

HLA genotyping and TaqMan genotyping of SNPs on chromosome 6

HLA A, *B* and *Cw* types were determined using sequencing-based methods, as described previously.[10] Representative SNPs of 6p21 (rs2734583, rs3099844, rs9263726 and rs3131643) were re-genotyped using TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA) (ID; C__27465749_10, C__27455402_10, C__30352071_10, C__26778946_20) according to the manufacturer's instruction using 5 ng of genomic DNA. We did not genotype rs9267445 and rs1634776 because TaqMan SNP genotyping assays for these SNPs were not available. Measurement of the linkage disequilibrium (LD) coefficient was performed using

the *HLA* types and 6p21 SNPs of the 141 Japanese SJS/TEN cases and an additional 65 Japanese individuals (non-SJS/TEN patients). The LD coefficient was calculated as previously described.[15,16]

Association analysis

Genome-wide SNPs data from allopurinol-related SJS/TEN cases and ethnically matched controls were used for association analysis using the Fisher's exact test based on the dominant genotype mode and minor allele frequencies of each SNP. Because there are no homozygotes of minor alleles of SNPs, which have significantly related to allopurinol-related SJS/TEN except rs3099844 and rs3131643 in 'Case group', other association analysis models such as trend test (Cochran–Armitage analysis) or recessive model analysis were not applied in this study. All association analyses were carried out with PLINK.[14] *P*-values were corrected for multiple testing according to the Bonferroni's correction. *P*-values $<5.62\times10^{-8}$ were regarded as statistically significant.

Results

Characteristics of study subjects

A total of 14 allopurinol-treated Japanese patients, who were diagnosed with definite SJS/TEN were recruited for the whole-genome association study (IDs 1–14 in Table 1). Patients, IDs 1, 2, 3, 9, 10, 13 and 14 were reported in our previous paper.[10] After the GWAS, an additional four allopurinol-treated Japanese SJS/TEN patients were recruited for *HLA* typing (IDs 15–18). Therefore, a total of 18 allopurinol-treated Japanese SJS/TEN patients participated in the study (Table 1). In all, 12 of 18 patients were male and 6 were female, and the average age was 72.3 ± 10.0 (mean $\pm$ s.d.) years. In all, 12 of 18 cases showed systemic complications of liver and/or renal dysfunction, and most patients had high fever. The average period of SJS/TEN onset after allopurinol treatment was 21.7 ± 11.9 days. Drug-induced lymphocyte stimulation tests were examined in 13 of 18 patients to determine the causative agent; however, in these tests, only two cases (IDs 1 and 5) were positive for allopurinol and only one (ID 16) was positive for oxipurinol, a metabolite of allopurinol. The patient (ID 1) who was positive for the drug-induced lymphocyte stimulation test for allopurinol was also positive for other co-administrated drugs (Table 1). On the other hand, patients who received a patch test showed positive reactions for allopurinol although only two patients were examined (ID 4, 10). The patient who was patch test positive for allopurinol (ID 4) was also patch test positive for other co-administrated drugs (Table 1). Four patients (ID 1, 2, 4 and 14) were co-administrated non-steroidal anti-inflammatory drugs, four (ID 7, 8, 11 and 15) were co-administrated angiotensin II receptor antagonists and three (ID 4, 7 and 17) were co-administrated statin anti-hyperlipemic agents.

Whole-genome association study of major determinants for allopurinol-related SJS/TEN

A total of 14 allopurinol-related SJS/TEN patients (IDs 1–14), who were diagnosed with definite SJS/TEN, and 991 ethnically matched controls, were genotyped with the use of the Illumina Human 1M-Duo BeadChip containing 11 63 218 SNPs. A series of quality-control steps resulted in the elimination of 2 72 897 polymorphisms. For each SNP, Fisher's exact tests were performed to compare the dominant genotype distributions and minor allelic frequencies in the allopurinol-related SJS/TEN patients (the case group) versus those in the ethnically matched healthy control group. The resulting *P*-values were adjusted with the Bonferroni's correction ($P<5.62\times10^{-8}$). The distribution of *P*-values from the Fisher's exact tests (dominant genotype mode) along each chromosome indicated that 21 SNPs were significantly associated with the cases, all of which were located on the chromosome 6: 6p21.3, 6p22.1 and 6p21.1 (Figures 1a and b). The quantile–quantile (Q–Q) plot for the distribution of *P*-values showed that observed *P*-values matched the expected *P*-values over the range of $0<-\log_{10}(p)<4.0$ (Figure 2). A departure was observed at the extreme tail ($-\log_{10}(p)>4.0$) of the distribution of test statistics for the allopurinol-related Japanese SJS/TEN, suggesting that the identified associations are likely due to true variants rather than potential biases such as genotyping error. These SNPs, with their associated genes, are described in Table 2. As is observed in all SNPs in Table 2, minor allele frequencies in the controls were quite small, ranging around 0.5–0.6%. The genotypic distributions of the case and control groups are identical among groups with the same *P*-value, suggesting that these SNPs might be linked. These SNPs also have ORs that are much higher than the ORs of SNPs commonly observed in sporadic cancer and other complex diseases, suggesting they are of higher penetrance. For example, the most significant SNPs (rs2734583, rs3094011 and GA005234) had an OR of 66.8 (95% confidence interval, 19.8–225.0), and the twentieth most significant SNPs (rs9263827 and rs1634776) had an OR of 60.9 (95% confidence interval, 18.3–202.5). Most SNPs in Table 2 are associated with known or predicted genes; of these, 13 are in known genes. Three SNPs (rs17190526, rs9263726 and rs2233945) were found in *PSORS1C1* (psoriasis susceptibility 1 candidate 1), which is considered as one of the potential psoriasis genes.[17–19] The *CCHCR1* (coiled coil α helical rod protein 1), which is a regulator of keratinocyte proliferation or differentiation and is over-expressed in keratinocytes in psoriatic lesions,[20–23] contained four SNPs (rs9263745, rs130077, rs9263781 and rs9263785). *HCP5* (HLA complex P5), which is involved in hypersensitivity to abacavir,[24–26] had three SNPs (rs3094011, rs3099844 and rs31431643). *TCF19* (transcription factor 19), which is a potential trans-activating factor that might play an important role in the transcription of genes required for the later stages of cell cycle progression,[27] contained two SNPs (rs9263794 and rs10448701). Two SNPs (rs9263796 and rs9263800) were also found in *POU5F1* (POU class 5 homeobox; alternative names for Oct4). *BAT1* (HLA-B

Table 1 Summary of clinical characteristics of Japanese patients with allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis

								Co-administered drugs	
Patient ID[a]	*ADR type*	*Sex/age (years)*	*Highest BT (°C)*	*Total area of blistering skin (%)*	*Systemic complications*	*DLST to allopurinol (PT)*	*Period of onset (days) by allopurinol*	*Drug name*	*DLST result/period of onset*
1	SJS	F/53	38.1	0.5	liver dysfunction renal dysfunction	+	26	loxoprofen clarithromycin	+/9 days +/26 days
2	TEN	M/58	37.1	15	neutropenia liver dysfunction	–	ca 10 days	loxoprofen levofloxacin	–/1 day –/1 day
3	SJS	M/77	unknown	unknown	none	not tested	16	none	
4	TEN	F/72	>37	20	none	–(PT+)	16	pitavastatin lansoprazole salicylamide, acetaminophen, caffeine, promethazine, methylenedisalicylate serrapeptase loxoprofen acetaminophen	–/16 days –/179 days –(PT+)/8 days –/1 day –/8 days (PT+)/8 days
5	TEN	M/82	39	35	none	+	52	none	
6	SJS	M/67	1	1	liver dysfunction	not tested	14	none	
7	SJS	M/76	38.8	unknown	GI tract disturbance liver dysfunction renal dysfunction	not tested	<26 days	losartan furosemide carbon atorvastatin	not tested/8 days not tested/3 days not tested/7 days not tested/8 days
8	SJS	M/83	>38	10	renal dysfunction	–	20	amlodipine olmesartan medoxomil	not tested/very long not tested/very long
9	TEN	M/75	>38	20	neutropenia liver dysfunction renal dysfunction	–	6	none	
10	SJS	M/75	38.4	6	neutropenia liver dysfunction renal dysfunction	–(PT+)	14	none	
11	SJS	M/74	37.8	8	neutropenia liver dysfunction renal dysfunction	–	38	cefazolin Furosemide Sodium polystyrene sulfonate olmesartan medoxomil	not tested/1 day not tested/53 day not tested/51 day not tested/59 day
12	SJS	M/67	38.9	2	liver dysfunction	not tested	17	none	
13	SJS	F/81	39.2	0.5	renal dysfunction	–	28	spironolactone	–/24 days
14	SJS	M/83	39	0	respiratory involvement	–	29	diclofenac	–/1 day
15	TEN	F/73	38	10	liver dysfunction renal dysfunction	–	27	valsartan epoetin β	–/18 days –/2 days
16	SJS	M/53	40	5	liver dysfunction	–(oxipurinol +)	19	none	
17	SJS	F/86	38	0	liver dysfunction renal dysfunction	–	30	rosuvastatin	–/43 days
18	TEN	F/66	37.8	15	none	not tested	2	none	

Abbreviations: ADR, adverse drug reaction; BT, body temperature; DLST; drug-induced lymphocyte stimulation test; F, female; M, male; PT, patch test; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

[a]Patients ID 1–14 were applied for whole genome analysis. ID 1–18 were for the *HLA* typing and the analysis of linkage disequilibrium.

Patients IDs 1, 2, 3, 9, 10, 13, and 14 were reported in our previous paper.[10]

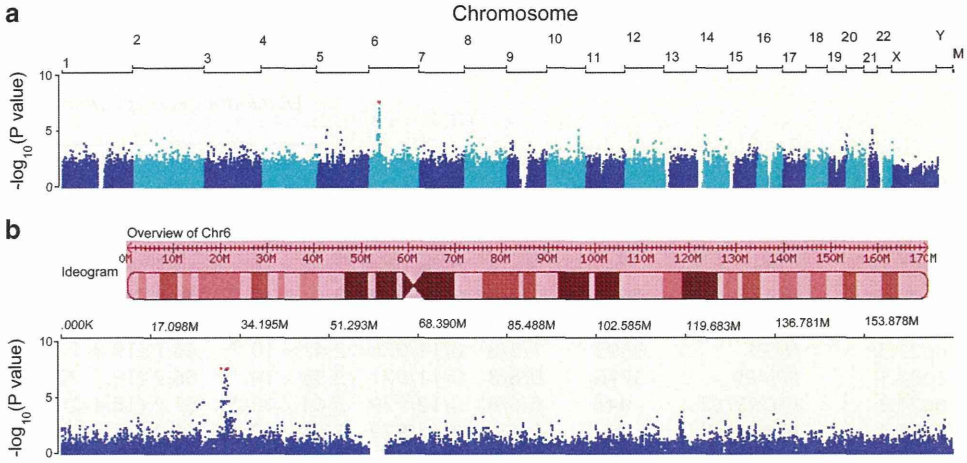

Figure 1 Genome-wide association study of allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis. Each dot represents a single nucleotide polymorphism (SNP). The *x* axis: the position of the SNP on chromosomes. The *y* axis: the $-\log_{10}$ of Fisher's exact test *P*-values (dominant genotype mode) of the SNP in the case–control association study. SNPs with *P*-values $<5.62 \times 10^{-8}$ are highlighted in red. (**a**) Whole genome. (**b**) Chromosome 6.

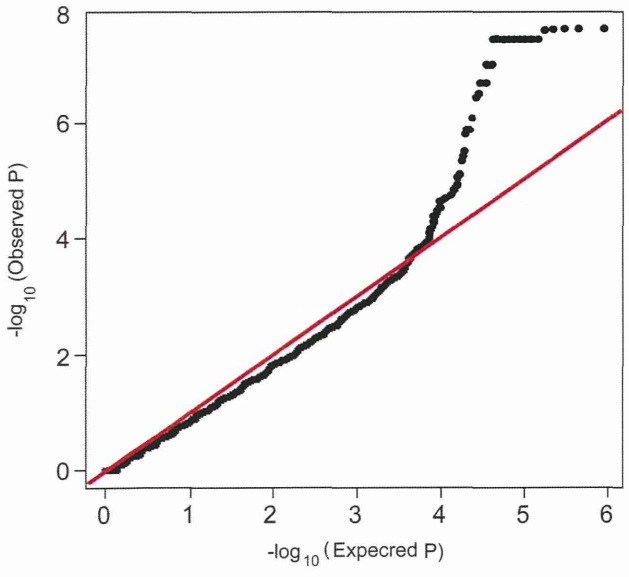

Figure 2 Quantile–quantile plot of Fisher's exact test statistics obtained from the genome-wide association study for allopurinol-related Stevens–Johnson syndrome or toxic epidermal necrolysis under dominant genotype mode. The solid red line represents the null model where observed Fisher's exact test values match the expected values. The dots represent observed versus the expected values from the case–control study.

associated transcript 1) and *PSORS1C3* each carried one SNP (rs2734583 and rs9263827). The SNPs, rs1634776 and rs4084090, were located in more than 10 kb away from the *HLA-B* and *HLA-C* genes, respectively. Two pseudo genes, *MICC* (major histocompatibility complex class I polypeptide-related sequence) and *PPIAP9* (peptidylprolyl isomerase A (cyclophilin A) pseudogene 9), had one SNP each (GA005234 and rs9267445). Previous report using Han-Chinese patients with allopurinol-induced SCAR indicated rs3117583 of *BAT3*, rs1150793 of *MSH5* and rs2855804 of *MICB*, which are located in *HLA* region, showed significant *P*-values ($P<1 \times 10^{-7}$).[7] In this study using Japanese patients, both rs3117583 and rs1150793 showed $P=6.34 \times 10^{-3}$ (allele frequency mode) and $P=6.14 \times 10^{-3}$ (dominant genotype mode). There was no data of rs2855804 in the Illumina Human 1M-Duo BeadChip.

HLA types of allopurinol-related SJS/TEN patients

Classical class I *HLA* types (*A*, *B* and *Cw*) of allopurinol-related SJS/TEN patients were determined because the *HLA-B*5801* type is associated with allopurinol-related SCARs in Han Chinese,[7] Caucasians[9] and Japanese[10] (Table 3). In this analysis, four patients with allopurinol-related SJS/TEN (IDs 15–18), who were recruited after BeadChip analysis, joined the case group (total of 18 allopurinol-related SJS/TEN patients). Eight cases of *HLA-A*3303* (allele frequency = 22.2%), 10 cases of *HLA-B*5801* (allele frequency = 27.8%) and 10 cases of *HLA-Cw*0302* (allele frequency = 27.8%) were found in 18 allopurinol-related SJS/TEN patients (Table 3). By comparison, the allelic frequencies of *HLA-A*3303*, *HLA-B*5801* and *HLA-Cw*0302* were 7.9%, 0.6% and 0%, respectively in Japanese general population (Tables 4a–c). The OR of *HLA-A*3303* was calculated as 3.32 (Table 4a). The OR of *HLA-B*5801* was calculated as 62.8 (Table 4b), which was a little larger than the previously reported OR in Japanese patients.[10] *HLA-Cw*0302* also showed significant association with allopurinol-related SJS/TEN (Table 4c). *HLA-A*3303* and *HLA-Cw*0302* are in LD with *HLA-B*5801* in the Japanese although the general frequency of *HLA-A*3303* is higher than other two types. Other *HLA-A*, *B* and *Cw* types, which were not listed in Tables 4a–c, showed very low frequencies in the general Japanese population, or were not found in 18 allopurinol-related SJS/TEN patients.

Table 2 The association of single nucleotide polymorphism with allopurinol-related Japanese patients with Stevens–Johnson syndrome or toxic epidermal necrolysis

							Dominant genotype mode		*Allelic frequency mode*	
Order	*SNP*	*Chromosome*	*Closest gene*	*Distance to gene (bp)*	*Case*[a]	*Control*[a]	P	*Odds ratio (95% CI)*	P	*MAF (%)*
1	rs2734583	6p21.3	*BAT1*	0	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
1	rs3094011	6p21.3	*HCP5*	6553	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
1	GA005234	6p22.1	*MICC*	0	0/6/8	0/11/980	2.44×10^{-8}	66.8 (19.8–225.0)	4.62×10^{-8}	0.55
4	rs3099844	6p21.3	*HCP5*	3693	1/5/8	0/11/978	2.47×10^{-8}	66.7 (19.8–224.5)	1.33×10^{-9}	0.56
5	rs9267445	6p21.1	*PPIAP9*	3776	0/6/8	0/11/971	2.58×10^{-8}	66.2 (19.7–222.9)	4.87×10^{-8}	0.56
6	rs17190526	6p21.3	*PSORS1C1*	−446	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263726	6p21.3	*PSORS1C1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs2233945	6p21.3	*PSORS1C1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263733	6p21.3	*POLR2LP*	139	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263745	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs130077	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263781	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263785	6p21.3	*CCHCR1*	0	0/6/8	0/12/979	2.44×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263794	6p21.3	*TCF19*	0	0/6/8	0/12/979	2.47×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs1044870	6p21.3	*TCF19*	0	0/6/8	0/12/979	2.58×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263796	6p21.3	*POU5F1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs9263800	6p21.3	*POU5F1*	0	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
6	rs4084090	6p21.3	*HLA-C*	17691	0/6/8	0/12/979	3.64×10^{-8}	61.2 (18.4–203.5)	6.87×10^{-8}	0.61
19	rs3131643	6p21.3	*HCP5*	0	1/5/8	0/12/977	3.68×10^{-8}	61.1 (18.4–203.1)	2.08×10^{-9}	0.61
20	rs9263827	6p21.3	*PSORS1C3*	−3369	0/6/8	0/12/974	3.75×10^{-8}	60.9 (18.3–202.5)	7.07×10^{-8}	0.61
20	rs1634776	6p21.3	*HLA-B*	12661	0/6/8	0/12/974	3.75×10^{-8}	60.9 (18.3–202.5)	7.07×10^{-8}	0.61

Abbreviations: CI, confidence interval; MAF; minor allelic frequency; SNP, ingle nucleotide polymorphism.
[a]Number of subjects in minor homo/hetero/major homo.

Table 3 *HLA* types and representative genotypes in 6p21 of allopurinol-related Japanese patients with Stevens–Johnson syndrome or toxic epidermal necrolysis

ID	HLA-A		HLA-B		HLA-Cw		*rs2734583*	*rs3099844*	*rs9267445*	*rs9263726*	*rs3131643*	*rs1634776*
1	2402	3303	4002	**5801**	0302	0304	T/**C**	C/**A**	G/**C**	G/**A**	C/**T**	G/**A**
2	2402	3101	1501	5601	0303	0401	T/T	C/C	G/G	G/G	C/C	G/G
3	2402	3101	5201	**5801**	0302	1202	T/**C**	C/**A**	G/**C**	G/**A**	C/**T**	G/**A**
4	1101	1101	4801	**5801**	0302	0803	T/**C**	**A**/**A**	G/**C**	G/**A**	**T**/**T**	G/**A**
5	2402	2602	4006	5101	0801	1402	T/T	C/C	G/G	G/G	C/C	G/G
6	0201	1101	1518	3501	0401	0801	T/T	C/C	G/G	G/G	C/C	G/G
7	2402	3303	5201	**5801**	0302	1202	T/**C**	C/**A**	G/**C**	G/**A**	C/**T**	G/**A**
8	0201	2402	1527	4003	0304	0401	T/T	C/C	G/G	G/G	C/C	G/G
9	2402	2402	3501	5201	0303	1202	T/T	C/C	G/G	G/G	C/C	G/G
10	0210	1101	4002	4006	0401	0801	T/T	C/C	G/G	G/G	C/C	G/G
11	0207	2402	4601	5101	0102	1402	T/T	C/C	G/G	G/G	C/C	G/G
12	2402	3101	3901	4001	0304	0702	T/T	C/C	G/G	G/G	C/C	G/G
13	0207	3303	4601	**5801**	0102	0302	T/**C**	C/**A**	G/**C**	G/**A**	C/**T**	G/**A**
14	3101	3303	3901	**5801**	0302	0702	T/**C**	C/**A**	G/**C**	G/**A**	C/**T**	G/**A**
15	2402	3303	5101	**5801**	0302	1402	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA
16	0201	3303	3802	**5801**	0302	0702	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA
17	2402	3303	0702	**5801**	0302	0702	T/**C**	C/**A**	NA	G/**A**	C/**T**	NA
18	2402	3303	5101	**5801**	0302	0304	T/**C**	C/**A**	NA	G/**A**	**T**/**T**	NA

Abbreviations: HLA, human leukocyte antigen; NA, not available.
Single nucleotide polymorphisms data of rs2734583, rs3099844, rs9263726 and rs3131643 are from BeadChip analysis and TaqMan genotyping analysis. Single nucleotide polymorphisms data of rs9267445 and rs1634776 are from BeadChip analysis.
Underlines of HLA types mean that these types are in linkage disequilibrium. HLA-B*5801s are expressed by bold types.
Bold types of the nucleotide mean the variant allele.

Table 4a Association between *HLA-A* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

HLA-A allele	*Number of alleles detected (allele frequency)*		P	*Odds ratio (95% CI)*
	Case, n = 36 (%)	*General population control (n = 986)[a] (%)*		
0201	3 (8.3)	10.9	0.7895	
0206	0 (0)	10.4	0.0426	
0207	2 (5.6)	3.4	0.3650	
0210	1 (2.8)	0.1	0.0692	
1101	4 (11.1)	8.1	0.5299	
2402	13 (36.1)	35.6	1.000	1.02 (0.51–2.04)
2601	0 (0)	9.8	0.0417	
2602	1 (2.8)	2.2	0.5657	
3101	4 (11.1)	7.7	0.5195	
3303	8 (22.2)	7.9	0.0077	3.32 (1.46–7.54)

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-A* types of which the allele frequencies in the Japanese population are more than 9% or which were detected in this study.
[a]General population control data are cited from Tanaka *et al.*[40]

Table 4b Association between *HLA-B* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

HLA-B allele	*Number of alleles detected (allele frequency)*		P	*Odds ratio (95% CI)*
	Case, n = 36 (%)	*General population control (n = 986)[a] (%)*		
0702	1 (2.8)	5.2	1.000	
1501	1 (2.8)	7.2	0.5076	
1518	1 (2.8)	0.9	0.3025	
1527	1 (2.8)	0	0.0352	
3501	2 (5.6)	8.6	0.7621	
3802	1 (2.8)	0.3	0.1338	
3901	2 (5.6)	4.0	0.6520	
4001	1 (2.8)	5.1	1.0000	
4002	2 (5.6)	8.2	0.7620	
4003	1 (2.8)	1.1	0.3512	
4006	2 (5.6)	5.3	0.7150	
4403	0 (0)	6.9	0.1648	
4601	2 (5.6)	3.8	0.6441	
4801	1 (2.8)	2.7	1.0000	
5101	4 (11.1)	7.9	0.5244	
5201	3 (8.3)	13.7	0.4624	
5401	0 (0)	6.5	0.1620	
5601	1 (2.8)	1.0	0.3273	
5801	10 (27.8)	0.6	5.388×10^{-12}	62.8 (21.2–185.8)

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-B* types of which the allele frequencies in the Japanese population are more than 6.5% or which were detected in this study.
[a]General population control data are cited from Tanaka *et al.*[40]

*LD of HLA-B*5801 with SNPs on chromosome 6*

We compared the genotypic distributions of six SNPs, which were significantly associated with SJS/TEN (Table 2), with *HLA* types because these SNPs are located near the *HLA-B* gene. These 6 SNPs listed in Table 3 represent 21 SNPs in Table 2 because the other 15 SNPs are in absolute LD with 1 of the 6 SNPs. Representative six variants of the significant SNPs on chromosome 6 were found in all of the SJS/TEN patients who carried the *HLA-B*5801* (10 patients) (Table 3). Therefore, in order to evaluate LD in the Japanese

Table 4c Association between *HLA-Cw* alleles and allopurinol-induced Stevens–Johnson syndrome or toxic epidermal necrolysis

	Number of alleles detected (allele frequency)			
HLA-Cw allele	*Case,* n = *36 (%)*	*General population control (*n = *234)*[a] *(%)*	P	*Odds ratio (95% CI)*
0102	2 (5.6)	17.0	0.0859	
0302	10 (27.8)	0	5.303×10^{-10}	
0303	2 (5.6)	7.8	1.000	
0304	4 (11.1)	11.3	1.000	
0401	4 (11.1)	6.5	0.2961	
0702	4 (11.1)	11.3	1.000	
0801	3 (8.3)	10.9	0.7777	
0803	1 (2.8)	2.6	1.000	
1202	3 (8.3)	10.4	1.000	
1402	3 (8.3)	5.7	0.4559	
1403	0 (0)	12.2	0.0192	

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen.
We listed the *HLA-Cw* types of which the allele frequencies in the Japanese population are more than 10% or which were detected in this study.
[a]General population control data are cited from Tokunaga *et al.*[41]

Table 5 The linkage disequilibrium between *HLA* types and representative single nucleotide polymorphisms on 6p21 of 206 Japanese individuals

HLA	*rs3099844*	*rs3131643*	*rs2734583*	*rs9267445*	*rs9263726*	*rs1634776*
A	0.821	0.621	0.835	0.798	0.847	0.803
B	0.973	0.873	1.000	1.000	1.000	0.996
Cw	0.984	0.773	1.000	1.000	1.000	0.909

Abbreviation: HLA, human leukocyte antigen.
Data are expressed in D'.

Table 6 The linkage disequilibrium between representative single nucleotide polymorphisms on 6p21 and *HLA-B*5801* of 206 Japanese individuals

SNP	D'	r^2
rs3099844	0.930	0.866
rs3131643	0.929	0.674
rs2734583	1.000	0.931
rs9267445	1.000	0.896
rs9263726	1.000	1.000
rs1634776	1.000	0.905

Abbreviation: SNP, single nucleotide polymorphism.

population, LD coefficients (D') were calculated between classical class 1 *HLA* types and six representative SNPs at 6p21, using the *HLA*-type and SNPs genotype data of 206 Japanese individuals, including 141 SJS/TEN cases and an additional 65 non-SJS/TEN Japanese subjects. As shown in Tables 5 and 6 representative SNPs on chromosome 6 showed LD for the *HLAs*. In particular, three SNPs (rs2734583, rs9267445 and rs9263726) showed a strong linkage with *HLA-B* and *Cw* alleles (Table 5). LD between six representative SNPs in 6p21 and *HLA-B*5801* are shown in Table 6. A novel observation was the absolute LD ($D' = 1$, $r^2 = 1$) between rs9263726 in *PSORS1C1* and the *HLA-B*5801* allele.

Discussion

In order to explore new genetic biomarkers associated with the occurrence of allopurinol-related SJS/TEN Japanese patients, we conducted a GWAS using 890321 SNPs from patients with allopurinol-related SJS/TEN and an ethnically matched control group. The GWAS data indicated that most SNPs significantly associated with allopurinol-related SJS/TEN are located on or close to genes that overlap the 6p21 region, especially the genes neighboring *HLA-B*. There was no significantly associated SNP in any other region of the genome (Figures 1 and 2 and Table 2), indicating that the 6p21 region has the most important role in the progress of allopurinol-related SJS/TEN. We expected to find SJS/TEN-associated SNPs, which are unrelated to *HLA-B*5801* from this GWAS study because the association of *HLA-B*5801* with SJS/TEN is incomplete (10/18) in Japanese patients in contrast to Han Chinese[7] and Thai patients.[8] However, most

of significant SNPs were closely linked with *HLA-B*5801* (Table 6). Previous studies have indicated that a SNP (rs2395029) in the *HCP5*, which is on 6p21.3, is strongly associated with human immunodeficiency virus-1 set points,[28–30] abacavir-induced hypersensitivity[24–26] and flucloxacillin-induced liver injury.[31] This SNP is in strong LD with *HLA-B*5701* in Caucasians.[25] Another SNP in 6p21 in *PSORS1C1*, a psoriasis-susceptibility candidate gene, was related with psoriasis in Swedish and Canadian populations[17,18] and exhibits LD with *HLA-Cw*0602* in Canadian populations.[18] These reports suggest that SNPs located in 6p21 link with a specific type of classical class I *HLA* that could be an alternative biomarker for the physiological phenomenon. Therefore, we examined the LD between these SNPs, shown in Table 2, and *HLA-B*5801*, which has been regarded as a genetic biomarker of SJS/TEN not only in Han Chinese,[7] but also in Caucasians[9] and Japanese.[10] We found that all of the Japanese patients with the allopurinol-related SJS/TEN who had the *HLA-B*5801* (10 patients) also had variant SNPs of genes that are located in 6p21, including *BAT1*, *HCP5*, *PPIAP9*, *PSORS1C1* and *HLA-B* (Table 3). The analysis of the LD coefficients between SNPs located in 6p21 and *HLA* types in the Japanese population indicated that these SNPs are in strong LD with *HLA* types (Table 5), and an absolute LD between rs9263726 in *PSORS1C1* and *HLA-B*5801* was observed in the Japanese population (Table 6). These results mean that all subjects (14 individuals including 10 with allopurinol-related SJS/TEN) who carry *HLA-B*5801* are in complete accord with all subjects with minor A allele of rs9263726 in the Japanese population. Therefore, rs9263726 in *PSORS1C1* is an alternative biomarker for *HLA-B*5801* in the Japanese population. Conventional genotyping of rs9263726 based on allelic discrimination offers several advantages over *HLA-B* typing, which is determined by genotyping of several SNPs forming the *HLA-B*5801* haplotype. Various broadly used technologies (for example, TaqMan genotyping) allow the standardized identification of two distinct alleles in one reaction tube, limiting the risk of contamination and allowing high-throughput genotyping with high sensitivity and specificity. In addition, the test is largely independent of both the performance of and interpretation by laboratory personnel. SNP genotyping is also less time consuming and cheaper than sequence-based *HLA* typing, and it does not require specialized laboratories. Therefore, the easy detection of these SNPs has a practical and economical advantage in clinical application for predicting the onset of allopurinol-related SJS/TEN. Although the previous report revealed that three SNPs in *HLA* region strongly associated with allopurinol-related SCAR in Han Chinese,[7] the two SNPs analyzed by the Illumina Human 1M-DUO BeadChip showed only weak association in the Japanese. This ethnic difference might be due to the difference of LD.

The functional analysis of genes that carry these SNPs—including *HCP5*, *BAT1*, *PSORS1C1*, *CCHCR1*, *TCF19* and *POU5F1*—in the pathogenesis of allopurinol-related SJS/TEN might be useful for determining their relevance. *CCHCR1* is a regulator of keratinocyte proliferation or differentiation and is overexpressed in keratinocytes in psoriatic lesions.[20–23] *TCF19* is a potential trans-activating factor that could play an important role in the transcription of genes required for the later stages of cell cycle progression.[27] Possible psoriasis candidate genes near *HLA-B* include *PSORS1C1*,[17–19] *CCHCR1*,[22,23] and *POU5F1*.[32,33] Mutations in *BAT1* may be associated with rheumatoid arthritis.[34–36] *HCP5* encodes an endogenous retroviral element mainly that is expressed in immune cells and there is evidence that the SNP in this gene is protective against human immunodeficiency virus-1 infection.[37–39] The functions and relevance of these genes suggest that the pathogenesis of allopurinol-related SJS/TEN might involve not only an immune system disorder, but also processes of cell proliferation and differentiation.

In conclusion, the results of this GWAS of allopurinol-related SJS/TEN in Japanese patients show that SNPs in genes located in 6p21, which are in LD with *HLA-B*5801*, are strongly associated with the cutaneous adverse reaction. Therefore, these SNPs, especially rs9263726, prove to be predictors for allopurinol-related SJS/TEN in Japanese, and their genes might be involved in the pathogenesis of allopurinol-related SJS/TEN. The OR of rs9263726 is extremely high from this case–control study and the typing cost of SNP is much cheaper than that of *HLA* typing. Moreover, the SJS/TEN has a very severe adverse reaction of allopurinol, which is high mortality. Therefore, we believe that the screening of rs9263726 genotype before allopurinol administration is necessary to prevent SJS/TEN in allopurinol-treated Japanese patients, although its allele frequency is very low in the Japanese. Association analyses of other ethnic populations are needed for confirming and comparing the results obtained in this study. *In vitro* functional studies of these genes are also necessary for identification of the physiological and molecular pathways leading to allopurinol-related SJS/TEN.

Conflict of interest

The authors declare no conflict of interest except one member of JPDSC, Mitsubishi Tanabe Pharma, which is a distributor of allopurinol in Japan.

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Appendix

Japan Pharmacogenomics Data Science Consortium (JPDSC)

The Japan Pharmacogenomics Data Science Consortium is composed of Astellas Pharma, Otsuka Pharmaceutical, Daiichi Sankyo, Taisho Pharmaceutical, Takeda Pharmaceutical and Mitsubishi Tanabe Pharma, and is chaired by Ichiro Nakaoka (Takeda Pharmaceutical).

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特集◆薬疹・薬物障害

HHV-6 抗体価の上昇をみたイソソルビドゼリーによる薬疹の1例

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要　約

34歳，女性。イソソルビドゼリー内服13日後に38℃台の発熱と頸部リンパ節腫脹，全身に紅斑と丘疹が出現。薬剤中止のみで数日で皮疹は改善。イソソルビドのDLSTは陰性，内服テストで陽性。イソソルビドによる播種状紅斑丘疹型薬疹から進展した紅皮症と診断。経過中，HHV-6 IgG抗体価が1280倍と著明に上昇していたが，臨床症状からはDIHSとは診断しえなかった。イソソルビドによる薬疹は本邦で4例しかなく，またHHV-6抗体価の上昇をみた症例はほかにないため貴重と考え報告する。

キーワード：イソソルビドゼリー，播種状紅斑丘疹型，薬疹，HHV-6，薬剤性過敏症症候群

Ⅰ．はじめに

重症型薬疹である薬剤性過敏症症候群（drug-induced hypersensitivity syndrome，以下DIHS）は特定の薬剤により生じ，経過中にhuman herpes virus 6（以下HHV-6）の再活性化をみることが知られている。今回我々は，HHV-6 IgG抗体価の上昇をみたイソソルビドゼリーによる薬疹の症例を経験した。近年，これまでにDIHSの原因として報告されていない薬剤による薬疹において，DIHSに類似した症状とHHV-6の再活性化をみる症例が報告されており，若干の考察を加えて報告する。

Ⅱ．症　　例

患　者　34歳，女性

初　診　2007年12月13日

家族歴　特記すべきことなし。

既往歴　メニエール病，過敏性腸炎

現病歴　2007年11月29日，某耳鼻科でめまいに対しイソソルビドゼリー（メニレット70%ゼリー®），メシル酸ベタヒスチン（メリスロン®），五苓散を処方された。その後37℃台の微熱が続き，12月11日には39℃の発熱がみられ，翌日には眼球結膜の充血と顔面，体幹の皮疹が出現した。

初診時現症　体温39℃，左右の頸部リンパ節を1cm触知した。顔面の腫脹と眼球結膜の充血を伴って全身に浸潤のある紅斑と丘疹が多発し，体幹では癒合していた（図1-a，b）。鼻汁と軽度の咽頭痛があったが，口腔粘膜疹はなかった。

臨床検査所見　血算・生化学：白血球6400/μl，ヘモグロビン445万/μl，血小板6.8万/μl，総

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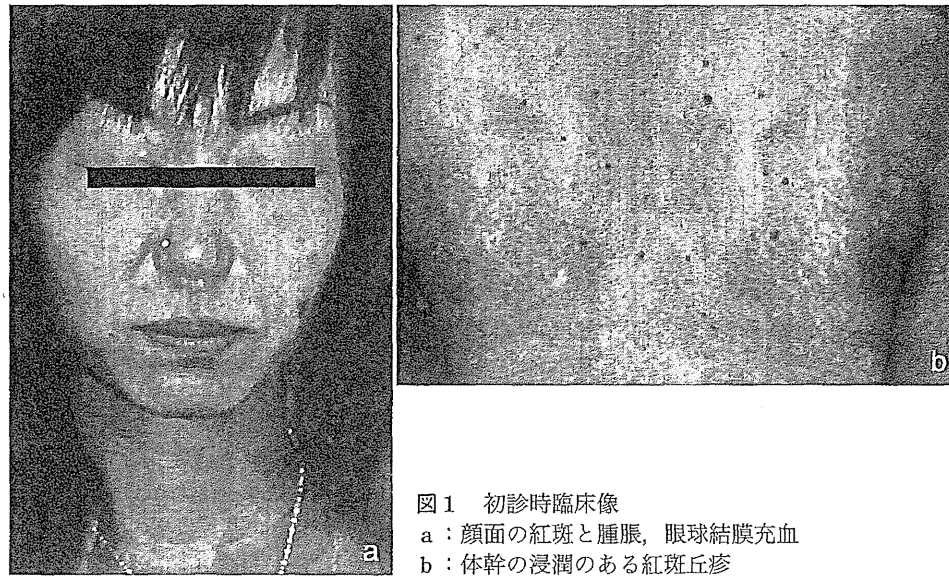

図1　初診時臨床像
a：顔面の紅斑と腫脹，眼球結膜充血
b：体幹の浸潤のある紅斑丘疹

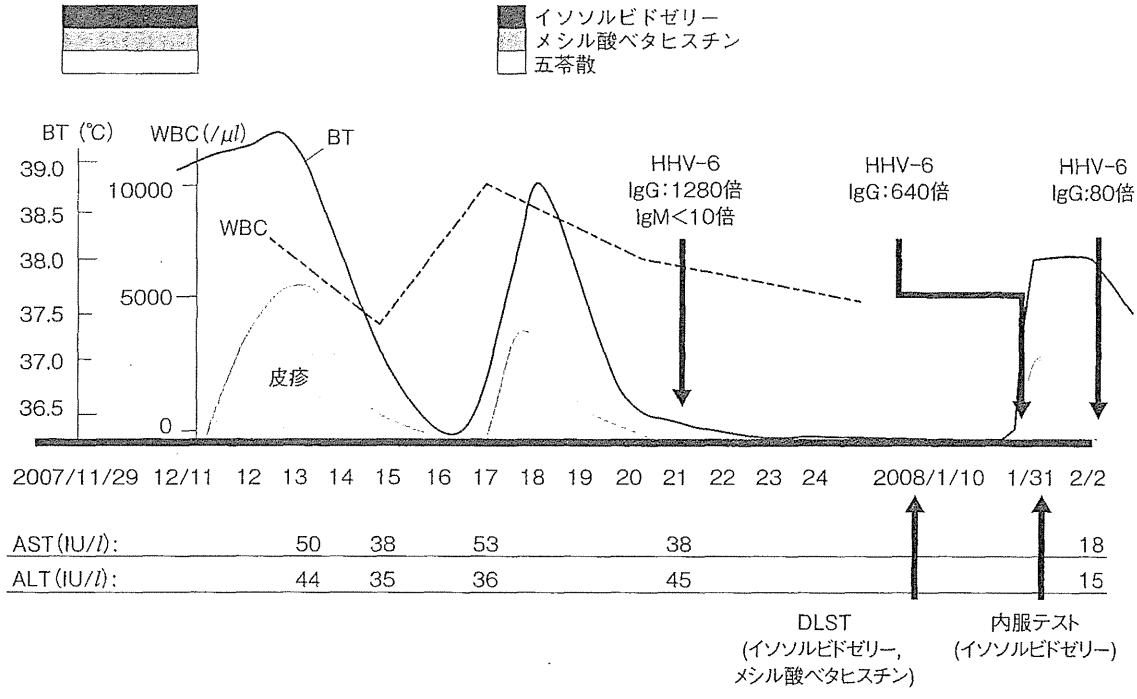

図2　臨床経過

蛋白 8.0 g/dl，AST 50 IU/l，ALT 44 IU/l，γ-GTP 21 IU/l，BUN 11 mg/dl，Cre 0.72 mg/dl，LDH 44 IU/l，CRP 7.75 mg/dl，IgA 172 mg/dl，IgG 1643 mg/dl，IgM 96 mg/dl，マイコプラズマ抗体価（PA）40 倍未満，ASO 40 倍以下，ASK 40 倍，サイトメガロウイルス，麻疹，風疹，EB ウイルス抗体価はすべて既感染パターンであった。

尿検査：ビリルビン 1+，ケトン体 2+，蛋白質

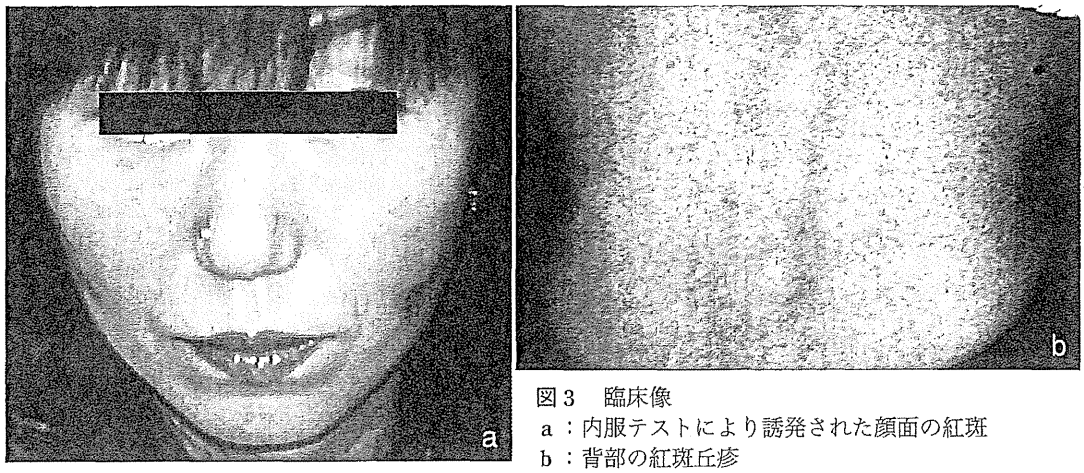

図3　臨床像
a：内服テストにより誘発された顔面の紅斑
b：背部の紅斑丘疹

2＋，潜血反応±。

治療および経過　ウイルス性中毒疹や薬疹を疑い，内服薬をすべて中止し，外来で補液とパモ酸ヒドロキシジンの点滴，塩酸フェキソフェナジンの内服，ステロイド軟膏の外用を開始した。12月15日には皮疹は速やかに改善し，12月17日には解熱した。しかし同日，めまい発作が出現，自己判断でイソソルビドゼリー，メシル酸ベタヒスチン，五苓散を内服した。内服30分後より悪寒と振戦が出現，発熱と皮疹を認めたため当科に再診した。来院時，体温37.5℃，顔面の腫脹と眼球結膜の充血あり。顔面，体幹に淡い紅斑を認めた。前回と同様の治療を行い，12月21日には症状は改善した。DIHSの鑑別のために，症状出現から10日目にHHV-6 IgG抗体価を測定したところ，1280倍と高値を示した。症状出現後42日目には640倍，44日目に80倍と低下した。

2008年4月より五苓散を再開したが，症状の再燃なく経過している（図2）。

1）パッチテスト（2007年1月7日）：イソソルビドゼリー，メシル酸ベタヒスチン

2）五苓散：すべて陰性

3）薬剤リンパ球刺激試験（drug lympocyte stimulation test，以下DLST）（2007年1月10日）：メシル酸ベタヒスチン最大stimulation index（以下SI）2.2（最大反応値330 cpm），イソソルビド最大SI 1.5（最大反応値227 cpm）

4）内服テスト：イソソルビドゼリーのみ施行した。イソソルビドゼリー30 g内服で発熱とともに皮疹が再現された（図3-a，b）。

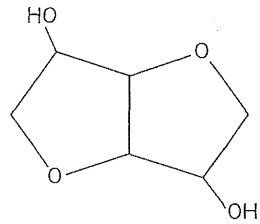

図4　イソソルビドの構造式

以上より，イソソルビドゼリーによる薬疹と診断した。

Ⅲ．考　　案

イソソルビドは浸透圧利尿剤として，メニエール病や脳圧降下，利尿，眼圧降下に使用されている。イソソルビドゼリーはイソソルビド70％と添加物としてカンテン末，サッカリン酸水和物，クエン酸水和物，カカオ末などを含有した製剤である。イソソルビドの構造式（図4）は循環器領域で広く使用されている硝酸イソソルビド（ニトロール®）と類似しており，硝酸イソソルビドの構造式はイソソルビドのエステル基が2つとも硝酸エステル化されたものである。これらはいずれも広く使用されている薬剤であるがイソソルビドによる薬疹は調べえた限り，本邦で自験例を含め4例[1)~3)]（表1）のみであり，海外では硝酸イソ

表1　イソソルビドによる薬疹の本邦報告例

症例	報告者	報告年	年齢/性別	皮疹型	投与から発症までの期間	検査	治療
1	立野ら[3]	2003	39/女性	播種状紅斑丘疹型	1カ月	PT（−） SPT（−） 内服テスト：1/10量で陽性	不明
2	鎌倉ら[2]	2004	35/男性	紅斑丘疹型	不明	不明	プレドニゾロン30 mg 2週間で皮疹軽快
3	井上ら[1]	2009	85/女性	多型紅斑型	18日	PT（＋？） SPT（＋？）	リンデロン®6 mg
4	自験例	2007	34/女性	播種状紅斑丘疹型 〜紅皮症型	13日	PT（−） DLST（−） 内服テスト：2/3量で陽性	補液 抗アレルギー薬 数日で軽快 HHV-6抗体価上昇

PT：パッチテスト　SPT：スクラッチパッチテスト

ソルビドがニトログリセリンと交叉反応を起こした1例[4]が報告されている。この4例中2例ではステロイドの全身投与を行っているが，自験例は抗アレルギー薬とステロイド薬の外用で軽快し，比較的軽症であったと考えられる。

被疑薬の1つであるメシル酸ベタヒスチンは使用頻度の高い薬剤であるにもかかわらず，それによる薬疹は極めてまれであり，海外で固定薬疹の1例[5]が報告されているのみであった。またメシル酸ベタヒスチンのDLSTのSIは2.2と陽性であったが非特異的反応も否定できず[6]，内服テストも施行しえなかったことから原因薬剤の1つであるかは明らかにはできなかった。

自験例では顔面体幹の紅斑と顔面の腫脹，HHV-6抗体価の著明な上昇がみられたが，血液検査で末梢血の異常所見や著明な肝機能障害はなかったこと，症状が数日で軽快し再燃もなかったことから，臨床的にDIHSとは診断できなかった。

近年，DIHSの原因薬剤として知られている抗けいれん薬やサルファ剤，メキシレチン，アロプリノール，塩酸ミノサイクリンなどとは異なる薬剤による薬疹でDIHSに酷似した臨床症状とHHV-6の再活性化を認めた症例報告が散見されている[7)〜14)]。それらのまれな症例は，薬疹発症時に何らかの要因が加わってHHV-6の再活性化をきたし，DIHS様の症状を呈するものと考えられる。自験例ではイソソルビド投与開始から皮疹出現までの約2週間，37°C台の微熱が続いたというエピソードや，咽頭痛や鼻汁がみられたことから，イソソルビドの投与と同時期に何らかのウイルス感染があった可能性が考えられる。すなわち，ウイルス感染による免疫変調とイソソルビドによる薬剤アレルギー反応が相まってHHV-6の再活性化をきたした可能性が示唆された。イソソルビドゼリー自体は，抗けいれん薬のようにB細胞の免疫グロブリン産生異常などの免疫変調をきたすという報告はなく，薬熱の報告もみられない。症状遷延のない薬疹でHHV-6抗体価の上昇を認める場合，抗体価は80倍程度であることが多いとされており[15]，自験例は1280倍と高値であったことから薬疹発症とHHV-6の再活性化は何らかの関係があったと推測される。イソソルビドの薬疹報告においてHHV-6抗体価の上昇をみた症例はほかになく，今後の症例の集積が待たれる。

（2011年2月25日受理）

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過去6年間における薬疹患者の統計的観察
—横浜市立大学附属病院受診例について—

渡邉裕子[1]　佐野沙織[1]　村田奈緒子[1]　長島真由美[1]　白田阿美子[1]　前田修子[1]
山根裕美子[2]　池澤善郎[1,3]　相原道子[1]

要旨

2003年4月から2009年3月までの過去6年間に横浜市立大学付属病院を受診した薬疹患者について解析した．総数は341名，原因薬は抗菌薬が最も多く29%，次いで抗腫瘍薬18%であった．抗菌薬では耐性菌増加に伴いグリコペプチド系が13%，カルバペネム系が10%と増加し，抗腫瘍薬では分子標的薬が19%と急増していた．発疹型は紅斑丘疹型がもっとも多かったが，Stevens-Johnson症候群/中毒性表皮壊死症が5%，1998年以前の統計では分類されていなかった薬剤性過敏症症候群（DIHS）が2%にみられた．抗腫瘍薬の発疹型は限局する紅斑丘疹や手足症候群，光線過敏型など多彩であり，特に分子標的薬は痤瘡型，水疱型，爪甲異常といった特徴的な発疹型を呈していた．好酸球増加は17%の患者でみられ，紅皮症型がもっとも高頻度であった．原因薬剤の検索では，パッチテスト，皮内テスト，薬剤添加リンパ球刺激試験がそれぞれ施行例の34%，68%，60%に陽性であった．治療は薬剤中止または継続したままで約80%が軽快し，抗腫瘍薬やインターフェロン製剤は薬疹を生じても継続できる症例が多かった．今後も新薬の開発とともに薬疹の原因薬や臨床像は変化していく．各時代の薬疹の調査と知識の集積は今後も薬疹に対処していくために重要である．

はじめに

薬疹は日常診療でしばしば遭遇する疾患であるが，その臨床像や頻度はそれぞれの時代で使用される薬剤や罹患する疾病により変遷する．近年，分子標的治療薬をはじめとする様々な薬剤が開発され，それらによる薬疹が急増している．それに伴い，薬疹の出現時期や臨床型がこれまで以上に多岐にわたるようになった．また，2000年以降薬剤性過敏症症候群（DIHS）の診断が広く皮膚科でなされるようになり[1]，それまでStevens-Johnson症候群（SJS）や紅皮症型薬疹に分類されていた症例の一部が新たに分類上独立した．

今回我々は，2003年4月から2009年3月までの過去6年間に当科で経験した薬疹患者について集計し，最近の薬疹の臨床的特徴や傾向について調査を行った．それらの結果を過去の報告と比較することにより，最近の薬疹の動向について検討した．

対象と方法

2003年4月から2009年3月の6年間に横浜市立大学附属病院皮膚科を受診した患者のうち，臨床経過から薬疹と診断された症例を対象とした．具体的には被疑薬の投与後発症し，薬剤の継続中に皮膚症状が軽快することが知られている抗悪性腫瘍薬やインターフェロン製剤を除き薬剤の中止で消褪または減量により軽快した症例を対象とした．調査項目は年齢，性別，原因薬剤，発疹型，薬疹発症までの薬剤投与期間，末梢血好酸球数，皮膚試験成績，薬剤誘発性リンパ球刺激

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図1 年齢・性別

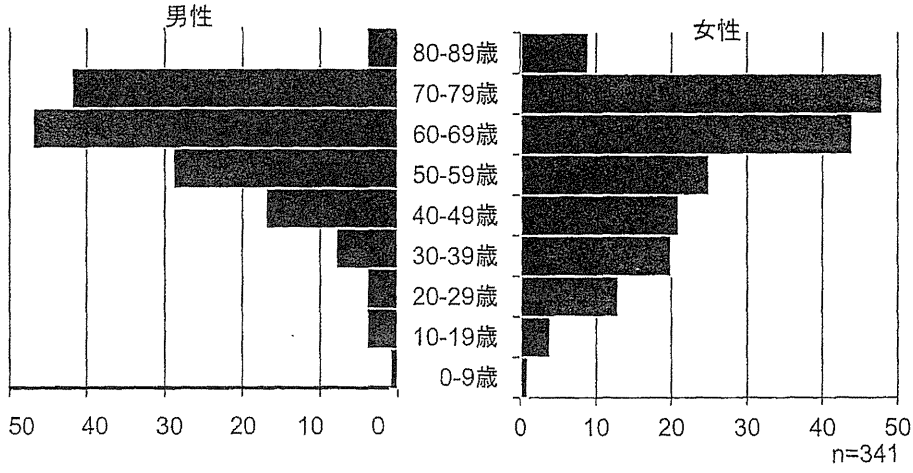

表1 薬剤分類における頻度

薬剤種別	人数 (%)
抗菌薬	150 (29)
抗腫瘍薬	93 (18)
循環器官用薬	38 (7)
抗けいれん薬	32 (6)
消化器官用薬	27 (5)
消炎鎮痛薬	23 (4)
神経系用薬	23 (4)
非イオン系造影剤	23 (4)
総合感冒薬	17 (3)
呼吸器官用薬	17 (3)
インターフェロン製剤	16 (3)
その他	62 (12)
	n=521

表2 薬剤別の頻度

原因薬剤	人数 (%)
カルバマゼピン	18 (3)
フルオロウラシル	16 (3)
インターフェロン製剤*	16 (3)
アモキシシリン	14 (3)
パクリタキセル	13 (2)
ゲムシタビン	13 (2)
バンコマイシン	12 (2)
テガフール・ギメラシル・オテラシルカリウム	12 (2)
*リバビリン併用を含む	n=521

試験(DLST)，治療とした．パッチテスト判定は International Contact Dermatitis Research Group (ICDRG) 基準に基づいて行われた．DLST は Stimulation Index (SI) 180% 以上を陽性とした．なお，原因薬剤は薬剤投与歴と臨床経過から推察されるものとし，複数の薬剤が投与されている場合は再投与により誘発されたもの，または皮膚試験や DLST の結果から総合的に判断して決定し集計した．原因薬剤が多剤の場合や発疹型が複数の場合，さらに複数回にわたり薬疹を生じた場合はそれぞれについて集計を行った．なお，薬剤による接触皮膚炎患者は除外した．

結果

1．薬疹患者数，性別，年齢（図1）

総数は 341 例，男性 156 例，女性 185 人(1：1.19)でやや女性が多かった．20～40 歳代では特に女性に多く，男女比 1：4.3 であった．患者年齢は平均 57.8 歳で，60～70 歳代にピークを認めた．

2．原因薬剤別頻度（表1）

原因薬剤の分類では抗菌薬(29%)と抗腫瘍薬(18%)が多くを占めた．次いで循環器官用薬剤が多く(7%)，抗けいれん薬 (6%) がそれに次いで多かった．循環器官用薬のなかでは降圧薬が 38 例中 25 例 (65.8%) を占めていた．

個々の薬剤については (表2)，頻度の高い順にカルバマゼピン(3%)，フルオロウラシル(3%)，インターフェロン製剤 (3%) の順であった．タキサン系植物ア

表4 発疹型と原因薬剤

発疹型	抗菌薬	抗腫瘍薬	抗けいれん薬	消炎鎮痛薬・総合感冒薬	循環器官用薬	消化器官用薬	呼吸器官用薬	神経系用薬	造影剤	その他	総計
MP型	101	30	14	17	19	19	5	8	17	32	262
EM型	26	8	6	3	7	5	3	7	4	17	86
湿疹型	7	8	3	4	7	3	2	3	2	8	47
SJS	9		6	9	5	3		4		8	44
蕁麻疹型	13	3			1		1		2	3	23
紫斑型	7	3		3	2		1		1	3	20
固定疹	1	2		7			5			2	17
紅皮症型	2	1	4	2	1	1		2		2	15
痤瘡型		13	1								14
TEN	5			3		1	2				11
DIHS			6					1		1	8
手足症候群		8									8
乾癬型					5			1		2	8
苔癬型				1	2					1	4
光線過敏型		2			3						5
血管性浮腫型	1			1			1				3
SLE型	1	1	1								3
水疱型		2									2
局所反応型										2	2
脂漏性皮膚炎型					1						1
その他	2	15					2		1	2	22
総計	175	96	41	50	53	33	22	26	27		605

数値は患者数

13.6%，ペニシリン系，マクロライド系の抗生剤がそれぞれ9.1%となっていた．蕁麻疹型では抗生剤(セフェム系)が27.3%を占めていた．手足症候群，痤瘡型はほとんどが抗腫瘍薬であった．局所反応型は全てインターフェロン製剤によるものであった．重症薬疹についてみると，SJSは抗菌薬（20.5%）に加え，降圧薬(11.4%，うちARB 3/5例)や総合感冒薬・消炎鎮痛薬(15.9%）が多かった．TENは抗菌薬が45%を占め，その内訳はアモキシシリン，メロペネム，イミペネム・シラスタチンナトリウムの3剤であった．DIHSは抗けいれん薬が75%であり，カルバマゼピンが3例，フェノバルビタールが2例，アレビアチン1例であった．

次に原因薬剤からみた発疹型について検討した．全体の発疹型別割合と比較した（χ二乗検定で$p<0.05$を有意差ありとした)．抗菌薬はMP型（57.7%）と蕁麻疹型(7.4%)，系統別にみるとセフェム系は蕁麻疹型(17.1%)，ペネム系はTEN(18.8%)，グリコペプチド系はMP型(78.9%)で有意差があった．抗てんかん薬はDIHS（15.4%）と紅皮症型（44.4%)，消炎鎮痛薬はSJS(25.0%)，TEN(10.7%)，降圧薬は湿疹型(19.4%)，乾癬型（9.7%)，苔癬型（6.5%）で有意差を認めた．

4. 発疹型と薬疹発症までの期間（表5）

発症までの期間は多くが2週間以内であった．発症までの期間に特徴がみられた発疹型は蕁麻疹型，苔癬型，乾癬型であった．蕁麻疹型は約50%が薬剤投与同日に皮疹が出現しており，苔癬型では1カ月以内と1年以上に2分されていた．乾癬型は投与から発症まで全例1カ月以上，最長5年1カ月であった．これらはいずれも最も頻度の高かったMP型の発症までの期間と比較して有意差を認めた(χ二乗検定，$P<0.05$)．

重症薬疹の発症までの期間は，SJSでは同日から1週間以内が23例（54%)，TENでは3日以内が6例(50%）であったのに対して，DIHSでは1カ月以上が4例(57%)を占めていた．SJS/TENの症例において，発症までの期間が3日以内の症例は26例あり，SJSでは42%，TENでは67%が3日以内の発症であった．その原因薬剤は抗菌薬が11例，鎮痛薬が8例，その他が7例であった．抗菌薬については以前に同系統の薬剤による薬疹歴があったり，感染症に対して長期にわたり抗菌薬を使用したのち，同系統の抗菌薬に切り替えてから薬疹を生じた症例が合わせて7例あった．

表6 皮膚検査・DLSTの結果（発疹型別）

発疹型	PT陽性率	皮内テスト陽性率	DLST陽性率
MP型	8/32(25)	7/11(64)	12/17(71)
EM型	5/13(38)	5/6 (67)	5/9 (56)
固定疹	2/10(20)	ND	2/3 (67)
DIHS	2/6 (33)	ND	2/5 (40)
蕁麻疹型	1/3 (33)	1/1 (100)	0/4 (0)
湿疹型	3/4 (75)	0/1 (0)	0/2 (0)
SJS	2/3 (67)	ND	2/3 (67)
紫斑型	0/3 (0)	1/1 (100)	1/1 (100)
紅皮症型	1/2 (50)	ND	2/2 (100)
苔癬型	0/2 (0)	ND	2/2 (100)
TEN	1/2 (50)	1/1 (100)	1/1 (100)
血管性浮腫型	1/1 (100)	ND	2/2 (100)
光線過敏型	1/1 (100)	ND	ND
痤瘡型	0/1 (0)	ND	ND
SLE型	0/1 (0)	ND	0/1 (0)
その他	2/2 (100)	1/1 (100)	ND
総計	29/86(34)	15/22(68)	31/52(60)

ND：Not done
数値は患者数，() 内は発疹型における％

表7 治療

	人数（%）
薬剤中止/継続	268 (82)
ステロイド薬の全身投与	46 (14)
ステロイド・パルス療法	10 (3)
ステロイドの全身療法	3 (1)
＋その他の治療	
（免疫グロブリン療法・血漿交換療法）	
	n=327

あった．3例以上施行した発疹型を比較すると，陽性率の高い順からMP型12/17例（71％），固定疹型，SJSはいずれも2/3例（67％），EM型は5/9例（56％），DIHSは2/5例（40％）であった．DLST陽性となった症例において，検査陽性となった時期はMPは11日～742日，SJSは42日～63日，EM型は19日～79日，DIHSは1例のみで79日であった．

7．治療（表7，8）

薬剤を継続したものおよび転院等で経過が不明なものを除いた327例のうち，薬剤中止または継続し，ステロイドの外用などの局所療法のみで治癒した症例が82％，ステロイドの全身投与を必要とした症例は14％，パルス療法3％であった（表7）．ステロイドの全身療法を施行したのはSJS，DIHS，EM型，紅皮症型などほとんどが発熱や肝障害などの全身症状や粘膜疹を伴った症例であった．パルス療法を施行した症例はSJS，TEN，DIHSのみであり，一部の症例では免疫グロブリンの投与や血漿交換療法が併用されていた（表8）．なお，抗腫瘍薬による薬疹では61/89例が，インターフェロン製剤によるものでは11/16例が薬剤の中止をせず継続可能であり，継続中に局所療法のみで軽快または消失した．

考察

近年，新薬が次々と臨床の場に登場するに伴って，薬疹の臨床像にも変化がみられるようになった．これまで我々は当科を受診した薬疹患者の調査結果を報告してきた[2) 3)]が，今回最近の薬疹の動向を明らかにする目的で2009年までの過去6年間に当科を受診した薬疹患者を集計した．その結果を1983年から1997年の当科の薬疹患者の調査結果[3)]と比較検討したところ，原因薬や発疹型などを中心に様々な変化が明らかになった．

原因薬剤の割合は抗菌薬，抗腫瘍薬，循環器官用薬，抗けいれん薬の順で，過去の集計[3)]と比較すると抗菌薬は41.9％から29％，消炎鎮痛薬が13.0％から4％と減少傾向にあり，抗腫瘍薬の薬疹が4.7％から18％と増加していた．薬疹の原因となった抗菌薬は過去の集計ではペニシリン系とセフェム系で約90％を占めて

的に表皮下の非自己免疫性水疱であり血管からの水分の漏出による真皮の浮腫が原因である可能性が考えられた[11].

インターフェロン製剤による薬疹は2000年にC型肝炎に対するインターフェロンα-2bとリバビリンの併用療法が保険適用されて以来増加し，今回の調査でも原因薬剤の3位を占めていた．主な発疹型は播種状紅斑丘疹型であり，サルコイドーシスの発生はみられなかった．これらの皮疹は多くが薬理作用によるものと考えられ，これまでにインターフェロン単剤では3.64%であった薬疹発症頻度がリバビリン併用により59.4%に急増したことが報告されている[12]．その機序としてはインターフェロンによる抗ウイルス作用がリバビリンによって増強されることが推察されており，リバビリンのTh1サイトカイン産生促進作用によりTh1優位の病態を促進することや，スーパーオキサイドやヒドロキシラジカルなどの活性酸素により誘発される酸化ストレスが皮疹の出現に関与している可能性が考えられている[13].

また，2011年度に本邦で承認となったテラプレビルは，インターフェロンα-2bとリバビリンとの3剤併用で使用することで再燃例や難治例のC型肝炎に対して高い有効率が得られる薬剤として期待されている．しかし，国内の第III相臨床試験では薬疹の発症率が80%以上と非常に高く，またSJSやDIHSといった重症薬疹の報告もあり[14]，今後3剤併用による薬疹の増加に注意が必要である．

薬疹の発疹型はMP型(41%)，EM型(13%)，湿疹型(8%)が上位を占め，過去の集計[3]がそれぞれ37%，13%，6%であったことから，変動はみられなかった．一方，大学病院という特殊性から一般病院と比較して重症薬疹患者の受診率は以前から高いものの，過去の調査と比較してSJS/TENが2.4%から6%に増加しており，過去には分類されていなかったDIHSも2%の患者で診断されていた．一方，減少した発疹型としては苔癬型があげられ，4.1%から1%に減少していた．これは原因薬の多くを占めたシンナリジンや塩酸ピリチオキシンが販売中止になったこと，カプトリルの使用が減少していることが大きな要因と考えられる．今回の調査ではアムロジピンとシルニジピン(Caブロッカー)を投与されていた1例と，ロキソプロフェン(NSAID)，ベザフィブラート(高脂血症薬)が投与されていた各1例の3例であった．

薬剤別に頻度の高い発疹型をみると，セフェム系抗菌薬は蕁麻疹型が，抗てんかん薬はDIHSと紅皮症型が，薬疹全体の平均と比較して発症頻度が高かった．抗てんかん薬のうち，使用頻度の高いカルバマゼピン，フェノバルビタール，フェニトインの3剤は代謝過程で形成されるarene oxideにより40～80%に交叉反応を生じることが知られており[15]，今回の調査でも薬疹発症後のてんかんの治療でこれらの薬剤に変更され，症状が持続した症例がみられた．総合感冒薬・消炎鎮痛薬では他剤と比較してSJS/TENが多くみられた．Yamaneら[16]の2000年～2006年の本邦報告例におけるSJSの最も多い原因薬は抗てんかん薬，TENでは消炎鎮痛薬とされており，今回の集計でもSJS/TENを合わせると抗菌薬14例，総合感冒薬・消炎鎮痛薬12例，抗痙攣薬6例と，上位を占めた．SJS/TENの発症までの薬剤投与期間は，欧州における調査ではアセトアミノフェンによるものを除き4日以上がほとんどであった[17]のにたいして，わが国の最近の調査では[18]SJSで22.9%，TENで28.3%の患者が3日以内に発症していた．今回の調査では，SJSでは42%，TENでは67%が3日以内に発症しており，わが国の全国調査よりさらに早期に発症していた．わが国においてSJS/TENの発症までの投薬期間が短い理由は，欧州と比較して医療機関で抗菌薬を投与される機会が多く，感作が成立しやすいことが関与していると推察される．

末梢血好酸球増加出現率は平均で17%であった．紅皮症型，TEN，苔癬型，乾癬型で50%以上の患者にみられたが，紅皮症型のみ平均の出現率と比較して有意に高かった．DIHSの患者より紅皮症患者に好酸球増加が多くみられたことは興味深かった．

パッチテストの結果は表皮変化が主体の湿疹型やSJS/TENで陽性率が50%以上と高く，真皮上層の炎症が主体の紅斑丘疹型では25%と低かった．石川らは本邦報告例におけるPT陽性例を集計し，多い順に湿疹型(75.4%)，EM型(69.1%)，ジベル型(66.7%)と報告しているが[19]，これらは原因薬剤が明らかになり報告された例を集計していることから，当科の集計よりも高い割合となったと考えられる．皮内テストは感作の危険性や全身反応を誘発する可能性[20]を配慮して，当科では使用可能な薬剤を決定するために最小限で行われ，EM型，MP型で60%以上の陽性率を示した．皮内テストを行った薬剤はほとんどが抗菌薬であり，抗菌薬による薬疹の診断に有用であることが示された．皮内テストがパッチテストと比較して陽性率が

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Clinical Analysis of Cutaneous Adverse Drug Reactions in Yokohama City University Hospital from 2003 to 2009

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A retrospective study was performed at Yokohama City University Hospital using medical records of patients with cutaneous adverse drug reactions (CADR) from April 2003 to March 2009. In total 341 patients were analyzed for clinical features and causative drugs. The two major causative drugs were antibiotics (29%) and anti-cancer drugs (18%). Among the causative anti-cancer drugs, we recorded a greater number of molecular target drugs than reported in previous studies. Although macropapular rash was the most common reaction, as reported previously, patterns of clinical manifestation differed from those previously recorded. Notably, in patients treated with anti-cancer drugs, localized macropapular rash, hand-foot syndrome, and severe acneciform eruption were seen. The severe types of CADR, Stevens-Johnson syndrome/toxic epidermal necrolysis and drug-induced hypersensitivity syndrome, accounted for 6% and 2%, respectively, of CADR-types. Positive reactions with causative drugs were observed in 34%, 68%, and 60% of patients as determined by patch, intradermal, and drug-induced lymphocyte stimulation tests, respectively. Almost 80% of patients were cured after discontinuing the causative drugs without any general treatments, including steroids, or could continue the drugs with just topical therapies. Clinical manifestations of CADR are changing with changing drug therapies. It is therefore important to continue clinical analysis of CADR.

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Key words: cutaneous adverse drug reactions, causative drugs, anti-cancer drugs, eosinophilia

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