IKZF1, a new susceptibility gene for cold medicine–related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement

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Background: Stevens-Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, including the ocular surface, oral cavity, and genitals. These reactions are very rare but are often associated with inciting drugs, infectious agents, or both.

Objective: We sought to identify susceptibility loci for cold medicine–related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement (SMI).

Methods: A genome-wide association study was performed in 808 Japanese subjects (117 patients with CM-SJS/TEN with SMI and 691 healthy control subjects), and subsequent replication studies were performed in 204 other Japanese subjects (16 cases and 188 control subjects), 117 Korean subjects (27 cases and 90 control subjects), 76 Indian subjects (20 cases and 56 control subjects), and 174 Brazilian subjects (39 cases and 135 control subjects).

Results: In addition to the most significant susceptibility region, *HLA-A*, we identified *IKZF1*, which encodes Ikaros, as a novel susceptibility gene (meta-analysis, rs4917014 [G vs T]; odds ratio, 0.5; $P = 8.5 \times 10^{-11}$). Furthermore, quantitative ratios of the *IKZF1* alternative splicing isoforms Ik1 and Ik2 were significantly associated with rs4917014 genotypes.

Conclusion: We identified *IKZF1* as a susceptibility gene for CM-SJS/TEN with SMI not only in Japanese subjects but also in Korean and Indian subjects and showed that the Ik2/Ik1 ratio might be influenced by *IKZF1* single nucleotide polymorphisms, which were significantly associated with susceptibility to CM-SJS/TEN with SMI. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Stevens-Johnson syndrome, toxic epidermal necrolysis, cold medicine, severe mucosal involvement, genome-wide association study,* IKZF1*, alternative splicing*

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Abbreviations used
CM-SJS/TEN: Cold medicine–related SJS/TEN
GWAS: Genome-wide association study
IRF: Interferon regulatory factor
KPUM: Kyoto Prefectural University of Medicine
NSAID: Nonsteroidal anti-inflammatory drug
OR: Odds ratio
SJS: Stevens-Johnson syndrome
SMI: Severe mucosal involvement
SNP: Single nucleotide polymorphism
TEN: Toxic epidermal necrolysis
TLR: Toll-like receptor

Stevens-Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are acute inflammatory vesiculobullous reactions of the skin and mucous membranes, including the ocular surface, oral cavity, and genitals. These reactions are often associated with inciting drugs and infectious agents.[1-3] Although they are rare, with an annual incidence of 1 to 6 cases per million persons,[3,4] these reactions carry high mortality rates of 3% for SJS and 27% for TEN,[5] and surviving patients often experience severe sequelae, such as vision loss caused by severe ocular surface complications.[6]

HLA genotypes are associated with SJS/TEN. In the Taiwanese Han Chinese the *HLA-B*15:02* allele exhibited a strong significant association with carbamazepine-induced SJS/TEN (cases, n = 44; control subjects [tolerant], n = 101; odds ratio [OR], 2504; $P_{corrected} = 3.1 \times 10^{-27}$).[7] Similarly, in Japanese (cases, n = 77; control subjects [tolerant], n = 420; OR, 9.5; $P = 1.1 \times 10^{-16}$)[8] and European (cases, n = 145; control subjects [normal]; n = 257; OR, 15.0; $P = 3.5 \times 10^{-8}$) subjects,[9] the *HLA-A*31:01* allele was significantly associated with carbamazepine-induced cutaneous adverse reactions, including SJS/TEN, drug-induced hypersensitivity syndrome, and others. Allopurinol, a uric acid–decreasing drug that induces severe cutaneous adverse reactions, including SJS/TEN, was significantly associated with *HLA-B*58:01* in Han Chinese (cases, n = 51; control subjects [tolerant], n = 135; OR, 580; $P_{corrected} = 4.7 \times 10^{-24}$),[10] white (cases, n = 27; control subjects [normal], n = 1822; OR, 80; $P_{corrected} < 10^{-6}$),[11] and Japanese (cases, n = 36; control subjects [normal], n = 986; OR, 62.8; $P = 5.4 \times 10^{-12}$)[12] patients. Allopurinol and anticonvulsants, such as carbamazepine, are the main inciting drugs for SJS/TEN[13]; in addition we[1,14] and others[2,15] have cited cold medicines, including nonsteroidal anti-inflammatory drugs (NSAIDs) and multi-ingredient medications, as causative drugs for SJS/TEN. We have also found that cold medicine–related SJS/TEN (CM-SJS/TEN) with severe mucosal involvement (SMI), including severe ocular complications, was significantly associated with *HLA-A*02:06* (cases, n = 151; control subjects [normal], n = 639; OR, 5.6; $P = 2.7 \times 10^{-20}$) and significantly associated with *HLA-B*44:03* in Japanese subjects (cases, n = 151; control subjects [normal], n = 639; OR, 2.0; $P = 1.3 \times 10^{-3}$), and this *HLA* genotype was irrelevant to patients with CM-SJS/TEN without SMI.[16] Thus genetic predisposition, including *HLA* genotype, might be different between patients with SJS/TEN with and without SMI. We also reported that CM-SJS/TEN with SMI was significantly associated with *HLA-B*44:03* in Indian (cases, n = 20; control subjects [normal], n = 55; OR, 12.3; $P = 1.1 \times 10^{-5}$) and Brazilian (especially Brazilian white; cases, n = 15; control subjects [normal], n = 62; OR, 6.2; $P = 3.7 \times 10^{-3}$) subjects.[17]

Here we performed a genome-wide association study (GWAS) to identify genetic factors associated with CM-SJS/TEN with SMI; cold medicines included NSAIDs and multi-ingredient cold medications, and SMIs included severe ocular surface complications. We identified *IKZF1* as a susceptibility gene for CM-SJS/TEN with SMI not only in Japanese subjects but also in Korean and Indian subjects.

METHODS

Patients

This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine (KPUM), the University of Tokyo, and other collaborating research institutes (see the Methods section in this article's Online Repository at www.jacionline.org).

Diagnosis of SJS/TEN by ophthalmologists was based on a confirmed history of acute onset of high fever, serious mucocutaneous illness with skin eruptions, and involvement of at least 2 mucosal sites, including the ocular surface.[1,14,16-23] Usually, ophthalmologists encounter patients with SJS/TEN in the chronic rather than acute stage, and therefore many of our patients had SJS/TEN many years before recruitment for this study. The samples from the National Institute of Health Sciences represented only patients with SJS/TEN in the acute stage, and the criteria proposed by Bastuji-Garin et al[24] were used for a diagnosis of SJS/TEN for these patients in the acute stage.

We defined patients with severe ocular complications as those who manifested pseudomembranes and epithelial defects on the ocular surface (cornea, conjunctiva, or both) in the acute stage[25] and as patients with ocular sequelae, such as severe dry eye, trichiasis, symblepharon, and conjunctival invasion into the cornea in the chronic stage.[6]

Moreover, we have focused here on CM-SJS/TEN, which can be induced by cold medicines, such as multi-ingredient cold medications and NSAIDs. The patients included in this study had taken cold medicines (eg, NSAIDs or multi-ingredient cold medications) after they had symptoms of the common cold a few to several days before disease onset; they were classified as having CM-SJS/TEN, although the specific drugs used were not named by each patient. We have also focused on patients with SJS/TEN with SMI because we previously found that the genetic predisposition might be different between patients with SJS/TEN with and without SMI.[16] Cases of NSAID-related SJS/TEN with SMI that did not involve symptoms of the common cold, such as involving rheumatoid arthritis or lumbago, were not included in this study. Detailed information on the patients with SJS/TEN with SMI and control subjects who were analyzed is shown in the Methods section in this article's Online Repository.

GWAS and single nucleotide polymorphism genotyping

In the GWAS we genotyped 820 samples, including 118 Japanese patients with SJS/TEN with SMI and 702 Japanese healthy control subjects (283 from KPUM and 419 from the University of Tokyo) by using the Affymetrix AXIOM Genome-Wide ASI 1 Array (Affymetrix, Santa Clara, Calif), according to the manufacturer's instructions. Because all genotyped samples passed the recommended sample quality control metric for the AXIOM arrays (Dish quality control > 0.82), we excluded 1 case sample with an overall call rate of less than 97%. We recalled the remaining 819 samples by using Genotype Console v4.1.4 software (Affymetrix). All samples used for GWASs passed a heterozygosity check, and 5 related samples were identified by using descendent testing. A principal component analysis found 6 outliers to be excluded by using the Smirnov-Grubbs test, and we showed that all cases (n = 117) and control subjects (n = 691) formed a single cluster with the HapMap Japanese (JPT) samples but not with the Chinese (CHB) samples (see Fig E1 in