Whole genome sequencing identifies genetic variants associated with co-trimoxazole hypersensitivity in Asians

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Background: Co-trimoxazole, a sulfonamide antibiotic, is used to treat a variety of infections worldwide, and it remains a common first-line medicine for prophylaxis against *Pneumocystis jiroveci* pneumonia. However, it can cause severe cutaneous adverse reaction (SCAR), including Stevens-Johnson syndrome, toxic epidermal necrolysis, and drug reaction with eosinophilia and systemic symptoms. The pathomechanism of co-trimoxazole–induced SCAR remains unclear.

Objective: We aimed to investigate the genetic predisposition of co-trimoxazole–induced SCAR.

Methods: We conducted a multicountry case-control association study that included 151 patients with of co-trimoxazole–induced SCAR and 4631 population controls from Taiwan, Thailand, and Malaysia, as well as 138 tolerant controls from Taiwan. Whole-genome sequencing was performed for the patients and population controls from Taiwan; it further validated the results from Thailand and Malaysia.

Results: The whole-genome sequencing study (43 case patients vs 507 controls) discovered that the single-nucleotide polymorphism rs41554616, which is located between the *HLA-B*

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and *MICA* loci, had the strongest association with co-trimoxazole–induced SCAR ($P = 8.2 \times 10^{-9}$; odds ratio [OR] = 7.7). There were weak associations of variants in co-trimoxazole–related metabolizing enzymes (*CYP2D6*, *GSTP1*, *GCLC*, *N*-acetyltransferase [*NAT2*], and *CYP2C8*). A replication study using HLA genotyping revealed that *HLA-B*13:01* was strongly associated with co-trimoxazole–induced SCAR (the combined sample comprised 91 case patients vs 2545 controls [$P = 7.2 \times 10^{-21}$; OR = 8.7]). A strong HLA association was also observed in the case patients from Thailand ($P = 3.2 \times 10^{-5}$; OR = 3.6) and Malaysia ($P = .002$; OR = 12.8), respectively. A meta-analysis and phenotype stratification study further indicated a strong association between *HLA-B*13:01* and co-trimoxazole–induced drug reaction with eosinophilia and systemic symptoms ($P = 4.2 \times 10^{-23}$; OR = 40.1). Conclusion: This study identified *HLA-B*13:01* as an important genetic factor associated with co-trimoxazole–induced SCAR in Asians. (J Allergy Clin Immunol 2021;147:1402-12.)

Key words: *Co-trimoxazole,* HLA-B*13:01*, severe hypersensitivity reactions, sulfonamide, whole-genome sequencing*

Abbreviations used

DRESS: Drug reaction with eosinophilia and systemic symptoms
EEF2: Eukaryotic translation elongation factor 2
GCLC: Glutamate cysteine ligase catalytic subunit
GSTP1: Glutathione S-transferase pi 1
IRB: Institutional review board
LAT: Lymphocyte activation test
MICA: MHC class I polypeptide–related sequence A
MPO: Myeloperoxidase
NAT2: *N*-acetyltransferase 2
NPV: Negative predictive value
OR: Odds ratio
PPV: Positive predictive value
SCAR: Severe cutaneous adverse reaction
SJS: Stevens-Johnson syndrome
SNP: Single-nucleotide polymorphism
TEN: Toxic epidermal necrolysis
WGS: Whole-genome sequencing

Sulfonamide antibiotics, such as co-trimoxazole (which comprises 5 parts sulfamethoxazole and 1 part trimethoprim), sulfadimethoxine, and sulfafurazole, are widely prescribed for the treatment of a variety of infections, such as pneumonia, bronchitis, traveler's diarrhea, shigellosis, and ear and urinary tract infections. Co-trimoxazole remains a common first-line medicine used for prophylaxis against *Pneumocystis jiroveci* pneumonia—especially in patients human with HIV or AIDS.[1,2] Although effective in treating infectious diseases, sulfonamide antibiotics may cause hypersensitivity reactions ranging from mild rash (maculopapular exanthema) 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), that hinder their clinical use. SJS and TEN are considered part of the same disease spectrum and often involve generalized blistering mucocutaneous reactions with relatively high mortality rates of approximately 5% to 12.5% for SJS and 50% for TEN.[3] DRESS is characterized by fever, skin rash, atypical lymphocytes, eosinophilia, and systemic involvement, and it has a mortality rate of up to 10%.[4,5] The incidence of co-trimoxazole–induced hypersensitivity is about in 1% to 3% among the exposed general population,[6] which rises in patients with HIV or AIDS.[7,8] We recently analyzed the SJS and/or TEN registration databases of multiple Asian countries for the period from 1998 to 2017. A total of 1028 case patients with SJS and/or TEN were analyzed for their drug causality; the results indicated that co-trimoxazole was the fourth most common causative drug for SJS and/or TEN.[9]

To date, there is no method available to prevent severe hypersensitivity reactions caused by sulfonamide antibiotics. SCAR is considered to be a delayed-type hypersensitivity reaction; its pathogenesis involves the activation of effector T lymphocytes by culprit drugs.[10-14] Advances in pharmacogenetic studies have revealed genetic links to drug hypersensitivity reactions. HLA has been found to be an important molecule involved in the pathomechanism of drug hypersensitivity. Our group first identified the strong genetic associations between *HLA-B*15:02* and carbamazepine–induced SJS and/or TEN[10] and between *HLA-B*58:01* and allopurinol–induced SCAR.[11] Other HLA alleles have also been found to have strong associations with specific drug-induced hypersensitivity reactions, such as *HLA-B*57:01* and abacavir hypersensitivity[15,16] and *HLA-B*13:01* and dapsone hypersensitivity syndrome.[17] As in the case of HLA alleles, our group also identified strong correlations among genetic variants of drug-metabolizing enzymes with phenytoin-induced hypersensitivity.[18] The discoveries of these strong genetic associations have been applied in clinical testing to prevent cases of severe drug hypersensitivity.

However, genetic susceptibilities to sulfonamide antibiotic–induced hypersensitivity remain unclear. Lonjou et al and Kongpan et al previously reported that *HLA-B*38* and *HLA-B*15:02/HLA-C*06:02/HLA-C*08:01* were found to be weakly associated with co-trimoxazole–induced SJS and/or TEN in the European population and Thai population, respectively.[19,20] Reinhart et al reported that no single-nucleotide polymorphisms (SNPs) were found to have genome-wide significance in sulfonamide antibiotic–induced delayed-type hypersensitivity among patients from America.[21] To investigate the genetic predisposition to hypersensitivity reactions induced by sulfonamide antibiotics in Asian populations, we using large-scale whole-genome sequencing (WGS) to carry out a multicountry case-control study of patients from Taiwan, Thailand, and Malaysia with co-trimoxazole–induced severe hypersensitivity reactions and then further validated the results in different populations.

METHODS

Subject enrollment

The case series used in this study consisted of 91, 52, and 8 patients with co-trimoxazole–induced SCAR from Taiwan (50 with SJS and/or TEN and 41 with DRESS), Thailand (41 with SJS and/or TEN and 11 with DRESS), and Malaysia (3 with SJS and/or TEN and 5 with DRESS), respectively. Phenotypes of SCAR were classified according to the consensus definitions of the RegiSCAR study criteria.[4,22-25] The drug causality for each enrolled case patient was determined by using the algorithm of drug causality for epidermal necrolysis score published by the RegiSCAR study group or the Naranjo algorithm.[5,26,27] Among all the patients from Taiwan, 52 available patients with co-trimoxazole–related SCAR had undergone an *in vitro* lymphocyte activation test (LAT)[28,29] to confirm their culprit drug.

TABLE I. Demographic and baseline clinical characteristics of patients with co-trimoxazole–induced severe hypersensitivity reaction and co-trimoxazole–tolerant controls from Taiwan, Thailand, and Malaysia

	Co-trimoxazole–induced SCAR							
	Taiwan		Thailand		Malaysia			
Characteristics	SJS/TEN (n = 50)	DRESS (n = 41)	SJS/TEN (n = 41)	DRESS (n = 11)	SJS/TEN (n = 3)	DRESS (n = 5)	Total (n = 151)	Co-trimoxazole–tolerant controls* (n = 138)
Age (y), mean ± SD	51.7 ± 19.9	40.0 ± 20.4	39.5 ± 12.3	42.3 ± 18.5	24.7 ± 11.7	33.8 ± 15.0	43.4 ± 18.7	55.8 ± 11.9
Sex, no. (%)								
Male	29 (58%)	19 (46%)	19 (46%)	6 (55%)	2 (67%)	5 (100%)	80 (53%)	79 (57%)
Female	21 (42%)	22 (54%)	22 (54%)	5 (45%)	1 (33%)	0 (0%)	71 (47%)	59 (43%)
Deceased case patients, no. (%)	3 (6%)	1 (2%)	0 (0%)	0 (%)	0 (0%)	0 (0%)	4 (3%)	0 (0%)
Internal organ involvement								
Hepatitis, GPT, IU/L, no. (%)								
Normal	44 (88%)	17 (42%)	32 (78%)	5 (45%)	3 (100%)	3 (60%)	104 (69%)	NA
>3-fold	6 (12%)	24 (59%)	9 (22%)	6 (55%)	0 (0%)	2 (40%)	47 (31%)	NA
Acute renal failure†	6 (12%)	4 (10%)	7 (17%)	2 (18%)	0 (0%)	3 (60%)	22 (15%)	NA
Hematologic abnormalities								
Eosinophilia, eosinophils (%)								
<5%	45 (90%)	10 (24%)	33 (80%)	2 (18%)	1 (33%)	1 (20%)	92 (61%)	NA
≥5%	5 (10%)	31 (76%)	8 (20%)	9 (82%)	2 (67%)	4 (80%)	59 (39%)	NA
Atypical lymphocytosis	8 (16%)	10 (24%)	NA	NA	0 (0%)	1 (20%)	—	NA
Skin detachment, no. (%)								
BSA ≥10%	15 (30%)	0 (0%)	13 (32%)	0 (0%)	1 (33%)	0 (0%)	29 (19%)	NA
BSA <10%	35 (70%)	41(100%)	28 (68%)	11(100%)	2 (67%)	5 (100%)	122 (81%)	NA
Mucosal involvement, no. (%)								
Oral	48 (96%)	3 (7%)	33 (89%)	1 (9%)	2 (67%)	0 (0%)	87 (59%)	NA
Ocular	32 (64%)	2 (5%)	21 (57%)	1 (9%)	2 (67%)	0 (0%)	58 (39%)	NA
Genital	23 (46%)	2 (5%)	10 (27%)	0 (0%)	1 (33%)	0 (0%)	36 (24%)	NA
Underlying diseases, no. (%)								
Opportunistic infections in patients with HIV	1 (2%)	1 (2%)	31 (76%)	8 (73%)	0 (0%)	2 (40%)	42 (28%)	7 (5%)
Other infections	49 (98%)	40 (98%)	11 (27%)	3 (27%)	3 (100%)	3 (60%)	109 (72%)	131 (95%)
Co-trimoxazole exposure								
Dosage (mg/d), mean ± SD (range)	1446 ± 313 (400-1600)	1429 ± 414 (200-2400)	1217 ± 1185 (800-4800)	1018 ± 374 (800-1600)	1600 ± 0 (1600)	1600 ± 566 (800-2400)	1375 ± 603 (240-4800)	1575 ± 284 (400-2400)
Duration (d), mean ± SD (range)	12.7 ± 9.0 (3-47)	14.3 ± 10.9 (2-41)	15.9 ± 12.4 (1-48)	28.6 ± 15. (14-65)	39 ± 27 (8-60)	18.4 ± 7.6 (10-30)	15.9 ± 12.3 (1-65)	145 ± 208 (75-1095)

BSA, Body surface area; *GPT*, glutamic pyruvic transaminase; *NA*, not applicable.
*In all, 138 Taiwanese patients who had received co-trimoxazole for more than 2.5 months without evidence of adverse reactions were enrolled as tolerant controls.
†The creatinine value was 1.5-fold higher than the normal value range (0.4-1.5 mg/dL) after drug intake.

Each subject who enrolled in this study signed a written informed consent form, and the study itself was approved by the institutional review board (IRB) and ethics committee of each institute and hospital involved in accordance with the laws of each country (IRB No.97-0509B, 100-4657A3, 103-2562C, 104-2664A3, 104-0291B, 201403073A3, 201601761B0, 201600224A3, 201901301B0A3, YM106026F-1, IRB00001189, HE510837 and R.1235-9). Informed consent was obtained from all the participants.

Ethical approval

Ethical approval for this study was provided by the ethics review boards of Chang Gung Memorial Hospital in Taiwan, various medical school hospitals in Thailand (including Khon Kaen University, Udon Thani Hospital, Khon Kaen Hospital, Ramathibodi Hospital, and King Chulalongkorn Memorial Hospital), and Hospital Sultanah Aminah Johor Bahru in Malaysia.

WGS, variant calling/functional annotation, HLA prediction and genotyping, and computer modeling/docking

We performed WGS analyses for 43 case participants with SCAR and 507 population controls from Taiwan as, well as for 45 case participants from Thailand with SCAR. A total of 13,601,921 and 12,576,698 variants in the individuals from Taiwan and Thailand, respectively, were identified by the WGS. To determine the functional relevance of the variations, we first focused on the exonic regions; 75,260 variants from Taiwan were determined to be exonic regions according to the ANNOVAR software tool and RefSeq database. We used these exonic variants to depict the Manhattan plot of the first cohort by making comparisons between the 43 case participants and 507 control participants.

Additional information regarding methods used to determine or perform the disease phenotype, drug causality, LAT, WGS DNA libraries quantification, WGS variant calling/functional annotation, HLA prediction and genotyping, and computer modeling and docking are provided in the Methods section in this article's Online Repository (available at jacionline.org).

Statistical analysis

We conducted the statistical analysis for any associations by comparing the allele or genotype frequencies between case participants and controls in models of inheritance (additive model, recessive model, or dominant models). The WGS associations were examined by logistic regression analysis and rank-ordered according to the lowest *P* value in these models. All *P* values were 2 tailed. The methods used for Bonferroni correction, odds ratio (OR) calculation, and meta-analysis are provided in the Methods section in this article's Online Repository.

RESULTS

Patient recruitment and LAT

A total of 151 patients with co-trimoxazole–induced SCAR from Taiwan, Thailand, and Malaysia were enrolled in this genetic association study. In addition, 138 drug-tolerant patients who had received co-trimoxazole for more than 2.5 months without evidence of adverse reactions were enrolled as tolerant controls. The demographic and clinical data of the enrolled patients are shown in Table I. Among these 151 patients with SCAR, 4 died as a result of the episode. The underlying diseases

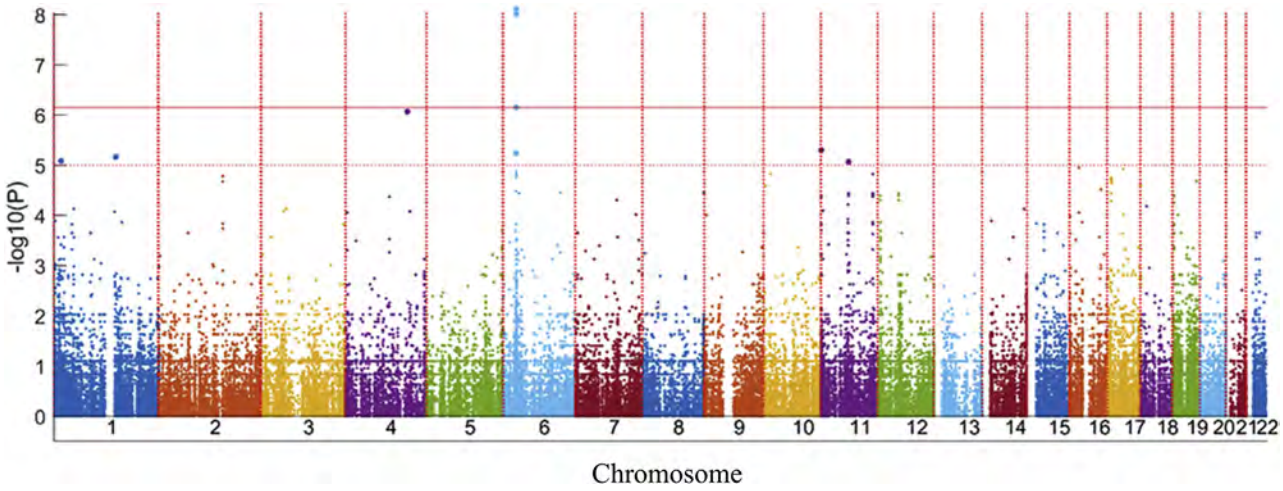

FIG 1. Whole-genome sequencing scan for the exonic regions associated with co-trimoxazole–induced severe hypersensitivity reactions. Manhattan plot showing associations between the exonic regions of genetic variants and co-trimoxazole–induced severe cutaneous adverse reactions. Each dot represents a negative $\log_{10}$ *P* value derived with a logistic regression model adjusted by sex, age, and principal components (PCs) in 43 case patients and 507 general population controls. The red horizontal solid line represents $P = 6.99 \times 10^{-7}$, indicating $P = .05$ by Bonferroni correction for the multiple comparisons (0.05 of 71440).

of the enrolled case patients and tolerant controls that led to the use of co-trimoxazole were mostly infectious diseases, including urinary tract infection, acne, pneumonia, unknown fever, wound infection, HIV infection, cellulitis, and urethritis (see Table E1 in the Online Repository at www.jacionline.org). The average initial daily drug doses were 1375 ± 603 mg and 1575 ± 284 mg for patients with SCAR and tolerant controls, respectively. The average onset time of cutaneous manifestation was 15.9 ± 12.3 days after exposure to co-trimoxazole (Table I). We also performed an *in vitro* LAT for each of the 52 patients with SCAR from Taiwan to confirm that the culprit drug was co-trimoxazole by measuring the $CD8^+$ cytotoxic T-lymphocyte activation marker granulysin, which is known as a key mediator for keratinocyte detachment in SJS and TEN[14,30] and as a specific cytotoxicity protein in DRESS.[31] Our results showed that the sensitivity and specificity of granulysin-based LAT for patients with co-trimoxazole–induced SCAR were 69.2% (36 of 52) and 100%, respectively (see Fig E1 in the Online Repository).

Case-control association study by WGS

For the initial WGS, we first used 43 case patients with co-trimoxazole–induced SCAR (n = 20 with SJS and/or TEN and n = 23 with DRESS) and 507 individuals in the general population of Taiwan without a history of adverse drug reactions as a control group. The flowchart and detailed information regarding the genetic analysis of co-trimoxazole–induced SCAR in the study are shown in Fig E2 (available in the Online Repository at www.jacionline.org). Through the WGS, we identified a total of 13,601,921 variants, including 11,226,208 SNPs and 2,375,713 insertion-deletions in the patients and population controls from Taiwan. We first focused on analyzing the functional exonic regions, as a result of which 75,260 variants (73,363 SNPs and 1,897 insertion-deletions) were identified. We further analyzed the data by using logistic regression modeling with the following covariates: sex, age, and ancestry-specific principal components. The principal component analysis map showed that there was no difference in the distribution of ancestry among the patients with SCAR and the population controls (see Fig E3 in the Online Repository at www.jacionline.org). The WGS discovered a cluster of exonic SNPs on chromosome 6 (Fig 1). Among them, the SNP rs41554616, which is located between the *HLA-B* and MHC class I polypeptide–related sequence A (*MICA*) loci, showed the strongest association with co-trimoxazole–induced SCAR ($P = 8.2 \times 10^{-9}$; OR = 7.7; 95% CI = 4.0-14.9) (Fig 1 and Table II). We then identified the top 20 functional variants (Table II) (for the definition of deleterious variants, please see the Methods section and in Table E2 in the Online Repository at www.jacionline.org). We found that the variants of *HLA-A*, *MICA*, metabolism of cobalamin associated A (*MMAA*), *CFL1*, eukaryotic translation elongation factor 2 (*EEF2*), and *PKM* (which may be involved in HLA- or immune-related pathways [see Fig E4 in the Online Repository at www.jacionline.org]), which are implicated by Phenolyzer, and genes such as *MYH4*, *RIF1*, *UCN3*, *NISCH*, *OR52R1*, *SLC9B1*, which are related to other pathways suggested to not be involved in HLA- or immune-related pathways (see Fig E4), are associated with co-trimoxazole–induced SCAR among patients in Taiwan.

We further performed a WGS for another replication cohort of 45 case patients with SCAR from Thailand (comparing them with data on 290 controls from the Thai general population that were obtained from the Thai biobank of Mahidol University in Thailand) and found that the identified variants, including *MICA*, *HLA-B*, *EEF2*, and *SLC9B1*, were also associated with co-trimoxazole–induced SCAR among patients in the Thai population (see Table E2).

Analysis of genetic variants of drug-metabolizing enzymes

Divergences in individual metabolism or drug clearance are also suspected to contribute to the development of SCAR. For

TABLE II. The deleterious variants associated with co-trimoxazole–induced severe cutaneous adverse reactions in WGS discovery cohort of patients from Taiwan

				WGS discovery cohort				
				MAF				
Gene SNP	Chr (location)	Fun	Minor allele	Case patients (n = 43)	Ctrls (n = 507)	OR (95% CI)	*P* value	HWE
HLA and immune-related pathway								
MICA rs41554616	6 (31379930)	NSV	G	0.244	0.058	7.7 (4.0-14.9)	8.2×10^{-9}	0.298
HLA-B rs12697943	6 (31324057)	NSV	A	0.233	0.070	5.4 (2.8-10.4)	1.1×10^{-8}	0.311
MMAA rs2270655	4 (146576418)	NSV	C	0.233	0.062	4.2 (2.4-7.4)	4.0×10^{-7}	0.020
CFL1 rs11550158	11 (65623512)	NSV	T	0.070	0.002	41.0 (8.0-210.0)	8.6×10^{-6}	0.964
EEF2	19 (3979893)	NSV	A	0.058	0.002	33.2 (6.2-177.0)	4.0×10^{-5}	0.965
EEF2	19 (3982302)	NSV	T	0.058	0.001	66.5 (7.6-584.4)	1.5×10^{-4}	0.982
HLA-A rs1059526	6 (29911218)	NSV	A	0.221	0.093	3.2 (1.7-5.9)	2.1×10^{-4}	0.021
PKM	15 (72494868)	NSV	C	0.047	0.002	25.9 (4.6-145.8)	2.2×10^{-4}	0.965
Other genetic associations								
NEB rs118191309	2 (152362730)	NSV	G	0.163	0.044	4.6 (2.3-9.1)	1.7×10^{-5}	0.999
NEB rs3732309	2 (152372974)	NSV	T	0.163	0.044	4.6 (2.3-9.1)	1.7×10^{-5}	0.999
MYH4 rs2277649	17 (10348354)	NSV	C	0.663	0.856	0.3 (0.2-0.5)	1.8×10^{-5}	0.592
RIF1 rs3732305	2 (152321090)	NSV	G	0.163	0.045	4.5 (2.2-8.9)	2.1×10^{-5}	0.965
UCN3 rs143074510	10 (5415798)	NSV	A	0.070	0.007	11.6 (3.7-36.2)	2.6×10^{-5}	0.876
SLC9B1 rs200075071	4 (103826769)	Stop gain	A	0.163	0.050	4.3 (2.2-8.7)	4.3×10^{-5}	0.233
PNPLA1 rs74946910	6 (36262153)	NSV	A	0.302	0.156	3.8 (2.0-7.2)	4.8×10^{-5}	0.917
MYH4 rs11651295	17 (10355371)	NSV	T	0.709	0.878	0.3 (0.2-0.6)	5.2×10^{-5}	0.557
NISCH rs866595152	3 (52521391)	NSV	A	0.058	0.005	13.2 (3.7-47.6)	8.0×10^{-5}	0.911
OR52R1 rs7941731	11 (4825225)	NSV	G	0.058	0.005	13.2 (3.7-47.6)	8.0×10^{-5}	0.911
OR2A7 rs201738556	7 (143955884)	NSV	C	0.293	0.080	3.4 (1.8-6.4)	9.6×10^{-5}	0.455
CLCA2 rs150305306	1 (86894204)	NSV	T	0.047	0.002	25.9 (4.6-145.8)	2.2×10^{-4}	0.965

CFL1, cofilin 1; *Chr*, chromosome; *Ctrl*, population control; *CLCA2*, chloride channel accessory 2; *EEF2*, eukaryotic translation elongation factor 2; *Fun*, functional variant type; *HLA-A*, HLA class I histocompatibility antigen-A; *HLA-B*, HLA class I histocompatibility antigen-B; *HWE*, Hardy-Weinberg equilibrium *P* values for 507 controls from the Taiwan general population; MAF, minor allele frequency; *MICA*, major histocompatibility complex class I chain–related protein A; *MMAA*, metabolism of cobalamin associated A; *MYH4*, myosin heavy chain 4; *NEB*, nebulin; *NISCH*, nischarin; *NSV*, nonsynonymous; *OR2A7*, olfactory receptor family 2 subfamily A member 7; *OR52R1*, olfactory receptor family 52 subfamily R member 1; *PKM*, pyruvate kinase M1/2; *PNPLA1*, patatin like phospholipase domain containing 1; *RIF1*, replication timing regulatory factor 1; *SLC9B1*; solute carrier family 9 member B1; *UCN*, urocortin.

cases involving co-trimoxazole, there are several metabolizing enzymes that have been reported; they include types of cytochrome P450 (eg, CYP1A2, CYP2D6, CYP2C8, and CYP2C9), myeloperoxidases (MPOs), flavin-containing monooxygenases, prostaglandin-endoperoxide synthases, glutathione, glutathione synthetase, glutamate-cysteine ligase modifier subunit, glutathione S-transferase mu 1, glutathione S-transferase pi 1 (GSTP1), glutathione S-transferase tau 1, glutamate cysteine ligase catalytic subunit (GCLC), mitochondrial amidoxime reducing component 1, mitochondrial amidoxime reducing component 2, *N*-acetyltransferase 1, and *N*-acetyltransferase 2 (NAT2).[21,32-35] However, the results of our WGS analysis of co-trimoxazole–induced SCAR from Taiwan for co-trimoxazole–metabolizing enzymes did not find significant exonic variants, except for *CYP2D6* rs1135822 ($P = .18$; OR = 5.4) (see Table E3 in the Online Repository at www.jacionline.org), which belongs to a nonsynonymous but not a deleterious variant. Similarly, for the Thai population, no significant exonic variant of co-trimoxazole–metabolizing enzymes was identified in our WGS analysis (data not shown).

We then analyzed the intronic variants of co-trimoxazole–metabolizing enzymes in the WGS results and found that the *GSTP1* rs8191438, *GSTP1* rs8191439, *NAT2* rs141791671, *CYP2C8* and *CYP1A2* deletion mutations, as well as *MPO rs14103145*, *GCLC* rs2397147, rs1980491, and rs761141, etc, were weakly associated with co-trimoxazole–induced SCAR ($P = .026$ to 4.8×10^{-4}; OR = 0.1-11.0) (see Table E4 in the Online Repository at www.jacionline.org).

Validation of the WGS results by HLA genotyping showed that *HLA-B*13:01* was strongly associated with co-trimoxazole–induced SCAR

To determine which HLA alleles were associated with co-trimoxazole–induced SCAR in the WGS discovery cohort, we determined the HLA-A, HLA-B, and HLA-C genotypes for case patients with SCAR by using sequencing–based typing ([see Table E5 in the Online Repository at www.jacionline.org) and those for the general population controls by using HLAscan software prediction (as described in the Methods section of the Online Repository at www.jacionline.org). The results showed that *HLA-B*13:01* ($P = 1.0 \times 10^{-9}$; $Pc = 2.5 \times 10^{-8}$; OR = 8.6) was strongly associated with co-trimoxazole–induced SCAR (see Tables E6-E8 in the Online Repository at www.jacionline.org).

The HLA genotyping results also revealed that *HLA-C*03:04* ($P = 3.3 \times 10^{-5}$; $Pc = 5.3 \times 10^{-4}$; OR = 4.0) was significantly associated with co-trimoxazole–induced SCAR. We found that most of the subjects carrying *HLA-B*13:01* also cocarried *HLA-C*03:04* allele (98.7% [543 of the 550 subjects]) but not vice versa. These 2 HLA alleles were in linkage disequilibrium ($D = 0.044$, $D' = 0.898$, $r^2 = 0.391$). *HLA-B*13:01* can eliminate the effect of *HLA-C*03:04* and is more significant to co-

trimoxazole–induced SCAR than is *HLA-C*03:04*, suggesting that *HLA-B*13:01* forms an extended haplotype with *HLA-C*03:04*.

HLA replication study of co-trimoxazole–induced SCAR in Taiwan, Thailand, and Malaysia

An additional 48 patients with co-trimoxazole–induced SCAR and 2038 population controls from Taiwan were enrolled and analyzed as a replication cohort to confirm the HLA associations (the detailed HLA genotypes are shown in Table E9 in the Online Repository at www.jacionline.org). The demographic and clinical data of the WGS discovery cohort in comparison with those of the replication cohort are shown in Table E10 (in the Online Repository at www.jacionline.org); there was no statistical difference in the clinical data between the 2 cohorts.

The replication cohort also showed a strong association between *HLA-B*13:01* and co-trimoxazole–induced SCAR ($P = 2.1 \times 10^{-12}$; OR = 9.1; 95% CI = 5.1-16.3 [Table III]). The combined analysis of the discovery and replication cohorts revealed a very strong association of *HLA-B*13:01* with co-trimoxazole–induced SCAR, with frequencies of 52.7% in the patients with SCAR and only 11.4% in the population controls ($P = 7.2 \times 10^{-21}$; $Pc = 1.8 \times 10^{-20}$; OR = 8.7, 95% CI = 5.7-13.4 [Table III and see Table E11 in the Online Repository at www.jacionline.org]). Furthermore, we analyzed the group with SCAR versus the co-trimoxazole–tolerant control group and found that the *HLA-B*13:01* genotype was significantly higher in the SCAR group than in the tolerant control group, with an OR of 11.7 (8.7% in the tolerant controls; $P = 1.3 \times 10^{-13}$; 95% CI = 5.7-24 [Table IV]).

We further found that the SNP rs41554616 in *MICA* and *HLA-B*13:01* allele were in strong linkage disequilibrium (D = 0.073; D' = 0.988; $r^2 = 0.958$) in the data sets of 91 case patients with SCAR, 507 population controls, and 138 tolerant controls (see Table E12 in the Online Repository at www.jacionline.org), suggesting that rs41554616 and *HLA-B*13:01* are regarded as the haplotype.

In addition to the associations involving *HLA-B*13:01*, we also identified an association between *HLA-B*38:02* and co-trimoxazole–induced SCAR in the combined cohort from Taiwan ($P = .003$; OR = 2.5; 95% CI = 1.4-4.3 [Table III]). The *HLA-B*38:02* allele was present in 17.6% of the patients with co-trimoxazole–induced SCAR, compared with in 7.9% of the population controls and 5.8% of the tolerant controls (Table IV). Through further phenotype stratification of co-trimoxazole–induced SCAR, we found that *HLA-B*13:01* had a stronger association with co-trimoxazole–induced DRESS, with 85.4% of the patients with DRESS found to carry *HLA-B*13:01* ($P = 1.1 \times 10^{-26}$; $Pc = 2.8 \times 10^{-25}$; OR = 45; 95% CI = 18.7-134) (Table IV), whereas *HLA-B*38:02* had a stronger association with co-trimoxazole–induced SJS and/or TEN, with *HLA-B*38:02* being observed in 24% of patients with SJS and/or TEN ($P = 5.0 \times 10^{-4}$; $Pc = .013$; OR = 3.7; 95% CI = 1.7-7.3) (Table IV). In addition, we found that *HLA-B*39:01* ($P = .010$; OR = 3.7) and *HLA-B*15:02* ($P = .008$; OR = 2.7) were weakly associated with co-trimoxazole–induced DRESS and SJS and/or TEN, respectively, with neither association reaching significance after Bonferroni adjustment.

We next examined the association between *HLA-B* and co-trimoxazole–induced SCAR by using samples from 52 patients with SCAR and 1135 population controls from Thailand and 8 patients with SCAR and 951 population controls from Malaysia. The results showed that *HLA-B*13:01* was significantly associated with co-trimoxazole–induced SCAR in both the Thai ($Pc = 8.0 \times 10^{-4}$; OR = 3.6; $P = 3.2 \times 10^{-5}$ [see Table E13 in the Online Repository at www.jacionline.org]) and Malaysian ($P = .002$; $Pc = .04$; OR = 12.8 [see Table E14 in the Online Repository at www.jacionline.org]) populations. We further analyzed the associations of other *HLA-B* alleles and found that *HLA-B*38:02* and *HLA-B*15:02* showed weak associations with co-trimoxazole–induced SJS and/or TEN in the Thai population (see Tables E13 and Table E14), with neither association reaching significance after Bonferroni adjustment. As a higher prevalence of patients with HIV or AIDs was observed in Thailand, we then analyzed the patients with HIV or /AIDs among the 39 case patients and 61 controls of the Thai population (see Table E15 in the Online Repository at www.jacionline.org). The results showed that *HLA-B*13:01* was also associated with co-trimoxazole–induced SCAR among the patients who were HIV-infected ($P = .004$; OR = 4.1).

Meta-analysis of *HLA-B* allele in co-trimoxazole–induced severe hypersensitivity

We further analyzed the association between *HLA-B*13:01* and co-trimoxazole–induced SCAR through a meta-analysis using a random-effects model and classified the case patients and controls according to their phenotype (SJS and/or TEN or DRESS) and area (Taiwan, Thailand, or Malaysia) (Fig 2). The results of this meta-analysis showed an overall OR of 6.62 ($z = 5.12$; $P = 1.5 \times 10^{-7}$) for the *HLA-B*13:01* association with co-trimoxazole–induced SCAR, a pooled OR of 3.47 ($z = 3.08$; $P = .001$) for the *HLA-B*13:01* association with co-trimoxazole–induced SJS and/or TEN, and a pooled OR of 40.11 ($z = 10.06$; $P = 4.2 \times 10^{-23}$) for the *HLA-B*13:01* association with co-trimoxazole–induced DRESS in Asians (Fig 2).

The PPV and NPV of *HLA-B*13:01*

The estimated incidence of co-trimoxazole–induced SCAR was 0.90% (with 45 cases of co-trimoxazole–induced SCAR, including 25 cases of DRESS and 20 cases of SJS and/or TEN) among 5005 new users from 2017 to 2019 at Chang Gung Memorial Hospital in Taiwan. Further analysis to determine the positive predictive value (PPV) and negative predictive value (NPV) of *HLA-B*13:01* for the prevention of co-trimoxazole–induced SCAR indicated that those values were 4.05% and 99.52%, respectively. Moreover, the PPV and NPV for co-trimoxazole–induced DRESS were 3.64% and 99.92%, respectively. Theoretically, 1 patient could be prevented from experiencing SCAR if at least 211 individuals were tested (see Table E16 in the Online Repository at www.jacionline.org). And 1 patient could be prevented from experiencing DRESS if at least 235 individuals were screened.

The binding mode and *in vitro* test of HLA-B*13:01 to sulfamethoxazole and its reactive metabolite

We then performed *in silico* modeling to investigate the potential interaction between HLA-B*13:01 and co-trimoxazole by using homology modeling of the SWISS-MODEL web server.

TABLE III. The significant HLA alleles associated with co-trimoxazole–induced severe cutaneous adverse reactions in WGS discovery, replication, and combined samples from Taiwan

	WGS discovery cohort				Replication cohort				Combination				
	Individual, no. %				Individual, no. %				Individual, no. %				
HLA	SCAR (n = 43)	Ctrls (n = 507)	OR (95% CI)	*P* value	SCAR (n = 48)	Ctrls (n = 2038)	OR (95% CI)	*P* value	SCAR (n = 91)	Ctrls (n = 2545)	OR (95% CI)	*P* value	*Pc* value
HLA-B*13:01	22 (51.2%)	55 (10.9%)	8.6 (4.2-17.5)	**1.0×10^{-9}**	26 (54.2%)	234 (11.5%)	9.1 (5.1-16.3)	**2.1×10^{-12}**	48 (52.7%)	289 (11.4%)	8.7 (5.7-13.4)	**7.2×10^{-21}**	**1.8×10^{-20}**
HLA-B*38:02	8 (18.6%)	48 (9.5%)	2.2 (0.8-5.2)	.066	8 (16.7%)	154 (7.6%)	2.4 (1.1-5.3)	**.029**	16 (17.6%)	202 (7.9%)	2.5 (1.4-4.3)	**.003**	—
HLA-C*03:04	22 (51.2%)	105 (20.7%)	4.0 (2.0-8.0)	**3.3×10^{-5}**	23 (16.7%)	437 (21.4%)	3.4 (1.9-6.0)	**6.0×10^{-5}**	45 (49.5%)	542 (20.4%)	3.6 (2.4-5.5)	**7.9×10^{-9}**	**1.3×10^{-7}**

The HLA data for the replication cohort were obtained by high-resolution sequencing-based typing or HLA next-generation sequencing of genotypes, which were carried out by using HOLOTYPE HLATM X2-96/7. The main ethnicity of the enrolled case patients with SCAR and controls from Taiwan was Chinese. *P* values were calculated by using the Fisher exact test. Boldface indicates statistical significance.

TABLE IV. Associations of *HLA-B* alleles with phenotypes of co-trimoxazole–induced severe cutaneous adverse reactions from Taiwan

Taiwan subgroup	Case no. (%)	General population Ctrl, no. (%)	Tolerant Ctrl, no. (%)	OR* (95% CI)*	*P* value*	*Pc* value*	OR† (95% CI)†	*P* value†	*Pc* value†
HLA-B*13:01									
SCAR	48/91 (52.7%)	289/2545 (11.4%)	12/138 (8.7%)	8.7 (5.7-13.4)	**7.2×10^{-21}**	**1.8×10^{-20}**	11.7 (5.7-24)	**1.3×10^{-13}**	**3.3×10^{-12}**
SJS/TEN	13/50 (26.0%)			2.7 (1.3-5.4)	**.006**	—	3.7 (1.6-8.8)	**.003**	—
DRESS	35/41 (85.4%)			45 (18.7-134)	**1.1×10^{-26}**	**2.8×10^{-25}**	61 (21.5-175)	**7.2×10^{-21}**	**1.8×10^{-19}**
HLA-B*38:02									
SCAR	16/91 (17.6%)	202/2545 (7.9%)	8/138 (5.8%)	2.5 (1.4-4.3)	**.003**	—	3.5 (1.4-8.5)	**.007**	—
SJS/TEN	12/50 (24.0%)			3.7 (1.7-7.3)	**5.0×10^{-4}**	**.013**	5.1 (2.0-13.5)	**8.9×10^{-4}**	**.02**
DRESS	4/41 (9.8%)			1.3 (0.3-3.5)	.564	—	1.5 (0.5-5.1)	.475	—
HLA-B*39:01									
SCAR	8/91 (8.8%)	114/2545 (4.5%)	5/138 (3.6%)	2.1 (0.8-4.4)	.069	—	2.6 (0.8-8.1)	.143	—
SJS/TEN	2/50 (4.0%)			0.9 (0.1-3.5)	1	—	1.1 (0.2-5.9)	1	—
DRESS	6/41 (14.6%)			3.7 (1.2-9.0)	**.010**	—	4.6 (1.3-15.8)	**.019**	—
HLA-B*15:02									
SCAR	12/91 (13.2%)	212/2545 (8.3%)	10/138 (7.2%)	1.7 (0.8-3.1)	.122	—	1.9 (0.8-4.7)	.170	—
SJS/TEN	10/50 (20.0%)			2.7 (1.2-5.7)	**.008**	—	3.2 (1.2-8.2)	**.017**	—
DRESS	2/41 (4.9%)			0.7 (0.1-2.6)	.766	—	0.7 (0.1-3.1)	.736	—

The main ethnicity of the enrolled case patients and controls from Taiwan was Chinese. *P* values were calculated by using the Fisher exact test. Boldface indicates statistical significance.

*Data obtained from comparison of case patients with the general population from Taiwan.

†Data obtained from comparison of case patients with relevant tolerant controls.

Because the crystal structure of HLA-B*13:01 has yet to be determined, we used HLA-B*44:03 (PDB entry 4JQX), which shares 96.04% identity with HLA-B*13:01 (the most similar alternative, which is shown in Table E17 in the Online Repository at www.jacionline.org), as a template. Docking was automatically performed after uploading of the coordinates and topology files of the compound and HLA-B*13:01. The docking models with the highest scores in terms of HLA-B*13:01 interacting with sulfamethoxazole and its reactive metabolite, 4-nitro sulfamethoxazole, are shown in Fig 3. In the HLA-B*13:01–sulfamethoxazole complex, the residues R121 and Y123 were found to be crucial for forming hydrogen bonds, and the residues Y31, Y33, T48, I90, and Y183 were found to be within contact distance for hydrophobic interactions. In the HLA-B*13:01–4-nitro sulfamethoxazole complex, however, the residues T48, S91, and N94 were hydrogen-bonded to 4-nitro sulfamethoxazole. The 2 additional oxygen atoms in 4-nitro sulfamethoxazole, as compared with in sulfamethoxazole, contributed all 3 hydrogen bonds to HLA-B*13:01. In addition, only 2 residues (I90 and Y123) were suggested by the program of the protein-ligand interaction profiler[36] to be involved in hydrophobic interactions. The docking models also revealed that HLA-B*13:01 could interact with trimethoprim and dapsone, but both of the binding pockets were different from those of sulfamethoxazole and 4-nitro sulfamethoxazole (see Fig E5 in the Online Repository at www.jacionline.org).

We then investigated the potential interaction between sulfamethoxazole and HLA-B*38:02 (by using HLA-B*39:01 [PDB entry 4O2C] as a template, as shown in Table E17). The docking models showed that residues Y31, R86, Y123, and Y183 of HLA-B*38:02 were hydrogen-bonded to sulfamethoxazole (see Fig E6 in the Online Repository at www.jacionline.org). HLA-B*38:02 shared 94% identity with HLA-B*13:01 in terms of amino acid sequence alignment (see Table E18 in the Online Repository at www.jacionline.org), and the proposed sulfamethoxazole interaction modes between HLA-B*13:01 and HLA-B*38:02 were found to be quite different.

We further determined the main drug antigen(s) related to co-trimoxazole–induced SCAR (for a total of 10 patients, including 5 patients who carried *HLA-B*13:01* and 2 patients who carried *HLA-B*38:02*) through an *in vitro* lymphocyte activation assay. Our results showed that both sulfamethoxazole and 4-nitro sulfamethoxazole, but not trimethoprim, could activate the T cells of patients with co-trimoxazole–induced SCAR (see Fig E7 in the Online Repository at www.jacionline.org), suggesting that sulfamethoxazole was the major causative drug for the enrolled patients with SCAR. We then investigated the cross-reactivity of co-trimoxazole and dapsone in another 6 patients with co-

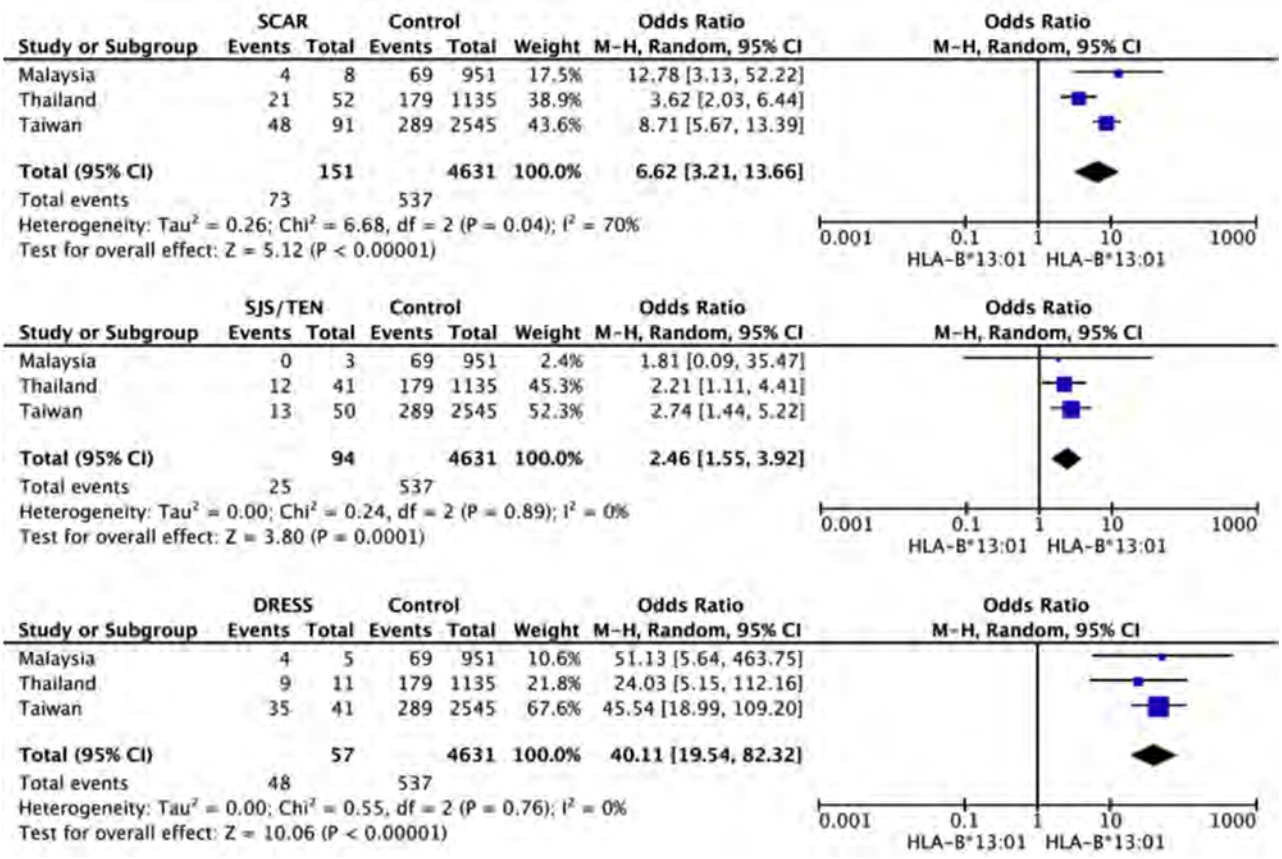

FIG 2. Distribution of the *HLA-B*13:01* in patients with co-trimoxazole–induced severe hypersensitivity reactions and general population controls. Patients with co-trimoxazole–induced severe hypersensitivity reactions who carried *HLA-B*13:01* were recruited in Taiwan, Thailand, and Malaysia. Study weighting (indicated by size of data markers) refers to the proportion of participants who were recruited from each study. The tau-squared and I^2 values represent measures of heterogeneity. The diamonds represent pooled ORs (Mantel-Haenszel [M-H] method, random effects), and the error bars indicate 95% CIs.

trimoxazole–induced SCAR who carried *HLA-B*13:01* (these patients were different from those in Fig E7). The results showed that the patients with co-trimoxazole–induced SCAR who carried *HLA-B*13:01* had a high cross-reactivity to dapsone (sensitivity = 83.3% [see Fig E8 in the Online Repository at jacionline.org]). This result was consistent with the genetic finding.

DISCUSSION

SCAR (including SJS and/or TEN and DRESS) is a life-threatening condition that is most commonly caused by medications. Although the incidence of SCAR is low at an estimated 0.4 to 6 cases per million persons per year, it may cause high mortality and permanent disabling complications.[37,38] Co-trimoxazole is used as an antibiotic treatment for a variety of infections worldwide. However, co-trimoxazole is one of the leading causative drugs for life-threatening SCAR.[9] The pathomechanism of co-trimoxazole–induced SCAR remains unknown. There have been a number of studies that only showed weak genetic associations of HLAs (*HLA-B*38*, *HLA-A*11:01*, *HLA-B*14:01/HLA-B*35:01*, and *HLA-B*15:02/HLA-C*06:02/HLA-C*08:01*) and drug-metabolizing enzymes with co-trimoxazole hypersensitivity reactions.[19,20,32,39-42] The failure to find strong and applicable genetic markers in those past studies may have been due to their limited sample sizes or lack of severe phenotypes among the studied cases. In this study, we conducted an international study to enroll patients with co-trimoxazole–induced severe hypersensitivity reactions, including SJS and/or TEN (94 cases) and DRESS (57 cases), from multiple centers in Asian countries. We used a WGS approach to determine a novel genetic marker, the SNP *rs41554616*, which is located between the *HLA-B* and *MICA* loci of exonic variants; it showed the strongest association with co-trimoxazole–induced SCAR. The significance of the genome-wide analysis may have been affected by the sample size because of the paucity of cases of co-trimoxazole–induced SCAR available for study. A further HLA genotyping revealed that *HLA-B*13:01* was strongly associated with co-trimoxazole–induced SCAR in the investigated Asian populations (including Chinese, Thai, and Malaysian populations).

*HLA-B*13:01* has also been reported to be associated with hypersensitivity induced by other drugs, including dapsone and salazosulfapyridine,[17,30,43,44] but the strength levels of the associations were quite different. Co-trimoxazole consists of sulfamethoxazole and trimethoprim. A few studies have revealed that trimethoprim may also be implicated as a culprit drug in some cases[45,46]; however, the sulfonamide moiety of co-trimoxazole–sulfamethoxazole or that its reactive metabolite (4-nitro sulfamethoxazole) has been reported as the major drug antigen(s) of

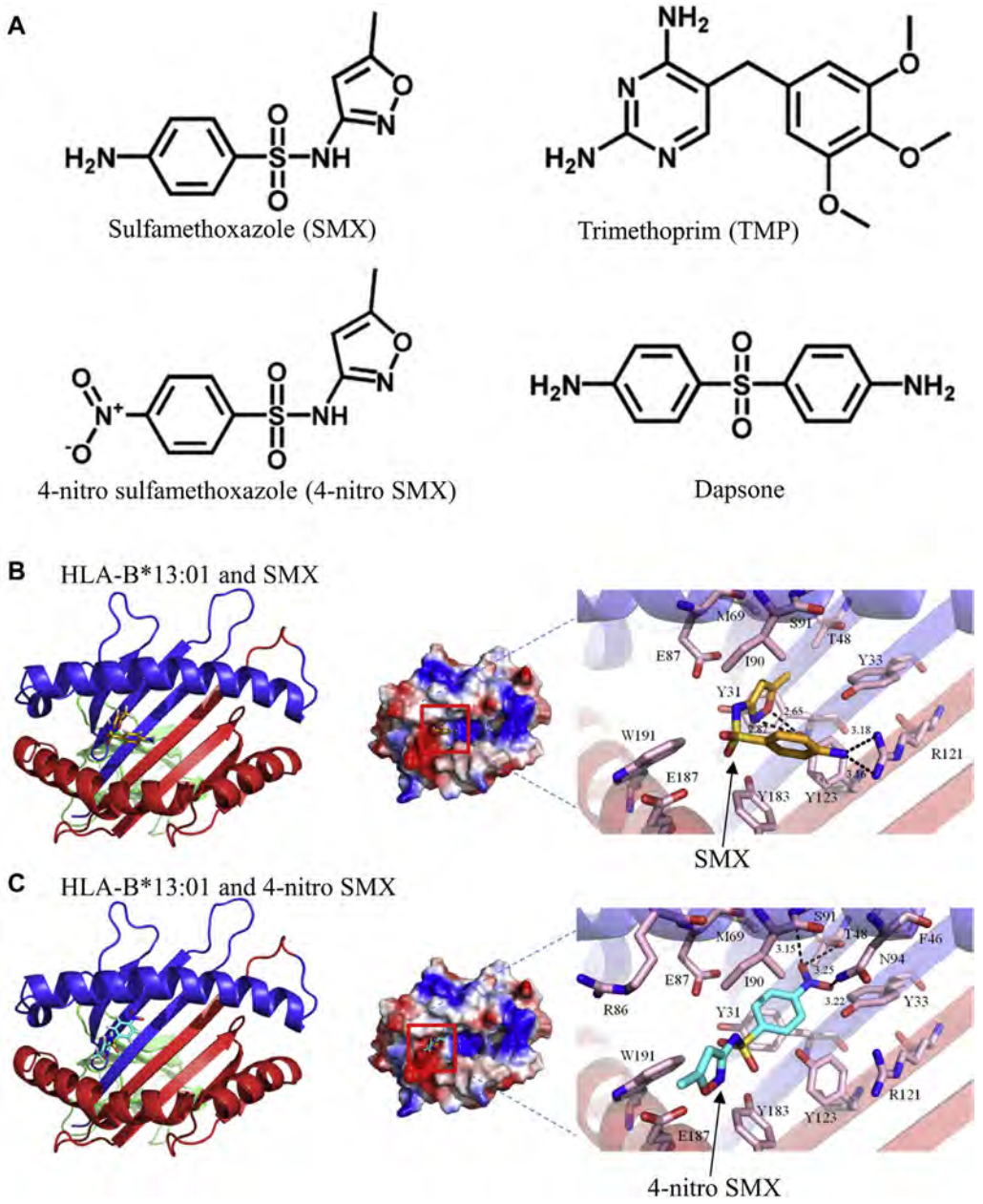

FIG 3. The docking modeling of HLA-B*13:01 binding with sulfamethoxazole (SMX) and its reactive metabolite (4-nitro SMX). **A,** Molecular structures of SMX, 4-nitro sulfamethoxazole (4-nitro SMX [the reactive metabolite of SMX]), trimethoprim (TMP), and dapsone. Residues within a contact distance (<4 Å) with the sulfonamide functional group of SMX/4-nitro SMX are labeled. **B,** Residues R121 and Y123 of HLA-B*13:01 were found to be crucial for forming hydrogen bonds to SMX. **C**, Residues T48, S91, and N94 of HLA-B*13:01 are hydrogen-bonded to 4-nitro SMX.

hypersensitivity reactions.[39,47] By performing an *in vitro* T-cell activation assay, we confirmed that the major drug antigen for triggering immune responses of co-trimoxazole–induced SCAR was sulfamethoxazole or its metabolite 4-nitro sulfamethoxazole, rather than trimethoprim. The docking models revealed that sulfamethoxazole and 4-nitro sulfamethoxazole were docked into the same A pocket in HLA-B*13:01, but the binding pocket was different from that of dapsone[17,48] and trimethoprim. Moreover, we found, through an *in vitro* LAT assay, that patients with co-trimoxazole–induced SCAR who carried *HLA-B*13:01* showed a high cross-reactivity to dapsone (83.3%). This may be a reason for the different effects of these small-molecule drugs on HLA-B*13:01 in terms of the hypersensitivity reactions.

The main ethnicity of people living in Taiwan is Han Chinese (>95%). Our previous genetic study involving a principal component analysis conducted by using Affymetrix 6.0 SNP chip genotyping data revealed that the genetic background of Taiwanese people is between Southern, Central, and Northern Han Chinese from Mainland China.[18] According to the Allele Frequency Net Database (http://www.allelefrequencies.net/), the frequency levels of *HLA-B*13:01* are relatively high (8%-15%) in Taiwan and China, as well

as in Hong Kong. Furthermore, the frequency levels of *HLA-B*13:01* are also high (>5%) in other areas of Southeast Asia (including Thailand, Indonesia, etc) and northern Australia but are lower in people of European, African, or American Indian ancestry. Co-trimoxazole–induced DRESS is still one of the most common forms of SCAR in European and African populations; therefore, it is likely that risk-related HLA alleles other than *HLA-B*13:01* are relevant in these populations. The specific risk allele(s) and genes present in these populations require further investigation.

Our recent study reported that TCR contributes to the pathogenesis of severe hypersensitivity reactions, especially in cases of SJS and/or TEN.[49] Furthermore, Watkins et al[50] previously used molecular dynamics modeling to demonstrate that TCR containing Vβ20-1 may play a role in sulfamethoxazole-induced hypersensitivity. In fact, the docking models revealed that sulfamethoxazole, 4-nitroso sulfamethoxazole, and trimethoprim can all bind with HLA-B*13:01, but the *in vitro* LAT results showed only that sulfamethoxazole and 4-nitroso sulfamethoxazole can activate the T cells of patients with co-trimoxazole–induced SCAR. Although the detailed interaction model of HLA and TCR in co-trimoxazole–induced SCAR is still unclear, the immune synapse of HLA and TCR should play an important role in the pathomechanism of co-trimoxazole–induced SCAR.

Phenotype-specific genetic associations have been found in drug hypersensitivity. We previously reported that HLA genotypes associated with specific phenotypes of carbamazepine-induced SCAR, such as *HLA-B*15:02* and *HLA-B*57:01*, were strongly associated with carbamazepine-induced SJS and/or TEN,[10,51] whereas *HLA-A*31:01* was associated with carbamazepine-induced DRESS.[52] In this study, we found that *HLA-B*13:01* is strongly associated with co-trimoxazole–induced SCAR, especially with DRESS. Interestingly, we also identified *HLA-B*38:02* as being weakly associated with co-trimoxazole–induced SJS and/or TENbut not with co-trimoxazole–induced DRESS. The docking models also confirmed that sulfamethoxazole could be docked into HLA-B*38:02. In addition, Ogese et al[53] previously reported that 4-nitroso sulfamethoxazole-specific $CD4^+$ T cells were activated in patients with mild maculopapular exanthema who carried *HLA-DQB1*05:01* and *HLA-DQB1*02:01*, suggesting that HLA-DQ–restricted, $CD4^+$ T-cell responses can also be observed under sulfamethoxazole stimulation. These results revealed that the different types of HLA may be linked to phenotype-specific associations in co-trimoxazole–induced hypersensitivity reactions.

In addition to *HLA-B*, our WGS-based study discovered that certain genetic deleterious variants, such as *EEF2* and *SLC9B1,* showed weak associations with co-trimoxazole–induced SCAR among cases from Taiwan and Thailand. EEF2 is an elongation factor that can control the activation of transcription factor NF-κB and regulate inflammation[54]; SLC9B1 is a transporter of glucose and other sugars, bile salts, and organic acids.[55] These associated genetic variants may be involved in immune-related or metabolic pathways, contributing to the pathogenesis of co-trimoxazole–induced SCAR. Through WGS results, we also identified the genetic variants of drug-metabolizing enzymes involved in co-trimoxazole–induced SCAR. One previous study suggested that the intronic variant of *GCLC* rs761142 is associated with co-trimoxazole–induced hypersensitivity.[32] Our WGS results demonstrated that only the exonic variant in *CYP2D6* rs1135822 showed a weak association with SCAR. Interestingly, we further found that several other intronic variants of drug-metabolizing enzymes, such as *GSTP1* rs8191438, rs8191439, *CYP2C8* and *CYP1A2* deletion variant, *GCLC* rs2397147, rs1980491, rs761141, *NAT2* rs141791671, *MPO* rs14103145, etc, were associated with co-trimoxazole–induced SCAR. Further investigation of the functional roles of these intronic variants in metabolizing enzymes of co-trimoxazole is needed.

In conclusion, this study identified a novel genetic marker, *HLA-B*13:01*, that is strongly associated with co-trimoxazole–induced SCAR in Asians, and it provided relevant information for the potential implementation of preemptive multiple genetic tests to identify patients who are at risk of development of these life-threatening severe hypersensitivity reaction conditions caused by sulfonamide antibiotics.

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Clinical implications: *HLA-B*13:01* has been identified as being strongly associated with co-trimoxazole–induced SCAR in Asians. A preemptive screening of genes may be applied preventatively to clinically test for sulfonamide antibiotic–induced severe hypersensitivity.

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