

Specific association of the rs6500265 and rs9933632 single-nucleotide polymorphisms in Japanese patients with antipyretic analgesic-related Stevens–Johnson syndrome and toxic epidermal necrolysis with severe ocular involvements

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A recent study using the microarray for single-nucleotide polymorphisms (SNPs) genotyping specifically designed for the Japanese population in combination with genome-wide imputation showed the association of several SNPs with cold medicine-related Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) with severe ocular complications. However, it remains to be determined whether these polymorphisms are associated with the onset of antipyretic analgesic (AA)-related SJS/TEN, the progression of severe ocular involvements (SOIs), or both AA-related SJS/TEN and SOI phenotypes. To gain a better understanding of the features of these genetic markers, we compared the allele and carrier frequencies of these SNPs among our original SJS/TEN patient groups: (a) AA-related SJS/TEN with SOIs, (b) AA-related SJS/TEN without SOIs, and (c) AA-unrelated SJS/TEN with SOIs. AA-related SJS/TEN with SOIs were found to be associated significantly with both rs6500265 [allele frequency: odds ratio (OR): 2.18; 95% confidence interval (CI): 1.30–3.65; $P = 0.0052$; carrier frequency: OR: 2.52; 95% CI: 1.33–4.78; $P = 0.058$] and rs9933632 (allele frequency: OR: 2.28; 95% CI: 1.37–3.79; $P = 0.0032$; carrier frequency: OR: 2.76; 95% CI: 1.46–5.22; $P = 0.0031$). In contrast, allele and carrier frequencies of

these SNPs in patients with AA-related SJS/TEN without SOIs or with SOIs not treated with any AAs were comparable with those in healthy Japanese controls. Collectively, our findings indicate that the rs6500265 and rs9933632 SNPs could be specific markers for AA-related SJS/TEN with SOIs, suggesting that certain genetic backgrounds contribute toward the etiology of this complex syndrome. *Pharmacogenetics and Genomics* 00:000–000 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions. Although they are very rare, associated mortality rates in Japan have been reported to be 3 and 19% for SJS and TEN, respectively [1]. Moreover, these disorders frequently result in severe sequelae, such as vision loss, which adversely affect the patient's quality of life [2]. Therefore, estimation of the clinical outcome and early intervention are important for the effective treatment of SJS/TEN.

We reported previously that patients with SJS/TEN related to antipyretic analgesics (AAs), including acetaminophen or NSAIDs, show a significantly higher rate of experiencing severe ocular involvements (SOIs) than those not related to

AAs [3,4], particularly when AAs were used for the treatment of common cold [3]. Previous studies have also shown that the susceptibility to cold medicine-related SJS/TEN with severe ocular complications is associated strongly with unique genetic polymorphisms such as those in human leukocyte antigens (HLAs; *HLA-A*02:06* and *HLA-A*44:03*), Toll-like receptor 3, IL-4 receptor, IL-13, Fas ligand, prostaglandin E receptor 3, and IKAROS family zinc finger 1 [5–9]. These findings indicate that there is a high-risk population with unique genetic factors contributing toward the onset of cold medicine (including AA)-related SJS/TEN with SOIs.

A recent genome-wide association study using the ethnic-specific Japonica array [10] with genome-wide imputation identified new susceptibility loci for cold medicine-related SJS/TEN with severe ocular complications on chromosomes 15 and 16 [11]. However, these loci have not been fully validated in other Japanese patients with AA-related SJS/TEN (AA-SJS/TEN) with SOIs. In addition, determination

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of single-nucleotide polymorphism (SNP) frequencies in patients with AA-SJS/TEN without SOIs or patients with SJS/TEN with SOIs who were not treated with AAs would provide a better understanding of whether these polymorphisms are associated with the onset of AA-SJS/TEN and/or SOI development. In this study, to gain a more comprehensive understanding of these SNPs, we investigated their frequencies in our independent SJS/TEN sample groups: AA-related SJS/TEN with SOIs, AA-related SJS/TEN without SOIs, and AA-unrelated SJS/TEN with SOIs.

Materials and methods

Study population

Through a nationwide case collection network and participating hospitals, we recruited 115 Japanese patients who experienced SJS/TEN from March 1983 to September 2013 [3]. AAs were categorized according to the therapeutic category, including acetaminophen, loxoprofen, ibuprofen, salicylamide, aspirin, diclofenac, etenzamide, mefenamic acid, aminopyrine, celecoxib, lornoxicam, tiaprofenic acid, ketoprofen, and flurbiprofen (Supplementary Table 1, Supplemental digital content 1, <http://links.lww.com/FPC/B305>). The numbers of cases of AA-SJS/TEN with SOIs, AA-SJS/TEN without SOIs, and AA-unrelated SJS/TEN with SOIs were 40, 41, and 34, respectively [3]. The diagnosis of SJS/TEN was made on the basis of the criteria proposed by Bastuji-Garin *et al.* [12]. According to the Japanese diagnostic criteria of SJS/TEN, TEN (the more severe dermatological phenotype) was diagnosed in cases in which skin covering more than 10% of the body surface area was detached. Acute-phase ocular surface involvement was graded from 0 to 3 on the basis of the status of hyperemia, pseudomembrane formation, and/or a defect/erosion of the conjunctiva/corneal epithelia, as described in detail previously [3], and cases with grades 2 and 3 (presence of at least either latter symptoms) were considered to feature SOIs. The Ethics Committees of the National Institute of Health Sciences and all of the participating institutes approved this study. Written informed consent was obtained from each patient. In the present study, we used the DNA samples collected from the SJS/TEN patients partially overlapping with our previous pharmacoepidemiological study [3], but not with Ueta *et al.* [11]. The data on the frequencies of the candidate SNPs in the healthy Japanese population reported by Ueta *et al.* [11] were used as a control.

Genotyping

Genomic DNA was extracted from blood leukocytes as described previously [13]. rs16957893, rs113301740, rs6500265, and rs9933632 were genotyped using the TaqMan Predesigned SNP genotyping assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA), in accordance with the manufacturer's instructions, with 5 ng of genomic DNA.

Statistical analysis

Allele and carrier frequencies in each SJS/TEN group were compared with those of 881 healthy Japanese

controls reported by Ueta *et al.* [11]. Fisher's exact test was carried out using GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, California, USA), and the significance level was set at a *P* value of less than 0.05. A logistic regression analysis was carried out using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results

To confirm the association between the SNPs on chromosomes 15 and 16 and AA-SJS/TEN with SOIs, we analyzed our independent 40 Japanese patients with AA-SJS/TEN with SOIs (15 men, mean age 43.4 ± 23.0). The numbers of cases with probable/definite SJS and definite TEN were 22 and 18, respectively. We found that the minor allele frequencies of rs6500265 and rs9933632 on chromosome 16 were significantly higher in the group with AA-SJS/TEN with SOIs than in the healthy Japanese population [for rs6500265, odds ratio (OR): 2.18; 95% confidence interval (CI): 1.30–3.65; *P* = 0.0052; for rs9933632, OR: 2.28; 95% CI: 1.37–3.79; *P* = 0.0032] (Table 1). Similarly, the carrier frequencies of these SNPs were significantly higher in AA-SJS/TEN patients than in the control population when using an analysis based on a dominant mode of inheritance, but not in one based on a recessive mode of inheritance, suggesting that these minor alleles exert their effects even if patients have only a single copy of them (for rs6500265, OR: 2.52; 95% CI: 1.33–4.78; *P* = 0.0058; for rs9933632, OR: 2.76; 95% CI: 1.46–5.22; *P* = 0.0031). These results are consistent with a previous study with 117 Japanese patients with cold medicine-related SJS/TEN with severe ocular complications [11]. Patients with AA-SJS/TEN with SOIs did not possess rare SNPs, rs16957893 or rs113301740, on chromosome 15 (data not shown). Because the minor allele frequencies (MAFs) of these SNPs were less than 0.01 [11], the sample size may not be sufficient to analyze the association between them and AA-SJS/TEN with SOIs. Therefore, we focused on the rs6500265 and rs9933632 SNPs in the following analysis.

To investigate whether rs6500265 and rs9933632 are associated with the groups with susceptibility to AA-SJS/TEN, irrespective of the ocular phenotype, we next genotyped these SNPs in 41 Japanese patients with AA-SJS/TEN without SOIs (22 men, mean age 57.9 ± 21.5). The probable/definite SJS and definite TEN cases numbered 29 and 12, respectively, in this group. The typing results showed that the allele and carrier frequencies of the rs6500265 and rs9933632 SNPs in the patients with AA-SJS/TEN without SOIs were comparable to those of the healthy control group (Table 2).

Furthermore, we speculated that these SNPs might be associated with the exacerbation of ocular symptoms in SJS/TEN patients irrespective of drug type. Therefore, we investigated the association between these SNPs and patients with SJS/TEN with SOIs not treated with AAs (*N* = 34, 15 males, mean

Table 1 Association between the two single-nucleotide polymorphisms and antipyretic analgesic-Stevens–Johnson syndrome/toxic epidermal necrolysis with severe ocular involvements

rs number chromosome: position	Genotypes	Genotype frequencies (%)		P value [OR (95% CI)]		
		Patients (n = 40)	Controls (11) (n = 883)	Allele 1 vs. allele 2	Genotype 11 vs. 12 + 22 (recessive)	Genotype 11 + 12 vs. 22 (dominant)
rs6500265 16: 49912759	11 T/T	2/40 (5.0)	15/883 (1.7)	0.0052* [2.18 (1.30–3.65)]	0.166 [3.05 (0.67–13.80)]	0.0058* [2.52 (1.33–4.78)]
	12 C/T	17/40 (42.5)	218/883 (24.7)			
	22 C/C	21/40 (52.5)	650/883 (73.6)			
rs9933632 16: 49906847	11 T/T	2/40 (5.0)	17/883 (1.9)	0.0032* [2.28 (1.37–3.79)]	0.197 [2.68 (0.60–12.03)]	0.0031* [2.76 (1.46–5.22)]
	12 G/T	18/40 (45.0)	218/883 (24.7)			
	22 G/G	20/40 (50.0)	648/883 (73.4)			

P values for allele and genotype frequency comparisons between cases and controls using Fisher's exact test.

CI, confidence interval; OR, odds ratio.

* $P < 0.05$.

Table 2 Association between the two single-nucleotide polymorphisms and antipyretic analgesic-Stevens–Johnson syndrome/toxic epidermal necrolysis without severe ocular involvements

rs number chromosome: position	Genotypes	Genotype frequencies (%)		P value [OR (95% CI)]		
		Patients (n = 41)	Controls (11) (n = 883)	Allele 1 vs. allele 2	Genotype 11 vs. 12 + 22 (recessive)	Genotype 11 + 12 vs. 22 (dominant)
rs6500265 16: 49912759	11 T/T	2/41 (5.0)	15/883 (1.7)	0.871 [1.05 (0.56–1.96)]	0.172 [2.97 (0.66–13.44)]	0.858 [0.90 (0.43–1.87)]
	12 C/T	8/41 (19.5)	218/883 (24.7)			
	22 C/C	31/41 (75.6)	650/883 (73.6)			
rs9933632 16: 49906847	11 T/T	2/41 (5.0)	17/883 (1.9)	0.872 [1.03 (0.55–1.93)]	0.205 [2.61 (0.58–11.71)]	0.858 [0.89 (0.43–1.84)]
	12 G/T	8/41 (19.5)	218/883 (24.7)			
	22 G/G	31/41 (75.6)	648/883 (73.4)			

P values for allele and genotype frequency comparisons between cases and controls using Fisher's exact test.

CI, confidence interval; OR, odds ratio.

age 57.7 ± 20.4 , Supplementary Table 2, Supplemental digital content 2, <http://links.lww.com/FPC/B306>, for the list of suspected drugs). The findings showed that SJS/TEN with SOIs without treatment by AAs was not associated with either rs6500265 or rs9933632 (Table 3).

Discussion

Genotyping of the rs6500265, rs9933632, rs16957893, and rs113301740 SNPs in 115 Japanese SJS/TEN patients showed that rs6500265T and rs9933632T are risk alleles for AA-SJS/TEN with SOIs, but not for AA-SJS/TEN without SOIs or AA-unrelated SJS/TEN with SOIs. Our findings suggest that these alleles are relevant to the onset of AA-related SJS/TEN associated with SOIs. Previous studies have shown that the susceptibility to cold medicine-related SJS/TEN with severe ocular complications is associated strongly with *HLA-A*02:06* and *HLA-A*44:03* in multiple ethnic groups [5,6]. Therefore, we also investigated the possibility that rs6500265 and rs9933632 SNPs are associated with the severity of dermatological phenotype in patients with AA-SJS/TEN with SOIs possessing *HLA-A*02:06* and/or *HLA-B*44:03*. However, these SNPs were not associated with the ratio of TEN (more severe dermatological phenotype) in *HLA-A*02:06*-positive patients

(for rs6500265, OR: 0.12; 95% CI: 0.12–8.31; $P = 1.00$; for rs9933632, OR: 1.87; 95% CI: 0.20–17.30; $P = 0.58$) and/or *HLA-B*44:03*-positive patients (for rs6500265, OR: 0.15; 95% CI: 0.01–2.05; $P = 0.16$; for rs9933632, OR: 1.87; 95% CI: 0.20–17.30; $P = 0.58$).

The differences in the carrier frequencies of rs6500265 and rs9933632 were significant in the dominant mode analysis, but not in the recessive mode analysis (Table 1); however, we could not rule out the possibility that the failure to achieve statistical significance in the analysis on the basis of a recessive mode of inheritance was because of the small sample size. Our results suggest that at least one minor allele of these SNPs would increase the risk of AA-SJS/TEN with SOIs. rs6500265 and rs9933632 are located in one linkage disequilibrium block on chromosome 16 that does not contain any protein-coding genes; however, one long noncoding RNA, RP11-305A4.2, is located within this block, indicating that this noncoding RNA might be involved in AA-related SJS/TEN with SOIs. Therefore, our findings would be useful not only for estimation of the clinical outcome and early intervention of AA-related SJS/TEN with SOIs but also for understanding the basic mechanisms of this complex syndrome.

Table 3 Association between the two single-nucleotide polymorphisms and Stevens–Johnson syndrome/toxic epidermal necrolysis with severe ocular involvements not treated with any antipyretic analgesics

rs number chromosome: position	Genotypes	Genotype frequencies (%)		P value [OR (95% CI)]		
		Patients (n = 34)	Controls (11) (n = 883)	Allele 1 vs. allele 2	Genotype 11 vs. 12 + 22 (recessive)	Genotype 11 + 12 vs. 22 (dominant)
rs6500265 16: 49912759	11 T/T	1/34 (2.9)	15/883 (1.7)	1.000 [0.93 (0.46–1.91)]	0.456 [1.75 (0.22–13.68)]	0.844 [0.86 (0.38–1.92)]
	12 C/T	7/34 (20.6)	218/883 (24.7)			
	22 C/C	26/34 (76.5)	650/883 (73.6)			
rs9933632 16: 49906847	11 T/T	1/34 (2.9)	17/883 (1.9)	1.000 [0.92 (0.45–1.87)]	0.497 [1.54 (0.20–11.96)]	0.844 [0.85 (0.38–1.90)]
	12 G/T	7/34 (20.6)	218/883 (24.7)			
	22 G/G	26/34 (76.5)	648/883 (73.4)			

P values for allele and genotype frequency comparisons between cases and controls using Fisher's exact test.
CI, confidence interval; OR, odds ratio.

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Conflicts of interest

There are no conflicts of interest.

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