Can the SNPs on *TCF19* and *POU5F1* predict risk in allopurinol-induced severe cutaneous adverse drug reactions (SCARs) in Thai patients, besides *HLA-B*58:01*?

Thawinee Jantararoungtong[1,2], Gaidganok Sornsamdang[1,2], Santirat Prommas[1,2], Napatrupron Koomdee[1,2], Apichaya Puangpetch[1,2], Patompong Satapornpong[1,2], Therdpong Tempark[3], Pawinee Rerknimitr[4,12], Jettanong Klaewsongkram[5,12], Papapit Tuchinda[6,12], Leena Chularojanamontri[6,12], Napatra Tovanabutra[7,12], Kumutnart Chanprapaph[8,12], Wareeporn Disphanurat[9,12], Panlop Chakkavittumrong[9,12], Chutika Srisuttiyakorn[10,12], Ticha Rerkpattanapipat[11,12], Chonlaphat Sukasem[1,2, 12]*

[1]Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
[2]Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Bangkok 10400, Thailand
[3]Division of Pediatric Dermatology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
[4]Division of Dermatology, Department of Medicine, Faculty of Medicine, Allergy and Clinical Immunology Research Group, Chulalongkorn University, Bangkok 10330, Thailand
[5]Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine, Allergy and Clinical Immunology Research Group, Chulalongkorn University, Bangkok 10330, Thailand
[6]Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[7]Dermatological Division, Department of Internal Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[8]Division of Dermatology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
[9]Division of Dermatology, Department of Medicine, Faculty of Medicine, Thammasat University, Pathumthani 10120, Thailand
[10]Division of Dermatology, Department of Medicine, Phramongkutklao Hospital, Phramongkutklao College of Medicine, Bangkok 10400, Thailand
[11]Division of Allergy Immunology and Rheumatology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
[12]The Thai Severe Cutaneous Adverse Drug Reaction (THAI-SCAR) Research Group, Thailand

**Corresponding author: chonlaphat.suk@mahidol.ac.th*

ABSTRACT

The aim of this study was to investigate the association of *Human leukocyte antigen B (HLA-B), Transcription Factor 19 (TCF19)* and *POU Class 5 Homeobox 1 (POU5F1*) genes with allopurinol-induced cutaneous adverse drug reactions (CADR), including Stevens-Johnson syndrome/ toxic epidermal necrosis (SJS/TEN; n=21), drug rash with eosinophilia and systemic symptoms (DRESS; n=16) and maculopapular exanthema (MPE; n=7) in Thai patients. This case-control association study compares 44 cases with allopurinol-induced CADR with allopurinol-tolerant control patients (n=100), and a population control group (n=1,095). The control group comprised patients who had received allopurinol for more than 6 months without any adverse cutaneous event. *HLA* alleles were genotyped using a two-stage sequence-specific oligonucleotide probe system (PCR-SSOP). Variants in *TCF19* (rs9263794 and rs1044870) and *POU5F1* (rs9263796) were genotyped. The risk of allopurinol-induced CADR was significantly higher in the patients with *HLA-B*58:01* allele with an odds ratio 240 (95%CI: 57.19–1007.08, p<0.0001). In addition, the single nucleotide polymorphisms (SNPs) were also significantly associated with the allopurinol-induced CADR (rs9263794; OR 57.20, p< 0.001, rs1044870; OR 77.31, p=0.003 and rs9263796; OR 84.14, p<0.0001). Furthermore, we found significant association between the SNPs and allopurinol-induced SJS/TEN, DRESS, and MPE (rs9263794; OR 304.4,

Received 8 November 2018, Revised 8 May 2020, Accepted 25 November 2020

p=0.0001, 31.7 p<0.0001, 18.3, p= 0.0011, rs1044870; OR 25.7, p=0.038, 124.4, p=0.0013, 258.4, p=0.0005 and rs9263796; OR 536.0, p<0.0001, 39.8, p<0.0001, 33.2, p=0.0001), respectively.

Keywords*: HLA-B*58:01*; allopurinol; Thai; SCAR; CADR; drug hypersensitivity

INTRODUCTION

Adverse drug reactions (ADRs) are common in clinical practice occurring in 6–10% of patients and remain an important public health problem as they are potentially life-threatening (Cheng *et al.*, 2014; Sukasem *et al.*, 2014). Cutaneous adverse drug reactions (CADR) occur in 1–3% of patients in hospital and vary in of clinical manifestation from mild symptom, such as rash, maculopapular exanthema (MPE), urticaria or exfoliative dermatitis, to severe symptom (severe cutaneous adverse drug reaction; SCAR)(Cheng *et al.*, 2014; Sukasem *et al.*, 2014). SCAR includes syndromes such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reaction with eosinophilia and systemic symptoms (DRESS) and drug induced hypersensitivity syndrome (DIHS) (Barvaliya *et al.*, 2011; Daly, 2014; Harr and French, 2012; Karlin and Phillips, 2014; Lonjou *et al.*, 2008; Sasidharanpillai *et al.*, 2015). SJS and TEN are severe manifestations of cutaneous hypersensitivity reactions affecting approximately 0.4–6 persons per million populations each year. Despite the low incidence, the mortality rate has been estimated at 5% for SJS and 30–50% for TEN (Aihara, 2011; Fleming and Marik, 2011). Clinical presentation of SJS and TEN is characterized by a rapidly progressive, blistering exanthema accompanied by mucosal involvement and systemic symptoms that may present as fever, mild elevation of hepatic enzymes, intestinal and pulmonary manifestations (Min *et al.*, 2015). DRESS is an extremely serious adverse effect caused by medications, characterized by skin rash, fever, and lymphadenopathy. It generally occurs 2 to 4 weeks after treatment with the reactive medication (Lam *et al.*, 2013).

The past decade, pharmacogenetic studies have shown strong associations between *human leukocyte antigen (HLA)* alleles and susceptibility to drug hypersensitivity reactions. A medical genetic study in recent years has focused on the area of *HLA* genotypes and their association with severe drug hypersensitivity (Tassaneeyakul *et al.*, 2009). To generate an immune reaction, HLA molecules function to present antigens to immune T-cells. HLA class I (HLA-A, HLA-B, HLA-C) molecules are ubiquitous and are found on all nucleated cell surfaces. They present intracellular antigens to $CD8^+$ cytotoxic T-cells. HLA class II (HLA-DP, HLA-DQ, HLA-DR) molecules are found on the immune cells and present extracellular antigens to $CD4^+$ helper T-cells. It has been suggested that major histocompatibility complex (MHC) presentation of drug-derived antigen plays a key role in the development of drug hypersensitivity. *HLA* associations that have been described in severe cutaneous adverse reactions include: *HLA-B*15:02* with carbamazepine-induced SJS/TEN, *HLA-B*58:01* with allopurinol-induced SJS/TEN and *HLA-B*57:01* with abacavir-induced drug hypersensitivity (Saokaew *et al.*, 2014).

Allopurinol is a generally well-tolerated urate-lowering agent but has a relatively high rate of hypersensitivity reactions and is known as one of the major causative agents of SCAR such as SJS, TEN, and DIHS (Goncalo *et al.*, 2013; Hung *et al.*, 2005; Kaniwa *et al.*, 2008; Puangpetch *et al.*, 2014). Allopurinol is widely used in the management of hyperuricemia in patients with impaired renal function and for the prevention of recurrent gout in clinical practice. It is also commonly used for the prevention of hyperuricemia associated with tumor lysis syndrome before starting chemotherapy in patients with hematologic malignancies (Choudhary *et al.*, 2013; Pichler *et al.*, 2002). Until now, many studies have shown strong associations between allopurinol-induced SCAR and the human leukocyte antigen *HLA-B*58:01* allele. *HLA-B*58:01* is also known as a risk marker for the development of allopurinol-induced SCAR in Koreans (Kang *et al.*, 2011). However, the risk of allopurinol-induced hypersensitivity reaction in patients with hematologic malignancies, especially in patients with *HLA-B*58:01* allele, has not yet been evaluated.

Currently, genome-wide association studies (GWAS) (Tohkin *et al.*, 2013) providing opportunities to uncover polymorphisms that influence susceptibility to allopurinol induced CADR are controversial. Therefore, we further conducted a retrospective pharmacogenetic case–control study of *HLA-B* genotyping by polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP) and single nucleotide polymorphisms (SNPs) by using real-time PCR to the reported the risk marker and to identify new and effective genetic biomarkers for allopurinol-related CADR in Thai population.

MATERIALS AND METHODS

Subjects and characteristics

In this study, we carried out research as a retrospective and prospective case-control study. From 2011 to 2014, patients with allopurinol-induced SCAR from The Thai Severe Cutaneous Adverse Drug Reaction *(*THAI-SCAR*)* research group were enrolled. A total of 30 samples were recruited from the previous study (Sukasem *et al.*, 2016), and 14 samples were additionally recruited for this study. Among them, 44 patients with allopurinol-induced CADR were categorized into SJS/TEN (n = 21), DRESS (n = 16), and MPE (n = 7). Meanwhile, patients who had been taking allopurinol for more than 6 months without evidence of cutaneous adverse effects were recruited as allopurinol-tolerant controls (n=100). In addition, healthy individuals comprising subjects not taking allopurinol and with no history of drug-induced cutaneous adverse reactions were included in this study. The population control group consisted of 1,095 subjects who were undergoing *HLA-B* genotyping at the Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Thailand. The clinical data of patients which was collected from medical records comprised (1) Demographic data: gender, nationality, age, address and phone number (2) Medical history: illness history, congenital disease and history of allergy (3) Adverse drug reactions data: information of drug use, clinical manifestation of allergy, type of ADR, suspected drugs, concomitant drugs, drug onset, treatment history and other laboratory results (4) Genetic data: *HLA-B* alleles and SNPs genotypes. The study was performed with approval from the Ramathibodi Hospital ethical review board (Approval No. MURA2015/300), and informed consent was obtained from all of the participants.

Diagnosis of cutaneous adverse drug reaction (CADR)

All CADR patients were assessed by a dermatologist and allergist who reviewed photographs, pathological slides, clinical morphology and medical records. The diagnosis of drug-induced DRESS, SJS and TEN was made according to the RegiSCAR criteria (Choudhary *et al.*, 2013). In brief, DRESS was diagnosed in patients presenting with fever, maculopapular rash with internal organ involvement, and hematologic abnormalities. SJS was diagnosed in patients with skin rash and mucosal erosion covering 3–10% of body surface area (BSA) whereas SJS/TEN overlap was diagnosed in patients with epidermal necrosis whose occur blistering skin lesions affected between 10–30% of BSA. Severe MPE was diagnosed in patients presenting with danger signs in drug-induced exanthema or covering 30% of BSA with or without associated systemic symptoms, but not fulfilling the criteria of DRESS (Pichler *et al.*, 2002).

Genomic DNA extraction

Blood samples were collected in EDTA tubes. DNA was isolated using the MagNA Pure Compact automated extraction system (Roche Diagnostics, USA) based on magnetic-bead technology. The quality and quantity of genomic DNA were assessed by using Nano Drop (ND-1000). All DNA was aliquoted and stored at -20 °C before analysis.

HLA-B genotyping

HLA-B genotyping was carried out using the Luminex™ Multiplex Technology (Luminex®IS 100, USA) based on PCR-SSOP method. Briefly, the PCR products were hybridized against a panel of oligonucleotide probes coated on polystyrene microspheres that have sequences complementary to stretches of polymorphic sequence within the target *HLA-B* alleles. The amplicon-probe complex was visualized using a colorimetric reaction and fluorescence detection technology. Data analysis for the *HLA-B* assays was performed with HLA fusion™ 2.0 software.

SNPs genotyping

TaqManSNP genotyping assay (ViiA™ 7 Real-Time PCR System) was used to genotype SNPs on *TCF19* gene (*rs9263794* and *rs1044870)* and *rs9263796* on *POU5F1 (POU class 5 homeobox 1)* gene. Briefly, TaqMan™ probe (quencher) with a dye label were hybridized to the target DNA, and release signal through fluorescence resonance energy transfer (FRET). During PCR, *Taq* polymerase extends the unlabeled primers and, when it reaches the TaqMan probe, cleaves the molecule, separating the dye from the quencher. The qPCR instrument detects fluorescence.

Statistical analysis

The association between *HLA-B*58:01* allele and allopurinol-induced CADR was evaluated by comparing the group of individuals with CADR with the allopurinol-tolerant control groups and the population control group. Data were counted by presence or absence of *HLA-B*58:01* allele. Chi-square test and Fisher's exact test were used to analyze the association between allopurinol-induced CADR and *HLA-B*58:01*. Demographic data of allopurinol-induced CADR and allopurinol-tolerant control group was compared by mean comparison and Student's t-test. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The strength of association was estimated by calculating the odds ratio (OR) with a 95% confidence interval (CI).

RESULTS

Subject clinical characteristics

From the 44 patients with CADR, 37 had underlying gout and 7 had hyperuricemia. Twenty-four of them were male and 20 were female, with age 66.82±14.33 years. Indication of drug uses and the results of *HLA-B* and SNPs genotyping are summarized in Table 1. The mean interval from allopurinol initiation to symptom onset was 22.2 days (range, 7–42 days). The onset of symptoms for all patients was within the first two months of allopurinol exposure. The underlying diseases, such as diabetes, hypertension, chronic kidney disease, and dyslipidemia, and the co-medication, such as colchicine, sodamint, prednisolone and simvastatin, were not associated with allopurinol-induced CADR. In contrast, the starting dosage of allopurinol between allopurinol-induced CADR and allopurinol tolerant control group were significantly different; OR 3.040 (95%CI: 20.63–97.37, p=0.0028). Laboratory results, eGFR, creatinine, AST and ALT were not significantly different between allopurinol-induced CADR and the tolerant control group, while BUN were significantly different between groups (p<0.001), as shown in Table 2.

*HLA-B*58:01* in CADR case-control study

To identify genetic markers for allopurinol-induced CADR (the subgroup of patients with DRESS, SJS, SJS/TEN and severe MPE), we carried out a case-controlled association study. Frequencies of *HLA-B*58:01* allele in the three groups are shown in Table 3. Of the 44 patients with allopurinol-induced CADR, 40 patients (90.9%) carried *HLA-B*58:01* allele while 4 of 100 (4.0%) allopurinol-tolerant controls and 111 of 1,095 (10.1%) population control carried this allele. The frequency of *HLA-B*58:01* in subjects with allopurinol-induced CADR was notably higher than that in the allopurinol-tolerant group (OR 240.00; 95%CI: 57.19–1007.08, p<*0.0001*). When compared with the incidence in the population control group, the frequency was still significantly higher (OR 88.65, 95%CI: 31.13–252.42, p<0.0001). In our *HLA-B* genotyping studies, no other alleles showed significantly differences for allopurinol-induced CADR.

*HLA-B*58:01* in SCAR case-control study

In the allopurinol-induced SCARs group, the subgroup of patients with SJS, SJS/TEN overlapping, TEN and DRESS contained 9 (20.5%), 9 (20.5%), 3 (6.8%) and 16 (36.4%) patients, respectively. *HLA-B*58:01* was carried by 34 of 37 (91.9%) patients with allopurinol-induced SCAR (data not shown). We found a significant *HLA-B*58:01* allele frequencies when comparing between allopurinol-induced SCAR and allopurinol-tolerant control (OR 272.0, 95%CI: 57.89–1277.98). Among 37 patients with allopurinol-induced SCAR, *HLA-B*58:01* alleles occurred at significantly high frequencies among the allopurinol-induced SJS/TEN patients compared to the tolerant-control groups, as shown in Table 4. In particular, the *HLA-B*58:01* allele was present in all 21 (100%) patients with allopurinol-induced SJS/TEN (OR 922.11, 95%CI 47.83–17776.18, P<0.0001), 13 (81.25%) patients with allopurinol-induced DRESS (OR 144.0, 95%CI: 20.89–517.77, p<0.0001) and 6 (85.71%) patients with allopurinol-induced MPE (OR 144.00 95%CI: 13.85–1497.03, p<0.001) (Table 4).

SNPs genotyping in CADRs case-control study

Three SNPs were genotyped including *rs9263794*, *rs1044870* and *rs9263796*. The results showed association between CADR and *rs9263794* (OR 57.20, 95% CI: 18.86–173.45, p<0.0001), *rs1044870* (OR 77.31, 95%CI: 4.45–1342.22, p=0.003) and *rs9263796* (OR 84.14, 95%CI: 26.54–266.78, p<0.0001) as shown in Table 5. Furthermore, Genotype frequencies of *TCF* polymorphism, *rs9263794*, were *AA* (64.58%), *AG* (30.56%), *GG* (4.86%), and *TCF* gene, *rs1044870*, were *CC* (91.67 %), *CT* (7.64 %), *TT* (0.69 %). Genotype frequencies of *POU5F1 rs9263796* were *CC* (68.75%), *CT* (27.78%), and *TT* (3.47%). Using the allopurinol tolerant control group as the control for diagnosing CADR, the *rs9263794* had 88.64% sensitivity and 88.0% specificity, *rs1044870* had 27.27% sensitivity and 100% specificity, and *rs9263796* had 86.36% sensitivity and 93.0% specificity (data not shown). In the subsequent analysis, the association between SNPs and allopurinol-induced CADR were categorized into three groups. The results showed significantly association between SJS/TEN and SNPs, *TCF19 rs9263794* (OR 304.4, 95%CI 17.3–5346.7, p=0.0001), *TCF19 rs1044870* (OR 25.7, 95%CI 1.1–557.8, p=0.038) and *POU5F1 rs9263796* (OR 536.0, 95%CI 29.4–9751.2, p<0.0001), as shown in Table 6. The association between DRESS and SNPs were significant, *TCF19 rs9263794* (OR 31.7, 95%CI 7.8–127.9, p<0.0001), *TCF19 rs1044870* (OR 124.4, 95%CI 6.5–2367.5, p=0.0013) and *POU5F1 rs9263796* (OR 39.8, 95%CI 10.1–156.4, p<0.0001), as shown in Table 7. The results also showed significant association with MPE, *TCF19 rs9263794* (OR 18.3, 95%CI 3.1–105.2, p=0.0011), *TCF19 rs1044870* (OR 258.4, 95% CI 11.5–5794.8, p=0.0005) and *POU5F1 rs9263796* (OR 33.2, 95%CI 5.4–203.1, p=0.0001) as shown in Table 8

Table 1. Characteristics and genotyping data of allopurinol-induced cutaneous adverse drug reactions (CADR) in Thai population.

No	Age/Sex	Type of CADR	Indication	*HLA-B*58:01*	*HLA-B* genotyping	*rs9263794 A>G*	*rs1044870 C>T*	*rs9263796 C > T*
1	72/F	SJS	gouty arthritis	positive	*57:01/**58:01***	***AG***	*CC*	***CT***
2	78/F	SJS	hyperuricemia	positive	*35:01/**58:01***	***AG***	*CC*	***CT***
3	48/F	SJS	gouty arthritis	positive	*13:01/**58:01***	***AG***	*CC*	***CT***
4	68/M	SJS	gouty arthritis	positive	*13:01/**58:01***	***AG***	*CC*	***CT***
5	72/F	SJS	gouty arthritis	positive	*40:01/**58:01***	***AG***	*CC*	***CT***
6	67/M	SJS	gouty arthritis	positive	*40:01/**58:01***	***AG***	*CC*	***CT***
7	54/M	SJS	gouty arthritis	positive	*15:01/**58:01***	***AG***	*CC*	***CT***
8	76/F	SJS	gouty arthritis	positive	***58:01/58:01***	***GG***	***TT***	***TT***
9	76/M	SJS	hyperuricemia	positive	*40:01/**58:01***	***AG***	***CT***	***CT***
10	68/F	SJS/TEN	gouty arthritis	positive	*46:01/**58:01***	***AG***	*CC*	***CT***
11	54/M	SJS/TEN	gouty arthritis	positive	*40:01/**58:01***	***AG***	*CC*	***CT***
12	78/M	SJS/TEN	gouty arthritis	positive	*44:03/**58:01***	***GG***	*CC*	***CT***
13	74/F	SJS/TEN	gouty arthritis	positive	*15:02/**58:01***	***AG***	*CC*	***CT***
14	84/F	SJS/TEN	gouty arthritis	positive	*15:32/**58:01***	***AG***	*CC*	***CT***
15	76/F	SJS/TEN	gouty arthritis	positive	*15:13/**58:01***	***AG***	*CC*	***CT***
16	73/F	SJS/TEN	gouty arthritis	positive	*46:01/**58:01***	***AG***	*CC*	***CT***
17	55/M	SJS/TEN	gouty arthritis	positive	*15:02/**58:01***	***AG***	*CC*	***CT***
18	63/F	SJS/TEN	gouty arthritis	positive	*40:02/**58:01***	***AG***	*CC*	***CT***
19	72/F	TEN	gouty arthritis	positive	*15:11/**58:01***	***AG***	*CC*	***CT***
20	68/M	TEN	gouty arthritis	positive	*40:01/**58:01***	***AG***	*CC*	***CT***
21	47/F	TEN	gouty arthritis	positive	*13:02/**58:01***	***AG***	*CC*	***CT***
22	56/M	DRESS	hyperuricemia	positive	*44:02/**58:01***	***AG***	***CT***	***CT***
23	74/M	DRESS	gouty arthritis	negative	*40:01/46:01*	*AA*	*CC*	*CC*
24	30/M	DRESS	hyperuricemia	positive	*39:15/**58:01***	***GG***	***CT***	***TT***
25	39/F	DRESS	hyperuricemia	positive	*08:01/**58:01***	***AG***	*CC*	***CT***
26	81/F	DRESS	hyperuricemia	positive	*13:01/**58:01***	*AA*	*CC*	*CC*
27	86/M	DRESS	gouty arthritis	negative	*44:03/51:02*	***AG***	*CC*	*CC*
28	62/M	DRESS	gouty arthritis	positive	*40:01/**58:01***	***AG***	*CC*	***CT***
29	73/M	DRESS	gouty arthritis	positive	*52:01/**58:01***	***AG***	*CC*	***CT***
30	51/M	DRESS	gouty arthritis	negative	*13:01/46:01*	*AA*	*CC*	*CC*
31	53/F	DRESS	gouty arthritis	positive	*13:02/**58:01***	***AG***	*CC*	***CT***
32	32/M	DRESS	gouty arthritis	positive	*54:01/**58:01***	***AG***	*CC*	***CT***
33	55/F	DRESS	gouty arthritis	positive	***58:01/58:01***	***GG***	***CT***	***TT***
34	57/F	DRESS	gouty arthritis	positive	***58:01/58:01***	***GG***	***CT***	***TT***
35	78/M	DRESS	gouty arthritis	positive	*15:02/**58:01***	***AG***	*CC*	***CT***
36	81/M	DRESS	gouty arthritis	positive	***58:01/58:01***	***GG***	***CT***	***TT***
37	61/M	DRESS	gouty arthritis	positive	*51:01/**58:01***	***GG***	***CT***	***CT***
38	85/F	MPE	hyperuricemia	positive	*46:01/**58:01***	***AG***	*CC*	***CT***
39	69/M	MPE	gouty arthritis	negative	*13:01/54:01*	*AA*	*CC*	*CC*
40	88/F	MPE	gouty arthritis	positive	*40:01/**58:01***	*AA*	*CC*	*CC*
41	79/M	MPE	gouty arthritis	positive	*40:01/**58:01***	***AG***	***CT***	***CT***
42	73/M	MPE	gouty arthritis	positive	*13:01/**58:01***	***AG***	***CT***	***CT***
43	72/M	MPE	gouty arthritis	positive	*18:02/**58:01***	***AG***	***CT***	***CT***
44	84/M	MPE	gouty arthritis	positive	*39:01/**58:01***	***AG***	***CT***	***CT***

Table 2 Demographic and clinical characteristics of allopurinol-induced cutaneous adverse drug reactions (CADR) and allopurinol-tolerant control group.

Characteristic	Allopurinol-induced CADR group (n=44)	Allopurinol tolerant control group (n=100)	p-value
Gender (n)			
Male	24 (54.5%)	77 (77%)	**0.007***
Female	20 (45.5%)	23 (23%)	
Age. Years (mean ± SD)	66.82 ± 14.33	62.03 ± 15.30	0.851
Allopurinol exposure			
Dosage, (mean± SD), mg/day	221 ± 96.4	162 ± 111.7	**0.0028***
Duration (Median (Range), days	19.5 (10–51)	846.5 (29–1465)	**<0.001***
Onset of ADR, mean (Range), days	22.2 (7–42)	-	
Underlying Disease (n)			
Diabetes	4 (9.1%)	13 (13%)	0.5053
Hypertension	27 (61.36%)	60 (60%)	0.879
Chronic Kidney Disease	18 (40.91%)	33 (33%)	0.364
Dyslipidemia	4 (9.09%)	16 (16%)	0.273
Co-Medication (n)			
Colchicine	20 (27.8%)	52 (52%)	0.473
Sodamint	3 (15.8%)	16 (16%)	0.136
Prednisolone	2 (4.5%)	9 (9%)	0.3631
Simvastatin	5 (23.8%)	16 (16%)	0.471
Clinical chemistry			
Creatinine, mean ± SD (mg/dl)	1.96 ± 1.43	1.72 ± 1.22	0.309
eGFR,mean ± SD	45.7 ± 28.12	52.74 ± 23.93	0.126
BUN,mean ± SD (mg/dl)	48.05 ± 31.52	21.9 ± 8.14	**<0.001***
AST,mean ± SD (U/L)	63.19 ± 88.120	58.40 ± 62.378	0.7104
ALT,mean ± SD (U/L)	71.26 ± 96.188	57.46 ± 62.095	0.3050

eGFR; Estimated Glomerular Filtration Rate, BUN; Blood Urea Nitrogen, AST; Aspartate Aminotransferase, ALT; Alanine Aminotransferase

Table 3 The association of individual *HLA-B* allele with allopurinol-induced cutaneous adverse drug reactions (CADR).

HLA-B allele	Allopurinol induced CADR (n=44)	Allopurinol tolerant group (n=100)	Population control group (n=1,095)	CADR cases versus Allopurinol tolerant group		CADR cases versus population control group	
				OR (95%CI)	p-value	OR (95%CI)	p-value
58:01	**40 (90.9)**	**4 (4.0)**	**111 (10.1)**	**240.00 (57.19–1007.08)**	**<0.0001**	**88.65 (31.13–252.42)**	**<0.0001**
13:01	5 (11.4)	9 (9.0)	137 (12.5)	1.30 (0.41–4.12)	0.660	0.90 (0.33–2.31)	0.821
15:02	3 (6.8)	20 (20.0)	161 (14.7)	0.29 (0.08–1.04)	0.058	0.42 (0.13–1.39)	0.156
40:01	9 (20.5)	29 (29.0)	162 (14.8)	0.63 (0.27–1.49)	0.286	1.48 (0.69–3.14)	0.305
46:01	4 (9.1)	25 (25.0)	227 (20.7)	0.30 (0.10–0.92)	0.035	0.38 (0.14–1.08)	0.069
51:01	1 (2.3)	12 (12.0)	65 (5.9)	0.17 (0.02–1.35)	0.094	0.37 (0.05–2.72)	0.328

Table 4 The association of individual *HLA-B* allele with cutaneous adverse drug reactions (CADR).

HLA-B	**Allopurinol-induced SJS/TEN (n=21)**		**Allopurinol-induced DRESS (n=16)**		**Allopurinol-induced MPE (n=7)**	
allele	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
58:01	**922.11 (47.83–17776.18)**	**<0.0001**	**104.00 (20.89–517.77)**	**<0.001**	**144.00 (13.85–1497.03)**	**<0.0001**
13:01	1.06 (0.21–5.32)	0.94	0.67 (0.08–5.71)	0.718	4.04 (0.68–23.91)	0.1233
15:02	0.42 (0.09–1.96)	0.27	0.27 (0.03–2.14)	0.214	0.44 (0.02–8.43)	0.583
40:01	0.77 (0.26–2.28)	0.631	0.35 (0.07–1.64)	0.182	2.45 (0.33–18.22)	0.382
46:01	0.32 (0.07–1.45)	0.139	0.43 (0.09–2.02)	0.284	0.33 (0.02–6.32)	0.461
51:01	0.17 (0.01–2.89)	0.217	0.49 (0.06–4.04)	0.507	0.79 (0.04–15.51)	0.875

Table 5 The association of SNPs with allopurinol-induced cutaneous adverse drug reactions (CADR)

SNPs	**Allopurinol-induced CADR (n=44)**	**Allopurinol-tolerant group (n=100)**	**Allopurinol-CADR versus Allopurinol tolerant group**	
			OR (95%CI)	**p-value**
***TCF19_rs9263794, A>G* (n, %)**				
Mutant (*AG,GG*)	39 (88.64%)	12 (12%)	57.20 (18.86–173.45)	**<0.0001**
Wild type (*AA*)	5 (11.36%)	88 (88%)		
***TCF19_rs1044870, C>T* (n, %)**				
Mutant (*CT,TT*)	12 (27.27%)	0 (0)	77.31 (4.45–1342.22)	**0.0028**
Wild type (*CC*)	32 (72.73%)	100 (100%)		
***POU5F1_rs9263796, C>T* (n, %)**				
Mutant (*CT,TT*)	38 (86.36%)	7 (7%)	84.14 (26.54–266.78)	**<0.0001**
Wild type (*CC*)	6 (13.64%)	93 (93%)		

Table 6 The association of individual SNP with allopurinol-induced SJS/TEN.

SNP	**Allopurinol-induced SJS/TEN (n=21)**	**Allopurinol-tolerant group (n=100)**	**SJS/TEN cases versus Allopurinol tolerant group**	
	n (%)	**n (%)**	**OR (95% CI)**	**p-value**
TCF19_rs9263794				
AG/GG	21 (100%)	12 (12%)	304.4 (17.3–5346.7)	**0.0001**
AA	0	88 (88%)		
TCF19_rs1044870				
CT/TT	2 (9.5%)	0	25.7 (1.1–557.8)	**0.0384**
CC	19 (90.5%)	100 (100%)		
POU5F1_rs9263796				
CT/TT	21 (100%)	7 (7%)	536.0 (29.4–9751.2)	**< 0.0001**
CC	0	93 (93%)		

Table 7 The association of individual SNP with allopurinol-induced DRESS.

SNP	Allopurinol-induced DRESS (n=16)	Allopurinol-tolerant (n=100)	DRESS cases versus Allopurinol tolerant control	
			OR (95% CI)	p-value
TCF19_rs9263794				
AG/GG	13 (81.3%)	12 (12%)	31.7 (7.8–127.9)	< **0.0001**
AA	3 (18.7%)	88 (88%)		
TCF19_rs1044870				
CT/TT	6 (37.5%)	0	124.4 (6.5–2367.5)	**0.0013**
CC	10 (62.5%)	100 (100%)		
POU5F1_rs9263796				
CT/TT	12 (75%)	7 (7%)	39.8 (10.1–156.4)	< **0.0001**
CC	4 (25%)	93 (93%)		

Table 8 The association of individual SNP with allopurinol-induced MPE.

SNP	Allopurinol-induced MPE (n=7)	Allopurinol-tolerant group (n=100)	MPE cases versus Allopurinol tolerant group	
			OR (95% CI)	p-value
TCF19_rs9263794				
AG/GG	5 (71.4%)	12 (12%)	18.3 (3.1–105.2)	**0.0011**
AA	2(28.6%)	88 (88%)		
TCF19_rs1044870				
CT/TT	4 (57.1%)	0	258.4 (11.5–5794.8)	**0.0005**
CC	3 (42.9%)	100 (100%)		
POU5F1_rs9263796				
CT/TT	5 (71.4%)	7 (7%)	33.2 (5.4–203.1)	**0.0001**
CC	2 (28.6%)	93 (93%)		

DISCUSSION

In current study, we conducted a case-control analysis including 44 cases of allopurinol-induced CADR which comprises DRESS (16 cases), SJS/TEN (21 cases) and severe MPE (7 cases). We confirmed an association between *HLA-B* 58:01* allele and allopurinol-induced SCAR including SJS/TEN (OR 922.1) and DRESS (OR 104.0). In addition, we confirmed an association between *HLA-B*58:01* allele and allopurinol-induced severe MPE with OR 144. Thus, *HLA-B* 58:01* allele is associated with allopurinol-induced CADR. This strong association also has been observed in other Asian countries (Hung *et al.*, 2005; Jung *et al.*, 2011; Kang *et al.*, 2011; Kaniwa *et al.*, 2008). Previous studies in Taiwan has shown that the risk for allopurinol-induced SCAR in chronic renal insufficiency increased (OR 4.7; 95%CI: 2.3–9.3, p<0.001) (Hung *et al.*, 2005). In the present study, an underlying disease, chronic kidney disease and level of creatinine and eGFR did not affect a different level of risk for allopurinol-induced CADR, but the level of BUN showed increased risk for allopurinol-induced CADR, as shown in Table 2.

Beside *HLA-B* genotyping, we also conducted SNP genotyping in *TCF19 (rs9263794, rs1044870)* and *POU5F1 (rs9263796)*. The results showed significant association between all 3 SNPs and allopurinol-induced CADR (OR 57.20, 77.31, 84.4, respectively), similar to the result from a previous study by Tohkin *et al.* which examined genome-wide association in Japanese patients and found representative SNPs in *6p21* and *HLA-B*58:01* (Tohkin *et al.*, 2013). The *TCF19* gene plays an important role in the transcription of genes required for the later stages of cell cycle progression (Teraoka *et al.*, 2000), and is important for cell survival (Krautkramer *et al.*, 2013). Furthermore, *POU5F1* gene

expression may cause dysplasia in epithelial cells (Chang *et al.*, 2007). For the *TCF19* SNPs (*rs95963794* and *rs1044870*) and *POU5F1* SNP (*rs9263796*), the results showed positive predictive value (PPV) of 76.5, 100 and 84.4%, and negative predictive value (NPV) of 94.6, 75.8 and 93.9%, respectively (data not shown). *TCF19 rs1044870* showed 100% PPV but 27.7% sensitivity, therefore it is not suitable to be used as a predictive marker for allopurinol-induced CADR. Nevertheless, *TCF19 rs9263794* and *POU5F1 rs9263796* which showed sensitivity of 88.6% and 86.4%, and specificity of 88% and 93%, respectively (data not shown) can be used as a predictive marker for allopurinol-induced SJS/TEN in Thailand.

CONCLUSION

Our results suggest that the screening tests for *TCF19 (rs9263794, rs1044870), POU5F1 (rs9263796)* in patients who will be treated with allopurinol will be clinically helpful in preventing development of SCAR. Regarding to our finding, the pharmacogenetic interpretation could be generalized to SCAR including DRESS, SJS and SJS/TEN. Physicians and national policy makers maybe concerned that our findings support the consideration for implementation of *HLA-B*58:01* genetic screening prior to initiation of allopurinol in Thai patients. Furthermore, Biomarker SNPs to predict allopurinol-induced CADR and functional studies for identification of the physiological and molecular pathways leading to allopurinol-induced CADR should be further studied.

ACKNOWLEDGEMENTS

This study was supported by grants from the (1) Faculty of Medicine, Ramathibodi Hospital, Mahidol University (2) THAISCAR project: WCU-002-HR-57, Chulalongkorn University. The authors thank the study participants and staff of the Pharmacogenomics and Personalized Medicine laboratory, Ramathibodi Hospital.

REFERENCES

Aihara M. Pharmacogenetics of cutaneous adverse drug reactions. J Dermatol. 2011;38*(3):*246-254.

Barvaliya M., Sanmukhani J, Patel T, Paliwal N, Shah H, Tripathi C. Drug-induced Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and SJS-TEN overlap: a multicentric retrospective study. J Postgrad Med. 2011;57(2):115-119.

Chang YT, Hsu CY, Chou CT, Lin MW, Shiao YM, Tsai CY, *et al.* The genetic polymorphisms of *POU5F1* gene are associated with psoriasis vulgaris in Chinese. J Dermatol Sci. 2007;46*(2):*153-156.

Cheng CY, Su SC, Chen CH, Chen WL, Deng ST, Chung WH. HLA associations and clinical implications in T-cell mediated drug hypersensitivity reactions: an updated review. J Immunol Res. 2014;2014:565320.

Choudhary S, McLeod M, Torchia D, Romanelli P. Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome. J Clin Aesthet Dermatol. 2013;6(6):31-37.

Daly AK. Human leukocyte antigen (HLA) pharmacogenomic tests: potential and pitfalls. Curr Drug Metab. 2014;15(2):196-201.

Fleming P, Marik PE. The DRESS syndrome: the great clinical mimicker. Pharmacotherapy. 2011;31(3):332.

Goncalo M, Coutinho I, Teixeira V, Gameiro AR, Brites MM, Nunes R, Martinho A. HLA-B*58:01 is a risk factor for allopurinol-induced DRESS and Stevens-Johnson syndrome/toxic epidermal necrolysis in a Portuguese population. Br J Dermatol. 2013;169(3):660-665.

Harr T, French LE. Stevens-Johnson syndrome and toxic epidermal necrolysis. Chem Immunol Allergy. 2012;97:149-166.

Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, Lin YL, Lan JL, Yang LC, Hong HS, *et al.* HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proc Natl Acad Sci U S A. 2005;102(11):4134-4139.

Jung JW, Song WJ, Kim YS, Joo KW, Lee KW, Kim SH, Park HW, Chang YS, Cho SH, Min KU, *et al.* HLA-B58 can help the clinical decision on starting allopurinol in patients with chronic renal insufficiency. Nephrol Dial Transplant. 2011;26(11):3567-3572.

Kang HR, Jee YK, Kim YS, Lee CH, Jung JW, Kim SH, Park HW, Chang YS, Jang IJ, Cho SH, *et al.* Positive and negative associations of HLA class I alleles with allopurinol-induced SCARs in Koreans. Pharmacogenet Genomics. 2011;21(5):303-307.

Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, Sawada J, Furuya H, Takahashi Y, Muramatsu M, *et al.* HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. Pharmacogenomics. 2008;9(11):1617-1622.

Karlin E, Phillips E. (2014). Genotyping for severe drug hypersensitivity. Curr Allergy Asthma Rep. 2014;14(3):418.

Krautkramer KA, Linnemann AK, Fontaine DA, Whillock AL, Harris TW, Schleis GJ, Truchan NA, Marty-Santos L, Lavine JA, Cleaver O, *et al.* Tcf19 is a novel islet factor necessary for proliferation and survival in the INS-1 beta-cell line. Am J Physiol Endocrinol Metab. 2013;305(5):E600-E610.

Lam MP, Yeung CK, Cheung BM. (2013). Pharmacogenetics of allopurinol--making an old drug safer. J Clin Pharmacol. 2013;53(7):675-679.

Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, Naldi L, Bouwes-Bavinck JN, Sidoroff A, de Toma C, *et al.* A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. Pharmacogenet Genomics. 2008;18(2):99-107.

Min HK, Lee B, Kwok SK, Ju JH, Kim WU, Park YM, Park SH. Allopurinol hypersensitivity syndrome in patients with hematological malignancies: characteristics and clinical outcomes. Korean J Intern Med. 2015;30(4):521-530.

Pichler W, Yawalkar N, Schmid S, Helbling A. Pathogenesis of drug-induced exanthems. Allergy. 2002;57(10):884-893.

Puangpetch A, Koomdee N, Chamnanphol M, Jantararoungtong T, Santon S, Prommas S, Hongkaew Y, Sukasem C. HLA-B allele and haplotype diversity among Thai patients identified by PCR-SSOP: evidence for high risk of drug-induced hypersensitivity. Front Genet. 2015;5:478.

Saokaew S, Tassaneeyakul W, Maenthaisong R, Chaiyakunapruk N. Cost-effectiveness analysis of HLA-B*5801 testing in preventing allopurinol-induced SJS/TEN in Thai population. PLoS One. 2014;9(4):e94294.

Sasidharanpillai S, Riyaz N, Khader A, Rajan U, Binitha MP, Sureshan DN. Severe cutaneous adverse drug reactions: a clinicoepidemiological study. Indian J Dermatol. 2015;60(1):102.

Sukasem C, Jantararoungtong T, Kuntawong P, Puangpetch A, Koomdee N, Satapornpong P, Supapsophon P, Klaewsongkram J, Rerkpattanapipat T. HLA-B (*) 58:01 for allopurinol-induced cutaneous adverse drug reactions: implication for clinical interpretation in Thailand. Front Pharmacol. 2016;7:186.

Sukasem C, Puangpetch A, Medhasi S, Tassaneeyakul W. Pharmacogenomics of drug-induced hypersensitivity reactions: challenges, opportunities and clinical implementation Asian Pac J Allergy Immunol. 2014;32(2):111-123.

Tassaneeyakul W, Jantararoungtong T, Chen P, Lin PY, Tiamkao S, Khunarkornsiri U, Chucherd P, Konyoung P, Vannaprasaht S, Choonhakarn C, *et al.* Strong association between HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. Pharmacogenet Genomics. 2009;19*(9):*704-709.

Teraoka Y, Naruse TK, Oka A, Matsuzawa Y, Shiina T, Iizuka M, Iwashita K Ozawa A, Inoko H. Genetic polymorphisms in the cell growth regulated gene, SC1 telomeric of the HLA-C gene and lack of association of psoriasis vulgaris. Tissue Antigens. 2000;55(3):206-211.

Tohkin M, Kaniwa N, Saito Y, Sugiyama E, Kurose K, Nishikawa J, Hasegawa R, Aihara M, Matsunaga K, Abe M, *et al.* A whole-genome association study of major determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. Pharmacogenomics J. 2013;13(1):60-69.