Research Article

Comparison between the HLA-B*58:01 Allele and Single-Nucleotide Polymorphisms in Chromosome 6 for Prediction of Allopurinol-Induced Severe Cutaneous Adverse Reactions

Niwat Saksit,1,2 Nontaya Nakkam,1 Parinya Konyoung,3 Usanee Khunarkornsiri,3 Wongwiwat Tassaneeyakul,4 Pansu Chumworathayi,5 Sirimas Kanjanawart,1 Chonlaphat Sukasem,6,7 Alisara Sangviroon,8 Oranuch Pattanacheewapull,9 and Wichittra Tassaneeyakul1

1Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
2School of Pharmaceutical Sciences, University of Phayao, Phayao, Thailand
3Pharmacy Unit, Udon Thani Hospital, Udon Thani, Thailand
4Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand
5Pharmacy Unit, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
6Department of Pathology, Division of Pharmacogenomics and Personalized Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
7Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
8Pharmacy Unit, Police General Hospital, Bangkok, Thailand
9Pharmacy Department, Khon Kaen Hospital, Khon Kaen, Thailand

Correspondence should be addressed to Wichittra Tassaneeyakul; wichittra.tassaneeyakul@gmail.com

Received 24 July 2017; Accepted 8 November 2017; Published 17 December 2017

Academic Editor: Ethan M. Shevach

Copyright © 2017 Niwat Saksit et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Severe cutaneous adverse drug reactions (SCARs) are life-threatening reactions. The strong association between the HLA-B*58:01 allele and allopurinol-induced SCARs is well recognized. Screening for HLA-B*58:01 allele before prescribing allopurinol in some populations has been recommended. Several single-nucleotide polymorphisms (SNPs) in chromosome 6 have been found to be tightly linked with the HLA allele, and these SNPs have been proposed as surrogate markers of the HLA-B*58:01 allele. This study aimed to evaluate the association between three SNPs in chromosome 6 and allopurinol-induced SCARs in a Thai population. The linkage disequilibrium between the HLA-B*58:01 allele and these SNPs was also evaluated. Results showed that three SNPs including rs9263726, rs2734583, and rs3099844 were significantly associated with allopurinol-induced SCARs but with a lower degree of association when compared with the HLA-B*58:01 allele. The sensitivity, specificity, PPV, and NPV of these SNPs were comparable to those of the HLA-B*58:01 allele. Although detection of the SNP is simpler and less expensive compared with that of the HLA-B*58:01 allele, these SNPs were not perfectly linked with the HLA-B*58:01 allele. Screening using these SNPs as surrogate markers of the HLA-B*58:01 allele to avoid SCARs prior to allopurinol administration needs caution because of their imperfect linkage with the HLA-B*58:01 allele.
1. Introduction

Allopurinol, a uric acid-lowering agent, is one of the most common culprit drugs for severe cutaneous adverse drug reactions (SCARs). These reactions range from Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) to drug reaction with eosinophilia and systemic symptoms (DRESS). Although the incidence of allopurinol-induced SCARs is rare, they are life-threatening reactions. Data from systematic reviews shows that although the prevalence of gout in Asian population is lower than that in Caucasian populations, the hypersensitivity caused by allopurinol reported in Asians was about 73% of the reported cases [1]. In Taiwan, the annual incidence rates for allopurinol hypersensitivity were 4.68 per 1000 new users, 2.02 per 1000 new users for related hospitalization, and 0.39 per 1000 new users for related mortality [2]. The mortality rate of allopurinol hypersensitivity in Taiwan was about 8.3% [2]. Similarly, the incidence rate of allopurinol-induced SCARs in Thailand reported from the biggest hospital in Thailand was about 2.13 per 1000 new users [3]. Compared with that of other drug-induced SCARs, the mortality rate of allopurinol-induced SCARs observed in a Thai population is the highest up to 11% [4].

Although allopurinol-induced SCARs are considered as idiosyncratic reactions, current studies have identified several risk factors of such fatal reactions that include both genetic and nongenetic factors [4–6]. For genetic factors, the specific allele of the human leukocyte antigen (HLA), namely, the HLA-B*58:01 allele, is the first genetic marker that was found to be strongly associated with allopurinol-induced SCARs in a Taiwanese population [7]. This association has been confirmed in Thai [4, 8, 9] and Han Chinese [10, 11] populations. Unlike the HLA-B*15:02 allele which is specific to Chinese and Southeast Asian population, the associations between the HLA-B*58:01 allele and allopurinol-induced SCARs were also demonstrated in Japanese [12, 13], Korean [14], and Caucasian populations [15]. The strength of association ranges from an odds ratio (OR) of 39 to 696 [16], and the sensitivity and specificity of the HLA-B*58:01 allele for the prediction of allopurinol-induced SCARs were 93% (95% CI: 85–97%) and 89% (95% CI, 87–91%) across Asian and Caucasian populations [16]. To date, the American College of Rheumatology recommends the testing for the HLA-B*58:01 allele in certain ethnicities with a high frequency of this allele and showing an elevated risk for allopurinol-induced SCARs in HLA-B*58:01 allele carriers such as Han Chinese, Thai, and Korean populations [17].

Due to the highly polymorphic nature of the HLA gene, a specific method is required in order to determine a specific HLA allele, particularly the HLA-B alleles in which exon 2 and 3 regions exhibit the highest variability. Several molecular techniques including specific sequencing primers (SSP) PCR, sequence-specific oligonucleotide (SSO) probes, and sequencing-based typing (SBT) have been demonstrated to be specific methods for the determination of HLA genotype; however, these techniques are quite expensive, time-consuming, and not commonly available in hospital laboratories. A recent genome-wide association study in a Japanese population has discovered a number of single-nucleotide polymorphisms (SNPs) in chromosome 6 that were strongly associated with allopurinol-induced SCARs [13]. These SNPs included rs2734583 in the HLA-B-associated transcript 1 (BAT1) gene, rs3099844 in the HLA complex P5 (HCP5) gene, and rs9263726 in the psoriasis susceptibility 1 candidate 1 (PSORS1C1) gene. Due to the absolute linkage disequilibrium between rs9263726 and the HLA-B*58:01 allele found in 27 Japanese patients who suffered from allopurinol-induced SCARs, the rs9263726 has been proposed as a surrogate marker for allopurinol-induced SJS/TEN [18]. Similarly, an absolute linkage disequilibrium between the rs9263726 and allopurinol-induced SCARs has also been recently reported in 17 Eastern Chinese patients [19]. Ethnic specificity of the associations between HLA alleles and drug-induced SCARs is well recognized [20]. Whether these SNPs are good surrogates of allopurinol-induced SCARs in other ethnic societies or not needs to be evaluated. The present study aimed to evaluate the degree of relationship between the three selected SNPs in chromosome 6 including rs9263726, rs2734583, and rs3099844 and allopurinol-induced SCARs in a Thai population that has a relatively high frequency of the HLA-B*58:01 allele. In addition, the sensitivity and specificity for these selected SNPs for the prediction of allopurinol-induced SCARs and their linkage disequilibrium with the HLA-B*58:01 allele are characterized in the present study.

2. Materials and Methods

2.1. Study Population Assessment and Enrollment. A total of 96 allopurinol-induced SCARs patients including 23 DRESS and 73 SJS/TEN patients were recruited for the study. These patients had been diagnosed with allopurinol-induced SCARs within the first 3 months of allopurinol exposure. The phenotype of SCARs in an individual patient was scored and assessed by the ALDEN [21] or the RegiSCAR algorithms [22] whereas the assessment of causative drugs was performed using Naranjo’s algorithm [23]. All of the SCARs patients who represented at least a probable score were recruited for the study.

For the control cohort comparison, 193 patients were recruited from patients who had used allopurinol for more than 6 months without any evidence of cutaneous reactions. Written informed consent was obtained from each patient. The study protocol within the hospital network of Khon Kaen University was approved by the Khon Kaen Ethics Committee for Human Research, Khon Kaen University, Thailand (HE510837). The study protocol was also approved by each hospital, where IRB function was available.

2.2. Genomic DNA Preparation. Leukocytes were separated from peripheral blood by centrifugation at 3500 rpm for 15 min or buccal swab. Genomic DNA (gDNA) was isolated from leukocytes using a QIAamp DNA Blood mini kit (QIAGEN GmbH, Hilden, Germany). The quantity and quality of gDNA were checked by a Nano Drop machine (Thermo Scientific NanoDrop 2000c) and kept at −20°C until used.
2.3. Detection of the HLA-B*58:01 Allele. The HLA-B*58:01 allele was detected using a commercial PG801 DNA detection kit (Pharmigene Inc., Taipei, Taiwan) as described previously [8]. The HLA-B genotypes of patients who had the HLA-B*58:01 allele were confirmed using the PCR sequence-specific oligonucleotide probe method as has been described in a previous study [24].

2.4. Detection of rs9263726, rs2734583, and rs3099844 SNPs. The rs9263726 in the PSORS1C1 gene was detected using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay as previously described [18]. The PCR conditions were set at 94°C for 5 min followed by 35 cycles of amplification at 94°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec and final extension of 72°C for 7 min. The 260 bp length PCR products were digested with Fok I endonuclease (New England Biolabs, Beverly, MA, USA), and the DNA fragments were detected by agarose gel electrophoresis.

The rs2734583 in the BAT1 gene (assay ID: C__26778946_20) and the rs3099844 in the HCP5 gene (assay ID: C__27455402_10) were detected by TaqMan® SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA).

2.5. Statistical Analysis. Two-tailed Student’s t-test and Fisher’s exact test were used to compare the differences among SCARs and tolerant control demographic data (SPSS for Windows; IBM Corp., New York, USA). The risks for allopurinol-induced SCARs were calculated using the dominant model and univariate logistic regression analysis by SPSS software (IBM Corp., New York, USA). The Haldane modification of Woolf’s formula was used among samples containing zero. The Bonferroni-corrected P value (Pc-value) was calculated by multiplying P value by 4 which is the number of multiple comparisons to account for the observed SNPs in this study, and P-value less than 0.05 was considered statistically significant. The individual sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of the HLA-B*58:01 allele or the selected SNPs for the screening of allopurinol-induced SCARs were calculated.

The estimated linkage disequilibrium coefficients (D') and coefficient of correlation ($r^2$) between the HLA-B*58:01 allele and the selected SNPs were calculated using the PLINK (V1.07) program.

3. Results

3.1. Comparisons of the Associations between the HLA-B*58:01 Allele and the Selected SNPs with Allopurinol-Induced SCARs. Ninety-six allopurinol-induced SCARs (i.e., 23 DRESS and 73 SJS/TEN patients) and 193 allopurinol-tolerant controls were recruited for the study. The demographic and clinical characteristics of the case and the control groups are shown in Table 1.

Of ninety-six allopurinol-induced SCARs patients, 90 patients (93.75%) carried the HLA-B*58:01 allele including 21/23 (91.30%) patients in the DRESS group and 69/73 (94.52%) patients in the SJS/TEN group (Table 2). Compared with the SCARs group, only 23 of 193 (11.92%) patients in the tolerant control group carried this allele (Table 2). Results from the univariate analysis show that the HLA-B*58:01 allele was strongly associated with allopurinol-induced SCARs with an OR of 110.87 (95% CI = 43.57–282.15, P-value = 2.05 × 10−22) (Table 2). The risk of allopurinol-induced DRESS was 77.61-fold (95% CI = 17.07–352.85, P-value = 7.12 × 10−8) in the patients who carried the HLA-B*58:01 allele compared with those who did not carry this allele. Similar to that observed in SJS/TEN, the risk of allopurinol-induced SJS/TEN in the HLA-B*58:01 allele carriers was 127.50-fold (95% CI = 42.53–382.27, P-value = 1.99 × 10−15) (Table 2).

Among the three selected SNPs in chromosome 6, the rs2734583 in the BAT1 gene showed the strongest association with allopurinol-induced DRESS with an OR of 64.56 (95% CI = 14.31–291.16, P-value = 2.35 × 10−7) followed by the rs3099844 in the HCP5 gene (OR = 59.38, 95% CI = 13.21–266.97, P-value = 4.04 × 10−7) and the rs9263726 in the PSORS1C1 gene (OR = 22.21, 95% CI = 7.10–69.46, P-value = 3.91 × 10−5) (Table 2). In contrast, the strongest association between allopurinol-induced SJS/TEN was observed with rs9263726 (OR = 63.60, 95% CI = 23.85–169.56, P-value = 4.22 × 10−16). When considering both DRESS and SJS/TEN as SCARs, these three SNPs were significantly associated with SCARs induced by allopurinol with the strength of association ranging from 45 to 60 (Table 2). All of these SNPs in the study populations were followed the Hardy−Weinberg equilibrium.

The sensitivity, specificity, NPV, and PPV of the HLA-B*58:01 allele compared with the three selected SNPs in chromosome 6 for the prediction of both DRESS and SJS/TEN caused by allopurinol are shown in Table 3. These parameters of the HLA-B*58:01 allele for the prediction of DRESS or SJS/TEN were the highest. The sensitivity, specificity, PPV, and NPV of these three SNPs for the prediction of SJS/TEN as well as SCARs were quite similar (Table 3). It should be noted that these values of the rs9263726 for the prediction of DRESS were relatively lower than those of the other two SNPs (Table 3).

Concerning the haplotypes of these three selected SNPs (rs9263726-rs2734583-rs3099844), 69.57% (16/23) of the patients in the DRESS group and 83.56% (61/73) of the patients in the SJS/TEN group carried the CA-TC-CA haplotype compared with 12.44% (24/193) in the controls (Table 2). About 80.83% (156/193) of the control patients carried the CA-TC-GA haplotype. A small number of patients in the case and the control groups carried other haplotypes (data not shown). Compared with the GG-TT-CC haplotype, the risk of allopurinol-induced DRESS in the patients who carried the CA-TG-CG haplotype was about 52.00-fold (95% CI = 11.24–240.51, Pc = 1.17 × 10−6) whereas that of allopurinol-induced SJS/TEN was about 99.13-fold (95% CI = 33.03–297.52, Pc = 9.90 × 10−16) (Table 2). Sensitivity, specificity, PPV, and NPV of the CA-TG-CG haplotype screening for the prediction of DRESS and SJS/TEN are shown in Table 3.
| Characteristic data                        | DRESS (n = 23) | SJS/TEN (n = 73) | SCARs (n = 96) | Control (n = 193) |
|------------------------------------------|----------------|-----------------|---------------|------------------|
| **Age (year)**                           |                |                 |               |                  |
| Mean (SD)                                | 65 (14)        | 65 (12)         | 65 (12)       | 64 (12)          |
| Median (range)                           | 68 (28–82)     | 68 (38–84)      | 68 (28–84)    | 66 (29–91)       |
| **Gender**                               |                |                 |               |                  |
| Female; n (%)                            | 10 (43.48)     | 40 (54.79)++++  | 50 (52.08)****| 49 (25.39)       |
| **Onset of SCARs (day)**                 |                |                 |               |                  |
| Mean (SD)                                | 29 (13)        | 19 (11)         | 21 (13)       | —                |
| Median (range)                           | 30 (10–60)++++ | 18 (2–60)++++   | 20 (2–60)++++ | —                |
| Allopurinol dose (mg)                    |                |                 |               |                  |
| Mean (SD)                                | 171 (78)       | 182 (106)       | 179 (99)      | 194 (90)         |
| Median (range)                           | 200 (100–300)  | 100 (50–600)    | 100 (50–600)  | 200 (100–600)    |
| **Indication of allopurinol**            |                |                 |               |                  |
| Hyperuricemia; n (%)                     | 11 (47.83)++++ | 13 (17.81)++++  | 24 (25.00)****| 3 (1.55)         |
| Gouty arthritis; n (%)                   | 12 (52.17)     | 60 (82.19)      | 72 (75.00)    | 190 (98.45)      |
| **Baseline kidney function**             |                |                 |               |                  |
| Blood urine nitrogen (mg/dL)             |                |                 |               |                  |
| Mean (SD)                                | 29.79 (21.14)  | 24.85 (20.09)   | 26.58 (20.33) | 18.63 (10.40)    |
| Median (range)                           | 21.35 (11.00–80.00) | 18.50 (10.00–105.00) | 21.00 (10.00–105.00)* | 16.20 (3.00–70.00) |
| Serum creatinine (mg/dL)                 |                |                 |               |                  |
| Mean (SD)                                | 2.20 (2.00)    | 1.54 (1.05)     | 1.69 (1.40)   | 1.47 (1.46)      |
| Median (range)                           | 1.40 (0.90–9.60) | 1.30 (0.50–8.82) | 1.30 (0.50–9.60) | 1.20 (0.70–14.80) |
| Estimated glomerular filtration rate (eGFR) (mL/min/1.73 m²)  | | | | |
| Mean (SD)                                | 44.05 (21.99)  | 48.61 (21.95)   | 47.58 (21.91) | 58.01 (20.41)    |
| Median (range)                           | 46.00 (6.54–88.61)*** | 45.27 (4.62–127.17)*** | 45.65 (4.62–127.17)*** | 57.45 (3.12–12.49) |
| eGFR < 30.00; n (%)                      | 4 (17.39)      | 14 (19.18)**    | 18 (21.43)**  | 13 (7.47)        |
| 30.00 ≤ eGFR < 60.00; n (%)              | 11 (47.83)     | 37 (50.68)*     | 48 (57.14)    | 78 (44.83)       |
| eGFR ≥ 60.00; n (%)                      | 4 (17.39)*     | 14 (19.18)***   | 18 (21.43)**** | 83 (47.70)       |

*EGR: estimated glomerular filtration rate (expressed as mL/min/1.73 m²) calculated by Modification of Diet in Renal the Disease (MDRD) study equation [39]. Indicated significant difference between case and tolerant control. *P value < 0.05, **P value < 0.01, ***P value < 0.001, ****P value < 0.0001, and *****P value < 0.00001.
Table 2: Univariate analysis of the association between HLA-B*58:01 allele and SNPs with allopurinol-induced SCARs.

| Allele/SNPs | Control (n = 193) | DRESS (n = 23) | SJS/TEN (n = 73) | SCARs (n = 96) |
|-------------|-------------------|----------------|-----------------|--------------|
|             | n (%)             | n (%)          | OR [95% CI]     | OR [95% CI] |
| HLA-B*58:01 |                   |                |                 |              |
| Negative    | 170 (88.08)       | 2 (8.70)       | Reference       | 6 (6.25)     |
| Positive    | 23 (11.92)        | 21 (91.30)     | 77.61 [17.07–352.85] | 110.87 [43.57–282.15] |
| rs9263726 (G/A) |          |                |                 |              |
| Negative (GG)| 159 (82.38)       | 4 (17.39)      | Reference       | 9 (9.38)     |
| Positive (GA/AA)| 34 (17.62)     | 19 (82.61)     | 22.21 [7.10–69.46] | 45.21 [20.73–98.60] |
| rs2734583 (T/C) |            |                |                 |              |
| Negative (TT)| 166 (86.01)       | 2 (8.70)       | Reference       | 9 (9.37)     |
| Positive (TC/CC)| 27 (13.99)     | 21 (91.30)     | 64.56 [3.91×10^7] | 59.43 [26.76–131.97] |
| rs3099844 (C/A) |            |                |                 |              |
| Negative (CC)| 164 (84.97)       | 2 (8.70)       | Reference       | 9 (9.38)     |
| Positive (CA/AA)| 29 (15.03)     | 21 (91.30)     | 59.38 [14.31–291.16] | 54.67 [22.77–120.66] |
| SNPs haplotypes |            |                |                 |              |
| GG-TT-CC    | 156 (80.83)       | 2 (8.70)       | 52.00 [4.04×10^7] | 83.42 [32.74–212.55] |
| GA-TC-CA    | 24 (12.44)        | 16 (69.57)     | 11.24–240.51] | 9.43×10^7 |
3.2. Concordance between the HLA-B*58:01 Allele and the Selected SNPs in Chromosome 6. Results from linkage disequilibrium (LD) analysis in the study population (combining data from the case group and the control group, \(n = 289\)) showed that each SNP showed strong linkage disequilibrium with the HLA-B*58:01 allele with \(D'\) values of more than 0.90 and \(r^2\) values of more than 0.8 (Table 4). Subgroup analysis of LD in the DRESS group revealed that these three SNPs were a complete LD with the HLA-B*58:01 allele (\(D' = 1.0\)) but perfect LD with an \(r^2\) of 1.0 was found only for the rs2734583 and rs3099844 but not for rs9263726 (\(r^2 = 0.4524\)) (Table 4). For the SJS/TEN and the SCARs groups, these three SNPs were complete LDs with the HLA-B*58:01 allele (\(D' = 1.0\)) but the \(r^2\) values were less than 0.8 (Table 4).

4. Discussion

Allopurinol is an effective and cheap drug for the treatment of gout; however, allopurinol-induced SCARs may be life-threatening adverse drug reactions. Therefore, prediction for patients who may be at risk of these reactions is necessary to increase the safety of the drug. In line with previous reports, the results from this study clearly show that the HLA-B*58:01 allele was strongly associated with both phenotypes of SCARs including DRESS and SJS/TEN caused by allopurinol. High sensitivity and high specificity of the HLA-B*58:01 allele for the prediction of these life-threatening reactions were observed (93.75% and 88.08%) with the PPV and NPV of 79.65% and 96.59%. Compared with the HLA-B*58:01 allele, the selected SNPs in chromosome 6 showed relatively low sensitivity and specificity as well as low PPV and NPV for the prediction of allopurinol-induced SCARs. In contrast to previous reports in a Japanese population, the rs9263726, rs2734583, and rs3099844 SNPs were not complete and perfect LDs with the HLA-B*58:01 allele in a Thai population.

Results from univariate analysis revealed that the HLA-B*58:01 allele was strongly associated with both DRESS and SJS/TEN caused by allopurinol (Table 2). The OR of the HLA*58:01 allele for the SJS/TEN was about 1.6-fold higher than that of DRESS. The overall OR of this HLA allele for the prediction of both phenotypes of allopurinol-induced SCARs was 110.87 (95% CI = 43.57–282.15, \(P_c = 2.05 \times 10^{-22}\)). It is noteworthy that 86 patients of the SJS/TEN and 182 patients in the control groups are the same patients as reported in the previous study [4]. A lower OR between the HLA-B*58:01 and allopurinol-induced SCARs observed in the present study and a previous study was due to the absence of the HLA-B*58:01 allele in some of the SCARs patients that were currently recruited for the present study. Due to the high sensitivity, high specificity, high PPV, and high NPV observed in the present study and in other previous studies [5, 7, 15, 25], the HLA-B*58:01 allele screening has been proposed as a valid genetic marker for screening patients who may be at a high risk of allopurinol-induced SCARs. To date, guidelines and recommendations for HLA-B*58:01 allele screening prior to allopurinol administration particularly in certain ethnicities with a high frequency of HLA-B*58:01 allele carriers such as Han Chinese, Thai, and Korean populations have been released [17, 26]. Moreover, data from several countries including Thailand and Taiwan suggest that the HLA-B*58:01 allele screening is a cost-effective intervention for preventing allopurinol-induced SCARs [27, 28].

A recent study using a genome-wide association study in a Japanese population (including 14 allopurinol-related SJS/TEN patients and 991 ethnically matched controls) has discovered a set of SNPs in chromosome 6, particularly rs9263726 in the PSORS1C1 gene, rs2734583 in the BAT1 gene, and rs3094011 in the HIP5 gene that were closely

Table 3: Properties of proposed genetic screening tests for prediction of allopurinol-induced SCARs in a Thai population.

| Allele/SNPs     | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------------|----------------|----------------|---------|---------|
|                 | DRESS          | SJS/TEN        | SCARs   | DRESS   | SJS/TEN | SCARs   | DRESS   | SJS/TEN | SCARs   |
| HLA-B*58:01     | 93.10          | 94.52          | 93.75   | 88.08   | 88.08   | 88.08   | 47.73   | 75.00   | 79.65   | 96.84   | 97.70   | 96.59   |
| rs9263726 (G/A) | 82.61          | 93.15          | 90.63   | 82.38   | 82.38   | 82.38   | 35.85   | 66.67   | 71.90   | 97.55   | 96.95   | 94.64   |
| rs2734583 (T/C) | 91.30          | 90.41          | 90.63   | 86.01   | 86.01   | 86.01   | 43.75   | 70.97   | 76.32   | 98.81   | 95.95   | 94.86   |
| rs3099844 (C/A) | 91.30          | 90.41          | 90.63   | 84.97   | 84.97   | 84.97   | 42.00   | 69.47   | 75.00   | 98.80   | 95.91   | 94.80   |

Data presented as percentage. PPV: positive predictive value; NPV: negative predictive value.

Table 4: Concordance between selected SNPs and HLA-B*58:01 allele in allopurinol-induced SCARs.

| SNP             | DRESS (n = 23) | SJS/TEN (n = 73) | SCARs (n = 96) | Control (n = 193) | DRESS (n = 23) | SJS/TEN (n = 73) | SCARs (n = 96) | Control (n = 193) |
|-----------------|----------------|------------------|----------------|-------------------|----------------|------------------|----------------|-------------------|
| rs9263726       | 1.0000         | 1.0000           | 1.0000         | 1.0000            | 0.4524         | 0.7884           | 0.6444         | 0.6327            |
| rs2734583       | 1.0000         | 1.0000           | 1.0000         | 1.0000            | 1.0000         | 0.5466           | 0.6444         | 0.8318            |
| rs3099844       | 1.0000         | 1.0000           | 1.0000         | 1.0000            | 1.0000         | 0.5466           | 0.6444         | 0.7651            |
linked with the HLA-B*58:01 allele [13]. Moreover, results from a Chinese population revealed that among the three SNPs including rs9263726, rs2734583, and rs3099844, the rs9263726 showed the highest degree of association with allopurinol-induced SJS/TEN (OR = 108.8, 95% CI = 13.9–850.5, P-value = 1.1 × 10^{-6}) which was the same value as that of the HLA-B*58:01 allele [19]. Different to a report of a Chinese population, the OR values of the rs9263726 for the prediction of allopurinol-induced SCARs both DRESS and SJS/TEN in a Thai population were 2- to 3.5-fold lower than that of the HLA-B*58:01 allele (Table 2). Sensitivity, specificity, PPV, and NPV of the rs9263726 for the prediction of allopurinol-induced SCARs in a Thai population were relatively lower than those of the HLA-B*58:01 allele (Table 3).

The rs9263726 in the PSORS1C1 gene has been reported to be complete LD ($D^\prime = 1$) and perfect LDs ($r^2 = 1$) with the HLA-B*58:01 allele in a Japanese population ($n = 206$) [13]. Similarly, a recent study in 120 Chinese from the Eastern region of China ($n = 120$) showed a complete LD between the HLA-B*58:01 allele and rs9263726 ($D^\prime = 1$) but their LD was not perfect ($r^2 = 0.92$) [19]. In contrast, it has been reported in an Australian admixture population that the rs9263726 was not linked with the HLA-B*58:01 allele ($D^\prime = 0.059; r^2 = 0.001$) suggesting that these two alleles within nearby genes are inherited independently from each other in an Australian admixture population [29]. It should be noted that results from the present study showed that although the rs9263726 was in complete LD ($D^\prime = 1$) with the HLA-B*58:01 allele in DRESS, SJS/TEN, SCARs, and the tolerant control groups but this SNP appeared to be not perfect LD with the HLA-B*58:01 because the $r^2$ values were less than 1 (range from 0.4524 to 0.7884, Table 4). The lowest $r^2$ values of this SNP were found with the allopurinol-induced DRESS. These results suggest that the rs9263726 may not be a good surrogate marker or good tag SNP of the HLA-B*58:01 allele for screening patients who are at risk of SCARs, particularly DRESS induced by allopurinol.

In comparison with the HLA-B*58:01 allele, it was found that the strength of association between allopurinol-induced DRESS and allopurinol-induced SJS/TEN and the rs2734583 or the rs3099844 were about 1.3–2.39-fold lower (Table 2). The sensitivity, specificity, PPV, and NPV of these two SNPs were comparable to those of the HLA-B*58:01 allele (Table 3). Although these two SNPs were complete ($D^\prime = 1$) and perfect LDs ($r^2 = 1$) with the HLA-B*58:01 allele in the allopurinol-induced DRESS, they were not perfect LD ($r^2$ of 0.5466–0.8318) with the HLA-B*58:01 allele in SJS/TEN and tolerant control groups (Table 4). These results suggest that neither the rs2734583 nor the rs3099844 is a good surrogate marker of the HLA-B*58:01 allele for screening Thai patients who are at risk of SCARs. The rs2734583 and the rs3099844 appeared to be complete and perfect LDs in the SCARs group but not perfect LDs in the control group (data not shown). Detecting the haplotypes of these three SNPs was not significantly increased for the sensitivity, specificity, PPV, and NPV for the prediction of allopurinol-induced SCARs than single SNP detection (Table 3).

To date, the definite role of these SNPs in the pathogenesis of drug-induced SCARs is still unknown. The PSORS1C1 gene encodes a psoriasis susceptibility 1 candidate gene 1 protein. Although the function of this protein is not clear, the PSORS1C1 gene polymorphism has been reported to be associated with the susceptibility to psoriasis, hyperproliferative skin disorder [30, 31], and rheumatoid arthritis [32] whereas the BAT1 gene encodes a protein that downregulated inflammatory cytokine production in splicing and RNA export mechanism such as tumor necrosis factor (TNF), interleukin-1, and interleukin-6 [33]. The polymorphism of the BAT1 gene has been reported to be associated with rheumatoid arthritis [34]. The HCP5 gene encodes a human endogenous retroviral element that sequences homology to retroviral pol genes. The HCP5 was expressed in lymphocytes and suggested to control retrovirus proliferation via antisense mechanism [35]. Of interest, HCP5 genetic polymorphism has previously been reported to be associated with nevirapine-induced SJS/TEN [36] and abacavir hypersensitivity [37]. It should be noted that the three SNPs investigated in the present study are located in chromosome 6p21.3 which is the same region as the MHC molecule well recognized as a key element for the pathogenesis of several drug-induced SCARs [38]. It is likely that the strong association between these three SNPs and allopurinol-induced SCARs may be due to linkage disequilibrium with the HLA-B*58:01 allele.

In summary, the three selected SNPs, rs926372, rs2734583, and rs3099844, were significantly associated with DRESS and SJS/TEN caused by allopurinol but the degree of associations was lower than that of the HLA-B*58:01 allele. The sensitivity, specificity, PPV, and NPV of these SNPs were comparable to those of the HLA-B*58:01 allele; however, these SNPs were not perfect LDs with the HLA-B*58:01 allele. Although the detection of the single SNP is more simple and less expensive compared with the detection of such polymorphic gene like the HLA-B*58:01 allele, results obtained from screening for the risk of allopurinol-induced SCARs using these SNPs as surrogate makers of the HLA-B*58:01 allele need to be carefully interpreted.

**Conflicts of Interest**

All authors declare no conflict of interest.

**Acknowledgments**

A scholarship support from Thailand Research Fund and the University of Phayao through the Royal Golden Jubilee Ph.D. Program (PHD55K0050) to Wichittra Tassaneeyakul and Niwat Saksit and a grant from the Faculty of Medicine, Khon Kaen University, are acknowledged. Supports from Sumitra Suttisai, Napacha Piriyachananusorn, Pawinee Tiwong, Supinya Phoomwanitchakit, and Patcharee Karnjanawat for sample collection are acknowledged. The authors thank Professor James A. Will, University of Wisconsin-Madison, for his valuable comments and critical review of the manuscript.
References

[1] S. N. Ramasamy, C. S. Korb-Wells, D. R. W. Kannangara et al., "Allopurinol hypersensitivity: a systematic review of all published cases, 1950–2012," *Drug Safety*, vol. 36, no. 10, pp. 953–980, 2013.

[2] C. Y. Yang, C. H. Chen, S. T. Deng et al., "Allopurinol use and risk of fatal hypersensitivity reactions: a nationwide population-based study in Taiwan," *JAMA Internal Medicine*, vol. 175, no. 9, pp. 1550–1557, 2015.

[3] S. Limkobpaiboon, D. Panomvana Na Ayudhya, N. Dhana, and K. Jongjareanprasert, "Prevalence and mortality rate of severe cutaneous adverse reactions in Siriraj hospital," *Chulalongkorn Medical Journal*, vol. 54, no. 5, pp. 467–478, 2010.

[4] N. Saksit, W. Tassaneeyakul, N. Nakam et al., "Risk factors of allopurinol-induced severe cutaneous adverse reactions in a Thai population," *Pharmacogenetics and Genomics*, vol. 27, no. 7, pp. 255–263, 2017.

[5] W. H. Chung, W. C. Chang, S. L. Stocker et al., "Insights into the poor prognosis of allopurinol-induced severe cutaneous adverse reactions: the impact of renal insufficiency, high plasma levels of oxypurinol and granulysin," *Annals of the Rheumatic Diseases*, vol. 74, no. 12, pp. 2157–2164, 2015.

[6] C. Y. Ng, Y. T. Yeh, C. W. Wang et al., "Impact of the HLA-B*58:01 allele and renal impairment on allopurinol-induced cutaneous adverse reactions," *Journal of Investigative Dermatology*, vol. 136, no. 7, pp. 1373–1381, 2016.

[7] S. I. Hung, W. H. Chung, L. B. Liou et al., "HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 11, pp. 4134–4139, 2005.

[8] W. Tassaneeyakul, T. Jantararoungtong, P. Chen et al., "Strong association between HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population," *Pharmacogenetics and Genomics*, vol. 19, no. 9, pp. 704–709, 2009.

[9] C. Sukasem, T. Jantararoungtong, P. Kuntawong et al., "HLA-B*5801 for allopurinol-induced cutaneous adverse drug reactions: implications for clinical interpretation in Thailand," *Frontiers in Pharmacology*, vol. 7, p. 186, 2016.

[10] M. L. S. Chiu, M. Hu, M. H. L. Ng et al., "Association between HLA-B*58:01 allele and severe cutaneous adverse reactions with allopurinol in Han Chinese in Hong Kong," *British Journal of Dermatology*, vol. 167, no. 1, pp. 44–49, 2012.

[11] Z.-h. Cao, Z.-y. Wei, Q.-y. Zhu et al., "HLA-B*58:01 allele is associated with augmented risk for both mild and severe cutaneous adverse reactions induced by allopurinol in Han Chinese," *Pharmacogenomics*, vol. 13, no. 10, pp. 1193–1201, 2012.

[12] N. Kaniwa, Y. Saito, M. Aihara et al., "HLA-B locus in Japanese patients with anti-epileptic and allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis," *Pharmacogenomics*, vol. 9, no. 11, pp. 1617–1622, 2008.

[13] M. Tohkin, N. Kaniwa, Y. Saito et al., "A whole-genome association study of major determinants for allopurinol-related Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients," *The Pharmacogenomics Journal*, vol. 13, no. 1, pp. 60–69, 2013.

[14] H. R. Kang, Y. K. Jee, Y. S. Kim et al., "Positive and negative associations of HLA class I alleles with allopurinol-induced SCARs in Koreans," *Pharmacogenetics and Genomics*, vol. 21, no. 5, pp. 303–307, 2011.

[15] M. Goncalo, I. Coutinho, V. Teixeira et al., "HLA-B*58:01 is a risk factor for allopurinol-induced DRESS and Stevens–Johnson syndrome/toxic epidermal necrolysis in a Portuguese population," *British Journal of Dermatology*, vol. 169, no. 3, pp. 660–665, 2013.

[16] R. Wu, Y. J. Cheng, L. L. Zhu et al., "Impact of HLA-B*58:01 allele and allopurinol-induced cutaneous adverse drug reactions: evidence from 21 pharmacogenetic studies," *Oncotarget*, vol. 7, no. 49, pp. 81870–81879, 2016.

[17] D. Khanna, J. D. Fitzgerald, P. P. Khanna et al., "2012 American College of Rheumatology guidelines for management of gout: Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia," *Arthritis Care & Research*, vol. 64, no. 10, pp. 1431–1446, 2012.

[18] K. Maekawa, J. Nishikawa, N. Kaniwa et al., "Development of a rapid and inexpensive assay for detecting a surrogate genetic polymorphism of HLA-B*58:01: a partially predictive but useful biomarker for allopurinol-related Stevens-Johnson syndrome/toxic epidermal necrolysis in Japanese," *Drug Metabolism and Pharmacokinetics*, vol. 27, no. 4, pp. 447–450, 2012.

[19] Z. Chen, S. Zhang, J. Zhang, Y. Zhang, L. Xue, and L. Miao, "rs9263726 is a specific genetic marker for allopurinol-induced severe cutaneous adverse reactions in Chinese patients," *Personalized Medicine*, vol. 12, no. 6, pp. 585–592, 2015.

[20] C. Lonjou, L. Thomas, N. Borot et al., "A marker for Stevens-Johnson syndrome : ethnicity matters," *The Pharmacogenomics Journal*, vol. 6, no. 4, pp. 265–268, 2006.

[21] B. Sassolas, C. Haddad, M. Mockenhaupt et al., "ALDEN, an algorithm for assessment of drug causality in Stevens–Johnson syndrome and toxic epidermal necrolysis: comparison with case–control analysis," *Clinical Pharmacology & Therapeutics*, vol. 88, no. 1, pp. 60–68, 2010.

[22] S. H. Kardaun, A. Sidoroff, L. Valerye-Allanore et al., "Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist?*, British Journal of Dermatology, vol. 156, no. 3, pp. 609–611, 2007.

[23] C. A. Naranjo, U. Busto, E. M. Sellers et al., "A method for estimating the probability of adverse drug reactions," *Clinical Pharmacology & Therapeutics*, vol. 30, no. 2, pp. 239–245, 1981.

[24] W. Tassaneeyakul, N. Prabmeechai, C. Sukasem et al., "Associations between HLA class I and cytochrome P450 2C9 genetic polymorphisms and phenytoin-related severe cutaneous adverse reactions in a Thai population," *Pharmacogenetics and Genomics*, vol. 26, no. 5, pp. 225–234, 2016.

[25] L. Cheng, Y. Xiong, C. Z. Qin et al., "HLA-B*58:01 is strongly associated with allopurinol-induced severe cutaneous adverse reactions in Han Chinese patients: a multicentre retrospective case-control clinical study," *British Journal of Dermatology*, vol. 173, no. 2, pp. 558–568, 2015.

[26] Y. Saito, L. K. Stamp, K. E. Caudle et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for human leukocyte antigen B (HLA-B) genotype and allopurinol dosing: 2015 update," *Clinical Pharmacology & Therapeutics*, vol. 99, no. 1, pp. 36–37, 2015.

[27] S. Saokaew, W. Tassaneeyakul, R. Maenthaisong, and N. Chaiyakunapruk, "Cost-effectiveness analysis of HLA-B*5801 testing in preventing allopurinol-induced SJS/TEN in Thai population," *PLoS One*, vol. 9, no. 4, article e94294, 2014.
[28] C. H. Ke, W. H. Chung, Y. H. Wen et al., “Cost-effectiveness analysis for genotyping before allopurinol treatment to prevent severe cutaneous adverse drug reactions,” *The Journal of Rheumatology*, vol. 44, no. 6, pp. 835–843, 2017.

[29] C. Vidal, J. Li, R. Fulton, and S. L. Fernando, “A polymorphism within the psoriasis susceptibility 1 candidate 1 (PSORS1C1) gene is not linked to *HLA-B*^∗^58:01 in an Australian cohort,” *Drug Metabolism and Pharmacokinetics*, vol. 31, no. 3, pp. 252–255, 2016.

[30] S. J. Holm, L. M. Carlen, L. Mallbris, M. Stahle-Backdahl, and K. P. O’Brien, “Polymorphisms in the SEEK1 and SPRI genes on 6p21.3 associate with psoriasis in the Swedish population,” *Experimental Dermatology*, vol. 12, no. 4, pp. 435–444, 2003.

[31] P. Rahman, C. Butt, F. Siannis et al., “Association of SEEK1 and psoriatic arthritis in two distinct Canadian populations,” *Annals of the Rheumatic Diseases*, vol. 64, no. 9, pp. 1370–1372, 2005.

[32] H. Sun, Y. Xia, L. Wang, Y. Wang, and X. Chang, “PSORS1C1 may be involved in rheumatoid arthritis,” *Immunology Letters*, vol. 153, no. 1-2, pp. 9–14, 2013.

[33] S. Limou, S. Le Clerc, C. Coulouges et al., “Genomewide association study of an AIDS-nonprogression cohort emphasizes the role played by *HLA* genes (ANRS Genomewide Association Study 02),” *The Journal of Infectious Diseases*, vol. 199, no. 3, pp. 419–426, 2009.

[34] A. Quinones-Lombrana, A. Lopez-Soto, F. J. Ballina-Garcia et al., “BAT1 promoter polymorphism is associated with rheumatoid arthritis susceptibility,” *The Journal of Rheumatology*, vol. 35, no. 5, pp. 741–744, 2008.

[35] J. Fellay, K. V. Shianna, D. Ge et al., “A whole-genome association study of major determinants for host control of HIV-1,” *Science*, vol. 317, no. 5840, pp. 944–947, 2007.

[36] P. Borgiani, D. Di Fusco, F. Erba et al., “HCP5 genetic variant (RS3099844) contributes to nevirapine-induced Stevens-Johnsons syndrome/toxic epidermal necrolysis susceptibility in a population from Mozambique,” *European Journal of Clinical Pharmacology*, vol. 70, no. 3, pp. 275–278, 2014.

[37] S. Colombo, A. Rauch, M. Rotger et al., “The HCP5 single-nucleotide polymorphism: a simple screening tool for prediction of hypersensitivity reaction to abacavir,” *The Journal of Infectious Diseases*, vol. 198, no. 6, pp. 864–867, 2008.

[38] W. H. Chung, C. W. Wang, and R. L. Dao, “Severe cutaneous adverse drug reactions,” *The Journal of Dermatology*, vol. 43, no. 7, pp. 758–766, 2016.

[39] A. S. Levey, J. P. Bosch, J. B. Lewis, T. Greene, N. Rogers, and D. Roth, “A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group,” *Annals of Internal Medicine*, vol. 130, no. 6, pp. 461–470, 1999.