Association of IKZF1 SNPs in cold medicine-related Stevens–Johnson syndrome in Thailand

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Abstract

Purpose: Our meta-analysis of several ethnic groups (Japanese, Korean, Indian, Brazilian) revealed a significant genome-wide association between cold medicine-related SJS/TEN (CM-SJS/TEN) with severe ocular complications (SOC) and IKZF1 SNPs, suggesting that IKZF1 might be a potential marker for susceptibility to CM-SJS/TEN with SOC. In this study, we examined the association between CM-SJS/TEN with SOC and the IKZF1 SNPs in the Thai population.

Methods: 57 CM-SJS/TEN with SOC and 171 control samples were collected at Chulalongkorn University and Mahidol University. Genomic DNA samples were genotyped for the IKZF1 SNPs at Kyoto Prefectural University of Medicine in Japan using the TaqMan SNP genotyping assay.

Results: The four SNPs previously reported to be associated with CM-SJS/TEN with SOC in the Japanese were examined in the Thai samples. Although the number of Thai cases (n = 57) was small, a significant association between CM-SJS/TEN with SOC and IKZF1 SNPs which included rs4917014 (T vs G, OR = 2.9, p = 0.0012, Pc = 0.0049), rs4917129 (T vs C, OR = 2.8, p = 0.0026, Pc = 0.010) and rs10276619 (G vs A, OR = 1.8, p = 0.012, Pc = 0.048) was identified.

Conclusion: In addition to the Japanese, Korean and Indian populations, Thai cases with CM-SJS/TEN and SOC were significantly associated with IKZF1 SNPs. With our previous report of the critical role of IKZF1 in mucocutaneous inflammation, these results suggest that IKZF1 is important in the pathogenesis of CM-SJS/TEN with SOC.

Keywords: Stevens–Johnson syndrome, Cold medicine, Severe ocular complications, SNPs, IKZF1
of several ethnic groups (Japanese, Korean, Indian, Brazilian) revealed a significant genome-wide association between CM-SJS/TEN with SOC and IKZF1, suggesting that IKZF1 might be a potential marker for susceptibility to CM-SJS/TEN with SOC [5]. In this study, we examined the association between Thai CM-SJS/TEN with SOC and the IKZF1 SNPs, known to be associated with the Japanese CM-SJS/TEN with SOC.

The CM-SJS/TEN with SOC and control samples were collected at Chulalongkorn University (King Chulalongkorn Memorial Hospital and Mahidol University (Ramathibodi Hospital and Siriraj Hospital). Genomic DNA samples were genotyped for the IKZF1 SNPs at Kyoto Prefectural University of Medicine in Japan. The study was approved by the institutional review board of all institutes. Protocol explanation and obtaining written informed consent were done in all participants before starting experimental procedures. All experimental processes were complied with the principles set forth in the Helsinki declaration. The diagnostic criteria of SJS/TEN were based on a confirmed history of acute onset of high fever, skin eruption with at least two sites of serious mucocutaneous involvement including the oral mucosa and the ocular surface [5]. Thai healthy volunteers were used as controls. CM was defined as the drug that patients took for relieving cold symptoms including nonsteroidal anti-inflammatory drugs (NSAIDS), acetylsalicylic acid, and other multi-ingredient cold medications [6]. We previously reported that in Japanese, for SJS/TEN with SOC, acetylsalicylic acid was a main drug of CM; 48% of the patients have taken acetylsalicylic acid before developing SJS/TEN with SOC [6]. In Thailand, paracetamol (which is equal to acetylsalicylic acid) might be also important causative drug of CM, because 20 of 57 (35%) patients have taken paracetamol before developing it. The patients were classified as having SOC if the following manifestations were detected; severe conjunctivitis, pseudomembrane, and epithelial defect on the ocular surface in the acute stage and/or ocular sequelae such as dry eye, trichiasis, symblepharon, and conjunctival invasion into the cornea in the chronic stage [6].

Of 57 CM-SJS/TEN with SOC, 23 were male and 34 were female; their age ranged from 6 to 73 years [median 42.3 ± 15.6 (SD) years]. The age at SJS/TEN onset ranged from 2 to 54 years (median 24.8 ± 13.8 years). The controls were 85 males and 86 females; their median age was 39.5 ± 14.3 years. Some of the CM-SJS/TEN patients and some of the controls had been included in our earlier studies [7].

Subjects were obtained DNA extraction from whole peripheral blood using the PAX gene blood DNA kits (Qiagen, Hilden, Germany) or from saliva using Oragene DNA (Kyodou International, Kanagawa, Japan). The TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) was used for the genotypes of the IKZF1 gene as previously reported [5]. Chi squared test was applied to a two-by-two contingency table for the allele frequency and the dominant and recessive models.

The 4 SNPs previously reported to be associated with CM-SJS/TEN with SOC in the Japanese were examined in the Thai samples. Although the number of Thai cases (n = 57) was small, we again found a significant association between CM-SJS/TEN with SOC and 3 IKZF1 SNPs which included rs4917014 (T vs G, OR = 2.9, p = 0.0012, Pc = 0.0049), rs4917129 (T vs C, OR = 2.8, p = 0.0026, Pc = 0.010) and rs10276619 (G vs A, OR = 1.8, p = 0.012, Pc = 0.048) (Table 1). Our previous results of meta-analysis in the Japanese, Korean, Indian and Brazilian showed the significant associations in 3 IKZF1 SNPs [rs4917014 (T vs G, OR = 2, p = 8.5 × 10−11) (which is equal to (G minor allele) vs T (major allele), OR = 0.5) in the previous paper], rs4917129 (T vs C, OR = 2, p = 8.0 × 10−9) (which is equal to (C minor allele) vs T (major allele), OR = 0.5 in the previous paper) and rs10276619 (G vs A, OR = 1.8, p = 4.3 × 10−8) [5]. Present results in the Thai population are in concordance with our previous report.

Our functional analysis of SNPs of the IKZF1 gene revealed that the ratio of the splicing isoforms Ik2/Ik1 could be affected by IKZF1 SNPs significantly associated with susceptibility to CM-SJS/TEN with SOC [5]. The quantity of the Ik2 isofrom is increased in disease-protective genotypes of IKZF1 (rs4917014 G/G and rs10276619 A/A) [5]. As ikaros 2, an Ik2 isofrom lacks the DNA-binding ability and seems to be dominant-negative. It is possible that the function of Ikaros, the protein of IKZF1, is enhanced in CM-SJS/TEN with SOC [5].

Ikaros is a transcription factor that regulates numerous biological events. It was reported that Ikaros-null mice lack B-lineage cells, NK cells, peripheral lymph node- and fetal T-cells, thus Ikaros family members regulate important cell fate decisions in the development of the adaptive immune system [8]. On the other hand, we have reported that epithelium might contribute to the pathobiology of CM-SJS/TEN with SOC [9]. Thus, we produced K5-Ikzf1-EGFP transgenic mice (Ikzf1 Tg) by introducing the Ik1 isofrom into cells expressing keratin 5, which is expressed in epithelial tissues such as the epidermis and conjunctiva and found that mucocutaneous inflammation was exacerbated in Ikzf1-Tg mice. They developed dermatitis with some having blepharoconjunctivitis [10]. Histological analysis showed not only dermatitis but also tissue inflammation in the blepharoconjunctiva, tongue, and paronychia [10], similar to the findings in patients in the acute state of SJS/TEN with SOC [9]. Our studies demonstrated that IKZF1 could play a critical role in...
maintaining mucocutaneous homeostasis [10] and suggested that it might be implicated in the aggravation of mucocutaneous inflammation seen in the presence of CM-SJS/TEN with SOC [10].

In addition to the Japanese, Korean and Indian in our previous report [5], CM-SJS/TEN with SOC was significantly associated with IKZF1 SNPs in the Thai cases. With our previous report of the critical role of IKZF1 in mucocutaneous inflammation [8], these results suggest that IKZF1 is important in the pathogenesis of CM-SJS/TEN with SOC.

Acknowledgements
We thank all the patients and volunteers who enrolled in our study. We also thank Ms. Hiromi Nishigaki for technical assistance.

Authors' contributions
MU made the experimental design, analyze the SNPs, wrote the text, and prepared the table. PC, PJ, PP and PP collected samples. PC, PJ, VP, KL, KS and MU made the experimental design, analyze the SNPs, wrote the text, and approved the final manuscript.

Funding
This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government, and by the JSPS Core-to-Core Program, A. Advanced Research Networks and also partly supported by grants-in-aid for scientific research from the Japanese Ministry of Health, Labor and Welfare.

Ethics approval and consent to participate
The study was approved by the institutional review board of Chulalongkorn University (King Chulalongkorn Memorial Hospital), Mahidol University (Ramathibodi Hospital and Siriraj Hospital) and Kyoto Prefectural University of Medicine.

Consent for publication
Protocol explanation and obtaining written informed consent were done in all participants before starting experimental procedures.

Competing interests
The authors declare that they have no competing interests.

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Received: 20 June 2019    Accepted: 13 November 2019

Published online: 22 November 2019

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Table 1 Woolf’s correction

| rs number of IKZF1 SNP | Genotypes Case (N = 57) | Control (N = 171) | Allele 1 vs 2 | Genotype 11 vs 12 + 22 | Genotype 11 + 12 vs 22 |
|-----------------------|------------------------|------------------|--------------|----------------------|----------------------|
| rs897693              | 11 T/T 36/57 (63.2%)   | 114/171 (66.7%)  | 0.69         | 0.63                 | 1.00                 |
| rs49171014            | 11 T/T 46/57 (80.7%)   | 100/171 (58.5%)  | 1.22E—03     | 2.46E—03             | 0.0619               |
| rs4917129             | 11 T/T 47/57 (82.5%)   | 109/171 (63.7%)  | 2.59E—03     | 8.48E—03             | 4.97E—02             |
| rs10276619            | 11 T/T 25/57 (43.9%)   | 52/171 (30.4%)   | 1.21E—02     | 3.39E—02             | 0.199                |
| rs12       | 12 T/C 10/57 (17.5%)   | 51/171 (29.8%)   | 1.03E—02     | 2.67 (1.26–5.66)     | 8.24* (0.48–142.08) |
| rs10       | 12 G/A 26/57 (45.6%)   | 78/171 (45.6%)   | 4.84E—02     | –                    | 0.119                |
| rs12       | 22 C/C 4/57 (7%)       | 12/171 (7%)      | –            | –                    | 2.68 (1.07–6.70)    |
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