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Regular Article

Associations of HLA and drug-metabolizing enzyme genes in co-trimoxazole-induced severe cutaneous adverse reactions

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ABSTRACT

Co-trimoxazole is mainly used as a first-line drug for treatment and prophylaxis against *Pneumocystis jiroveci* pneumonia. This drug, however, has been reported as the most common causative drug for severe cutaneous adverse reactions (SCARs). This study aimed to extensively elucidate the associations between genetic polymorphisms of *HLA* class I and genes involved in bioactivation and detoxification of co-trimoxazole on co-trimoxazole-induced SCARS in a large sample size and well-defined Thai SCARs patients. A total of 67 patients with co-trimoxazole-induced SCARs, consisting of 51 SJS/TEN patients and 16 DRESS patients, and 91 co-trimoxazole tolerant controls were enrolled in the study. The results clearly demonstrated that the *HLA-B*13:01* allele was significantly associated with co-trimoxazole-induced SCARs, especially with DRESS (OR = 8.44, 95% CI = 2.66–26.77, P = 2.94×10^{-4}, Pc = 0.0126). Moreover, the *HLA-C*08:01* allele was significantly associated with co-trimoxazole-induced SJS/TEN in the HIV/AIDS patients with an OR of 8.51 (95% CI = 2.18–33.14, P = 8.60×10^{-4}, Pc = 0.0241). None of the genes involved in the bioactivation and detoxification of co-trimoxazole investigated in this study play any major role in the development of all phenotypes of SCARs.

1. Introduction

Co-trimoxazole is a fixed-dose combination of sulfamethoxazole (SMX) and trimethoprim (TMP) in a 5:1 ratio. This drug is a broad-spectrum antimicrobial drug that is widely prescribed for the treatment of a variety of bacterial, protozoal, and fungal infections. Co-trimoxazole is a first-line drug used for treatment and prophylaxis of *Pneumocystis jiroveci* pneumonia (PJP) in patients with human immunodeficiency virus infection (HIV) or acquired immunodeficiency syndrome (AIDS) [1]. The use of co-trimoxazole for treatment of infections other than *Pneumocystis jiroveci* pneumonia is currently restricted due to its high incidence of cutaneous adverse drug reactions which range from mild cutaneous reactions to life-threatening severe cutaneous adverse reactions (SCARs) including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug reactions with eosinophilia and systemic symptoms (DRESS) [2,3]. SJS and TEN are the most severe cutaneous reactions presenting with a prodrome of fever, sore throat and eyes (mucosal involvement), followed by blister formation, epidermal necrosis and skin detachment, with relatively high mortality rates of approximately 5–10% for SJS and 30–50% for TEN. While DRESS is characterized by generalized maculopapular

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eruptions, high fever, eosinophilia, atypical lymphocytes and systemic manifestations with a mortality rate of approximately 10% [4].

A recent study of 1078 SJS/TEN patients who have been admitted to hospitals during 1998–2017 from registration databases revealed that sulfamethoxazole or co-trimoxazole is one of the most common causative drugs for SJS/TEN in several Asian countries including Thailand [3]. Moreover, this drug has also been reported as a common causative drug of SJS/TEN in European countries [2,5,6].

SCARs are considered as unpredictable adverse drug reactions (ADRs), delayed-type hypersensitivity and immune mediated-ADRs, however, recent evidence revealed that genetic polymorphisms of *HLA* as well as some drug-metabolizing enzymes may play a role in the risk of SCARs induced by co-trimoxazole. Our group is the first to report the association between co-trimoxazole-induced SJS/TEN and *HLA* alleles including *HLA-B*15:02*, *HLA-C*06:02*, and *HLA-C*08:01* in a Thai population [7]. Moreover, the association between co-trimoxazole-induced SCARs and *HLA* alleles in Thai patients was later confirmed in another cohort of 30 co-trimoxazole-induced SCARs patients (including 18 SJS/TEN and 12 DRESS patients) in which *HLA-B*15:02* and *HLA-C*08:01* alleles were significantly associated with co-trimoxazole-induced SJS/TEN whereas the *HLA-B*13:01* allele was significantly associated with co-trimoxazole-induced DRESS, thus this study proposed that the associations of *HLA* alleles and SCARs induced by this drug are specific for certain phenotype of SCARs [8]. A recent whole-genome sequencing study in multi-Asian populations including Chinese, Thai, and Malaysian populations, however, revealed that *HLA-B*13:01* is an important genetic predisposing factor of both SJS/TEN and DRESS caused by co-trimoxazole [9]. Whether or not these *HLA* alleles are specific to SCARs phenotypes caused by co-trimoxazole needs to be explored further in larger studies and in various ethnic groups.

Sulfamethoxazole (SMX), a major active compound in co-trimoxazole, is bioactivated by several enzymes including cytochromes P450 2C9 (CYP2C9) to toxic N^{4-}-hydroxylamine-SMX (HA-SMX) [10,11]. HA-SMX is auto-oxidized via nitroxide-SMX to nitroso-SMX. This highly reactive nitroso-SMX subsequently binds to cellular proteins, forming neo-antigens, and triggers the human major histocompatibility complex (HMC) restricted T-cell mediated immune response. Detoxification of nitroso-SMX occurs by conjugation with glutathione (GSH) [12]. Glutamate cysteine ligase catalytic subunit (GCLC) is a rate-limiting enzyme for the biosynthesis of glutathione (GSH) which is the main cellular antioxidant scavenging reactive metabolites of a number of drugs. The previous study has demonstrated in Caucasian population that the genetic polymorphisms of *GCLC* that reduce GCLC expression may play an important role in the increasing risk of developing SMX-induced hypersensitivity [11]. In addition, inactivation of the parent drug, SMX, to an inactive metabolite can occur via two polymorphic enzymes, N-acetyltransferase 1 (NAT1) and N-acetyltransferase 2 (NAT2) [13]. Although there are some studies showing that genetic polymorphisms of enzymes involved in bioactivation and detoxification of this drug such as *CYP2C9*, *NAT2*, *GSTT1, GSTM1* and *GSTP1* may play a role in the susceptibility to sulfamethoxazole hypersensitivity [14–18], but discrepancies in these findings are noted in several reports [19,20].

Since co-trimoxazole is the most common causative drug for SCARs in several countries, the identification of patients who are at high risk of co-trimoxazole-induced SCARS is of clinical importance. We therefore conducted a case-control study in large sample sizes and well-defined Thai SCARs patients in order to extensively characterize the association between co-trimoxazole-induced SCARS and genetic polymorphisms of *HLA* class *I* and genes involved in bioactivation and detoxification of co-trimoxazole.

2. Materials and methods

2.1. Study population

Patients with co-trimoxazole-induced SCARs who had been admitted to local hospitals in Thailand from 1981 to 2021 were enrolled in the study. Patients hospitalized between 1981 and 2008 were identified retrospectively by reviewing their medical records, whereas patients who were hospitalized between 2009 and 2021 were prospectively enrolled in the study. Patients who had been diagnosed as co-trimoxazole-induced SCARs including SJS, TEN, SJS/TEN overlap and DRESS were enrolled in the study. The diagnosis of SCARs was made by pharmacists who were responsible for pharmacovigilance and dermatologists in each hospital based on the clinical morphology of the patients' skin lesions and other clinical manifestations. The criteria for SJS, TEN and DRESS were as previously described [21]. The drug causality for each patient was evaluated based on the Naranjo algorithm (Naranjo score) [22] and the algorithms for assessment of drug causality for Stevens-Johnson syndrome and toxic epidermal necrolysis (ALDEN) [6]. Only patients with the ALDEN or Naranjo score for co-trimoxazole but not other concomitant drugs, in the categories of probable or definite (ALDEN score $\geq$4 for SJS/TEN and Naranjo algorithm $\geq$5 for DRESS) were included as cases.

The tolerant control patients were defined as patients who tolerated co-trimoxazole for more than 6 months without any evidence of cutaneous reactions. All participants were informed both verbally and in writing about the experimental procedures and the purpose of the study. Written informed consent was obtained from each participant. The study protocol within the hospital networks of Khon Kaen University was approved by the Khon Kaen Ethics Committee for Human Research, Khon Kaen University, Thailand (HE510837).

2.2. Genotyping of HLA class I alleles

The *HLA* class I (*HLA-A, HLA-B* and *HLA-C* alleles) was genotyped from gDNA using the LIFECODES HLA Typing Kits (Immucor GTI Diagnostics, Waukesha, Wisconsin, USA), which are based on the reverse sequence-specific oligonucleotide probes (SSO) method coupled with xMAP technology designed to be used with the Luminex® system, as previously described [21]. The *HLA* alleles were analyzed using LIFECODES MATCH IT DNA Software version 1.2 based on Allele Database version 3.41. In addition, the *HLA* genotype data of these cases were confirmed by SeCore HLA sequence–based typing (SBT) (Invitrogen, Life Technologies, Carlsbad, CA).

2.3. Genotyping of genes involving in bioactivation and detoxification of co-trimoxazole

*CYP2C9*3* (rs1057910), *NAT2*5* (rs1801280), *NAT2*6* (rs1799930), *NAT2*7* (rs1799931), *GSTP1* (rs1695) and *GCLC* (rs761142) alleles were genotyped by TaqMan Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, California, USA on a QuantStudio™ 6 Flex Machine). While genotyping of *GSTT1* and *GSTM1* polymorphisms was performed by multiplex polymerase chain reactions as previously described [23,24].

2.4. Statistical analysis

The allele frequencies and genotype frequencies of the *HLA* class I, *CYP2C9*3*, *NAT2*5*, *NAT2*6*, *NAT2*7*, *GSTP1* (rs1695), *GSTT1 (null)*, *GSTM1 (null)* and *GCLC* (rs761142), were determined by direct counting. The *NAT2* metabolizer status (phenotype) was classified according to the *NAT2* genotype of each patient, including rapid (*NAT2*4/*4*), intermediate (*NAT2*4/*5, NAT2*4/*6 or NAT2*4/*7*) and slow (*NAT2*5/*5, NAT2*5/*6, NAT2*5/*7, NAT2*6/*6, NAT2*6/*7 or NAT2*7/*7)* metabolizers. The statistical analyses between cases and controls for the clinical characteristics were carried out using Student's t-test and Fisher's exact test. The strength of associations was estimated by calculating the odds ratios (ORs) and 95% confidence intervals (CIs) using SPSS statistical software, version 25.0 for Windows (IBM, Armonk, New York, USA). ORs were determined using Haldane's modification, which adds 0.5 to all cells to accommodate possible zero counts. The corrected P-values (Pc) for the multiple comparisons of all alleles were calculated using Bonferroni's correction. The Pc value less than 0.05 was considered statistically significant. The estimated linkage disequilibrium coefficients (D′) and coefficient of correlation (r^2) among identified *HLA* alleles were calculated using the PLINK (V1.07) program [25]. Moreover, the conditional analysis using a logistic regression was performed for determining the independence among the identified risk alleles of co-trimoxazole-induced SCARs.

3. Results

3.1. Characteristics of the study population

A total of 67 patients with co-trimoxazole-induced SCARs, consisting of 40 (59.70%) SJS, 1 (1.49%) SJS/TEN overlap, 10 (14.93%) TEN and 16 (23.88%) DRESS and 91 co-trimoxazole tolerant controls were enrolled in the study. All patients in the case group were admitted to the hospital for the treatment of SCARs. It is noteworthy that 38 patients in the SJS/TEN group, 3 patients in the DRESS group and 91 tolerant controls are recruited previous studies [7,9].

Demographic data of co-trimoxazole-induced SCARs patients and co-trimoxazole-tolerant patients are summarized in Table 1. The patients in the SCARs group were mostly middle-aged, with a mean age of 39.58 ± 13.44 years (39.51 ± 13.76 in the SJS/TEN and 39.81 ± 12.78 in the DRESS groups) and that the ages of patients in the SCARs groups were significantly younger than those of the tolerant control group. Among the 67 patients with co-trimoxazole-induced SCARs, 45 patients (67.16%) had been diagnosed with HIV/AIDs. Similar to the SCARs group, 61/91 co-trimoxazole-tolerant patients (67.03%) had been diagnosed with HIV/AIDs. The use of co-trimoxazole was mostly indicated for treatment and prophylaxis of *Pneumocystis jiroveci* pneumonia (PJP) in both SCARs cases (64.18%) and tolerant controls (67.03%).

The mean exposure time to co-trimoxazole until the first signs of SCARs was significantly longer in the DRESS group (37.44 ± 30.64 days, range from 8 to 108 days) compared with those of the SJS/TEN group (19.53 ± 18.48, range from 1 to 70 days). There were no significant differences in the mean dose of co-trimoxazole in the SJS/TEN, DRESS and tolerant control groups. The mean duration of hospital stay for SCARs treatment was 10.40 ± 8.85 (range from 2 to 51) days. The mean duration of hospital stay of the TEN group was longest compared with those of other SCARs groups. The mucosal involvements were mainly noted in the SJS/TEN patients whereas the hematological abnormalities were noted in the DRESS patients. Of these 67 cases, none of them died in the hospital as a result of the SCARs episodes.

3.2. Associations between genetic polymorphisms of HLA class I and co-trimoxazole-induced SCARs

For *HLA* class I genotyping results, 31 alleles for *HLA-A*, 43 alleles for *HLA-B* and 33 alleles for *HLA-C* were detected in the study population. For the 2-field or 4-digit resolution of *HLA* genotypes, results from LIFECODES HLA typing were 100% concordant with SeCore HLA sequence-based typing (SBT) method. *HLA* genotyping data of the SCARs patients and the tolerant control patients revealed that the carrier frequencies of four *HLA* alleles including *HLA-A*11:01*, *HLA-B*13:01*, *HLA-B*15:02* and *HLA-C*08:01* were higher in the SCARs group ($P < 0.05$) (Table 2). Among these four alleles, the *HLA-B*13:01* shows the highest OR, followed by *HLA-C*08:01*, *HLA-B*15:02* and *HLA-A*11:01*. The frequency of patients who carried the *HLA-B*13:01* allele in the co-trimoxazole-induced SCARs group was about 2.26-fold higher (25/67, 37.31%) compared with the tolerant control group (15/91, 16.48%) and the risk of SCARs for the patients with *HLA-B*13:01* was 3.02 (95% CI = 1.43–6.34, P = 0.0051, Pc = 0.2187), however, this did not reach a statistically significant difference after Bonferroni's correction. Interestingly, the frequency of patients who carried the *HLA-B*13:01* allele in the co-trimoxazole-induced SCARs group was about 2.79-fold higher (25/67, 37.31%) compared with that of the general population control group (87/653, 13.38%). The risk of co-trimoxazole-induced SCARs was significantly higher in the patients who carried *HLA-B*13:01* allele with an OR of 3.87 (95% CI = 2.25–6.67; $P = 4.00 \times 10^{-6}$, $Pc = 2.96 \times 10^{-4}$) compared with the general population controls who did not carry this allele (Table 2).

Moreover, higher risk of co-trimoxazole-induced SCARs were observed in patients who carried the *HLA-B*15:02* allele (OR = 2.54, 95% CI = 1.07–6.03, P = 0.0344, Pc = 1.4792), the *HLA-C*08:01* allele (OR = 2.63, 95% CI = 1.07–6.44, P = 0.0426, Pc = 1.4070) or the *HLA-A*11:01* allele (OR = 2.10, 95% CI = 1.10–4.00, P = 0.0252, Pc = 0.7811) (Table 2). Interestingly, the risk of co-trimoxazole-induced SCARs was lower in patients who carried either the *HLA-A*02:07* allele (OR = 0.35, 95% CI = 0.15–0.80, P = 0.0133, Pc = 0.4112) or the *HLA-C*14:02* allele (OR = 0.22, 95% CI = 0.05–1.05, P = 0.0443, Pc = 1.4604). However, it should be noted that these associations did not reach a statistically significant difference after Bonferroni's correction.

The results from linkage disequilibrium (LD) analysis of the study population (combining data from the case group and the control group, $n = 158$) showed that the linkage disequilibrium (LD) of any two (A - B, B - C, and C - A) or three (A - B - C) of these four *HLA* alleles (*HLA-A*11:01*, *HLA-B*13:01*, *HLA-B*15:02* and *HLA-C*08:01)* was not complete LD (D' < 1, range from 0.1023 to 0.8670) and perfect LD ($r^2 < 1$, range from 0.0045 to 0.5827). However, the *HLA-B*15:02 - HLA-C*08:01* and *HLA-A*11:01 - HLA-B*15:02 - HLA-C*08:01* may considered as significantly strong LD due to high D′ and r^2 values (D' = 0.8005, $r^2 = 0.5827$ and D' = 0.8670, $r^2 = 0.4907$) [25].

In order to demonstrate that these identified risk alleles are independent association with SCARs, logistic regression for analyzing the independent association of *HLA-A*11:01*, *HLA-B*15:02*, and *HLA-C*08:01* with SCARs using conditional analysis with *HLA-B*13:01* were performed and the results revealed that *HLA-B*15:02* and *HLA-C*08:01* were still showed the association with co-trimoxazole-induced SCARs with OR of 3.11 (95% CI = 1.27–7.61, P = 0.0131) and 3.58 (95% CI = 1.41–9.11, P = 0.0073). Whereas there is no significant correlation between *HLA-A*11:01* and co-trimoxazole-induced SCARs after conditional analysis.

In the subgroup analysis of DRESS phenotype, the *HLA-B*13:01* allele was about 3.79-fold significantly higher than those of the

Table 1
Demographic, medical, and clinical data of patients with co-trimoxazole-induced SCARs and co-trimoxazole tolerant controls.

Characteristics	SJS	SJS/TEN overlap	TEN	Total SJS/TEN	DRESS	Total SCARs	Tolerant controls
Demographic data							
n	40	1	10	51	16	67	91
Age, years							
Mean (SD)	40.45 (13.70)*	57	34 (12.89)*	39.51 (13.76)*	39.81 (12.78)	39.58 (13.44)*	45.31 (12.82)
Median [range]	38 [3–71]	57	37 [8–50]	37 [3–71]	37.5 [20–59]	37 [3–71]	45 [13–74]
Sex, n (%)							
Male	21 (52.50)	NA	3 (30)	24 (47.06)	12 (75)	36 (53.73)	54 (59.34)
Female	19 (47.50)	1 (100)	7 (70)	27 (52.94)	4 (25)	31 (46.27)	37 (40.66)
Comorbidity [n (%)]							
Chronic kidney disease stage 3 or worse	2 (5.00)	NA	NA	2 (3.92)	NA	2 (2.99)	1 (1.10)
Diabetes mellitus	2 (5.00)	NA	NA	2 (3.92)	3 (18.75)	5 (7.46)	10 (10.99)
Hypertension	1 (2.50)	NA	NA	1 (1.96)	2 (12.5)	3 (4.48)	3 (3.30)
Liver impairment	1 (2.50)	1 (100)	NA	2 (3.92)	1 (6.25)	3 (4.48)	1 (1.10)
Cardiovascular diseases	NA	NA	NA	NA	NA	NA	2 (2.20)
Pulmonary tuberculosis	5 (12.50)*	NA	1 (10)	6 (11.76)*	NA	6 (8.96)	2 (2.20)
HIV/AIDs	29 (72.50)	NA	7 (70)	36 (70.59)	9 (56.25)	45 (67.16)	61 (67.03)
Medical data							
Co-trimoxazole exposure							
n (available data)	29	0	7	36	7	43	91
Dose SMX (mg/day)							
Mean (SD)	1393.10 (1258.66)	NA	857.14 (151.19)	1288.89 (1147.86)	2057.14 (1868.03)	1413.95 (1295.70)	1301.10 (889.63)
Median [range]	800 [400–4800]	NA	800 [800–1200]	800 [400–4800]	800 [400–4800]	800 [400–4800]	800 [800–3200]
Dose TMP (mg/day)							
Mean (SD)	278.62 (251.73)	NA	171.43 (30.24)	257.78 (229.57)	411.43 (373.61)	282.79 (259.14)	260.22 (177.93)
Median [range]	160 [80–960]	NA	160 [800–1200]	160 [80–960]	160 [80–960]	160 [80–960]	160 [160–640]
n (available data)	40	1	10	51	16	67	91
Duration (day)							
Mean (SD)	19.68 (18.42)**	14	19.50 (20.59)	19.53 (18.48)*****	37.44 (30.64)	23.81 (23.05)	>90
Median [range]	14.5 [1–70]	14	12.5 [1–66]	14 [1–70]	27.5 [8–108]	16 [1–108]	NA
Indication of co-trimoxazole [n (%)]							
n (available data)	40	1	10	51	16	67	
Treatment and prevention of PJP	29 (72.50)	0	7 (70)	36 (70.59)	7 (43.75)	43 (64.18)	61 (67.03)
Bacterial infections	11 (27.5)	1 (100)	3 (30)	15 (29.41)	9 (56.25)	24 (35.82)	30 (32.97)
Internal organ involvement							
Liver function, no. (%)							
n (available data)	27	1	7	35	14	49	
AST (units/l)							
<100	17 (62.96)	0 (0)	4 (57.14)	21 (60)	10 (71.43)	31 (63.27)	NA
100–500	9 (33.33)	1 (100)	3 (42.86)	13 (37.14)	4 (28.57)	17 (34.69)	NA
501–1000	1 (3.70)	0 (0)	0 (0)	1 (2.86)	0 (0)	1 (2.04)	NA
ALT (units/l)							
<100	17 (62.96)	1 (100)	4 (57.14)	22 (62.86)	10 (71.43)	32 (65.31)	NA
100–500	6 (22.22)	0 (0)	3 (42.86)	9 (25.71)	4 (28.57)	13 (26.53)	NA
501–1000	4 (14.81)	0 (0)	0 (0)	4 (11.43)	0 (0)	4 (8.16)	NA
Kidney function, no. (%)							
n (available data)	32	1	7	40	14	54	
Acute renal failure[a]	4 (12.5)	0 (0)	1 (14.29)	5 (12.50)	0 (0)	5 (9.26)	NA
Hematologic function, no. (%)							
n (available data)	30	1	7	38	14	52	
Eosinophilia, eosinophils (%)							
<5%	18 (60)	1 (100)	6 (85.71)	25 (65.79)	3 (21.43)	28 (53.85)	NA
≥5%	12 (40)	0 (0)	1 (14.29)	13 (34.21)	11 (78.57)	24 (46.15)	NA
Mucosal involvement, no. (%)							
n (available data)	40	1	10	51	16	67	
Oral	31 (77.50)**	1 (100)	7 (70)***	39 (76.47)*****	4 (25)	43 (64.18)	NA
Ocular	24 (60.00)**	1 (100)	8 (80)***	33 (64.71)*****	3 (18.75)	36 (53.73)	NA
Genital	7 (17.50)	0 (0)	4 (40)***	11 (21.57)	0 (0)	11 (16.42)	NA
Outcome from SCARs							
Duration of hospital stay for treatment of SCARs, days							
n (available data)	32	1	9	42	8	50	
Mean (SD)	8.56 (5.52)****	8	17.67 (13.64)	10.50 (8.59)	9.88 (10.78)	10.40 (8.85)	NA
Median [range]	7 [2-25]	8	13 [8–51]	9 [2–51]	5.5 [2-34]	8 [2–51]	NA
Deceased cases, *n* (%)	0	0	0	0	0	0	NA

*P-value < 0.05 compared with the tolerant controls.
**P-value < 0.05 compared between SJS and DRESS.
***P-value < 0.05 compared between TEN and DRESS.
****P-value < 0.05 compared between SJS and TEN.
*****P-value < 0.05 compared between total SJS/TEN and DRESS.
[a] Creatinine value was higher than 2.25 mg/dl when SCARs occurred.; NA, not available.

Table 2
List of odds ratios and 95%confidence intervals for phenotypes of co-trimoxazole−induced severe cutaneous adverse reactions in individuals who carried different *HLA* alleles compared with the co-trimoxazole tolerant controls and the general Thai population controls.

Co-trimoxazole-induced SCARs							
HLA alleles	Tolerant controls (n = 91)	General population[a] (n = 653)	SCARs (n = 67)	SCARs cases vs tolerant controls		SCARs cases vs General Thai population	
	Carriers (%)	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	OR [95% CI]	P-value
A*11:01	39 (42.86)	289 (44.32)	41 (61.19)	**2.10 [1.11−4.00]**	**0.0252**	**1.99 [1.19−3.32]**	**0.0097**
A*02:07	28 (30.77)	127 (19.38)	9 (13.43)	**0.35 [0.15−0.80]**	**0.0133**	0.64 [0.31−1.33]	0.2560
B*13:01	15 (16.48)	87 (13.38)	25 (37.31)	**3.02 [1.43−6.34]**	**0.0051**	**3.87 [2.25−6.67]**	**4.00 x 10^{-6} ##**
B*15:02	10 (10.99)	95 (14.59)	16 (23.88)	**2.54 [1.07−6.03]**	**0.0344**	**1.84 [1.01−3.37]**	**0.0467**
B*07:05	6 (6.59)	34 (5.22)	8 (11.94)	1.92 [0.63−5.83]	0.2685	**2.47 [1.09−5.58]**	**0.0476**
C*08:01	9 (9.89)	122 (18.68)	15 (22.39)	**2.63 [1.07−6.44]**	**0.0426**	1.26 [0.68−2.30]	0.5127
C*04:06	2 (2.20)	14 (2.14)	5 (7.46)	3.59 [0.67−19.09]	0.1351	**3.68 [1.28−10.56]**	**0.0249**
C*14:02	11 (12.09)	41 (6.28)	2 (2.99)	**0.22 [0.05−1.05]**	**0.0443**	0.46 [0.11−1.94]	0.4166
Co-trimoxazole-induced SJS/TEN							
HLA alleles	Tolerant controls (n = 91)	General population[a] (n = 653)	SJS/TEN (n = 51)	SJS/TEN cases vs tolerant controls		SJS/TEN cases vs General Thai population	
	Carriers (%)	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	OR [95% CI]	P-value
A*02:07	28 (30.77)	127 (19.38)	6 (11.76)	**0.30 [0.11−0.78]**	**0.0134**	0.55 [0.23−1.32]	0.1985
B*13:01	15 (16.48)	87 (13.38)	15 (29.41)	2.11 [0.93−4.78]	0.0873	**2.71 [1.42−5.16]**	**0.0035**
B*15:02	10 (10.99)	95 (14.59)	13 (25.49)	**2.77 [1.12−6.88]**	**0.0326**	**2.01 [1.03−3.91]**	**0.0440**
C*08:01	9 (9.89)	122 (18.68)	13 (25.49)	**3.12 [1.23−7.92]**	**0.0170**	1.49 [0.77−2.88]	0.2663
C*04:06	2 (2.20)	14 (2.14)	4 (7.84)	3.79 [0.67−21.44]	0.1878	**3.88 [1.23−12.27]**	**0.0350**
Co-trimoxazole-induced DRESS							
HLA alleles	Tolerant controls (n = 91)	General population[a] (n = 653)	DRESS (n = 16)	DRESS cases vs tolerant controls		DRESS cases vs General Thai population	
	Carriers (%)	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	OR [95% CI]	P-value
A*68:01	1 (1.10)	13 (1.95)	2 (12.5)	12.86 [0.94−151.34]	0.0578	**7.03 [1.45−34.15]**	**0.0470**
B*13:01	15 (16.48)	87 (13.38)	10 (62.50)	**8.44 [2.66−26.77]**	**2.94 x 10^{-4} ##**	**10.84 [3.84−30.58]**	**1.00 x 10^{-6} ##**
C*03:04	21 (23.08)	112 (17.15)	7 (43.75)	2.59 [0.86−7.80]	0.1203	**3.76 [1.37−10.30]**	**0.0133**

P-values were calculated using Fisher's exact test. P-value < 0.05 was highlighted in bold.
The corrected P-values (Pc) for the multiple comparisons of all alleles were calculated using Bonferroni's correction (31 for *HLA-A*, 43 for *HLA-B* and 33 for *HLA-C*).
The corrected P-value (Pc) of less than 0.05 was considered statistically significant.
[a] Data obtained from the published information [27,28].

tolerant control group (10/16, 62.50% vs 15/91, 16.48%) and the risk of co-trimoxazole-induced DRESS in patients who carried *HLA-B*13:01* was 8.44-fold (95% CI = 2.66−26.77, P = 2.94 × 10^{-4}, Pc = 0.0126) which significantly higher compared with the tolerant control patients who did not carry this allele (Table 2). When compared with the general population control group, the results were still consistent with those observed in the tolerant control group (OR = 10.84, 95% CI = 3.84−30.58, P = 1.00 × 10^{-6}, Pc = 7.40 × 10^{-4}) (Table 2). None of the *HLA-A* and *HLA-C* alleles were significantly associated with co-trimoxazole-induced DRESS.

Different from the DRESS group, only the frequencies of *HLA-B*15:02* and *HLA-C*08:01* alleles in the co-trimoxazole-induced SJS/TEN group were higher than those of the tolerant controls (P < 0.05) (Table 2). Regardless of the control groups, an increased risk of co-trimoxazole-induced SJS/TEN was noted in the patients who carried the *HLA-B*15:02* allele. Apart from *HLA-B* alleles, patients with *HLA-C*08:01* allele were apparently at a higher risk of SJS/TEN (OR = 3.12, 95% CI = 1.23−7.92, P = 0.0170, Pc = 0.5613), whereas patients with *HLA-A*02:07* allele were apparently at a lower risk of co-trimoxazole-induced SJS/TEN (OR = 0.30, 95% CI = 0.11−0.78, P = 0.0134, Pc = 0.4152). However, these associations did not reach a statistically significant difference after Bonferroni's correction.

Results from subgroup analysis of HIV/AIDs patients revealed that the patients with *HLA-A*11:01*, *HLA-B*13:01*, *HLA-B*15:02* or *HLA-C*08:01* were at a higher risk of co-trimoxazole-induced SCARs when compared with the tolerant control group (Table 3). The highest OR was noted in *HLA-C*08:01* group (OR = 7.85, 95% CI = 2.08−29.62, P = 8.56 × 10^{-4}, Pc = 0.0240), followed by the *HLA-B*15:02* (OR = 4.14, 95% CI = 1.44−11.86, P = 0.0107,

Table 3
List of odds ratios and 95%confidence intervals for phenotypes of co-trimoxazole−induced severe cutaneous adverse reactions in the subgroup analysis of HIV/AIDS patients who carried different *HLA* class I alleles compared with the tolerant controls and general Thai patients with HIV infection [26].

HIV/AIDS patients (compared with the tolerant controls)

HLA Alleles	Tolerant Controls (n = 61)	SCARs (n = 45)			SJS/TEN (n = 36)			DRESS (n = 9)		
	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value
A*11:01	22 (36.07)	26 (57.78)	**2.43 [1.10−5.34]**	**0.0312**	21 (58.33)	**2.48 [1.07−5.77]**	**0.0372**	5 (55.56)	2.22 [0.54−9.12]	0.2921
A*02:07	21 (34.43)	6 (13.33)	**0.29 [0.11−0.80]**	**0.0146**	5 (13.89)	**0.31 [0.10−0.91]**	**0.0334**	1 (11.11)	0.24 [0.03−2.03]	0.2553
B*15:02	6 (9.84)	14 (31.11)	**4.14 [1.44−11.86]**	**0.0107**	11 (30.56)	**4.03 [1.34−12.13]**	**0.0132**	3 (33.33)	4.58 [0.91−23.21]	0.0843
B*13:01	9 (14.75)	14 (31.11)	**2.61 [1.01−6.73]**	**0.0474**	10 (27.78)	2.22 [0.80−6.14]	0.1843	4 (44.44)	**4.62 [1.04−20.57]**	**0.0445**
B*51:01	10 (16.39)	2 (4.44)	0.24 [0.05−1.14]	0.0673	1 (2.78)	**0.15 [0.02−1.19]**	**0.0497**	1 (11.11)	0.64 [0.07−5.68]	1.0000
C*08:01	3 (4.92)	13 (28.89)	**7.85 [2.08−29.62]**	**8.56 x 10^{-4} ##**	11 (30.56)	**8.51 [2.18−33.14]**	**8.60 x 10^{-4} ##**	2 (22.22)	5.52 [0.78−38.96]	0.1204

HIV/AIDS patients (compared with general Thai patients with HIV infection)

HLA Alleles	Thai HIV Controls[a] (n = 557)	SCARs (n = 45)			SJS/TEN (n = 36)			DRESS (n = 9)		
	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value
A*11:01	327 (58.71)	26 (57.78)	0.96 [0.52−1.78]	1.0000	21 (58.33)	0.98 [0.50−1.95]	1.0000	5 (55.56)	0.88 [0.23−3.31]	1.0000
A*02:07	107 (19.21)	6 (13.33)	0.65 [0.27−1.57]	0.4284	5 (13.89)	0.68 [0.26−1.79]	0.5162	1 (11.11)	0.53 [0.07−4.25]	1.0000
B*15:02	71 (12.75)	14 (31.11)	**3.09 [1.57−6.09]**	**0.0026**	11 (30.56)	**3.01 [1.42−6.39]**	**0.0098**	3 (33.33)	3.42 [0.84−13.99]	0.1007
B*13:01	99 (17.77)	14 (31.11)	**2.09 [1.07−4.07]**	**0.0445**	10 (27.78)	1.78 [0.83−3.81]	0.1791	4 (44.44)	3.70 [0.98−14.03]	0.0543
B*51:01	52 (9.34)	2 (4.44)	0.45 [0.11−1.92]	0.4145	1 (2.78)	0.28 [0.04−2.07]	0.2384	1 (11.11)	1.21 [0.15−9.90]	0.5900
C*08:01	102 (18.31)	13 (28.89)	1.81 [0.92−3.58]	0.1119	11 (30.56)	1.96 [0.94−4.12]	0.0800	2 (22.22)	1.27 [0.26−6.23]	0.6731

P-values were calculated using Fisher's exact test. P-value < 0.05 was highlighted in bold.
The corrected P-values (Pc) for the multiple comparisons of all alleles were calculated using Bonferroni's correction (31 for *HLA-A*, 43 for *HLA-B* and 33 for *HLA-C*).
The corrected P-value (Pc) of less than 0.05 was considered statistically significant.
[a] Data obtained from published information [26].

Pc = 0.4589), *HLA-B*13:01* (OR = 2.61, 95% CI = 1.01−6.73, P = 0.0474, Pc = 2.0382) and *HLA-A*11:01* (OR = 2.43, 95% CI = 1.10−5.34, P = 0.0312, Pc = 0.9687). It should be noted that only the *HLA-C*08:01* allele showed the significant association with co-trimoxazole-induced SCARs in HIV/AIDs patients after Bonferroni's correction. When compared with 557 general Thai patients with HIV infection [26], only the associations of co-trimoxazole-induced SCARs and *HLA-B*15:02* and *HLA-B*13:01* alleles were noted (*HLA-B*15:02,* OR = 3.09, 95% CI = 1.57−6.09, P = 0.0026, Pc = 0.1118; *HLA-B*13:01,* OR = 2.09, 95% CI = 1.07−4.07, P = 0.0445, Pc = 1.9135).

Similar to the SCARs group, the risk of SJS/TEN induced by co-trimoxazole was statistically significantly higher in HIV/AIDs patients who carried *HLA-C*08:01* compared with those who did not carry this allele with an OR of 8.51 (95% CI = 2.18−33.14, P = 8.60 × 10^{-4}, Pc = 0.0241). In addition, the increased risk of co-trimoxazole-induced SJS/TEN was also observed in HIV/AIDs patients who carried either *HLA-B*15:02* allele (OR = 4.03, 95% CI = 1.34−12.13, P = 0.0132, Pc = 0.5676) or *HLA-A*11:01* allele (OR = 2.48, 95% CI = 1.07−5.77, P = 0.0372, Pc = 1.1532). The association of co-trimoxazole-induced SJS/TEN and *HLA-B*15:02* was also found when compared with the general Thai patients with HIV infection, with an OR of = 3.01, 95% CI = 1.42−6.39, P = 0.0098, Pc = 0.4214). On the other hand, the lower risk of both SCARs and SJS/TEN was noticed in the HIV/AIDs patients who carried the *HLA-A*02:07* allele (Table 3).

For the DRESS group, only HIV/AIDs patients who carried the *HLA-B*13:01* allele were apparently at a higher risk of co-trimoxazole-induced DRESS when compared with the tolerant control group (OR = 4.62, 95% CI = 1.04−20.57, P = 0.0445, Pc = 1.9135) (Table 3).

3.3. Associations between genetic polymorphism of genes involved in bioactivation and detoxification process and co-trimoxazole-induced SCARs

The relationships between genetic polymorphisms of several enzymes involved in bioactivation and detoxification of co-trimoxazole are summarized in Table 4. Only *GSTP1* variant (c.313A > G, rs1695) showed an association with co-trimoxazole-induced SCARs, the increased risk of SCARs was noted in the AG genotype patients (OR = 2.12, 95% CI = 1.07−4.20, P = 0.0310) and patients who carried G allele (OR = 1.95, 95% CI = 1.03−3.70, P = 0.0417). However, this association did not reach a statistically significant difference after Bonferroni's correction and conditional analysis. In subgroup analysis, the results in SJS/TEN group were consistent with those observed in the SCARs group, whereas the significant association was not found in the DRESS group (Table 4).

Apart from the *GSTP1* variant, the risks of SCARs neither DRESS nor SJS/TEN in patients who carried other gene variants were not statistically significant. In addition, predicted metabolizer status from *NAT2* genotypes revealed that neither *NAT2* intermediate metabolizer nor the slow metabolizer group significantly increased the risk of all types of SCARs induced by co-trimoxazole. Similar results were observed with the *GSTT1* and *GSTM1* intermediate and poor metabolizer groups.

Table 4
List of odds ratios and 95%confidence intervals for phenotypes of co-trimoxazole−induced severe cutaneous adverse reactions in individuals who carried different variants of genes involved in the drug metabolism process compared with the tolerant controls.

Genes	Controls (n = 91)	SCAR cases (n = 67)			SJS/TEN cases (n = 51)			DRESS cases (n = 16)		
	Carriers (%)	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value	Carriers (%)	OR [95% CI]	P-value
CYP2C9*3										
CYP2C9*1/*1	82 (90.11)	62 (92.54)	Reference		46 (90.20)	Reference		16 (100.00)	Reference	
CYP2C9*1/*3	9 (9.89)	5 (7.46)	0.73 [0.23−2.30]	0.7786	5 (9.80)	0.99 [0.31−3.13]	1.0000	0 (0)	0.49 [0.06−4.07]	0.6890
CYP2C9*3/*3	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA	0 (0)	NA	NA
GSTP1 (rs1695, c.313 A > G)										
AA	57 (62.64)	31 (46.27)	Reference		22 (43.14)	Reference		9 (56.25)	Reference	
AG	26 (28.57)	30 (44.78)	**2.12 [1.07−4.20]**	**0.0310**	24 (47.06)	**2.39 [1.14−5.02]**	**0.0212**	6 (37.50)	1.46 [0.47−4.54]	0.5561
GG	8 (8.79)	6 (8.96)	1.38 [0.44−4.33]	0.5668	5 (9.80)	1.62 [0.48−5.49]	0.5142	1 (6.25)	0.79 [0.09−7.11]	1.0000
G allele carriers	34 (37.36)	36 (53.73)	**1.95 [1.03−3.70]**	**0.0417**	29 (56.86)	**2.21 [1.10−4.44]**	**0.0260**	7 (43.75)	1.30 [0.44−3.82]	0.7812
GSTT1										
GSTT1-Active	63 (69.23)	42 (62.69)	Reference		31 (60.78)	Reference		11 (68.75)	Reference	
GSTT1-Null	28 (30.77)	25 (37.31)	1.34 [0.69−2.61]	0.3998	20 (39.22)	1.45 [0.71−2.97]	0.3567	5 (31.25)	1.02 [0.32−3.22]	1.0000
GSTM1										
GSTM1-Active	29 (31.87)	23 (34.33)	Reference		20 (39.22)	Reference		3 (18.75)	Reference	
GSTM1-Null	62 (68.13)	44 (65.67)	0.89 [0.46−1.75]	0.8641	31 (60.78)	0.73 [0.35−1.48]	0.4621	13 (81.25)	2.03 [0.54−7.67]	0.3826
GSTT1/GSTM1 Phenotypes										
GSTT1Active/GSTM1-Active	24 (26.37)	14 (20.90)	Reference		12 (23.53)	Reference		2 (12.50)	Reference	
One null allele present	44 (48.35)	37 (55.22)	1.44 [0.65−3.18]	0.4290	27 (52.94)	1.23 [0.53−2.85]	0.6760	10 (62.50)	2.73 [0.55−13.48]	0.3189
GSTT1-Null/GSTM1-Null	24 (25.27)	16 (23.88)	1.19 [0.48−2.98]	0.8162	12 (23.53)	1.04 [0.39−2.79]	1.0000	4 (25.00)	2.09 [0.35−12.51]	0.6687
NAT2 Polymorphisms										
NAT2*5										
NAT2*4/*4	77 (84.62)	57 (85.07)	Reference		43 (84.31)	Reference		14 (87.50)	Reference	
NAT2*4/*5	14 (15.38)	10 (14.93)	0.96 [0.40−2.33]	1.0000	8 (15.69)	0.84 [0.34−2.11]	0.8200	2 (12.50)	0.79 [0.16−3.84]	1.0000
NAT2*5/*5	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA	0 (0)	NA	NA
NAT2*6										
NAT2*4/*4	32 (35.16)	31 (46.27)	Reference		25 (49.02)	Reference		6 (37.50)	Reference	
NAT2*4/*6	52 (57.14)	23 (34.33)	0.46 [0.23−0.92]	0.0355	17 (33.33)	0.42 [0.20−0.89]	0.0361	6 (37.50)	0.62 [0.18−2.07]	0.5319
NAT2*6/*6	7 (7.69)	13 (19.40)	1.92 [0.68−5.44]	0.3046	9 (17.65)	1.65 [0.54−5.03]	0.4097	4 (25.00)	3.05 [0.68−13.75]	0.2011
NAT2*6 carriers	59 (64.84)	36 (53.73)	0.63 [0.33−1.20]	0.1893	26 (50.98)	0.56 [0.28−1.13]	0.1127	10 (62.50)	0.90 [0.30−2.72]	1.0000
NAT2*7										
NAT2*4/*4	60 (65.93)	45(67.16)	Reference		35 (68.63)	Reference		10 (62.50)	Reference	
NAT2*4/*7	23 (25.27)	21(31.34)	1.22 [0.60−2.47]	0.5932	15 (29.41)	1.12 [0.52−2.42]	0.8438	6 (37.50)	1.57 [0.52−4.80]	0.5492
NAT2*7/*7	8 (8.79)	1(1.49)	0.17 [0.02−1.38]	0.0818	1 (1.96)	0.21 [0.03−1.79]	0.1580	0 (0.00)	0.62 [0.07−5.36]	1.0000
NAT2*7 carriers	31 (34.07)	22(32.84)	0.95 [0.48−1.85]	1.0000	16 (31.37)	0.88 [0.42−1.84]	0.8530	6 (37.50)	1.16 [0.39−3.49]	0.7825
NAT2*5, *6, or *7 carriers	81 (89.01)	59 (88.06)	0.91 [0.34−2.45]	1.0000	44 (86.27)	0.78 [0.28−2.18]	0.7883	15 (93.75)	1.85 [0.22−15.55]	1.0000
NAT2 Phenotypes										
Rapid metabolizer	10 (10.99)	8 (11.94)	Reference		7 (13.73)	Reference		1 (6.25)	Reference	
Intermediate metabolizer	44 (48.35)	36 (53.73)	1.02 [0.37−2.86]	1.0000	28 (54.90)	0.91 [0.31−2.67]	1.0000	8 (50.00)	1.82 [0.20−16.23]	1.0000
Slow metabolizer	37 (40.66)	23 (34.33)	0.78 [0.27−2.26]	0.7845	16 (31.37)	0.62 [0.20−1.91]	0.5536	7 (43.75)	1.89 [0.21−17.22]	1.0000
GCLC (rs761142, T > G)										
TT	37 (40.66)	27 (40.30)	Reference		24 (47.06)	Reference		3 (18.75)	Reference	
TG	43 (47.25)	34 (50.75)	1.08 [0.55−2.12]	0.8653	22 (43.14)	0.79 [0.38−1.63]	0.5807	12 (75.00)	3.44 [0.90−13.14]	0.0866
GG	11 (12.09)	6 (8.96)	0.75 [0.25−2.27]	0.7825	5 (9.80)	0.70 [0.22−2.27]	0.7727	1 (6.25)	1.12 [0.11−11.89]	1.0000
G allele carriers	54 (59.34)	40 (59.70)	1.02 [0.53−1.93]	1.0000	27 (52.94)	0.77 [0.39−1.54]	0.4841	13 (81.25)	2.97 [0.79−11.15]	0.1595

NA, not available.
P-values were calculated using Fisher's exact test. P-value < 0.05 was highlighted in bold.
The corrected P-values (Pc) for the multiple comparisons of all alleles were calculated using Bonferroni's correction (2 for *CYP2C9, GSTP1, GSTT1, GSTM1, GCLC* and 4 for *NAT2*). No statistically significant associations between all alleles of drug-metabolizing enzyme genes and co-trimoxazole-induced SCARs were found after Bonferroni's correction.

4. Discussion

In this study, the association between several phenotypes of SCARs induced by co-trimoxazole and genetic polymorphisms of the *HLA* class I and drug metabolism process of co-trimoxazole including *CYP2C9*3*, *GSTP1*, *GSTT1* and *GSTM1* null polymorphisms, *NAT2*5*, *NAT2*6*, *NAT2*7*, and *GCLC* were extensively elucidated in 67 SCARs patients, 91 co-trimoxazole tolerant control patients and 653 general population controls [27,28]. To the best of the authors' knowledge, this study is the largest well-defined case-control study that has reported all genetic variants previously proposed to be associated with SCARs caused by co-trimoxazole. Of these genetic variants determined, the *HLA-B*13:01* allele showed the strongest association with co-trimoxazole-induced DRESS, whereas *HLA-B*15:02* and *HLA-C*08:01* alleles were apparently associated with co-trimoxazole-induced SJS/TEN. Only the *HLA-B*13:01* allele was statistically significantly associated with all SCARs phenotypes induced by co-trimoxazole whereas other risk alleles including *HLA-A*11:01, HLA-B*15:02* and *HLA-C*08:01* alleles did not reach a statistically significant difference after Bonferroni's correction.

Results from this study showed that the *HLA-B*13:01* allele was statistically significantly associated with DRESS induced by co-trimoxazole in which the risk of developing DRESS in patients who carried *HLA-B*13:01* was about 8 to 11-fold higher compared with those who did not carry this allele. This finding was consistent with previous study in a Thai population (12 co-trimoxazole-induced DRESS patients and 91 tolerant controls) [8], that the risk of development of DRESS induced by co-trimoxazole was significantly higher in patients who carried *HLA-B*13:01* compared with those who did not carry this allele, with an OR of 15.20 (95% CI = 3.68−62.83; Pc = 7.2×10^{-5}). Moreover, this finding was

consistent with the report in Taiwanese population in which the study showed the strong association between the *HLA-B**13:01* allele and co-trimoxazole-induced DRESS (OR = 61; 95% CI = 21.5–175; Pc = 1.8×10^{-19}) [9]. Although the OR and strength levels of the associations obtained from this present study (OR: 8–11, Table 2) was lower than those reported in previous results in Thai [8] and Taiwanese [9] patients (OR = 15–61), the results obtained from the present study clearly showed and confirmed that the *HLA-B**13:01* allele is the most valid pharmacogenetic marker for the prediction of DRESS induced by co-trimoxazole.

According to the co-trimoxazole-induced SJS/TEN, the *HLA-B**15:02* or *HLA-C**08:01* alleles have been previously reported to be associated with these SCAR phenotype in Thai population [7,8], however, this study clearly showed that these associations did not reach statistical significance after Bonferroni's correction (Table 2). On comparison between general patients and HIV/AIDs patients, the associations of *HLA-B**15:02* or *HLA-C**08:01* and the risk of SJS/TEN induced by co-trimoxazole in HIV/AIDs patients was apparently higher than that observed in overall general SJS/TEN cases but only the *HLA-C**08:01* allele was statistically significantly associated with co-trimoxazole-induced SJS/TEN in HIV/AIDs patients after Bonferroni's correction (OR = 8.51, 95% CI = 2.18–33.14, P = 8.60×10^{-4}, Pc = 0.0241) (Tables 2 and 3). Currently, co-trimoxazole is mainly used as a first-line drug for treatment and prophylaxis against *Pneumocystis jiroveci* pneumonia in HIV/AIDs patients and several lines of evidence reported that host genetic factors particularly in *HLA* genetic polymorphisms have also been associated with HIV-1 pathogenesis and some certain alleles in *HLA* class I play a role in susceptibility to or protection against HIV infection [26,29,30]. The prevalence of *HLA* alleles in HIV/AIDs patients should be considered because those certain alleles might be prevalent or might be linked to both HIV pathogenesis and co-trimoxazole-induced SJS/TEN. The prevalence of these two identified *HLA* risk alleles observed in general Thai population [27,28] used as controls in the present study were compared to those reported in a larger cohort of HIV-infected Thai patients [26] (12.75% vs 14.59% for *HLA-B**15:02* carriers; 18.31% vs 18.68% for *HLA-C**08:01* carriers) (Tables 2 and 3).

Moreover, the risk of co-trimoxazole-induced SJS/TEN in HIV-infected patients who carried the risk allele was determined using the HIV-infected Thai cohort [26] as the control group and results remained the same for the *HLA-B**15:02* in which the risk of SJS/TEN induced by co-trimoxazole was higher in the HIV/AIDS patients who carried the *HLA-B**15:02* allele with an OR of 3.01 (95% CI = 1.42–6.39, P = 0.0098), however, its association did not reach a statistically significant difference after Bonferroni's correction. Results obtained from both HIV/AIDS patients and general patients revealed that *HLA-B**15:02* allele showed only a weak association with co-trimoxazole-induced SJS/TEN in Thai patients regardless of the host disease factor. Moreover, this weak association between *HLA-B**15:02* and the risk of SJS/TEN in this present study was consistent with those recently reported in a Taiwanese population (OR = 2.7 to 3.2) [9]. Regarding the weak association of *HLA-B**15:02* and co-trimoxazole-induced SJS/TEN, this association deserves further exploration in larger sample size and other ethnicity.

According to SCARs phenotypes, it is well-recognized in the case of carbamazepine that the *HLA-B**15:02* is strongly associated with the SJS/TEN phenotype but not DRESS phenotype [21,31,32]. Sukasem et al. have proposed that the *HLA-B**13:01* allele is a selective pharmacogenetic marker for prediction of DRESS induced by co-trimoxazole while the *HLA-B**15:02* and *HLA-C**08:01* alleles are selective pharmacogenetic markers of co-trimoxazole-induced SJS/TEN [8]. Whether *HLA-B**13:01, HLA-B**15:02* or *HLA-C**08:01* exhibit specificity toward SCARs phenotypes caused by co-trimoxazole as previously proposed by Sukasem et al. needs to be explored further in a larger cohort and other ethnic groups.

It should be noted that there is no significant correlation between *HLA-A**11:01* and co-trimoxazole-induced SCARs in our study population after conditional analysis with *HLA-B**13:01.* This observation was inconsistent with the report of 15 Japanese patients with SCARs induced by sulfonamides (6 patients with co-trimoxazole-induced SCARs and 9 patients with salazosulfapyridine-induced SCARs) in which the *HLA-A**11:01* has been shown to be significantly associated with sulfonamide-induced SCARs with an OR of 9.84 (95%CI = 3.35–28.9, Pc = 2.14×10^{-4}) [33]. The frequency of the *HLA-A**11:01* allele in a Japanese population [33] (8.2%–11.1%) is lower than those observed (22.16%) in our population. Therefore, the association between *HLA-A**11:01* and co-trimoxazole-induced SCARs deserves to be explored further in subsequent prospective studies.

Apart from *HLA* genetic polymorphisms, inter-individual variations in the bioactivation as well as detoxification of SMX may be linked to a difference in susceptibility to SMX hypersensitivity [14–17]. Since SMX is bioactivated by CYP2C9, producing the hydroxylamine and its subsequent auto-oxidation to the nitroso metabolite which is the toxic metabolite that can trigger the T-cell mediated immune response [34]. The nitroso metabolite subsequently undergoes detoxification of by GSH [11]. The inactivation process of SMX also occurs through N-acetylation by two polymorphic enzymes, NAT1 and NAT2 [13]. It has been reported that patients with *NAT2* slow acetylator genotypes were at a high risk with co-trimoxazole or SMX hypersensitivity [10,14]. Similarly, *CYP2C9**3,* loss of function alleles, decreases bioactivation of SMX, potentially protecting against sulfamethoxazole hypersensitivity [35]. Moreover, the GCLC enzyme is a catalytic enzyme that is a critical step in GSH biosynthesis and the genetic polymorphism of the *GCLC* gene (rs761142) was recently found to be associated with reduced GCLC mRNA expression and with SMX-induced hypersensitivity in HIV/AIDs patients [11]. The results from the previous genome-wide association study (GWAS) [36], however, showed no association between these genetic variants of drug-metabolizing enzymes and co-trimoxazole-induced hypersensitivity. Similar results were observed in this present study in that the *NAT2* slow acetylator genotypes, *CYP2C9**3* and *GCLC* variant were not significantly associated with co-trimoxazole-induced SCARs. Moreover, the variants of *GSTT1* and *GSTM1* null polymorphisms that showed the absence or decrease of enzyme activity were not associated with co-trimoxazole-induced SCARs in this study population, which was consistent with the previous reports [20,36]. Interestingly, only the *GSTP1* (rs1695, c.313 A > G) variant was observed to be risk allele for co-trimoxazole-induced SCARs in which about a 2-fold increased risk of SCARs was noted in patients who carried the G allele (Table 4). Moreover, this risk was increased in SJS/TEN patients who carried the G allele of *GSTP1* variant, with an OR of 2.21 (95% CI, 1.10–4.44; P = 0.0260) (Table 4).

This variant is an exonic variant and the decreased enzyme activity in the G alleles was reported in the previous *in vitro* study [37,38]. The previous reports in patients with co-trimoxazole hypersensitivity showed no association between this *GSTP1* variant and several phenotypes of co-trimoxazole hypersensitivity [20,36]. The recent whole genome sequencing (WGS) study showed that only intronic variants of the *GSTP1* gene including rs8191438 and rs8191439 were associated with co-trimoxazole-induced SCARs [9]. The present results which were performed in a well-defined SCARs population suggest that the detoxification enzyme, *GSTP1,* may have some contribution to the development of SCARs induced by co-trimoxazole.

In addition, it was found that the risk of co-trimoxazole-induced SCARs was higher in patients who carried both *HLA-B*13:01* and *GSTP1* risk allele compared with those who did not carry this *HLA-B*13:01-GSTP1* risk allele (Carrier frequency of *HLA-B*13:01-GSTP1* risk allele: 13/67 (19.40%) SCARs cases vs 7/91 (7.69%) tolerant controls; OR = 2.89, 95% CI, 1.08−7.70; P = 0.0339; data not shown). Interestingly, the carrier frequency of the *HLA-B*15:02-GSTP1* risk allele was higher in SJS/TEN group (7/51, 13.73%) compared to the tolerant controls (3/91, 3.30%) and the risk of co-trimoxazole-induced SJS/TEN was increased in patients who carried both *HLA-B*15:02* and *GSTP1* risk allele (OR = 4.67, 95% CI, 1.15−18.93; P = 0.0310; data not shown). Similarly, the carrier frequency of the *HLA-C*08:01-GSTP1* risk allele was also higher in the SJS/TEN group (7/51, 13.73%) compared to the tolerant controls (2/91, 2.20%) and this *HLA-C*08:01-GSTP1* risk allele was associated with SJS/TEN, with an OR of 7.08 (95% CI, 1.41−35.51; P = 0.0174) (data not shown). These findings suggested that *GSTP1* genetic polymorphism may play some role in the development of co-trimoxazole-induced SCARs, especially in the SJS/TEN group in patients who carried the *HLA* risk alleles.

In this study, the *HLA-B*13:01* allele has been identified as the strongest genetic marker of co-trimoxazole-induced SCARs, particularly DRESS. The pharmacological interaction of drugs with immune receptors (p-i concept) has been proposed to describe how sulfamethoxazole induces T cell−mediated immune responses [39,40]. Moreover, the potential interaction between the *HLA-B*13:01* allele and sulfamethoxazole and its reactive metabolite has recently been investigated using *in silico* modeling and the results revealed that residues R121 and Y123 of *HLA-B*13:01* were found to be crucial for forming hydrogen bonds to sulfamethoxazole and residues T48, S91, and N94 of *HLA-B*13:01* were hydrogen-bonded to 4-nitro sulfamethoxazole [9]. Similarly, the p-i concept has also been proposed as a mechanism for carbamazepine-induced hypersensitivity [39,40]. The previous study of *HLA-B*15:02*-bound peptides involved in carbamazepine-induced SJS/TEN showed that the preferable use of serine residues at the nonanchoring positions P5, P6, P7, and P8 appeared to be a unique feature of the *HLA-B*15:02*-bound peptides and the interaction between peptides and carbamazepine or its metabolites might have been due to non-covalent binding [41]. Unlike the binding between *HLA-B*15:02* and carbamazepine, information about the interaction between *HLA-B*15:02* and sulfamethoxazole, trimethoprim or their reactive metabolites is very limited.

In conclusion, this study clearly demonstrated that the *HLA-B*13:01* allele was significantly associated with co-trimoxazole-induced SCARs, especially with DRESS and the *HLA-C*08:01* allele was significantly associated with SJS/TEN induced by co-trimoxazole in the HIV/AIDS patients. Whereas major genetic polymorphisms of enzymes involved in the bioactivation and detoxification of co-trimoxazole may not play an important role in this SCARs. However, it should be noted that the associations between co-trimoxazole-induced SCARs and identified *HLA* alleles were weaker than the previously observed associations of *HLA* risk alleles and SCARs induced by carbamazepine or allopurinol, suggesting that other molecules, such as the T-cell receptor (TCR) repertoire may be involved in the molecular pathogenesis of these SCARs and these issues are worth to explore further.

Author contributions

N.N., N.S. and W.T. designed the study. N.N., N.S., P.K, W.A., U.K., D.P., O.P. and P.C. enrolled the patients and collected the samples and clinical data. N.N., N.S., T.N., P.W. and K.K. performed the experiments. N.N., N.S. and W.T. analyzed and interpreted the data. All authors contributed to the manuscript drafting.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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