Genetic Association of Co-Trimoxazole-Induced Severe Cutaneous Adverse Reactions Is Phenotype-Specific: HLA Class I Genotypes and Haplotypes

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Co-trimoxazole (CTX) causes various forms of severe cutaneous adverse reactions (SCARs). This case-control study was conducted to investigate the involvement between genetic variants of human leukocyte antigen (*HLA*) and *CYP2C9* in CTX-induced SCARs, including Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and drug reaction with eosinophilia and systemic symptoms (DRESS) in Thai patients. Thirty cases of CTX-induced SCARs were enrolled and compared with 91 CTX-tolerant controls and 150 people from the general Thai population. Cases comprised 18 SJS/TEN and 12 DRESS patients. This study demonstrated that genetic association of CTX-induced SCARs was phenotype-specific. *HLA-B*15:02* and *HLA-C*08:01* alleles were significantly associated with CTX-induced SJS/TEN, whereas the *HLA-B*13:01* allele was significantly associated with CTX-induced DRESS. In addition, a significant higher frequency of *HLA-A*11:01-B*15:02* and *HLA-B*13:01-C*03:04* haplotypes were detected in the group of CTX-induced Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and DRESS cases, respectively. Genetic association of CTX-induced SCARs is phenotype-specific. Interestingly, these association was observed only in HIV-infected patients but not in non-HIV-infected patients.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Until now, only one study reported the association of human leukocyte antigen (HLA) alleles and co-trimoxazole (CTX)-induced Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) in the Thai population. The relationship between HLA alleles and CTX-induced severe cutaneous adverse reactions (SCARs) remains unclear. There is no published data on the genetic association with other SCAR phenotypes, such as drug reaction with eosinophilia and systemic symptoms (DRESS) in the Thai population.

WHAT QUESTIONS DID THIS STUDY ADDRESS?

☑ Which HLA genes are associate with CTX-induced SJS/TEN and DRESS? Do they have different biomarkers in different CTX-induced manifestation?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ HLA-B*15:02 and HLA-C*08:01 alleles were significantly associated with CTX-induced SJS/TEN. The HLA-B*13:01 allele was significantly associated with CTX-induced DRESS. In addition, a significant higher frequency of HLA-A*11:01-B*15:02 and HLA-B*13:01-C*03:04 haplotypes were detected in the group of CTX-induced SJS/TEN and DRESS cases, respectively.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Genetic association of CTX-induced SCARs is phenotype-specific. Screening of the risk alleles is recommended for Thai patients before initiating CTX therapy.

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Co-trimoxazole (CTX), a combination of sulfamethoxazole and trimethoprim, sequentially blocks bacterial folate synthesis.[1] CTX is useful in the treatment of a variety of bacterial, fungal, and protozoal infections.[2,3] The World Health Organization (WHO) have recommended the use of CTX prophylaxis in infants, children, and adolescents with HIV to prevent opportunistic infections.[4] However, CTX is associated with severe cutaneous adverse reactions (SCARs), such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reaction with eosinophilia and systemic symptoms (DRESS), and fixed drug eruption.[5–8] Sulfamethoxazole is metabolized to sulfamethoxazole hydroxylamine, which is further oxidized to nitrosulfamethoxazole. Nitrosulfamethoxazole binds covalently to host proteins, eliciting an IgE and/or a T cell mediated response to modified proteins manifesting in different forms of drug hypersensitivity.[9]

Human leukocyte antigen (HLA) is the human major histocompatibility complex responsible for presentation of processed peptide antigens to T cells to initiate the adaptive immune response.[10] There are three regions of the human MHC: class I region consists of *HLA-A*, *HLA-B*, and *HLA-C* genes, class II region consists of *HLA-DR*, *HLA-DQ*, and *HLA-DP* genes, and class III region, which does not encode HLA molecules, consists of genes for complement components (C2, C4, and factor B), 21-hydroxylase, tumor necrosis factors, and others.[11] Several *HLA* alleles have been reported to be associated with CTX-induced SCARs. A recent case-control study in the Thai population reported an association between CTX-induced SJS/TEN and *HLA-B*15:02* (odds ratio (OR) = 3.91; 95% confidence interval (CI): 1.42–10.92; $P = 0.0037$), *HLA-C*06:02* (OR = 11.84; 95% CI: 1.24–566.04; $P = 0.0131$) and *HLA-C*08:01* (OR = 3.53; 95% CI: 1.21–10.40; $P = 0.0108$).[12] In European patients, the presence of *HLA-B*38* was significantly associated with sulfamethoxazole-related SJS/TEN, but the different allelic variants of *HLA-B*38* did not show significant association with sulfamethoxazole-related SJS/TEN.[13] Additionally, a strong association between SJS/TEN and trimethoprim alone was observed. (OR = 9.44; 95% CI = 3.83–23.25).[14] This finding suggests that not only the most common culprit sulfamethoxazole but also trimethoprim induce SJS/TEN in patients. A retrospective genomewide association study in European subjects did not find any single-nucleotide polymorphisms with genomewide significance when CTX-induced SJS/TEN cases were compared with controls.[15] Highly polymorphic gene, CYP2C9 is a key factor in enzyme activity variation in various drug once this variant occurred. Recently, the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommended guidelines for *CYP2C9* and HLA-B genotype and phenytoin dosing.[16] There was the report of *CYP2C9*3* association to phenytoin-induced SCARs in Thai children with epilepsy with an OR = 14.52 (95% CI = 1.18–∞; P value = 0.044).[17] However, no study on the association between *CYP2C9* and CTX was performed.

Until now, although one study has reported the association of *HLA* alleles and CTX-induced SJS/TEN in the Thai population, there are no data on the association with other SCAR phenotypes, such as DRESS. In the present study, our aim was to identify and compare the HLA alleles associated with CTX-induced SJS/TEN and DRESS in Thai patients.

METHODS

Patient recruitment

For our study, we included a total of 30 patients with CTX-induced SCARs (18 men and 12 women). These patients were admitted to the hospital for treatment of CTX-induced SCARs between 2014 and 2017 and were prospectively enrolled in our study. The patients recruited in the present study are different from those previously reported by Kongpan *et al*.[13]

The RegiSCAR criteria were used to diagnose and classify SCARs,[18,19] and a dermatologist and allergist confirmed the diagnoses based on the photographs, pathological slides, clinical morphology, and medical records. SJS was defined as a mucocutaneous disorder characterized by skin rash and skin detachment affecting < 10% of the body surface area. TEN was defined as skin detachment > 30%. SJS-TEN overlap was defined as skin detachment of 10–30%. DRESS was characterized by acute skin rash, fever above 38°C, enlarged lymph nodes, internal organ involvement, and hematological abnormalities, including lymphocytosis or lymphocytopenia, eosinophilia, and thrombocytopenia. We evaluated that CTX was the causative drug of SJS/TEN or DRESS using the Naranjo algorithm,[20] the score of the algorithm of drug causality for epidermal necrolysis,[21] and DRESS score. The cases defined as possible, probable, and definite were recruited in this study. In some cases, *in vitro* assay (such as ELISpot) was performed. Two groups of control were used in the present study: 91 CTX-tolerant patients (which are the same group as previously reported by Kongpan *et al*.[13]) and 150 healthy Thai subjects, which were obtained from five regional groups of unrelated healthy Thai individuals. The individuals lived in the abovementioned regions for more than three generations.[22]

This study was approved by the Ramathibodi Hospital Ethical Review Board, and the Khon Kaen Ethics Committee for Human Research, Khon

Kaen University, Thailand. Written informed consent was obtained from all the participants.

HLA-A, HLA-B and HLA-C alleles genotyping

For all the study subjects, DNA extraction using MagNAPure Compact Nucleic Acid Isolation kits was performed based on magnetic-bead technology (Roche Applied Science, Mannheim, Germany). DNA was aliquoted and stored at −20°C before HLA typing. Genotyping of *HLA-A*, *HLA-B*, and *HLA-C* alleles were determined by the polymerase chain reaction with sequence-specific oligonucleotide probes hybridization method using the Luminex IS 100 system (Luminex, Austin, TX) with well-established protocols.

CYP2C9 genotyping

The *CYP2C9* variant alleles are routinely tested and include *CYP2C9*2* and **3*, *CYP2C9*2* (*430C>T*, *rs1799853*), and *CYP2C9*3* (*1075A>C*, *rs1057910*) were genotyped by pre-designed TaqMan single-nucleotide polymorphism genotyping assays using sequence primers and TaqMan MGB probes of *CYP2C9*2* (assay ID: C_25625805_10) and *CYP2C9*3* (assay ID: C_27104892_10; Applied Biosystems, Foster City, CA).

Statistical analysis

Statistical analyses were performed using SPSS statistical software package, version 18 (SPSS, Chicago, IL). To detect differences in the demographic of study population, an independent *t*-test was used for continuous variables. The ORs with corresponding 95% CIs were calculated to determine the association between the presence of the HLA loci and CTX-induced SCARs. The Fisher's exact test was used to compare allele frequencies between cases and controls. Bonferroni correction was applied to adjust for multiple comparisons. P values < 0.05 (two-sided) indicated a statistical significance.

In this study, we pooled data from a previous study by Kongpan *et al.*[13] and the present study and compared the frequencies of *HLA* alleles in CTX-SJS/TEN cases and tolerant controls.

Haplotype association analysis was carried out using the "haplo.stats" package.

RESULTS

The demographic data of all the case are shown in **Table 1**. Our cohort comprised 13 cases (43.33%) of CTX-induced SJS, 4 cases (13.33%) of CTX-induced TEN, 1 case (3.33%) of CTX-induced SJS/TEN overlap, and 12 cases (40.00%) of CTX-induced DRESS (a total of 30). The mean age of CTX hypersensitive patients was 40.90 ± 13.94 and 60.00% were men. Most cases received CTX for prophylactic use among HIV-infected persons. There were no significant differences in sex, age, indication of drug administration, onset of reaction, and comorbidity.

Mucosal involvement, including eyes, oral and genital mucosa, abnormal liver, and renal function test, were observed in patients with SCARs (**Tables S1** and **S2**). Nine cases received monotherapy with CTX, whereas the other 21 cases received concomitant medications. For nine individuals, the CTX doses administered were not available in their medical records. Among 30 CTX-related cases of SCARs, we found no *CYP2C9*2* and **3* alleles, which possibly interpreted as *CYP2C9*1/*1* (extensive metabolizer) base on the frequency of this genotype in the study population. The genotypes of *HLA-A*, *HLA-B*, *HLA-C*, and *CYP2C9* in the 30 cases with CTX-induced SCARs are shown in **Table 2**.

Table 1 Study population's demographics

	CTX hypersensitive patients (n = 30)	CTX tolerant patients (n = 91)
Type of SCARs, *n* (%)		
SJS	13 (43.33)	0
TEN	4 (13.33)	0
SJS/TEN overlap	1 (3.33)	0
DRESS	12 (40.00)	0
Sex, *n* (%)		
Male	18 (60.00)	54 (59.34)
Female	12 (40)	37 (40.66)
Age		
Mean ± SD	40.90 ± 13.94	45.31 ± 12.28
Median (range)	41 (19–69)	45 (13–74)
Indication of drug administration *n* (%)		
HIV	23 (76.67)	61 (67.03)
Other diseases	7 (23.33)	30 (32.97)
Onset of reaction		
Mean ± SD	23.88 ± 15.82	0
Median (range)	17 (3–65)	0
Comorbidity *n* (%)		
HIV	23 (76.67)	61 (67.03)
TB	3 (10.00)	2 (2.20)
Other diseases	4 (13.33)	19 (20.88)
No comorbidity	3 (10.00)	17 (18.68)

CTX, co-trimoxazole; DRESS, drug reaction with eosinophilia and systemic symptoms; SCARs, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TB, tuberculosis; TEN, toxic epidermal necrolysis.

Association of all CTX-induced SCARs with *HLA* alleles

We compared the carrier frequencies of the *HLA* class I alleles in the 30 CTX-induced SCARs cases with the 91 CTX-tolerant controls and 150 healthy Thai population controls. The results are summarized in **Table 3**.

*HLA-B*13:01* was observed in 43.33% of cases (13/30), 16.48% of tolerant controls (15/91), and 15.33% of healthy controls (23/150). *HLA-B*15:02* was observed in 30.00% of cases (9/30), 10.99% of tolerant controls (10/91), and 18.00% of healthy controls (27/150). *HLA-B*13:01* and *HLA-B*15:02* were significantly over-represented in patients with CTX-induced SCARs as compared with CTX-tolerant controls with ORs of 3.88 (95% CI = 1.56–9.63; P = 0.0025) and 3.47 (95% CI = 1.25–9.63; P = 0.0201), respectively. Of the other HLA alleles, *HLA-A*11:01* allele showed the most significant association with CTX-induced SCARs cases with an OR of 3.25 (95% CI = 1.34–7.89; P = 0.0073). The frequency of *HLA-A*02:07* allele was significantly decreased among the CTX-induced SCARs cases and was thus designated protective allele. *HLA-C*07:27* and *HLA-C*08:01* were also significantly associated with CTX-induced SCARs compared with CTX-tolerant controls with ORs of 31.08 (95% CI = 1.62–595.88; P = 0.0032) and 3.91 (95% CI = 1.38–11.06; P = 0.0149), respectively.

Table 2 *HLA* class I genotyping, *CYP2C9* and clinical data of Thai patients with CTX-induced SCARs

No.	Sex/age[a]	Type of SCAR	Indication for CTX	*HLA* class I genotyping			*CYP2C9*
				HLA-A	*HLA-B*	*HLA-C*	
1	M/57	DRESS	HIV	*02:07/**11:01***	***13:01**/46:01*	*01:02/**03:04***	**1/*1*
2	F/21	DRESS	HIV	*24:02/24:02*	***13:01**/40:01*	***03:04** /**07:27***	**1/*1*
3	M/21	SJS	HIV	***11:01**/24:07*	***15:02**/**38:02***	***07:27** /08:01*	**1/*1*
4	M/29	SJS	HIV	***11:01**/32:01*	***15:02**/44:02*	*05:01/08:01*	**1/*1*
5	M/39	SJS	HIV	*02:03/74:01*	***13:01**/51:01*	*04:06/14:02*	**1/*1*
6	M/63	DRESS	HIV	***11:01**/11:01*	*07:05/**38:02***	*07:02/07:02*	**1/*1*
7	F/19	DRESS	N/A	***11:01**/33:03*	***13:01**/58:01*	*03:02/**03:04***	**1/*1*
8	F/30	SJS	HIV, disseminated TB	***11:01**/33:01*	*44:03/58:01*	*03:02/07:01*	**1/*1*
9	M/52	SJS	HIV, HCV infection	***11:01**/31:01*	*35:03/**38:02***	*04:01/18:01*	**1/*1*
10	M/33	SJS	HIV, pulmonary TB	*02:02/24:02*	*46:01/48:01*	*01:02/08:03*	**1/*1*
11	M/48	TEN	HIV	*02:01/02:03*	***13:01**/**38:02***	***03:04** /**07:27***	**1/*1*
12	F/32	DRESS	HIV	***11:01**/24:02*	*15:01/40:01*	*04:01/04:03*	**1/*1*
13	F/23	SJS	HIV, pulmonary TB	***11:01/11:01***	*15:25/**38:02***	*04:03/07:02*	**1/*1*
14	M/20	DRESS	HIV	***11:01**/24:10*	***13:01**/18:02*	***03:04** /07:04*	**1/*1*
15	M/36	SJS	HIV	***11:01/11:01***	*07:05/**15:02***	*07:02/08:01*	**1/*1*
16	F/69	DRESS	HIV	*02:07/11:02*	***13:01**/46:01*	*01:02/**03:04***	**1/*1*
17	F/31	DRESS	PCP in HIV	***11:01**/24:02*	***13:01**/**15:02***	***03:04** /08:01*	**1/*1*
18	M/47	SJS	PCP in HIV	*02:03/33:03*	*18:01/44:03*	*07:01/07:04*	**1/*1*
19	M/48	DRESS	PCP in HIV	***11:01/11:01***	***13:01**/46:01*	*01:02/04:03*	**1/*1*
20	F/59	DRESS	Melioidosis	***11:01**/68:01*	*08:01/**13:01***	*04:03/07:02*	**1/*1*
21	M/46	DRESS	Melioidosis	*02:07/**11:01***	***13:01**/46:01*	*01:02/**03:04***	**1/*1*
22	M/57	DRESS	PCP in HIV	***11:01**/24:07*	*07:05/**15:02***	***07:27** /08:01*	**1/*1*
23	F/	SJS	N/A	***11:01/11:01***	***15:02**/15:13*	*08:01/08:01*	**1/*1*
24	F/30	SJS/TEN overlap	N/A	*02:03/**11:01***	***15:02**/18:01*	*07:04/08:01*	**1/*1*
25	F/46	TEN	Melioidosis	***11:01**/26:01*	*08:01/51:01*	*07:02/14:02*	**1/*1*
26	M/50	SJS	PCP in HIV	*24:02/74:01*	*07:05/38:02*	*07:02/07:02*	**1/*1*
27	M/33	SJS	PCP in HIV	*24:02/29:01*	*07:05/**15:02***	*08:01/15:05*	**1/*1*
28	F/57	TEN	Melioidosis	***11:01/11:01***	***13:01**/40:02*	*04:06/15:02*	**1/*1*
29	M/37	TEN	PCP in HIV	*02:03/31:01*	***13:01**/38:02*	*04:06/07:02*	**1/*1*
30	M/43	SJS	PCP in HIV	***11:01**/26:01*	***15:02**/27:07*	*08:01/15:02*	**1/*1*

CTX, co-trimoxazole; *CYP2C9*, cytochrome P450 2C9; DRESS, drug reaction with eosinophilia and systemic symptoms; HCV, hepatitis C virus; *HLA*, human leucocyte antigenN/A, not available; PCP, Pneumocystis carinii pneumonia; SCARs, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TB, tuberculosis; TEN, toxic epidermal necrolysis.
[a]Age at the development of CTX -induced SCARs.

Haplotype analysis revealed that only the frequencies of the *HLA-A*11:01-B*15:02* and *HLA-B*13:01-C*03:04* haplotypes in the CTX-induced SCAR group were significantly different from those in the control groups. The OR for CTX-induced SCARs among patients with the *HLA-A*11:01-B*15:02* haplotype was 4.36 (95% CI = 1.43–13.34; P =0.0108), whereas the OR among those with the *HLA-B*13:01-C*03:04* haplotype was 3.77 (95% CI = 1.27–11.19; P = 0.0251). However, the significant associations of the HLA alleles and haplotypes disappeared after corrections for multiple testing ($P < 0.0025$).

Association of CTX-induced DRESS and *HLA* alleles

We compared the carrier frequencies of *HLA* class I alleles in the 12 CTX-induced patients with DRESS, in 91 CTX-tolerant controls, and in 150 healthy Thai population controls. The results are summarized in **Table 4** (only the alleles and haplotypes of *HLA* that showed a significant association when compared with the CTX-tolerant controls and/or healthy population controls) are shown.

As shown in **Table 4**, four alleles were significant in the CTX-induced DRESS group: *HLA-A*11:01*, *HLA-B*13:01*,

Table 3 The association of *HLA* class I alleles with CTX-induced SCARs (*n* = 30)

HLA class I alleles	CTX-induced SCARs (*n* = 30)	Tolerant controls (*n* = 91)	Thai population (*n* = 150)	CTX-induced SCARs cases vs. tolerant controls		CTX-induced SCARs cases vs. Thai population	
				OR (95% CI)	*P* value < 0.05	OR (95% CI)	*P* value < 0.05
HLA-A							
02:03	5 (16.67%)	19 (20.88%)	32 (21.33%)	0.76 (0.26–2.24)	0.6158	0.74 (0.26–2.08)	0.5637
02:07	**3 (10.00%)**	**28 (30.77%)**	26 (17.33%)	**0.25 (0.07–0.89)**	**0.0238**	0.53 (0.15–1.88)	0.4209
11:01	**21 (70.00%)**	**38 (41.76%)**	**68 (45.33%)**	**3.25 (1.34–7.89)**	**0.0073**	**2.81 (1.21–6.55)**	**0.0136**
24:02	6 (20.00%)	17 (18.68%)	32 (21.33%)	1.09 (0.39–3.07)	0.8732	0.92 (0.35–2.45)	0.8702
24:07	2 (6.67%)	5 (5.49%)	14 (9.33%)	1.23 (0.23–6.69)	1.0000	0.69 (0.15–3.23)	1.0000
33:03	2 (6.67%)	18 (19.78%)	32 (21.33%)	0.29 (0.06–1.33)	0.1539	0.26 (0.06–1.17)	0.0609
HLA-B							
07:05	**5 (16.67%)**	6 (6.59%)	**8 (5.33%)**	2.83 (0.79–10.07)	0.1379	**3.55 (1.07–11.73)**	**0.0446**
13:01	**13 (43.33%)**	**15 (16.48%)**	**23 (15.33%)**	**3.88 (1.56–9.63)**	**0.0025**	**4.22 (1.81–9.86)**	**4.7×10^{-4}[a]**
15:02	**9 (30.00%)**	**10 (10.99%)**	27 (18.00%)	**3.47 (1.25–9.63)**	**0.0201**	1.95 (0.81–4.73)	0.1336
38:02	**7 (23.33%)**	10 (10.99%)	**12 (8.00%)**	2.47 (0.85–7.19)	0.1273	**3.50 (1.25–9.82)**	**0.0210**
40:01	2 (6.67%)	13 (14.29%)	18 (12.00%)	0.43 (0.09–2.02)	0.3529	0.52 (0.12–2.39)	0.5352
46:01	5 (16.67%)	23 (25.27%)	40 (26.67%)	0.59 (0.20–1.72)	0.3323	0.55 (0.19–1.54)	0.2482
58:01	2 (6.67%)	11 (12.09%)	12 (8.00%)	0.52 (0.11–2.49)	0.5152	0.82 (0.17–3.87)	1.0000
HLA-C							
01:02	5 (16.67%)	23 (25.27%)	48 (32.00%)	0.59 (0.20–1.72)	0.3323	0.43 (0.15–1.18)	0.0926
03:02	2 (6.67%)	13 (14.29%)	23 (15.33%)	0.43 (0.09–2.02)	0.3529	0.39 (0.09–1.77)	0.2612
03:04	8 (26.67%)	21 (23.08%)	21 (14.00%)	1.21 (0.47–3.12)	0.6896	2.23 (0.88–5.67)	0.1027
04:01	2 (6.67%)	5 (5.49%)	20 (13.33%)	1.23 (0.23–6.69)	1.0000	0.46 (0.10–2.10)	0.5398
04:03	4 (13.33%)	13 (14.29%)	11 (7.33%)	0.92 (0.28–3.08)	1.0000	1.94 (0.58–6.58)	0.2821
07:02	7 (23.33%)	26 (28.57%)	26 (17.33%)	0.76 (0.29–1.99)	0.5764	1.45 (0.56–3.74)	0.4382
07:27	**4 (13.33%)**	**0 (0%)**	**2 (1.33%)**	**31.08 (1.62–595.88)**	**0.0032**	**11.39 (1.98–65.37)**	**0.0076**
08:01	**9 (30.00%)**	**9 (9.89%)**	33 (22.00%)	**3.91 (1.38–11.06)**	**0.0149**	1.52 (0.64–3.63)	0.3443
Haplotype							
*HLA-A*11:01/-B*15:02*	**8 (26.67%)**	**7 (7.69%)**	**16 (10.67%)**	**4.36 (1.43–13.34)**	**0.0108**	**3.05 (1.17–7.96)**	**0.0343**
*HLA-B*13:01/-C*03:04*	**8 (26.67%)**	**8 (8.79%)**	**10 (6.67%)**	**3.77 (1.27–11.19)**	**0.0251**	**5.09 (1.81–14.29)**	**0.0032**

P values were calculated by Fisher's exact test. Statistically significant values are highlighted in bold.
Significant difference *P* value < 0.05.
CI, confidence interval; CTX, co-trimoxazole; *HLA*, human leucocyte antigen; OR, odds ratio; SCARs, severe cutaneous adverse reactions.
[a]Corrected *P* value was obtained after Bonferroni correction ($P_c < 0.0025$).

Table 4 The association of *HLA* class I alleles with CTX-induced DRESS (*n* = 12)

HLA class I alleles	CTX-induced DRESS (*n* = 12)	Tolerant controls (*n* = 91)	Thai population (*n* = 150)	CTX-induced DRESS cases vs. tolerant controls		CTX-induced DRESS cases vs. Thai population	
				OR (95% CI)	*P* value < 0.05	OR (95% CI)	*P* value < 0.05
HLA-A							
02:03	0	19 (20.88%)	32 (21.33%)	0.15 (0.01–2.62)	0.1167	0.15 (0.01–2.53)	0.1259
02:07	3 (25.00%)	28 (30.77%)	26 (17.33%)	0.75 (0.19–2.98)	1.0000	1.59 (0.40–6.28)	0.4516
11:01	**10 (83.33%)**	**38 (41.76%)**	**68 (45.33%)**	**6.97 (1.45–33.67)**	**0.0067**	**6.03 (1.28–28.46)**	**0.0112**
24:02	3 (25.00%)	17 (18.68%)	32 (21.33%)	1.45 (0.36–5.94)	0.6978	1.23 (0.31–4.81)	0.7231
24:07	1 (8.33%)	5 (5.49%)	14 (9.33%)	1.56 (0.17–14.64)	0.5338	0.88 (0.11–7.36)	1.0000
33:03	1 (8.33%)	18 (19.78%)	32 (21.33%)	0.37 (0.05–3.04)	0.4579	0.34 (0.04–2.69)	0.4622
HLA-B							
07:05	2 (16.67%)	6 (6.59%)	8 (5.33%)	2.83 (0.50–15.97)	0.2341	3.55 (0.66–18.99)	0.1622
13:01	**9 (75.00%)**	**15 (16.48%)**	**23 (15.33%)**	**15.20 (3.68–62.83)**	**7.2×10^{-5}[a]**	**16.57 (4.17–65.85)**	**2.4×10^{-5}[a]**
15:02	2 (16.67%)	10 (10.99%)	27 (18.00%)	1.62 (0.31–8.47)	0.6286	0.91 (0.19–4.39)	1.0000
38:02	1 (8.33%)	10 (10.99%)	12 (8.00%)	0.74 (0.09–6.32)	1.0000	1.05 (0.12–8.80)	1.0000
40:01	2 (16.67%)	13 (14.29%)	18 (12.00%)	1.20 (0.24–6.11)	0.6860	1.47 (0.29–7.24)	0.6452
46:01	4 (33.33%)	23 (25.27%)	40 (26.67%)	1.48 (0.41–5.37)	0.5084	1.38 (0.39–4.82)	0.7365
58:01	1 (8.33%)	11 (12.09%)	12 (8.00%)	0.66 (0.08–5.63)	1.0000	1.05 (0.12–8.80)	1.0000
HLA-C							
01:02	4 (33.33%)	23 (25.27%)	48 (32.00%)	1.48 (0.41–5.37)	0.5084	1.06 (0.31–3.70)	1.0000
03:02	1 (8.33%)	13 (14.29%)	23 (15.33%)	0.55 (0.07–4.59)	1.0000	0.50 (0.06–4.08)	1.0000
03:04	**7 (58.33%)**	**21 (23.08%)**	**21 (14.00%)**	**4.67 (1.34–16.24)**	**0.0162**	**8.60 (2.49–29.63)**	**9.7×10^{-4}[a]**
04:01	1 (8.33%)	5 (5.49%)	20 (13.33%)	1.56 (0.17–14.64)	0.5338	0.59 (0.07–4.83)	1.0000
04:03	3 (25.00%)	13 (14.29%)	11 (7.33%)	2.00 (0.48–8.38)	0.3930	4.21 (0.99–17.84)	0.0711
07:02	2 (16.67%)	26 (28.57%)	26 (17.33%)	0.50 (0.10–2.44)	0.5050	0.95 (0.19–4.61)	1.0000
07:27	**2 (16.67%)**	**0 (0%)**	**2 (1.33%)**	**43.57 (1.96–969.96)**	**0.0126**	**14.80 (1.88–116.35)**	**0.0279**
08:01	2 (16.67%)	9 (9.89%)	33 (22.00%)	1.82 (0.34–9.65)	0.6133	0.71 (0.15–3.39)	1.0000
Haplotype							
*HLA-A*11:01/-B*15:02*	2 (16.67%)	7 (7.69%)	16 (10.67%)	2.40 (0.44–13.17)	0.2813	1.68 (0.34–8.33)	0.6255
*HLA-B*13:01/-C*03:04*	**7 (58.33%)**	**8 (8.79%)**	**10 (6.67%)**	**14.53 (3.74–56.47)**	**1.8×10^{-4}[a]**	**19.60 (5.26–72.99)**	**2.3×10^{-5}[a]**

Significant difference *P* value < 0.05. *P* values were calculated by Fisher's exact test. Statistically significant values are highlighted in bold.
CI, confidence interval; CTX, co-trimoxazole; DRESS, drug reaction with eosinophilia and systemic symptoms; *HLA*, human leucocyte antigen; OR, odds ratio.
[a]Corrected *P* value was obtained after Bonferroni correction (P_c < 0.0025).

*HLA-C*03:04*, and *HLA-C*07:27*. Only the *HLA-B*13:01* allele reached statistical significance after Bonferroni correction (75.00% vs. 16.48%; $P = 7.2 \times 10^{-5}$; OR = 15.20; 95% CI = 3.68–62.83).

Haplotype analysis revealed that the carrier rate of *HLA-B*13:01-C*03:04* haplotype was significantly higher among patients with CTX-induced DRESS as compared with the CTX-tolerant controls (OR = 14.53; 95% CI = 3.74–56.47; $P = 1.8 \times 10^{-4}$).

Association of CTX-induced SJS/TEN and *HLA* alleles

We compared the carrier frequencies of *HLA* class I alleles in the 18 CTX-induced patients with SJS/TEN, 91 CTX-tolerant controls, and 150 healthy Thai population controls. The results are summarized in **Table 5** (only the alleles and haplotypes of *HLA* are presented that showed a significant association when compared with the CTX-tolerant controls and/or healthy population controls).

None of the *HLA-A* alleles were significantly associated with CTX-induced SJS/TEN. Interestingly, we identified an increased frequency of *HLA-A*02:07* and *HLA-A*33:03* alleles in the controls as compared with the CTX-induced SJS/TEN cases.

A strong association between *HLA-B*15:02* and CTX-induced SJS/TEN was confirmed with an OR of 5.16 (95% CI = 1.63–16.33; $P = 0.0075$). The *HLA-B*38:02* allele, which was observed in 33.33% (6/18) of CTX-induced SJS/TEN cases, showed statistically significant differences (OR = 4.05; 95% CI = 1.25–13.18; $P = 0.0249$).

We found that *HLA-C*08:01* allele was detected more frequently in cases with 7 of 18 CTX-induced SJS/TEN possessing *HLA-C*08:01* allele vs. 9 of 91 of the CTX-tolerant controls (OR = 5.79; 95% CI = 1.79–18.70; $P = 0.0049$). In addition, we also found a significant association between *HLA-C*07:27* allele and CTX-induced SJS/TEN with OR of 27.73 (95% CI = 1.27–604.11; $P = 0.0259$).

The *HLA-A*11:01-B*15:02* haplotype was found with a higher frequency in the CTX-induced patients with SJS/TEN group than in the CTX-tolerant control group (33.33% vs. 7.69%; $P = 0.0074$; OR = 6.00; 95% CI = 1.72–20.88).

Association of CTX-induced SCARs and *HLA* alleles among the different subgroups

In subgroup analysis of patients with HIV, 23 patients in the CTX case group and 61 patients in the CTX-tolerant group were analyzed. The significant association between *HLA* class I and CTX-induced SCARs is shown in **Table 6**. For DRESS group, 9 patients in the case group and 61 patients in the tolerant group were analyzed. The *HLA-A*11:01*, *HLA-B*13:01*, and *HLA-C*07:27* were statistically associated with CTX-induced DRESS with OR of 5.78; 95% CI = 1.11–30.25; $P = 0.0376$; OR of 11.55; 95% CI = 2.44–54.78; $P = 0.0021$; and OR of 41.00; 95% CI = 2.44–54.78; $P = 0.0200$, respectively. Furthermore, patients with HIV were found in 14 patients in the SJS/TEN case group and 61 patients in the tolerant group. The *HLA-B*15:02* and *HLA-B*38:02* showed significant difference with CTX-induced SJS/TEN (OR = 5.09; 95% CI = 1.28–20.25; $P = 0.0208$; OR = 8.40; 95% CI = 2.07–34.03; $P = 0.0029$, respectively). Additionally, the association between *HLA-C*07:27* and *HLA-C*08:01* and CTX-induced SJS/TEN was also observed with OR of 24.60 (95% CI = 1.11–544.28; $P = 0.0427$) and OR of 10.74 (95% CI = 2.18–52.90; $P = 0.0035$, respectively. Although there was no association between *HLA-A*, *HLA-B*, and *HLA-C* alleles and CTX-induced SCARs in HIV-negative patients.

Pooled-data analysis

The allele frequencies of *HLA-A*11:02*, *HLA-B*15:02*, and *HLA-C*08:01* in CTX-induced SJS/TEN and tolerant controls are shown in **Table 7** after pooling our results with those obtained by Kongpan *et al*. The frequency of *HLA-B*15:02* and *HLA-C*08:01* in CTX-SJS/TEN were significantly higher than the frequency found in tolerant controls (OR = 4.25; 95% CI = 1.83–9.88; $P = 0.0008$ and OR = 4.12; 95% CI = 1.72–9.90; $P = 0.0015$, respectively). Nevertheless, no significant difference was observed when the frequency of *HLA-A*11:02* was compared between CTX-SJS/TEN cases and tolerant controls (OR = 1.64; 95% CI = 0.86–3.16; $P = 0.1361$).

In subgroup analysis of HIV-positive patients, two HLA alleles, *HLA-B*15:02* and *C*08:01*, were significantly associated with CTX-SJS/TEN with OR of 5.77 (95% CI = 2.04–16.30; $P = 0.0009$) and 11.05 (95% CI = 2.97–41.07; $P = 0.0003$), respectively. Although there was no association among *HLA-A*11:01*, *HLA-B*15:02*, and *HLA-C*08:01* and CTX-induced SCARs in HIV-negative patients (data not shown).

DISCUSSION

SJS, TEN, and DRESS are potentially fatal SCARs affecting multiple organs and systems, and CTX has been implicated as a trigger for these adverse drug reactions.[23,24] The hypersensitivity reaction should be occurred ~ 2–7 weeks after first exposure. The previous studies presented the range of drug exposure time of CTX patients was 1–74 days.[12] However, some of our patients seem to be outside of a recognized window of drug exposure. The latency was more than 7 weeks. Several studies have provided evidence of the genetic predisposition to SCARs in various populations.[25] In this study, we examined HLA risk factors for CTX-induced SCARs among Thai patients. We identified that carriers of *HLA-B*15:02* and *HLA-C*08:01* alleles are significantly more likely to develop SJS/TEN, whereas the *HLA-B*13:01* allele was associated with an increased risk of developing DRESS in patients taking CTX. None of the *HLA-A* alleles showed a significant association with CTX-induced SCARs. The haplotype analysis revealed a significant increase in the frequency of *HLA-A*11:01-B*15:02* and *HLA-B*13:01-C*03:04* haplotypes in the CTX-induced SJS/TEN and DRESS cases, respectively. However, the haplotype data was imputed by statistical program.

The association between the *HLA* class *I* and *HLA-DRB1* polymorphisms and CTX-induced SJS/TEN was first reported in a Thai population by Kongpan *et al*.[12] in which they demonstrated an increased frequency of *HLA-B*15:02*, *HLA-C*06:02*, and *HLA-C*08:01* in CTX-induced patients with SJS/TEN with risk being about fourfold higher among patients with the *HLA-B*15:02* or *HLA-C*08:01* alleles and 12-fold higher

Table 5 The association of *HLA* class I alleles with CTX-induced SJS-TEN (n = 18)

HLA class I alleles	CTX-induced SJS-TEN (n = 18)	Tolerant controls (n = 91)	Thai population (n = 150)	CTX-induced SJS-TEN cases vs. tolerant controls		CTX-induced SJS-TEN cases vs. Thai population	
				OR (95% CI)	P value < 0.05[a]	OR (95% CI)	P value < 0.05[a]
HLA-A							
02:03	5 (27.78%)	19 (20.88%)	32 (21.33%)	1.46 (0.46–4.59)	0.5397	1.42 (0.47–4.27)	0.5511
02:07	0	28 (30.77%)	26 (17.33%)	0.06 (0.01–1.03)	0.0058	0.13 (0.01–2.17)	0.0788
11:01	11 (61.11%)	38 (41.76%)	68 (45.33%)	2.19 (0.78–6.17)	0.1315	1.89 (0.69–5.15)	0.2051
24:02	3 (16.67%)	17 (18.68%)	32 (21.33%)	0.87 (0.23–3.35)	1.0000	0.74 (0.20–2.71)	0.7679
24:07	1 (5.56%)	5 (5.49%)	14 (9.33%)	1.01 (0.11–9.22)	1.0000	0.57 (0.07–4.62)	1.0000
33:03	1 (5.56%)	18 (19.78%)	32 (21.33%)	0.24 (0.03–1.91)	0.1893	0.22 (0.03–1.69)	0.2043
HLA-B							
07:05	3 (16.67%)	6 (6.59%)	8 (5.33%)	2.83 (0.64–12.58)	0.1669	3.55 (0.85–14.83)	0.0987
13:01	4 (22.22%)	15 (16.48%)	23 (15.33%)	1.45 (0.42–5.01)	0.5139	1.58 (0.48–5.22)	0.4959
15:02	**7 (38.89%)**	**10 (10.99%)**	**27 (18.00%)**	**5.16 (1.63–16.33)**	**0.0075**	2.89 (1.03–8.16)	0.0576
38:02	**6 (33.33%)**	**10 (10.99%)**	**12 (8.00%)**	**4.05 (1.25–13.18)**	**0.0249**	**5.75 (1.83–18.05)**	**0.0054**
40:01	0	13 (14.29%)	18 (12.00%)	0.16 (0.01–2.77)	0.1206	0.19 (0.01–3.35)	0.2230
46:01	1 (5.56%)	23 (25.27%)	40 (26.67%)	0.17 (0.02–1.38)	0.1151	0.16 (0.02–1.26)	0.0769
58:01	1 (5.56%)	11 (12.09%)	12 (8.00%)	0.43 (0.05–3.54)	0.6862	0.68 (0.08–5.53)	1.0000
HLA-C							
01:02	**1 (5.56%)**	23 (25.27%)	**48 (32.00%)**	0.17 (0.02–1.38)	0.1151	**0.13 (0.02–0.97)**	**0.0197**
03:02	1 (5.56%)	13 (14.29%)	23 (15.33%)	0.35 (0.04–2.88)	0.4581	0.33 (0.04–2.56)	0.4749
03:04	1 (5.56%)	21 (23.08%)	21 (14.00%)	0.19 (0.03–1.56)	0.1149	0.36 (0.05–2.86)	0.4729
04:01	1 (5.56%)	5 (5.49%)	20 (13.33%)	1.01 (0.11–9.22)	1.0000	0.38 (0.05–3.03)	0.7037
04:03	1 (5.56%)	13 (14.29%)	11 (7.33%)	0.35 (0.04–2.88)	0.4581	0.74 (0.09–6.12)	1.0000
07:02	5 (27.78%)	26 (28.57%)	26 (17.33%)	0.96 (0.31–2.97)	0.9456	1.83 (0.60–5.59)	0.3323
07:27	**2 (11.11%)**	**0 (0%)**	2 (1.33%)	**27.73 (1.27–604.11)**	**0.0259**	9.25 (1.22–70.19)	0.0573
08:01	**7 (38.89%)**	**9 (9.89%)**	33 (22.00%)	**5.79 (1.79–18.70)**	**0.0049**	2.26 (0.81–6.28)	0.1417
Haplotype							
*HLA-A*11:01/-B*15:02*	**6 (33.33%)**	**7 (7.69%)**	**16 (10.67%)**	**6.00 (1.72–20.88)**	**0.0074**	**4.19 (1.38–12.69)**	**0.0165**
*HLA-B*13:01/-C*03:04*	1 (5.56%)	8 (8.79%)	10 (6.67%)	0.61 (0.07–5.20)	1.0000	0.82 (0.09–6.84)	1.0000

Statistically significant values are highlighted in bold.
Significant different P value < 0.05. P values were calculated by Fisher's exact test.
CI, confidence interval; CTX, co-trimoxazole; *HLA*, human leucocyte antigen; OR, odds ratio; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.
[a]Corrected P value was obtained after Bonferroni correction (P_c < 0.0025).

Table 6 List of HLA alleles that showed a significant association with CTX-induced SCARs among the different subgroup

	HIV-positive patients				HIV-negative patients			
HLA class I alleles	CTX-induced SCARs+/ total	Tolerant controls+/ total	OR (95% CI)	*P* value	CTX-induced SCARs+/total	Tolerant controls+/total	OR (95% CI)	*P* value
SCAR								
HLA-A*02:07	**2/23**	**21/61**	**0.18 (0.04–0.85)**	**0.0302**	1/7	7/30	0.54 (0.06–5.35)	0.6047
*HLA-A*11:01*	14/23	23/61	2.57 (0.96–6.88)	0.0602	7/7	17/30	11.57 (0.61–220.97)	0.1037
HLA-B*07:05	**5/23**	**3/61**	**5.37 (1.17–24.70)**	**0.0308**	0/7	3/30	0.52 (0.02–11.30)	0.6799
HLA-B*13:01	**9/23**	**9/61**	**3.71 (1.24–11.12)**	**0.019**	4/7	6/30	5.33 (0.93–31.51)	0.0599
HLA-B*15:02	**7/23**	**6/61**	**4.01 (1.18–13.64)**	**0.0262**	2/7	4/30	2.60 (0.37–18.25)	0.3365
HLA-B*38:02	**7/23**	**5/61**	**4.90 (1.37–17.54)**	**0.0146**	0/7	5/30	0.31 (0.02–6.25)	0.4442
HLA-C*07:27	**4/23**	**0/61**	**28.38 (1.46–550.99)**	**0.0270**	0/7	0/30	N/A	N/A
HLA-C*08:01	**7/23**	**3/61**	**8.46 (1.96–36.47)**	**0.0042**	2/7	6/30	1.60 (0.24–10.36)	0.6219
DRESS								
HLA-A*11:01	**7/9**	**23/61**	**5.78 (1.11–30.25)**	**0.0376**	3/3	17/30	N/A	N/A
HLA-B*13:01	**6/9**	**9/61**	**11.55 (2.44–54.78)**	**0.0021**	3/3	6/30	N/A	N/A
HLA-C*07:27	**2/9**	**0/61**	**41.00 (1.79–937.48)**	**0.0200**	0/3	0/30	N/A	N/A
SJS/TEN								
*HLA-A*11:01*	7/14	23/61	1.65 (0.51–5.32)	0.3997	4/4	17/30	N/A	N/A
HLA-B*15:02	**5/14**	**6/61**	**5.09 (1.28–20.25)**	**0.0208**	2/4	4/30	N/A	N/A
HLA-B*38:02	**6/14**	**5/61**	**8.40 (2.07–34.03)**	**0.0029**	0/4	5/30	N/A	N/A
HLA-C*07:27	**2/14**	**0/61**	**24.60 (1.11–544.28)**	**0.0427**	0/4	0/30	N/A	N/A
HLA-C*08:01	**5/14**	**3/61**	**10.74 (2.18–52.90)**	**0.0035**	2/4	6/30	N/A	N/A

Data were obtained from two studies with high-resolution *HLA-B* typing results.
Significantly different *P* value < 0.05. *P* values were calculated by Fisher's exact test.
Statistically significant values are highlighted in bold.
CI, confidence interval; CTX, co-trimoxazole; *HLA*, human leucocyte antigen; OR, odds ratio; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

Table 7 Pooled-data analysis comparing the frequencies of *HLA-A*11:01*, *HLA-B*15:02*, and *C*08:01* in CTX-induced SJS/TEN and tolerant controls

HLA genotype	All patients				HIV-positive patients			
	CTX-SJS/TEN cases (n = 61)	Tolerant controls (n = 91)	OR (95% CI)	*P* value	CTX-SJS/TEN cases (n = 44)	Tolerant controls (n = 61)	OR (95% CI)	*P* value
*A*11:01*	33	38	1.64 (0.86–3.16)	0.1361	23	22	1.94 (0.88–4.28)	0.0995
Without *A*11:01*	28	53			21	39		
*B*15:02*	21	10	**4.25 (1.83–9.88)**	**0.0008**	17	6	**5.77 (2.04–16.30)**	**0.0009**
Without *B*15:02*	40	81			27	55		
*C*08:01*	19	9	**4.12 (1.72–9.90)**	**0.0015**	16	3	**11.05 (2.97–41.07)**	**0.0003**
Without *C*08:01*	42	82			28	58		

Data were obtained from two studies with high-resolution *HLA-B* typing results.
Significantly different *P* value < 0.05. *P* values were calculated by Fisher's exact test. Statistically significant values are highlighted in bold.
CI, confidence interval; CTX, co-trimoxazole; *HLA*, human leucocyte antigen; OR, odds ratio; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

among patients with *HLA-C*06:02*.[12] In the present study, we replicated the association with *HLA-B*15:02* and *HLA-C*08:01* but not with *HLA-C*06:02*. The *HLA-A*02:07* and *HLA-A*33:03* alleles were more common in the controls than in cases suggesting that they may protect against the development of CTX-induced SJS/TEN in Thai patients. However, these alleles did not reach statistical significance after Bonferroni correction ($P > 0.0025$).

*HLA-B*15:02* is primarily associated with carbamazepine-induced SJS/TEN in patients of certain Asian ethnicities.[26] Interestingly, our study provides evidence that the same HLA allele may predispose to SJS/TEN caused by either carbamazepine or CTX. It should be noted that *HLA-C*08:01* was the only allele significantly associated with SJS/TEN after Bonferroni's correction for multiple comparisons in the previous study by Kongpan *et al*.[12] In our present study, *HLA-C*08:01* allele showed a significant association with SJS/TEN, suggesting that this allele may also have a role in SJS/TEN induced by CTX. Whether *HLA-C*08:01* is a clinically relevant marker for CTX-induced SJS/TEN needs to be investigated in other populations.

The carrier frequency of the *HLA-B*13:01* allele in CTX-induced SCAR cases did not differ significantly from the tolerant controls after corrections for multiple testing ($P > 0.0025$). However, *HLA-B*13:01* showed a significant association with CTX-induced DRESS ($P < 0.0025$). This observation suggests that *HLA-B*13:01* is a risk factor that is specific for CTX-induced DRESS. It indicates that the individual phenotypes do not share the same risk locus in the HLA system. The frequency of *HLA-C*03:04* allele was also significantly higher in CTX-induced DRESS as compared with the healthy controls, and the haplotype analysis revealed a significant increase in the frequency of the *HLA-B*13:01-C*03:04* haplotype in the DRESS group. These haplotype results are different from the previous report in Thai population.[11] *HLA-B*13:01* has convincingly been shown to be a risk factor for dapsone hypersensitivity syndrome in Han Chinese and Thai populations.[27,28] *HLA-B*13:01* has a well-defined subpocket within the antigen-binding site, which fits the dapsone molecule and potentially alters self-peptides upon binding.[29] Given the structural similarities between sulfamethoxazole and dapsone, and in their respective metabolic pathways, our finding of an association with *HLA-B*13:01* is thus biologically plausible.

Nonetheless, *HLA-A* alleles; *HLA-A*11:01*, *HLA-A*11:02*, *HLA-A*31:01*, *HLA-A*33:03*, *HLA-A*68:01*, and *HLA-A*74:01*, have shared peptide binding specificities[30] and 26 of 30 of the cases carry one or more of these alleles. It is notable that the immune response of the drug interact to HLA binding groove can be shared across HLA molecules with similar peptide binding specificity.[31] In present study, seven patients (100%) who are reported HIV-negative carried *HLA-A*11:01*. Two of four HIV-negative patients with SJS/TEN have *HLA-B*15:02* and *HLA-C*08:01*, whereas three of three HLA-negative patients with DRESS also carried *HLA-B*13:01*. Interestingly, the association between *HLA* alleles and CTX-induced SCARs was found in HIV-infected patients but not HIV-negative patients. It is possibly that HIV itself could be related to this association due to systemic reduction of glutathione in HIV-infected patients.[32]

Pharmacological interaction of drugs with immune receptors, the so-called p-i concept, has been proposed to explain how T cells can be stimulated by the interaction of sulfamethoxazole with T-cell receptors, and resulting in clinical symptoms of drug hypersensitivity.[10,11] However, whether a specific T-cell function leads to a specific clinical phenotypes is unclear, and so further research is needed and should be based on well-characterized phenotypes and drug causality, considering the heterogeneity of T-cell function.[33] There is a theoretical possibility of cross-reactivity between sulfonamide antibiotics and nonantibiotic sulfonamides,[34] which has led precautionary advice in several countries.[35]

Some of the limitations of our study should be considered before interpreting the results. First, this research only studied the role of class I *HLA* alleles and *CYP2C9*, but not other HLA and drug metabolism enzyme genes and host factors. Apart from CYP2C9 enzyme, N-acetyltransferases and glutathione-S-transferases play important roles in sulfamethoxazole metabolism to reactive metabolites and toxicity detoxification, respectively.[36,37] Chang *et al*. reported that high daily doses of CTX were an independent risk factor for cutaneous and other adverse drug reactions.[38] We did not measure the serum concentrations of CTX, so it is possible that plasma and tissue concentrations of CTX might have influenced the adverse outcomes in our study. However, a previous case-control study by Pirmohamed *et al*. failed to demonstrate an association between genetic polymorphisms in drug metabolizing enzymes with CTX hypersensitivity in patients with HIV.[39] Nonetheless, the *in vitro* study on sulfamethoxazole hypersensitive patients demonstrated *HLA-DQ* plays a critical role in the activation of drug metabolite (SMX-NO)-specific CD4+ T cells. Further validation of other HLA and genetic polymorphisms in drug metabolism enzyme genes and host factors, or even *in vitro* study on drug specific T cells should be conducted. Second, there were few cases of CTX-induced SJS/TEN and DRESS. Nevertheless, despite limited power, we have identified phenotype-specific HLA associations, and present patient-level data for the SJS/TEN and DRESS cases in our study to allow for further replication. Third, there was evidence that a strong association between SJS/TEN and trimethoprim alone was found.[14] However, no *in vitro* or *in vivo* patch test was performed to validate the culprit drug as sulfamethoxazole or trimethoprim in this study. The number needed to test to prevent one case of CTX-induced SCARs have been done in the study. The estimated number needed to test of *HLA-B*13:01*-induced CTX-DRESS and *HLA-B*15:02*-induced CTX-SJS/TEN was 23 and 31, respectively.

In conclusion, this study has highlighted the importance of HLA class I alleles or haplotypes in predisposing to evaluating their influence on the susceptibility to CTX-induced SCAR in Thai patients. Most importantly, our data indicates that the association with HLA class I alleles may be phenotype-specific. Of course, our data need further replication in larger numbers of patients and in different ethnic groups. Given the public health drive to prevent SCAR caused by drugs in Thailand through the use of genotyping, we need to consider whether the alleles identified in this study should be utilized in the future to prevent SCAR from CTX.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.Su., W.C., and M.P. wrote the manuscript. S.Su, W.C., and M.P. designed the research. J.P., P.S., A.P., J.K., T.R., P.R., P.L., P.K., U.K., W.D., C.S., O.P., S.K., T.K., P.C., N.S., J.B., and W.T. performed the research. J.P., P.S., A.P., J.K., P.R., P.L., and N.N. analyzed the data.

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