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Effects of Long-Term Topical Prostaglandin Therapy on Central Corneal Thickness

Yuko Maruyama, Kazuhiko Mori, Yoko Ikeda, Morio Ueno, and Shigeru Kinoshita

Abstract

Purpose: Recent studies have shown that prostaglandin analogues can decrease the central corneal thickness (CCT), however, most of those studies followed the patient's CCT for only approximately 2 years. The purpose of this present study was to perform a long-term follow-up investigation of CCT in patients who underwent only topical prostaglandin monotherapy over 4 years, and then analyze the CCT changes and the correlation between intraocular pressure (IOP) changes and CCT changes.
Methods: This retrospective study involved 52 eyes of 52 glaucoma patients who consulted with glaucoma specialists at the Glaucoma Clinic of Kyoto Prefectural University of Medicine, Kyoto, Japan and underwent latanoprost eye drop monotherapy for more than 4 years in at least 1 eye between 2005 and 2011. In all patients, CCT was evaluated by the Pentacam® Scheimpflug system at pretreatment, midpoint, and final follow-up. The Student's *t*-test was used to analyze the CCT changes.
Results: The mean CCT significantly decreased from 537 ± 34 μm at pretreatment to 526 ± 32 μm at the final follow-up ($P < 0.0001$). Interestingly, no significant difference was found between the mean CCT at midpoint and that at final follow-up ($P = 0.17$), yet the mean CCT significantly decreased to 529 ± 32 μm in the first 2 years ($P = 0.0015$). No correlation was found between IOP and CCT reduction.
Conclusions: The findings of this study show that latanoprost eye drops significantly reduce CCT during the initial stage of use, however, CCT reduction does not clinically affect IOP values.

Introduction

GLAUCOMA IS ONE of the most devastating diseases worldwide, as it is the main cause of blindness in adults. Moreover, it is reported that the estimated prevalence of glaucoma and suspected glaucoma is around 5% in Japanese over 40 years of age.[1] Studies have shown that intraocular pressure (IOP) is the most important risk factor for glaucoma and the progression of glaucoma.[2,3] Reduction of IOP is reportedly the most effective treatment for glaucoma,[4,5] and topical antiglaucoma medications are usually chosen for the primary treatment. Recently, many new antiglaucoma eye drops have been developed, including prostaglandin analogues,[6] thus providing clinicians with a wider choice of eye drops for treatment. However, the long-term use of antiglaucoma eye drops is known to be associated with side effects. Recent studies have shown that antiglaucoma medications can decrease the central corneal thickness (CCT).[7–15] Especially in glaucoma patients, the CCT is clinically important, as it affects the measurement of IOP. One study reported that IOP measurement may change by as much as 7 mmHg from the true IOP for every 100 μm variation in CCT from a normal CCT of 520 μm.[16] Another study showed a 1.9 mmHg per 100 μm tonometric measurement error.[17]

IOP reduction associated with the use of prostaglandins has been reported in all forms of glaucoma.[8–15] Moreover, there have been no reports of corneal thinning associated with other antiglaucoma medications.[10,11] However, most of those studies have been short-term and have only followed the patient's CCT for a period of approximately 2 years.[8–15]

Several questions have been raised about the relationship between IOP and CCT changes, as well as the influence of the prostaglandin analogue therapy on this relationship. Another question that intrigues researchers is whether or not the CCT reduction continues unabated with the lifelong use of prostaglandin analogue medications. The purpose of this present study was to evaluate the correlation between IOP and CCT changes in patients who underwent long-term (>4 years) use of only topical latanoprost monotherapy. To perform this long-term follow-up investigation, anterior segment tomography, suitable for screening and providing a noncontact quantitative evaluation of CCT, was used.

Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Patients and Methods

This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the tenets set forth in the Declaration of Helsinki. This retrospective study involved 52 eyes of 52 glaucoma patients who consulted with glaucoma specialists at the Glaucoma Service of Kyoto Prefectural University of Medicine, Kyoto, Japan, and who underwent latanoprost eye drop monotherapy for more than 4 years in at least 1 eye between 2005 and 2011. The only patients enrolled in this study were those who were first diagnosed as glaucoma by glaucoma specialists at our university hospital and who had no previous history of using glaucoma medications. The diagnostic criteria for glaucoma were glaucomatous changes in the visual field with optic nerve cupping, as well as the absence of other optic neuropathies in accordance with the guidelines of the Japan Glaucoma Society[16] and the European Glaucoma Society.[17] Patients with a past history of other treatments such as laser surgery or glaucoma surgery were excluded from the study.

In all patients, CCT changes were evaluated by use of the Pentacam® (OCULUS Optikgeräte GmbH, Wetzlar, Germany) Scheimpflug camera system at pretreatment, at midpoint [at approximately 2 years (24±2 months)], and at final follow-up (more than 4 years from the first observations, ie, 48–72 months). The thinnest values were used as the CCT values in the central area of the corneal thickness map. In patients who underwent latanoprost treatment in both eyes, only the right-eye CCT values were used. In patients who underwent latanoprost eye drop treatment in only 1 eye, the CCT values of that eye were used. Patients in whom the image could not be obtained clearly due to blinking or other reasons were excluded from the study. The Student's *t*-test was used to analyze the CCT changes, and a *P* value of <0.05 was considered statistically significant.

Results

Patient characteristics

This retrospective study involved 52 eyes (36 right eyes, 16 left eyes) of 52 glaucoma patients (mean age: 60.6±15.5 years). Of the 36 right-eye patients, 24 patients had used latanoprost monotherapy for both eyes and 12 patients had used latanoprost only for the right eye. The mean observation period of the patients was 55.0±6.7 months (mean± SD, range: 48–72 months). Of the 52 patients, the most prevalent type of glaucoma observed was normal-tension glaucoma (34 cases), followed by primary open-angle glaucoma (11 cases). The other types of glaucoma included primary angle-closure glaucoma (1case), secondary glaucoma (1 case), developmental glaucoma (2 cases), and mixed types of glaucoma (3 cases) (Table 1).

TABLE 1. DEMOGRAPHIC DATA OF THE GLAUCOMA PATIENTS

Characteristic	*Value (mean±SD)*
Age at first consultation (years)	60.6±15.5
Sex [*n* (%)]	
Male	23 (44%)
Female	29 (56%)
IOP values (without medications) (mmHg)	17.9±4.8
Refractive errors (D)	−3.5±3.6
Eye used for the CCT measurements [*n* (%)]	
Right	36 (69%)
Left	16 (31%)
Mean observation period (months)	55.0±6.7
Glaucoma type [*n* (%)]	
Primary open-angle glaucoma	11 (21%)
Normal-tension glaucoma	34 (65%)
Primary angle-closure glaucoma	1 (2%)
Secondary glaucoma	1 (2%)
Other types of glaucoma	5 (10%)

CCT, central corneal thickness; IOP, intraocular pressure.

Correlation between IOP reduction and CCT reduction

The relationship between IOP and CCT changes was examined in glaucoma patients treated only with topical latanoprost monotherapy, and IOP and CCT reduction was defined as follows: (1) IOP reduction: final follow-up IOP measurements minus the pretreatment IOP measurements, and (2) CCT reduction: final follow-up CCT measurements minus the pretreatment CCT measurements. No correlation was found between IOP reduction and CCT reduction from all plotted data (Fig. 1).

CCT changes during the total observation period

CCT reduction was examined in the patients who underwent only long-term topical latanoprost monotherapy to investigate whether the CCT reduction continued unabated during the use of the latanoprost eye drops. All CCT data obtained by the Pentacam measurements showed that the use of latanoprost eye drops had the tendency of reducing CCT over time, however, CCT was found to decrease rapidly during the beginning of treatment (Fig. 2).

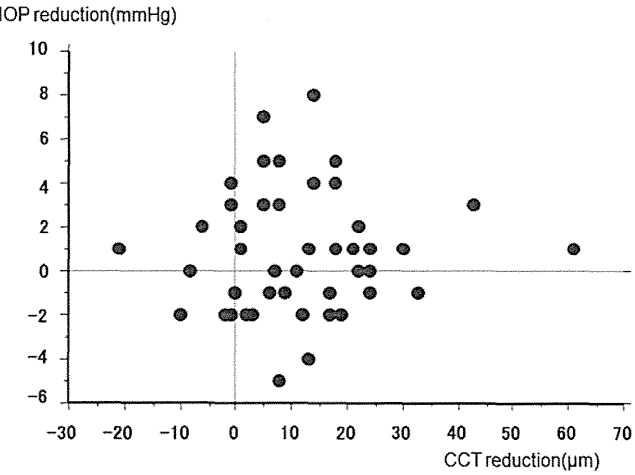

FIG. 1. The relationship between intraocular pressure (IOP) and central corneal thickness (CCT) changes. The relationship between IOP and CCT changes in the glaucoma patients using only topical latanoprost monotherapy ($n=52$). All IOP reduction (*x*-axis) and CCT reduction (*y*-axis) data are shown in the graph. No correlation was found between IOP reduction and CCT reduction.

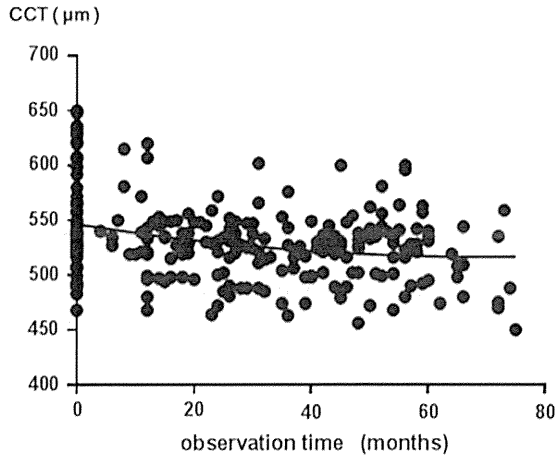

FIG. 2. CCT changes during the total observation period. All CCT data obtained by Pentacam® measurements are shown in the graph. With the use of latanoprost eye drops, CCT seemed to decrease rapidly at the beginning of use and then level off.

Mean CCT changes

To investigate the mean CCT changes, CCT changes that occurred were examined and compared; a representative case from 2006 to 2011 is shown in Fig. 3. In the representative Pentacam images shown in that figure, CCT was significantly decreased from 489 μm at pretreatment to 474 μm at the final follow-up. Most of the studies investigating whether latanoprost eye drop use decreased the CCT were performed over a period of approximately 2 years. In this present study, the CCT changes were evaluated at pretreatment, at midpoint (at 2 years from first consultation), and at final follow-up. The findings showed that mean CCT significantly decreased from 537 ± 34 μm at pretreatment to 526 ± 32 μm at the final follow-up ($P < 0.0001$). Interestingly, no significant difference was found between the mean CCT at midpoint and that at final follow-up ($P = 0.17$), however, the mean CCT significantly decreased to 529 ± 32 μm in the first 2 years of treatment ($P = 0.0015$) (Fig. 4).

Discussion

The findings of this study show that latanoprost eye drops significantly reduce CCT during the initial stage of use. In this study, no significant difference was found between the mean CCT at midpoint and that at final follow-up. However, the mean CCT significantly decreased in the first 2 years of treatment, thus confirming the findings of the previous studies that reported that prostaglandin analogue eye drops decreased the CCT for a period of approximately 2 years.[8–15] To confirm the CCT changes over the first 2 years of treatment, a comparison was made of the CCT at pretreatment, at 1 and 2 years of treatment, and also at the final follow-up in a subpopulation of patients ($n = 29$) in whom the CCT could also be observed at approximately 1 year (12 ± 1 months) of treatment. We found that the mean CCT gradually decreased in the first 2 years of treatment (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/jop).

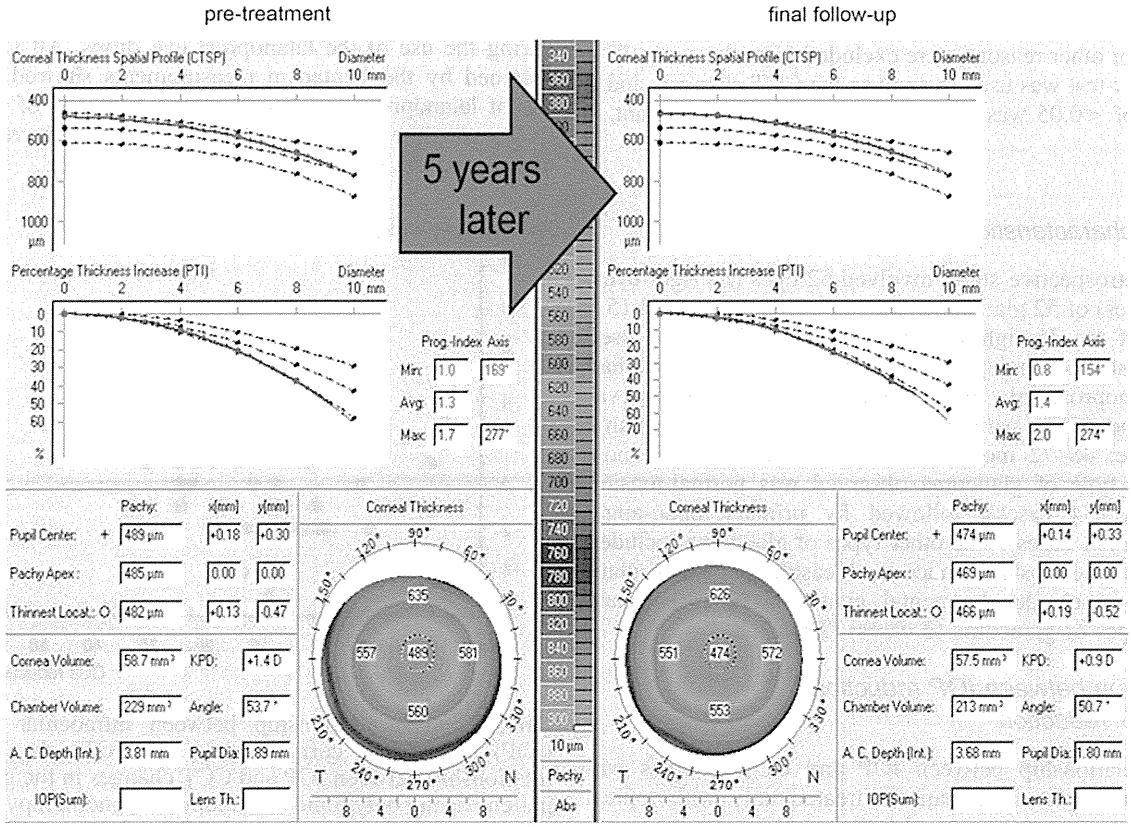

FIG. 3. Comparison of Pentacam images. As can be seen in these representative Pentacam images, CCT significantly decreased from 489 μm at pretreatment to 474 μm at the final follow-up.

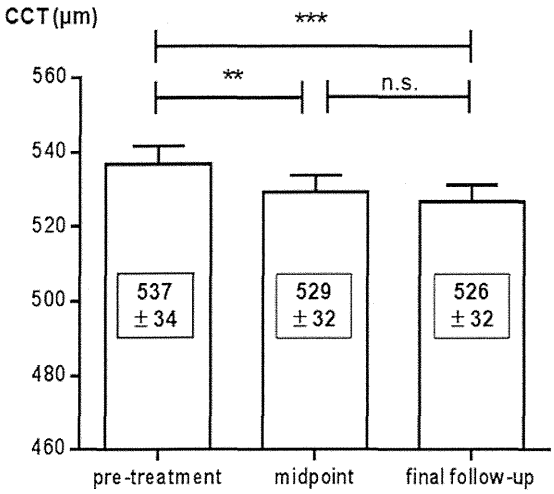

FIG. 4. Mean CCT changes. The mean CCT (*x*-axis) at pretreatment, at midpoint (approximately 2 years), and at final follow-up (more than 4 years after the first observations) (*y*-axis) is shown in the graph. The mean CCT significantly decreased from 537 ± 34 μm at pretreatment to 526 ± 32 μm at the final follow-up (***$P < 0.0001$). No significant difference was found between the mean CCT at midpoint and that at final follow-up ($P = 0.17$), however, the mean CCT significantly decreased to 529 ± 32 μm in the first 2 years of treatment (**$P < 0.01$).

In this study, no correlation was found between the IOP reduction and CCT reduction, thus showing that the CCT decrease induced by the instillation of latanoprost eye drops did not clinically affect IOP values. In the cases involved in this study, CCT was significantly reduced through the use of latanoprost eye drops, however, the IOP values were not considered to be in need of correction in relation to the CCT reduction. Previous studies have reported that IOP measurements may change by as much as 7 mmHg from the true IOP for every 100 μm variation in CCT from a normal CCT of 520 μm[18] or 1.9 mmHg[19] per 100 μm of tonometric measurement error. In this present study, only about 10 μm of CCT decrease was observed during the 4-year follow-up period, and IOP values were unaffected. There was a large variation in pretreatment CCT values, so those values were considered rather important in relation to the correction of IOP. As is shown in Fig. 1, some patients had zero IOP decrease or, in fact, IOP increase, thus indicating that those patients might be nonresponders to latanoprost. However, there are usually daily (and seasonal) changes of IOP, so it is difficult to determine whether or not those patients were nonresponders since their IOP was compared for just 2 time points—at pretreatment and at final follow-up. In most patients, their IOP was found to have decreased the next time it was measured. However, in some patients, their IOP was, indeed, increasing, due to their response to latanoprost, and some of those patients were started on a different glaucoma medication after the final follow-up examination. It should be noted that there were few patients in this study in whom the IOP was either increasing or remained the same at 2 consecutive time points in response to latanoprost, so it was difficult to analyze their CCT changes. Thus, those nonresponders may have different results for the CCT changes.

Studies have reported that the IOP reduction effect of latanoprost is achieved through a uveoscleral outflow increase by means of binding mainly with the prostaglandin F (FP) receptors, activating matrix metalloproteinases (MMPs), and reducing the constituents of the stromal extracellular matrix such as collagen that fill the spaces between the bundles of ciliary muscle fibers.[20–24] On the ocular surface, FP receptors are expressed mainly in the corneal epithelium, therefore, latanoprost eye drops may affect the corneal thinning through the corneal epithelial function.[25] Latanoprost may act through not only FP receptors but also through prostaglandin E (EP) receptors, such as EP3 and EP4,[26,27] which are mainly expressed in the corneal stroma,[25] where collagen is the main component. Therefore, CCT reduction may be caused by the reduction of collagens from the activation of EP receptors in the corneal stroma. Recently, it has been reported that human corneal epithelial cells manifest the mRNA expression of EP3 and EP4.[28] Latanoprost eye drops may affect the corneal thinning through these corneal epithelial EP3 and EP4 receptors. Many questions still remain in regard to the CCT reduction that occurs through the use of prostaglandin analogues. One question is about the main cause of the CCT reduction, that is, is it caused by MMP activations or corneal epithelial function changes? In addition, the question remains unanswered as to why these changes occur during the initial stage of the eye drop usage. It may be because all changes in the extracellular matrix usually occur in the first 2 years of treatment, with little or no change occurring after 2 years. Or it may be because the extracellular matrix changes gradually become less due to the responses to latanoprost lessening. The measurement of corneal hysteresis might help to more fully elucidate the extracellular matrix changes that affect CCT through the use of latanoprost. In future studies, attention should be focused on measuring the corneal hysteresis to shed light on what changes of the extracellular matrix, such as collagen, are involved in CCT reduction and help clarify the mechanisms of prostaglandin analogues.

It should be noted that this present study did include some limitations. First, there may have been some selection bias in regard to the samples, as the glaucoma patients were recruited from the glaucoma clinic of our university hospital. Second, treatment compliance by the patients might have influenced the CCT changes, that is, the lack of CCT decrease over the last 2 years might have been the result of some patients occasionally skipping or simply forgetting to instill the eye drops due to the long period of treatment. Thus, the patient's diligent adherence to the treatment regimen needs to be carefully followed.

In conclusion, the findings of this study show that the administration of latanoprost eye drops significantly reduces CCT during the initial stage of use, however, CCT did not continue to decrease over the long-term period. In addition, the CCT reduction caused by latanoprost eye drops was found to not clinically affect IOP values, thus showing that IOP correction does not need to be considered in patients undergoing latanoprost eye drop therapy.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Dr. Kazuhiko Mori
Department of Ophthalmology
Kyoto Prefectural University of Medicine
465 Kajii-cho, Hirokoji-agaru
Kawaramachi-dori, Kamigyo-ku
Kyoto 602-0841
Japan

E-mail: kmori@koto.kpu-m.ac.jp

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

Mayumi Ueta, MD, PhD,[a,b,c] Hiromi Sawai, PhD,[c] Chie Sotozono, MD, PhD,[a] Yuki Hitomi, PhD,[c] Nahoko Kaniwa, PhD,[d] Mee Kum Kim, MD, PhD,[e] Kyoung Yul Seo, MD, PhD,[f] Kyung-Chul Yoon, MD, PhD,[g] Choun-Ki Joo, MD, PhD,[h] Chitra Kannabiran, PhD,[i] Tais Hitomi Wakamatsu, MD, PhD,[j] Virender Sangwan, MD, PhD,[k] Varsha Rathi, MD, PhD,[k] Sayan Basu, MD, PhD,[k] Takeshi Ozeki, PhD,[l] Taisei Mushiroda, PhD,[l] Emiko Sugiyama, PhD,[d] Keiko Maekawa, PhD,[d] Ryosuke Nakamura, PhD,[d] Michiko Aihara, MD, PhD,[m] Kayoko Matsunaga, MD, PhD,[n] Akihiro Sekine, PhD,[o] José Álvaro Pereira Gomes, MD, PhD,[j] Junji Hamuro, PhD,[a] Yoshiro Saito, PhD,[d] Michiaki Kubo, MD, PhD,[l] Shigeru Kinoshita, MD, PhD,[a] and Katsushi Tokunaga, PhD[c]

Kyoto, Tokyo, Yokohama, and Toyoake, Japan, Seoul and Gwangju, Korea, Hyderabad, India, and São Paulo, Brazil

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*

From [a]the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto; [b]the Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto; [c]the Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo; [d]the Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo; [e]the Department of Ophthalmology, Seoul National University College of Medicine, Seoul; [f]the Department of Ophthalmology, Severance Hospital, Institute of Vision Research, Yonsei University College of Medicine, Seoul; [g]the Department of Ophthalmology, Chonnam National University, Gwangju; [h]the Department of Ophthalmology & Visual Science, Seoul St Mary's Hospital, College of Medicine, Catholic University of Korea, Seoul; [i]Prof Brien Holden Eye Research Centre and [k]Cornea and Anterior Segment Services, L V Prasad Eye Institute, Hyderabad; [j]the Department of Ophthalmology, Federal University of São Paulo; [l]the Research Group for Pharmacogenomics, RIKEN Center for Integrative Medical Sciences, Yokohama; [m]the Department of Environmental Immuno-Dermatology, Yokohama City University Graduate School of Medicine, Yokohama; [n]the Department of Dermatology, Fujita Health University School of Medicine, Toyoake; and [o]the EBM Research Center, Kyoto University Graduate School of Medicine, Kyoto.

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Corresponding author: Katsushi Tokunaga, PhD, Department of Human Genetics, Graduate School of Medicine, University of Tokyo 7-3-1 Hongo Bunkyoku, Tokyo 113-0033, Japan. E-mail: tokunaga@m.u-tokyo.ac.jp. Or: Mayumi Ueta, MD, PhD, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Hirokoji, Kawaramachi, Kamigyoku, Kyoto 602-0841, Japan. E-mail: mueta@koto.kpu-m.ac.jp.

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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

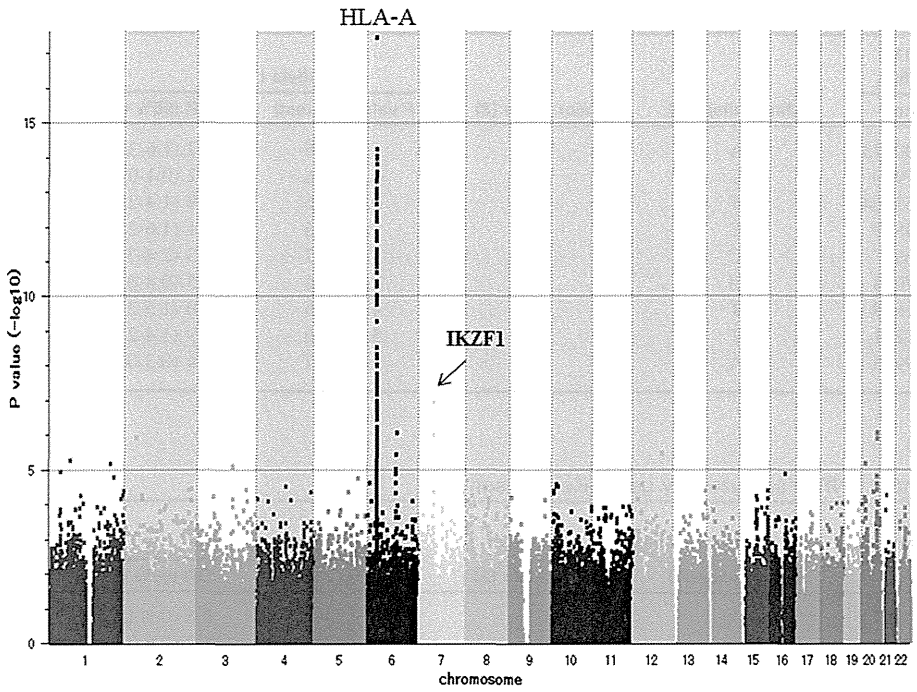

FIG 1. Results of the GWAS (Manhattan plot). *P* values were calculated with a χ^2 test for allele frequencies by using 117 cases and 691 control subjects.

this article's Online Repository at www.jacionline.org). The average overall call rate for the 117 patients with SJS/TEN and 691 control subjects reached 99.01% and 99.49%, respectively. We then applied the following threshold for single nucleotide polymorphism (SNP) quality control in data cleaning: SNP call rate of 95% or greater, minor allele frequency of 3% or greater, and Hardy-Weinberg equilibrium *P* value of .001 or greater in control subjects. A total of 449,205 SNPs on autosomal chromosomes passed the quality control filters and were used for the association study. A quantile-quantile plot of the distribution of test statistics for the comparison of genotype frequencies in cases and control subjects also showed that the inflation factor λ was 1.044 for all of the tested SNPs and decreased to 1.036 when SNPs in the *HLA* region were excluded (see Fig E2 in this article's Online Repository at www.jacionline.org). The χ^2 test was applied to an allele frequency model, all cluster plots for SNPs with *P* values of less than 10^{-3} were checked visually, and SNPs with ambiguous genotype calls were excluded.

The TaqMan SNP genotyping assay (Applied Biosystems, Foster City, Calif) or the DigiTag2[26,27] assay were used to confirm the genotypes at each SNP. For typing of validation by using the DigiTaq2 assay, 2 of the 691 control subjects were excluded because the quality of their data was insufficient. The remaining 689 subjects served as control subjects for typing by using the DigiTaq2 assay.

Some of the patients with CM-SJS/TEN and control subjects in the present study were subjects in our earlier studies. Fifty-seven candidate SNPs might have been associated with SJS/TEN ($P < 10^{-3}$). In the subsequent replication stage we selected 9 SNPs with *P* values of less than 10^{-5} from the results of the χ^2 test with allele frequency in the GWASs. SNP genotyping in 2 independent sets of samples (16 Japanese cases and 188 Japanese control subjects, 27 Korean cases and 90 Korean control subjects) was completed for the 8 SNPs for which functional TaqMan probes were available by using the TaqMan SNP genotyping assay, and 2 other independent sets of samples (20 Indian cases and 56 Indian control subjects, and 39 Brazilian cases and 135 Brazilian control subjects) were genotyped for the 4 IKZF1 SNPs. We used the Cochran-Mantel-Haenszel method as implemented in SAS (JMP Genomics; SAS Institute, Cary, NC) to conduct a meta-analysis.

In the TaqMan SNP genotyping assay PCR amplification was performed in a 10-μL reaction mixture containing 1 μL of genomic DNA, 5.0 μL of TaqMan GTXpress Master Mix (Applied Biosystems), and 40× TaqMan SNP Genotyping Assay probe (Applied Biosystems) for each SNP. The quantitative PCR thermal cycling program was 95°C for 20 seconds, followed by 50 cycles of 95°C for 3 seconds and 60°C for 20 seconds on the Applied Biosystems Step-one plus System.

For the replication study, a χ^2 test was applied to a 2 × 2 contingency table in the allele frequency.

Semiquantitative RT-PCR of *IKZF1* transcripts isoforms

Healthy volunteers were recruited from the University Hospital at KPUM. All subjects provided informed consent for genetic testing and quantification of gene transcripts under the approval of the ethics committee of KPUM. Venous blood samples were collected from the volunteers. DNA was extracted from whole blood samples by SRL (Tokyo, Japan). PureLine Total RNA Blood Purification Kits (Invitrogen, Carlsbad, Calif) were used to extract total RNA from whole blood samples.

Genotyping of rs4917014, rs10276619, and rs4917129 was performed with the TaqMan genotyping method. The ratios of Ik1 (full-length *IKZF1* isoform) and each *IKZF1* splicing isoform were estimated by using semiquantitative RT-PCR. RT-PCR was performed with the primer sets shown in Table E1 in this article's Online Repository at www.jacionline.org and Fast-Start Taq DNA polymerase (Roche, Mannheim, Germany) to detect Ik2, Ik3, and Ik4 isoforms (see the Methods section in this article's Online Repository). The nonparametric Jonckheere-Terpstra test and Kruskal-Wallis test were used for statistical analysis.

RESULTS

GWAS of CM-SJS/TEN with SMI in Japanese subjects

Fig 1 shows a genome-wide view of the SNP association data for 117 Japanese patients with CM-SJS/TEN with SMI and 691 control subjects, in which these association were based on allele frequencies. We found that the *HLA-A* region showed the strongest association with susceptibility to CM-SJS/TEN with

TABLE I. Nine SNPs associated with CM-SJS/TEN with SMI in Japanese patients ($P < 10^{-5}$)

Patients with SJS, n = 117; control subjects, n = 689				Allele (1 vs 2)		Minor allele frequency	
Gene symbol	rs no.	Minor allele (1)	Major allele (2)	*P* value (χ^2 test)	OR (95% CI)	Cases	Control subjects
LOC148709	rs10800873	A	C	4.24E-06	2.2 (1.6-3.2)	0.222	0.113
IGSF11	rs4687960	C	T	4.59E-06	0.2 (0.1-0.4)	0.030	0.135
FUT9	rs11153964	T	G	6.75E-06	1.9 (1.4-2.6)	0.385	0.244
FUT9	rs2294839	C	T	1.05E-06	2.1 (1.6-2.9)	0.323	0.184
IKZF1	rs897693	C	T	1.23E-07	4.3 (2.4-7.8)	0.085	0.021
IKZF1	rs4917014	G	T	2.12E-06	0.5 (0.4-0.7)	0.316	0.483
TMCC3	rs4761639	T	C	4.58E-06	2.0 (1.5-2.7)	0.355	0.217
SPTLC3	rs6041271	T	C	5.31E-06	1.9 (1.4-2.6)	0.616	0.455
TSHZ2	rs4809905	A	G	5.60E-07	0.4 (0.3-0.6)	0.188	0.355

TABLE II. Results of the 8 SNPs* analyzed by using Korean samples

Patients with SJS, n = 27; control subject, n = 90				Allele (1 vs 2)		Minor allele frequency	
Gene symbol	rs no.	Minor allele (1)	Major allele (2)	*P* value (χ^2 test)	OR (95% CI)	Cases	Control subjects
LOC148709	rs10800873	A	C	.574	0.8 (0.3-1.9)	0.130	0.161
IGSF11	rs4687960	C	T	.597	1.2 (0.6-2.6)	0.204	0.172
FUT9	rs11153964	T	G	.723	1.1 (0.5-2.4)	0.300	0.272
FUT9	rs2294839	C	T	.422	1.3 (0.7-2.7)	0.278	0.225
IKZF1	rs897693	C	T	.706	0.7 (0.1-5.8)	0.019	0.028
IKZF1	rs4917014	G	T	**3.97E-04**	**0.3 (0.1-0.6)**	0.222	0.494
TMCC3	rs4761639	T	C	.421	0.7 (0.4-1.6)	0.212	0.267
TSHZ2	rs4809905	A	G	.383	1.3 (0.7-2.6)	0.346	0.283

Values in boldface indicate statistical significance.
*The 8 SNPs showed *P* values of less than 10^{-5} in a GWAS with samples from Japanese subjects and for which functional TaqMan probes were available.

SMI ($P = 3.5 \times 10^{-18}$; OR, 4.4). This finding is consistent with findings from our previous studies, which showed strong association between SJS/TEN with SMI and *HLA-A*02:06*.[16,21-23]

Outside the *HLA* region, there were 57 SNPs with *P* values of less than 10^{-3} in allele frequency in the GWAS. Of the 57 SNPs, 45 had *P* values of less than 10^{-4}, and 9 of these 45 had *P* values of less than 10^{-5}. Although 2 loci, *IKZF1* and *TSHZ2*, among the 9 SNPs with *P* values of less than 10^{-5} showed relatively low *P* values (Table I), we could not find any associations that reached genome-wide significance in the GWAS.

Replication analysis with other Japanese and Korean subjects

Functional TaqMan probes were available for 8 of the 9 SNPs with *P* values of less than 10^{-5}. An independent set of 204 Japanese samples (16 Japanese patients with CM-SJS/TEN with SMI and 188 Japanese healthy control subjects) was used in a subsequent replication analysis to further evaluate these 8 SNPs. In this first replication study the SNPs had no significant association after applying Bonferroni correction because of the relatively small sample size (see Table E2 in this article's Online Repository at www.jacionline.org). However, the ORs of 7 of the 8 SNPs showed the same direction of association as those in the GWAS (rs11153964 [T vs G]: OR, 2.2 [$P = .029$] and rs2294839 [C vs T]: OR, 2.5 [$P = .013$] for *FUT9* and rs4917014 [G vs T]: OR, 0.5 [$P = .074$] for *IKZF1*).

Moreover, we genotyped these 8 SNPs in samples from the Korean population (27 patients with CM-SJS/TEN with SMI and 90 control subjects). Although the number of Korean cases was small, we found a significant association between Korean patients with CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.3; $P = 4.0 \times 10^{-4}$; Table II). Furthermore, the meta-analysis with Japanese and Korean samples showed a genome-wide significant association with *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 9.5 \times 10^{-10}$; see Table E3 in this article's Online Repository at www.jacionline.org).

Additional *IKZF1* SNP analysis with Japanese, Korean, Indian, and Brazilian subjects

Because the meta-analysis indicated that *IKZF1* rs4917014 was significantly associated with CM-SJS/TEN with SMI, we genotyped 2 additional *IKZF1* SNPs (rs10276619 and rs4917129); each of these SNPs was among the 57 SNPs with *P* values of less than 10^{-3} outside the *HLA* region in the GWAS. Notably, we could include additional samples in the analysis of 4 *IKZF1* SNPs (rs897693, rs4917014, rs4917129, and rs10276619); 16 additional samples from Japanese patients with CM-SJS/TEN with SMI collected at KPUM and 4 additional samples from Korean patients with CM-SJS/TEN with SMI were included. With all Japanese samples (149 from patients with SJS and 877 from control subjects), each of the 4 *IKZF1* SNPs (including rs10276619 and rs4917129) was significantly associated with CM-SJS/TEN with SMI (rs897693 [C vs T]: OR, 3.2; $P = 2.2 \times 10^{-6}$; rs4917014 [G vs T]: OR, 0.5; $P = 3.0 \times 10^{-8}$; rs4917129 [C vs T]: OR, 0.5; $P = 4.1 \times 10^{-6}$; and rs10276619 [G vs A]: OR, 1.8; $P = 1.3 \times 10^{-6}$; see

TABLE III. Meta-analysis of the 4 *IKZF1* SNPs using samples from Japanese, Korean, Indian, and Brazilian subjects

Patients with SJS, n = 239; control subjects, n = 1158		Minor	Major	Allele (1 vs 2)	
Gene symbol	rs number	allele (1)	allele (2)	*P* value*	OR (95% CI)
IKZF1	rs897693	C	T	7.98E-04	1.8 (1.3-2.5)
	rs4917014	G	T	**8.46E-11**	0.5 (0.4-0.6)
	rs4917129	C	T	**8.05E-09**	0.5 (0.4-0.7)
	rs10276619	G	A	**4.27E-09**	1.8 (1.5-2.3)

Values in boldface indicate statistical significance in the genome-wide association.
*Cochran-Mantel-Haenszel method.

Table E4 in this article's Online Repository at www.jacionline.org). With all Korean samples (31 from patients with SJS and 90 from control subjects), each of 3 of the 4 SNPs was significantly associated with CM-SJS/TEN with SMI (rs4917014 [G vs T]: OR, 0.4; $P = 1.2 \times 10^{-3}$; rs4917129 [C vs T]: OR, 0.4; $P = 4.3 \times 10^{-3}$; and rs10276619 [G vs A]: OR, 2.7; $P = 1.2 \times 10^{-3}$; see Table E5 in this article's Online Repository at www.jacionline.org). We also genotyped the 4 *IKZF1* SNPs in a set of Indian samples (20 from patients with CM-SJS/TEN with SMI and 56 from control subjects). Despite the small sample size, we found significant associations between Indian patients with CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.3; $P = .016$), although the result ceased to be significant when we corrected the P value for the number of alleles (ie, 4; see Table E6 in this article's Online Repository at www.jacionline.org). Furthermore, we also analyzed these 4 *IKZF1* SNPs in a set of Brazilian samples (39 from patients with CM-SJS/TEN with SMI and 135 from control subjects). There were no significant associations with these *IKZF1* SNPs; however, the ORs for these 4 SNPs with the Brazilian sample set showed the same direction of association as with Japanese samples (rs897693 [C vs T]: OR, 1.2; $P = .57$; rs4917014 [G vs T]: OR, 0.9; $P = .58$; rs4917129 [C vs T]: OR, 0.7; $P = .112$; and rs10276619 [G vs A]: OR, 1.5; $P = .12$; see Table E7 in this article's Online Repository at www.jacionline.org).

A meta-analysis that combined data from the Japanese, Korean, Indian, and Brazilian samples showed a significant genome-wide association between CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 8.5 \times 10^{-11}$; rs4917129 [C vs T]: OR, 0.5; $P = 8.1 \times 10^{-9}$; and rs10276619 [G vs A]: OR, 1.8; $P = 4.3 \times 10^{-9}$; Table III and see Fig E3 in this article's Online Repository at www.jacionline.org, which is a Forest plot summarizing the findings).

Association of *IKZF1* SNPs with the relative quantity of alternatively spliced isoforms

IKZF1 rs4917014 genotypes are not known to affect *IKZF1* mRNA expression levels.[28] Our analysis performed with the GENEVAR (Gene Expression Variation; Wellcome Trust Sanger Institute) database[29] also indicated that there was no relationship between the *IKZF1* rs4917014 genotype and gene expression levels (see Fig E4 in this article's Online Repository at www.jacionline.org; JPT, $P = .74$; Chinese, $P = .13$). Additionally, nonsynonymous substitutions, stop-gain, stop-loss, or splice site variants in the *IKZF1* gene region have never been registered in the Asian dbSNP database. Together, this evidence indicated that rs4917014, rs10276619, or other polymorphisms in strong linkage equilibrium with rs4917014 or rs10276619 did not influence *IKZF1* gene expression levels or functional amino acid replacements.

The human *IKZF1* gene encodes at least 11 protein isoforms through alternative mRNA splicing in human subjects. Although these isoforms share the C-terminus domain required to interact with other proteins, the number of N-terminus zinc-finger domains, which bind to DNA, differs among isoforms.[30-33] Ik1 (the full-length *IKZF1* isoform), Ik2 (lacking exon 4–encoded amino acid), Ik3 (lacking exon 6 and exon 7 amino acids), and Ik4 (lacking exon 4 and exon 6 amino acids) are reportedly abundantly expressed in human peripheral blood leukocytes,[32] and individual isoforms have 4, 3, 3, or 2 N-terminus zinc-finger domains, respectively (Fig 2, *A-D*). The location of rs4917014 and accession numbers of IKZF1 isoforms were shown in Fig E5 in this article's Online Repository at www.jacionline.org. Semiquantitative RT-PCR was performed to assess expression of these 4 *IKZF1* mRNA isoforms in each whole blood sample taken from 33 healthy subjects with different *IKZF1* SNP (rs4917014, rs10276619, and rs4917129) genotypes. The quantity of Ik2, Ik3, and Ik4 isoforms was normalized to the quantity of the Ik1 isoform for each subject to standardize the difference in the numbers of *IKZF1*-positive cells among individual samples. As shown in Fig 2, *E*, the Ik2/Ik1 ratio was significantly associated with the rs4917014 genotype ($P < .01$, Jonkheere-Terpstra test; $P < .05$, Kruskal-Wallis test). Notably, the rs10276619 and rs4917129 genotypes were also significantly associated with the Ik2/Ik1 ratio (Fig 2, *F* and *G*; $P < .05$, Jonkheere-Terpstra test; not significant by using the Kruskal-Wallis test). Probably because of the relatively low expression levels of Ik3 and Ik4 isoforms, associations of *IKZF1* SNPs with Ik3/Ik1 or Ik4/Ik1 ratios did not reach statistical significance (data not shown). These results indicated that the Ik2/Ik1 ratio might be influenced by *IKZF1* SNPs that are significantly associated with susceptibility to CM-SJS/TEN with SMI.

DISCUSSION

Here, we found that an *IKZF1* polymorphism was significantly associated with CM-SJS/TEN with SMI (including severe ocular surface complications) by performing a GWAS with Japanese samples and subsequent replication analyses with Korean, Indian, and Brazilian samples. We should acknowledge potential inflation because of population stratification in the replication studies, especially for Brazilian samples. Nevertheless, the data from individual population replication sets showed the same significant associations (Korean and Indian) or the same direction of association (Brazilian), and our meta-analysis that combined data from the Japanese, Korean, Indian and Brazilian replication sets showed a significant association between CM-SJS/TEN with SMI and *IKZF1* (rs4917014 [G vs T]: OR, 0.5; $P = 8.5 \times 10^{-11}$; Table III).

In the GWAS the *HLA-A* region clearly showed the strongest association with susceptibility to CM-SJS/TEN with SMI. Regarding *IKZF1*, statistical power of the present GWAS sample size was calculated to be 86% with the following conditions: risk allele OR of 2.02, prevalence of 0.00001, and risk allele frequency in control subjects of 0.51. These findings indicated that *IKZF1*

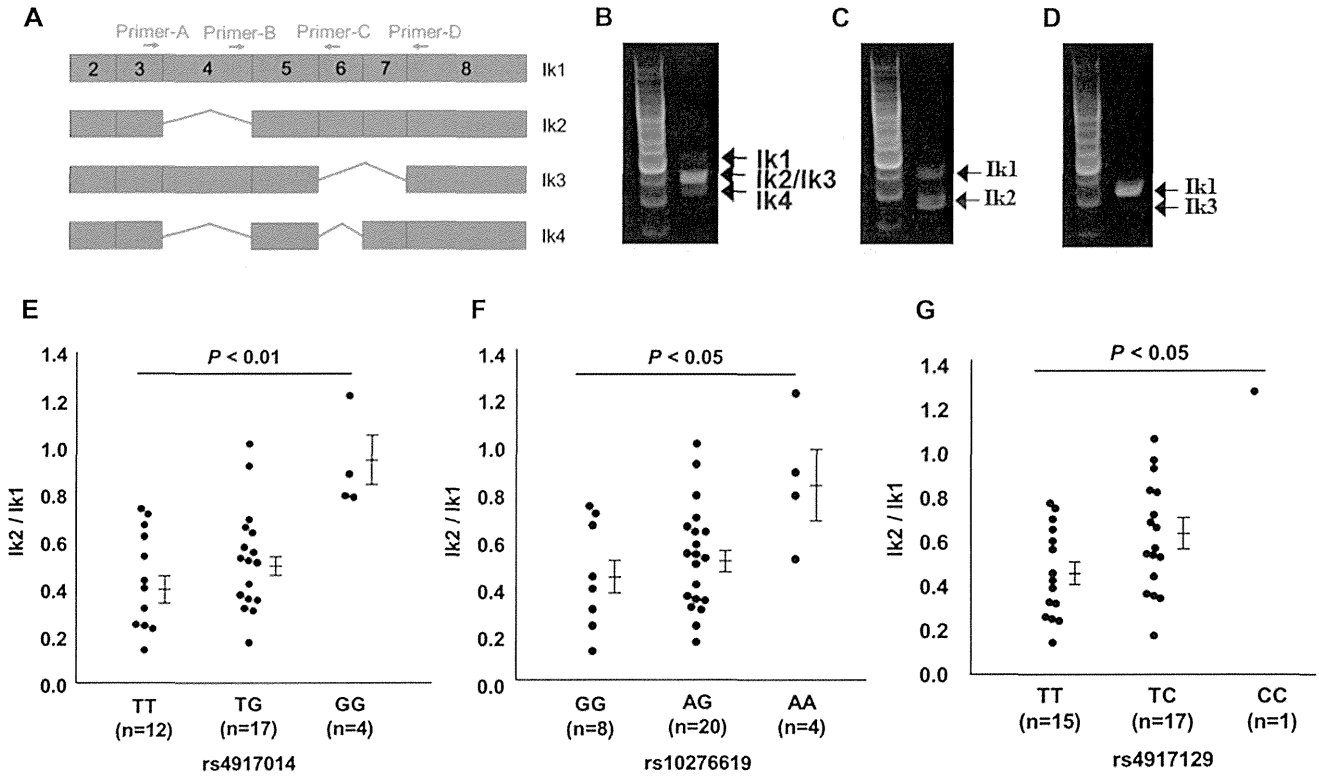

FIG 2. *IKZF1* SNPs (rs4917014, rs10276619, and rs4917129) were significantly associated with the efficiency of alternative splicing of *IKZF1* mRNA. **A,** There are 4 major splicing isoforms in *IKZF1* mRNA. Primers for semiquantitative RT-PCR are shown with *red arrows*. **B-D,** Band patterns of each amplicon of *IKZF1* alternative splicing isoforms using quantitative RT-PCR. Ik1, Ik2/Ik3 (same molecular size), and Ik4 can be detected by primer A and primer D (Fig 2, *B*). Ik1 and Ik2 can be detected by primer A and primer C (Fig 2, *C*). Ik1 and Ik3 can be detected by primer B and primer D (Fig 2, *D*). **E-G,** Ik2/Ik1 ratios are increased in whole blood cells from healthy subjects with the rs4917014-GG (Fig 2, *E*), rs10276619-AA (Fig 2, *F*), or rs4917129-CC (Fig 2, *G*). *Dots* show the Ik2/Ik1 ratio in each subject, and means ± SEMs are shown. $P <$ .01 (rs4917014) and $P <$ .05 (rs10276619 and rs4917129), Jonkheere-Terpstra test. Representative results from 3 independent experiments are shown.

might be a universal marker for susceptibility to CM-SJS/TEN with SMI.

We also found that the relative quantity of Ik2 (*IKZF1* isoform lacking exon 4–encoded amino acids) was significantly lower in subjects with the susceptible genotypes at *IKZF1* SNPs.

We previously reported that Toll-like receptor 3 *(TLR3)*,[1,23,34] IL-4 receptor *(IL4R)*,[18,19] *IL13*,[19] Fas ligand *(FASL)*,[20] and prostaglandin E receptor 3 *(PTGER3)*[14,34] SNPs showed significant associations with SJS/TEN with severe ocular surface complications. Moreover, we also reported that about 80% of our patients with CM-SJS/TEN had taken cold medicines, such as NSAIDs and multi-ingredient cold medications, for common cold symptoms within several days before disease onset.[14] Here we focused on CM-SJS/TEN with SMI (including severe ocular surface complications) and performed a GWAS followed by replication studies, concluding that *IKZF1* was significantly associated with CM-SJS/TEN with SMI. A meta-analysis with Japanese, Korean, Indian, and Brazilian subjects confirmed the significant association between the SNP rs4917014 in the *IKFZ1* locus and CM-SJS/TEN with SMI. To our knowledge, this is the first report of a genome-wide significant association between a non-*HLA* gene and CM-SJS/TEN with SMI.

SJS/TEN comprises various phenotypes, including both with SMI and without SMI, and the causative drugs also vary; for example, carbamazepine, allopurinol, and cold medicines, including NSAIDs and multi-ingredient cold medications, can independently elicit SJS/TEN. We reported that HLA-mediated genetic predispositions for SJS/TEN with SMI might differ from those for SJS/TEN without SMI.[16] The *IKZF1* SNP rs4917014 was not significantly associated with CM-SJS/TEN without SMI, such as severe ocular surface complications (cases, n = 16; control subjects, n = 877; rs4917014 [G vs T]: OR, 1.34; P = .41), and the OR of this SNP showed the opposite direction of association with CM-SJS/TEN with SMI, suggesting that the *IKZF1* SNPs are significantly associated with CM-SJS/TEN with SMI but not CM-SJS/TEN without SMI.

Each causative drug has different genetic predispositions; for example, carbamazepine-induced SJS/TEN is associated with *HLA-B*15:02*[7] or *HLA-A*31:01*,[8,9] and allopurinol-induced SJS/TEN is associated with *HLA-B*58:01*[10-12] (see Fig E6 in this article's Online Repository at www.jacionline.org). Interestingly, the *IKZF1* SNPs were not significantly associated with mild or moderate cold medicine–related adverse cutaneous reactions (not severe types; see Table E8 in this article's Online Repository at www.jacionline.org). It is possible that additional genetic factors could be identified by focusing on each distinct clinical phenotype, as in the case of the present study

on CM-SJS/TEN with SMI, including severe ocular surface complications.

In the present study we found that CM-SJS/TEN with SMI was highly significantly associated with the *IKZF1* gene. Previous studies reported that Ikaros, the protein product encoded by *IKZF1*, is a member of the DNA-binding protein family and works as a transcription factor in the thymus, spleen, peripheral blood lymphocytes, and lymph nodes. Ikaros plays an important role in the development of several lymphocytes, such as T and B cells.[35] There are dominant-negative forms of Ikaros that cannot bind to DNA.[36] Ikaros 2 (Ik2 isoform) and Ikaros 4 (Ik4 isoform) lack the DNA-binding ability and seem to be dominant-negative forms. In our present study the quantity of the Ik2 isoform is increased in disease-protective genotypes of *IKZF1* (rs4917014 G/G and rs10276619 A/A). As shown in Fig 2, the number of N-terminus zinc-finger domains of the Ik2 isoform is less than that of the Ik1 isoform. Taken together, these results indicated that the *IKZF1* Ik2 isoform might work as a dominant-negative form against the Ik1 isoform by having fewer N-terminus Zinc-finger domains in effector cells in the immune system, and the failure of immune tolerance by excess signaling in the antigen receptors is prevented by this dominant-negative isoform. Furthermore, interferon regulatory factors (IRFs) are implicated in regulating Ikaros.[36,37] IRF-5 and IRF-8 control the expression of *IKZF1* and Ikaros, thereby regulating the induction of inflammatory cytokines and type 1 interferons. Because TLRs are located upstream of IRFs, innate immunity might be associated with the regulation of Ikaros; this hypothesis is consistent with our previous findings that SJS/TEN might be associated with abnormalities of innate immunity.[1,34,38]

Although rs4917014, rs10276619, and rs4917129 are located in the 5′ region of *IKZF1* and the distances between these SNPs and *IKZF1* exon 1 are greater than 50 kb, our present results indicate that these SNPs play some role in the efficiency of *IKZF1* alternative splicing. These SNPs and the 5′ part of the *IKZF1* gene are located in the same linkage disequilibrium block. Therefore some polymorphisms that are in the same linkage disequilibrium block with rs4917014, rs10276619, and rs4917129 are probably located in a splice site or splicing regulatory motif (ie, exonic splicing enhancer, exonic splicing silencer, intronic splicing enhancer, or intronic splicing silencer) that regulates *IKZF1* alternative splicing, and these polymorphisms might alter the efficiency of alternative splicing. Further investigations and analysis are needed to elucidate the role of *IKZF1* in *the* pathogenesis of CM-SJS/TEN with SMI and to identify the causal variants related to the regulation of alternative splicing.

Interestingly, it is reported that the *IKZF1* SNP (rs4917014) has been associated with systemic lupus erythematosus in a Chinese population.[39] However, to the best of our knowledge, our patients with CM-SJS/TEN with SMI have not had systemic lupus erythematosus.

Previously and currently, we found that the *HLA-A* region showed the strongest association with susceptibility to CM-SJS/TEN with SMI[16]; *HLA-A*02:06* was strongly associated with CM-SJS/TEN with SMI, including severe ocular complications, in Japanese populations.[16] Here we use KPUM samples from the GWAS to perform an analysis examining the relationship between the 4 relevant *IKZF1* SNPs and *HLA-A*02:06*; we found that the *IKZF1* SNPs showed significant associations in both *HLA-A*02:06*–positive and *HLA-A*02:06*–negative subjects (see Tables E9 and E10 in this article's Online Repository at www.jacionline.org).

Notably, some infectious agents can, like cold medicine, trigger SJS/TEN.[40,41] We previously reported that rs3775296T/T, an SNP genotype at *TLR3*, is a risk factor for SJS/TEN with SMI, including severe ocular complications,[1] and that the interaction between *HLA-A*02:06* and rs3775296T/T manifests more than additive effects.[23] *TLR3* is a pattern recognition receptor and is related to virally activated innate immunity, which often cause common cold symptoms.

Here, we conducted a GWAS for CM-SJS/TEN with SMI in Japanese subjects and performed replication studies with Korean, Indian, or Brazilian subjects to identify host genetic factors for CM-SJS/TEN with SMI. Our findings might expand our knowledge of pathogenic pathways in SJS/TEN with SMI.

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Clinical implications: Ikaros might be one of the key players in the pathogenesis of CM-SJS/TEN with SMI and could be a target for the prevention or treatment of this disease.

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METHODS

Patients

This study was approved by the Institutional Review Board of KPUM, the University of Tokyo, and the National Institute of Health Sciences. Moreover, this study was also approved by the Institutional Review Boards of Seoul National University College of Medicine; Yonsei University College of Medicine; Chonnam National University Medical School and College of Medicine; the Catholic University of Korea; the L V Prasad Eye Institute in Hyderabad, India; and the Federal University of São Paulo, Brazil.

All experimental procedures were conducted in accordance with the principles of the Declaration of Helsinki. The purpose of the research and the experimental protocols was explained to all participants, and each provided prior written informed consent for their participation.

Samples from 117 patients with CM-SJS/TEN with SMI used for the GWAS and DigiTaq2 assay were collected at KPUM. Of the 117 patients with CM-SJS/TEN with SMI, 46 were male and 71 were female, and their ages ranged from 6 to 85 years (median age, 41.5 ± 17.1 [SD] years). The age at onset of SJS/TEN ranged from 1 to 70 years (median age at onset, 24.6 ± 16.2 [SD] years). For some patients, the specific drug or drugs used are not known. Healthy Japanese volunteers served as the control subjects for the GWAS (n = 691) and DigiTaq2 assay (n = 689). They were independently recruited by the University of Tokyo (n = 419; 350 female and 69 male subjects; unknown ages) and by KPUM (n = 270; 158 female and 112 male subjects; median age, 33.9 ± 10.1 [SD] years).

For the replication study, samples from 16 Japanese patients (11 female and 5 male patients) who had recently developed CM-SJS/TEN with SMI, including pseudomembrane formation and epithelial defects of the ocular surface (median age, 35.6 ± 18.1 [SD] years, which was the same as the median age at onset), were collected by participating institutes or through a nationwide blood sampling network operated by the National Institute of Health Sciences 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 were used for the diagnosis of SJS/TEN. Additional healthy Japanese volunteers recruited by the University of Tokyo (n = 188; 95 female and 93 male subjects; median age, 54.1 ± 8.0 [SD] years) served as control subjects.

For analysis of the 4 *IKZF1* SNPs, we could include 16 additional samples from Japanese patients with CM-SJS/TEN collected at KPUM, 6 from male and 10 from female patients (median age, 50.8 ± 18.3 [SD] years). The age at onset of SJS/TEN ranged from 4 to 64 years (median age at onset, 26.0 ± 17.0 [SD] years).

For the replication study, samples from Korean patients with CM-SJS/TEN with SMI were collected from the Seoul National University College of Medicine, Yonsei University, Chonnam National University, and the Catholic University of Korea. Samples were collected from 27 patients (10 male and 17 female patients) ranging in age from 4 to 66 years (median age, 34.2 ± 18.1 [SD] years), and the age at onset of SJS/TEN ranged from 3 to 63 years (median age at onset, 22.6 ± 16.2 [SD] years). Moreover, for analysis of the 4 *IKZF1* SNPs, we could include 4 additional samples from Korean patients with CM-SJS/TEN. We analyzed the 4 *IKZF1* SNPs in 31 samples from Korean patients with CM-SJS/TEN with SMI (11 male and 20 female patients; median age, 36.0 ± 19.1 [SD] years; median age at onset, 25.2 ± 17.4 [SD] years). Healthy Korean volunteers (n = 90; 35 male and 55 female subjects; median age, 31.7 ± 7.9 [SD] years) served as control subjects.

For the replication study, samples from Indian patients with CM-SJS/TEN with SMI were collected from the L V Prasad Eye Institute (n = 20; 12 male and 8 female patients; age range, 7-63 years; median age, 27.1 ± 13.4 [SD] years). The age at onset of SJS/TEN ranged from 3 to 42 years (median age at onset, 19.2 ± 12.2 [SD] years; unknown age of onset for 8 patients). Healthy Indian volunteers (n = 58; 31 male and 27 female subjects; median age, 36.3 ± 12.3 [SD] years) served as control subjects.

For the replication study, samples from Brazilian patients with CM-SJS/TEN with SMI were collected from the Federal University of São Paulo (n = 39; 15 male and 24 female patients; age range, 13-69 years; median age, 36.5 ± 15.7 [SD] years). Age at onset of SJS/TEN ranged from 3 to 69 years (median age at onset, 23.6 ± 16.9 [SD] years). Healthy Brazilian volunteers (n = 135; 55 male and 79 female subjects; median age, 41.1 ± 12.8 [SD] years) served as control subjects.

For Japanese cases and control subjects from KPUM, genomic DNA was isolated from peripheral blood by SRL (Tokyo, Japan). For Japanese cases from the National Institute of Health Sciences, genomic DNA was isolated from peripheral blood by Mitsubishi Chemical Medience Corporation (Tokyo, Japan). For samples from Korean subjects, the PAXgene Blood DNA kit (Qiagen, Hilden, Germany) was used to extract DNA from whole peripheral blood. For sampled from Indian subjects, DNA was extracted from whole peripheral blood with the phenol chloroform method.

For Japanese GWAS control samples from the University of Tokyo, a commercial kit (QIAamp Blood Kit; Qiagen, Hilden, Germany) was used to extract genomic DNA from peripheral blood samples. All 419 blood and DNA samples were deidentified. Verbal informed consent was obtained from all participants before 1990. In this study written informed consent was not obtained because blood sampling was conducted before the "Ethical guidelines for human genome and genetic sequencing research" were established in Japan. Under the condition that the DNA samples were permanently delinked from the subjects, this study was approved by the Research Ethics Committee of Graduate School of Medicine, University of Tokyo. For Japanese replication control samples, genomic DNA samples were provided by Health Science Research Resources Bank (Osaka, Japan).

Semiquantitative RT-PCR of *IKZF1* transcript isoforms

Healthy volunteers were recruited from the University Hospital at KPUM. All subjects provided informed consent for genetic testing and quantification of gene transcripts under the approval of the ethics committee of KPUM. Venous blood samples were collected from the volunteers. DNA was extracted from whole blood samples by SRL. PureLine Total RNA Blood Purification Kits (Invitrogen, Carlsbad, Calif) were used to extract total RNA from whole blood samples.

Genotyping of rs4917014, rs10276619, and rs4917129 was performed with the TaqMan genotyping method. The ratios of Ik1 (full-length *IKZF1* isoform) and each *IKZF1* splicing isoform were estimated by means of semiquantitative RT-PCR. RT-PCR was performed with the primer sets shown in Fig 2, *A*, and Table E1 and Fast-Start Taq DNA polymerase (Roche) to detect Ik2, Ik3, and Ik4 isoforms. To achieve linear amplification for the detection of Ik2 (exon 4–skipping isoform), Ik3 (exon 6 and exon 7–skipping isoform), or Ik4 (exon 4 and exon 6–skipping isoform), 28, 30, or 29 amplification cycles were found to be optimal in preliminary experiments, respectively; each cycle comprised incubations at 98°C for 5 seconds, 55°C for 10 seconds, and 72°C for 5 seconds in the GeneAmp PCR system 9700 (Perkin-Elmer Applied Biosystems). After electrophoresis of PCR amplicons through 1% agar gel, band intensities were quantified with Image J software (National Institute of Health, Bethesda, Md). These experiments were repeated 3 times with essentially identical results. The nonparametric Jonckheere-Terpstra test and Kruskal-Wallis test were used for statistical analysis.

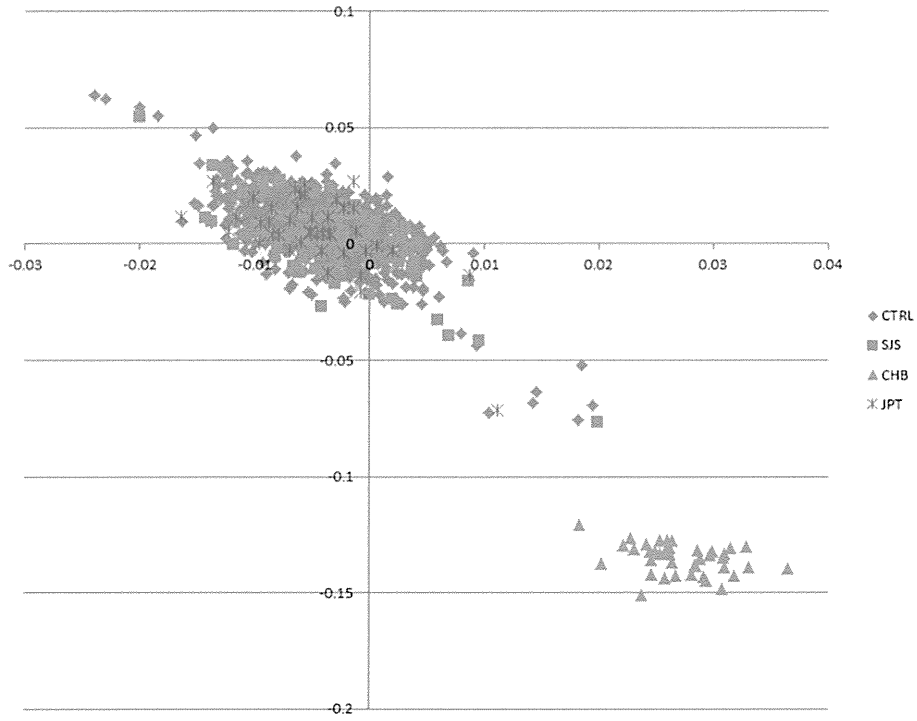

FIG E1. Principal component analysis.

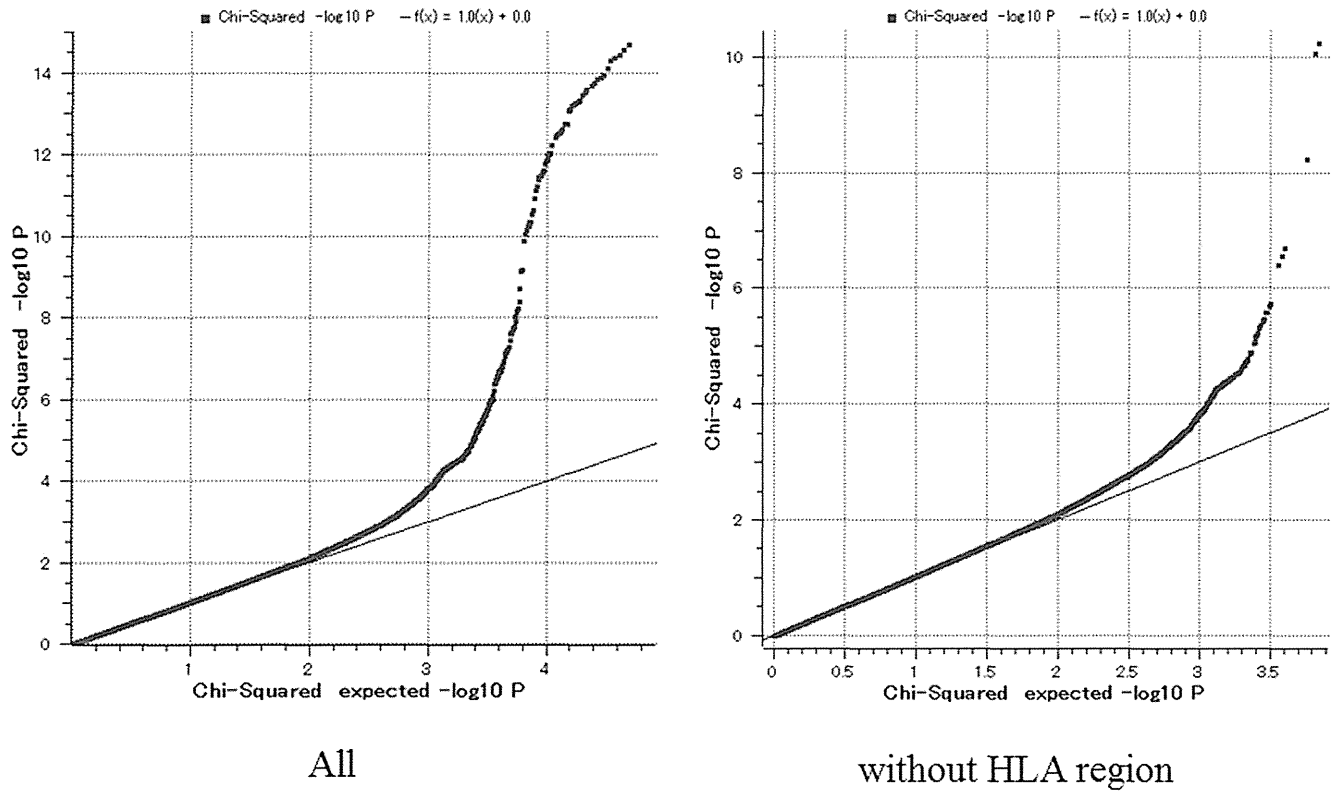

FIG E2. Quantile-quantile plot of the distribution of test statistics for comparison of genotype frequencies in cases and control subjects.

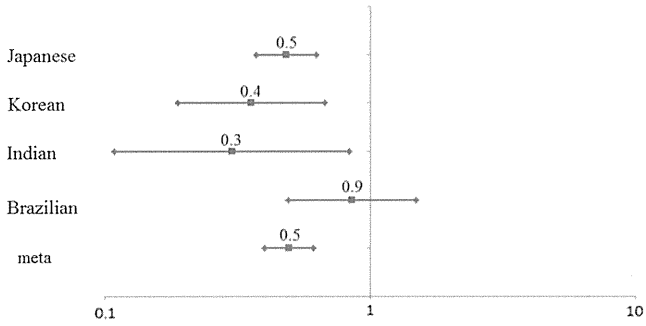

FIG E3. Forest plot presenting ORs in 4 populations and meta-analysis on rs4917014 in *IKZF1*. *Horizontal lines* represent 95% CIs.

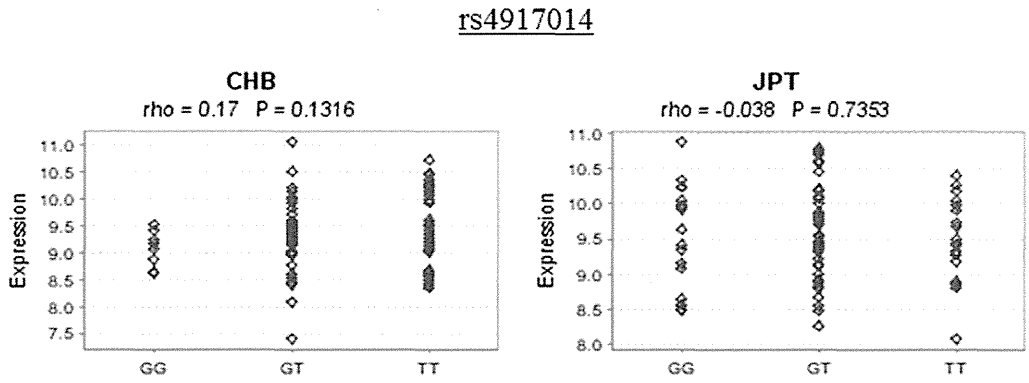

FIG E4. No significant association between rs4917014 genotype and *IKZF1* gene expression level based on GENEVAR (GENe Expression VARiation, Wellcome Trust Sanger Institute) database.

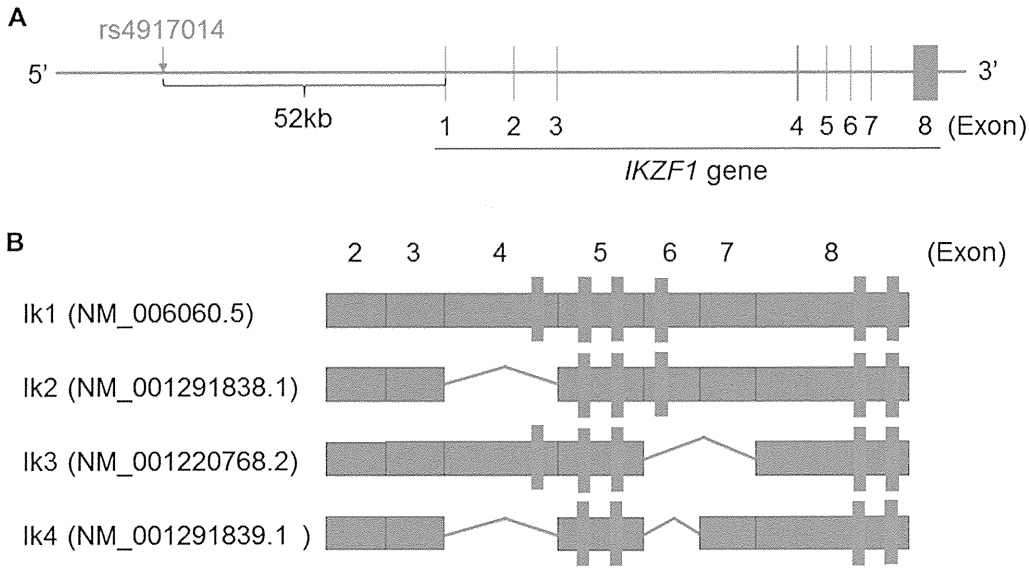

FIG E5. The location of rs4917014 and accession numbers of each *IKZF1* isoform.

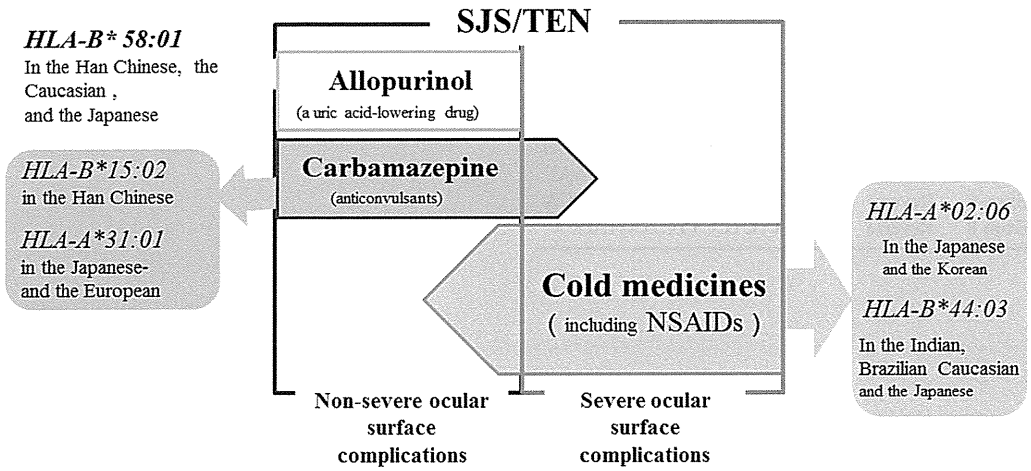

FIG E6. Particular *HLA* alleles are associated with different causative drugs.