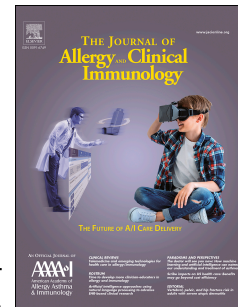


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Whole Genome Sequencing Identifies Genetic Variants Associated with Co-trimoxazole Hypersensitivity in Asians

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Abstract (249 words)

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 reactions (SCAR), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reactions with eosinophilia and systemic symptoms (DRESS). 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 multi-country case-control association study including 151 cases of co-trimoxazole-induced SCAR and 4631 population controls from Taiwan, Thailand, and Malaysia, as well as 138 tolerant controls from Taiwan. A whole-genome sequencing (WGS) was performed for the patients and population controls from Taiwan and further validated the results from Thailand and Malaysia.

Results: The WGS study (43 cases vs. 507 controls) discovered that SNP rs41554616, located between the *HLA-B* 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*, *NAT2*, and *CYP2C8*). A replication study using HLA genotyping revealed that *HLA-B*13:01* was strongly associated with co-trimoxazole-induced SCAR (combined samples: 91 cases vs. 2545 controls, $P=7.2\times 10^{-21}$; OR=8.7). The strong HLA association was also observed in the cases from Thailand ($P=3.2\times 10^{-5}$; OR=3.6) and Malaysia ($P=0.002$; 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 DRESS ($P=4.2\times 10^{-23}$, OR=40.1).

Conclusion: This study identified *HLA-B*13:01* is an important genetic factor associated with co-trimoxazole-induced SCAR in Asians.

Clinical Implications

This study identified *HLA-B*13:01* is 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.

Capsule Summary

We identified a novel genetic marker, *HLA-B*13:01*, as being strongly associated with co-trimoxazole-induced SCAR. Furthermore, a phenotype-specific association was found. A meta-analysis showed a pooled odds ratio of 40.1 ($P=4.2\times 10^{-23}$) for an *HLA-B*13:01* association with co-trimoxazole-induced DRESS.

Key words

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

Abbreviations:

- 149 95% CI, 95% confidence interval
- 150 AIDS, acquired immune deficiency syndrome
- 151 ALDEN, algorithm of drug causality for epidermal necrolysis
- 152 CGMH, Chang Gung Memorial Hospital
- 153 CTL, cytotoxic T lymphocyte
- 154 CYP, cytochrome P450
- 155 GCLC, glutamate cysteine ligase catalytic subunit
- 156 GCLM, glutamate-cysteine ligase modifier subunit
- 157 GSH, glutathione
- 158 GSS, glutathione synthetase
- 159 GSTM1, glutathione S-transferase mu 1
- 160 GSTP1, glutathione S-transferase pi 1
- 161 GSTT1, glutathione S-transferase tau 1
- 162 DP, depth
- 163 DRESS, drug reaction with eosinophilia and systemic symptoms
- 164 ELISA, enzyme-linked immunosorbent assay
- 165 FMO, flavin-containing monooxygenase
- 166 HIV, human immunodeficiency virus
- 167 HLA, human leukocyte antigen
- 168 HWE, Hardy-Weinberg equilibrium
- 169 InDel, insertion-deletion
- 170 IRB, institutional review board

- 171 GQ, quality score
- 172 LAT, lymphocyte activation test
- 173 MARC, mitochondrial amidoxime reducing component
- 174 MICA, MHC class I polypeptide-related sequence A
- 175 MMAA, metabolism of cobalamin associated A
- 176 MPE, maculopapular exanthema
- 177 NAT, N-acetyltransferase 1
- 178 NNT, Number needed to test
- 179 NPV, negative predictive value
- 180 MPO, myeloperoxidases
- 181 OR, odds ratio
- 182 PJP, *Pneumocystis jiroveci* pneumonia
- 183 PPV, positive predictive value
- 184 SBT, HLA sequencing-based typing
- 185 SCAR, severe cutaneous adverse reactions
- 186 SKAT, SNP-set kernel association test
- 187 SNP, single nucleotide polymorphism
- 188 SPR, surface plasmon resonance
- 189 SJS, Stevens-Johnson syndrome
- 190 PCA, principal component analysis
- 191 PTGS, prostaglandin-endoperoxide synthase
- 192 TEN, toxic epidermal necrolysis
- 193 WGS, whole-genome sequencing

194 INTRODUCTION

195 Sulfonamide antibiotics, such as co-trimoxazole (which comprises 5 parts of sulfamethoxazole
196 and 1 part trimethoprim), sulfadimethoxine, and sulfafurazole, are widely prescribed for the
197 treatment of a variety of infections, such as pneumonia, bronchitis, traveler's diarrhea,
198 shigellosis, and ear/urinary tract infections. Co-trimoxazole remains a common first-line
199 medicine used for prophylaxis against *Pneumocystis jiroveci* pneumonia (PJP), especially in
200 human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) patients¹.
201 ². Although effective in treating infectious diseases, sulfonamide antibiotics may cause
202 hypersensitivity reactions ranging from mild rash (maculopapular exanthema) to
203 life-threatening severe cutaneous adverse reactions (SCAR), including Stevens-Johnson
204 syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reactions with eosinophilia and
205 systemic symptoms (DRESS), that hinder their clinical use. SJS and TEN are considered as the
206 same disease spectrum and often involve generalized blistering mucocutaneous reactions with
207 relatively high mortality rates of approximately 5-12.5% for SJS and 50% for TEN³. DRESS is
208 characterized by fever, skin rash, atypical lymphocytes, eosinophilia, and systemic involvement
209 and has a mortality rate of up to 10%^{4, 5}. The incidence of co-trimoxazole-induced
210 hypersensitivity is about in 1–3% among the exposed general population⁶, which rises in
211 HIV/AIDS patients^{7, 8}. We recently analyzed the SJS/TEN registration databases of multiple
212 Asian countries for the period from 1998–2017. A total of 1028 SJS/TEN cases were analyzed
213 for their drug causality, and the results indicated that co-trimoxazole was the 4th most common
214 causative drug for SJS/TEN⁹.

215 To date, there is no method available to prevent severe hypersensitivity reactions caused by
216 sulfonamide antibiotics. SCAR is considered as delayed-type hypersensitivity, and its

pathogenesis involves the activation of effector T lymphocytes by culprit drugs¹⁰⁻¹⁴. Advances in pharmacogenetic studies have revealed genetic links to drug hypersensitivity reactions. Human leukocyte antigen (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/TEN¹⁰ and *HLA-B*58:01* and allopurinol-induced SCAR¹¹. Other HLA alleles have also been found 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 syndrome¹⁷. In addition to HLA alleles, our group also identified strong correlations among genetic variants of drug metabolizing enzymes with phenytoin-induced hypersensitivity¹⁸. 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 antibiotics-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/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 America²¹. To investigate the genetic predisposition to hypersensitivity reactions induced by sulfonamide antibiotics in Asian populations, we carried out a multi-country case-control study of patients with co-trimoxazole-induced severe hypersensitivity reactions from Taiwan, Thailand, and Malaysia by using large-scale

239 whole-genome sequencing (WGS) and then further validated the results in different
240 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/TEN and 41 with DRESS), Thailand (41 with SJS/TEN and 11 with DRESS), and Malaysia (3 with SJS/TEN and 5 with DRESS), respectively. Phenotypes of SCAR were classified according to the consensus definitions of RegiSCAR study criteria²²⁻²⁶. The drug causality for each enrolled case was determined using the algorithm of drug causality for epidermal necrolysis (ALDEN) score published by the RegiSCAR study group or the Naranjo algorithm²⁷⁻²⁹. Among all the patients from Taiwan, 52 available patients with co-trimoxazole-related SCAR had undergone an *in vitro* lymphocyte activation test (LAT)^{30, 31} to confirm their culprit drug.

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

Whole-genome sequencing (WGS), variant calling/functional annotation, HLA prediction and genotyping, and computer modeling/docking

We performed WGS analyses for 43 SCAR cases and 507 population controls from Taiwan as well as for 45 SCAR cases from Thailand. 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, and 75,260 variants from Taiwan were determined to be exonic regions according to ANNOVAR and RefSeq. 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, lymphocyte activation test (LAT), WGS DNA libraries quantification, WGS variant calling/functional annotation, HLA prediction and genotyping, computer modeling and docking are provided in the Methods section in this article's Online Repository.

Statistical Analysis

We conducted the statistical analysis for any associations by comparing the allele or genotype frequencies between cases 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 (ORs) calculation, and meta-analysis are provided in the Methods section in this article's Online Repository.

RESULTS

Patient recruitment and lymphocyte activation test (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 1**. Among these 151 patients with SCAR, 4 died as a result of the episode. The underlying diseases of the enrolled cases 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 (Table E1 in the Online Repository). The average initial daily drug dosages were 1375 ± 603 mg and 1575 ± 284 mg for SCAR patients and tolerant controls, respectively. The average onset time of cutaneous manifestation was 15.9 ± 12.3 days after exposure to co-trimoxazole (**Table 1**). We also performed an *in vitro* lymphocyte activation test (LAT) for each of the 52 SCAR patients from Taiwan to confirm that the culprit drug was co-trimoxazole by measuring the CD8⁺ cytotoxic T lymphocyte (CTL) activation marker-granulysin, which is known as a key mediator for keratinocyte detachment in SJS/TEN^{14, 32} and as a specific cytotoxicity protein in DRESS³³. Our results showed that the sensitivity and specificity of granulysin-based LAT for co-trimoxazole-induced SCAR patients were 69.2% (36/52) and 100%, respectively (Figure E1 in the Online Repository).

Case-control association study by whole-genome sequencing

For the initial WGS study, we first used 43 cases of co-trimoxazole-induced SCAR (n=20

SJS-TEN and n=23 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 Figure E2 in the Online Repository. Through the WGS, we identified a total of 13,601,921 variants (including 11,226,208 SNPs and 2,375,713 insertion-deletion [InDel]) in the patients and population controls from Taiwan. We first focused on analyzing the functional exonic regions, and 75,260 variants (73,363 SNPs and 1,897 InDel) 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 (PCA) map showed that there was no difference in the distribution of ancestry among the SCAR patients and the population controls (Figure E3 in the Online Repository). The WGS discovered a cluster of exonic SNPs on chromosome 6 (**Figure 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 ($P=8.2\times 10^{-9}$; odds ratio [OR]=7.7, 95% confidence interval [CI]=4.0-14.9) (**Figure 1** and **Table 2**). We then identified the top 20 functional variants (**Table 2**, the definition of deleterious variants is described in the Methods and Table E2 in the Online Repository), and found that the variants of *HLA-A*, *MICA*, *MMAA*, *CFL1*, *EEF2*, and *PKM*, which may be involved in HLA- or immune-related pathways (Figure E4 in the Online Repository, implicated by Phenolyzer), and *MYH4*, *RIF1*, *UCN3*, *NISCH*, *OR52R1*, *SLC9B1*, etc., which are related to other pathways that are suggested to not be involved in HLA- or immune-related pathways (Figure E4 in the Online Repository), are associated with co-trimoxazole-induced SCAR among patients in Taiwan.

We further performed a WGS study for another replication cohort of 45 SCAR cases from

Thailand (comparing them to 290 Thai general population controls 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 (Table E2 in the Online Repository).

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 cases involving co-trimoxazole, there are several metabolizing enzymes have been reported, such as cytochrome P450s (e.g., *CYP1A2*, *CYP2D6*, *CYP2C8*, and *CYP2C9*), myeloperoxidases (MPO), flavin-containing monooxygenases (FMOs), prostaglandin-endoperoxide synthases (PTGSs), glutathione (GSH), glutathione synthetase (GSS), glutamate-cysteine ligase modifier subunit (GCLM), glutathione S-transferase mu 1 (GSTM1), glutathione S-transferase pi 1 (GSTP1), glutathione S-transferase tau 1 (GSTT1), glutamate cysteine ligase catalytic subunit (GCLC), mitochondrial amidoxime reducing component 1 (mARC1), mARC2, N-acetyltransferase 1 (NAT1), and NAT2^{21, 34-37}. 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 *CYP2D6* rs1135822 ($P=0.018$; OR=5.4) (Table E3 in the Online Repository), 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,

rs761141, etc., were weakly associated with co-trimoxazole-induced SCAR (P =range from 0.026 to 4.8×10^{-4} , OR=range from 0.1 to 11.0) (Table E4 in the Online Repository).

Validation of WGS study by HLA genotyping showed *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, B, and C genotypes for SCAR cases (Table E5 in the Online Repository; by sequencing-based typing) and the general population controls (by HLAsCan software prediction, described in the Methods of the Online Repository), and the results showed that *HLA-B*13:01* ($P=1.0 \times 10^{-9}$, $P_c=2.5 \times 10^{-8}$; OR=8.6) was strongly associated with co-trimoxazole-induced SCAR (Table E6~E8 in the Online Repository).

The HLA genotyping results also revealed that *HLA-C*03:04* ($P=3.3 \times 10^{-5}$, $P_c=5.3 \times 10^{-4}$; OR=4.0) was significantly associated with co-trimoxazole-induced SCAR. We found that most of the subjects carrying *HLA-B*13:01* also co-carried *HLA-C*03:04* allele (98.7%, 543 of the 550 subjects), but not vice versa. These two 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 *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 co-trimoxazole-induced SCAR patients 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). The demographic and clinical data of the WGS discovery cohort in comparison to the replication cohort are shown in Table E10 in the Online Repository, and there was no statistical difference in the clinical data between the two 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 3**). 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 SCAR patients and only 11.4% in the population controls ($P=7.2\times 10^{-21}$, $P_c=1.8\times 10^{-20}$; OR=8.7, 95% CI=5.7-13.4; **Table 3** and Table E11 in the Online Repository). Furthermore, we analyzed the SCAR group in comparison to the co-trimoxazole-tolerant control group, and found that the *HLA-B*13:01* genotype was significantly higher in the SCAR group compared with 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 4**).

We further found that the *MICA* rs41554616 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 SCAR cases, 507 population controls, and 138 tolerant controls (Table E12 in the Online Repository), suggesting that rs41554616 and *HLA-B*13:01* are regarded as the haplotype.

In addition to *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 ($P=0.003$; OR=2.5, 95% CI=1.4-4.3; **Table 3**). The *HLA-B*38:02* allele was present in 17.6% of co-trimoxazole-induced SCAR patients, compared with 7.9% of population controls and 5.8% of tolerant controls (**Table 4**). Through further phenotype stratification of co-trimoxazole-induced SCAR, we found

*HLA-B*13:01* had a stronger association with co-trimoxazole-induced DRESS, with 85.4% of the DRESS patients found to carry *HLA-B*13:01* ($P=1.1\times 10^{-26}$, $P_c=2.8\times 10^{-25}$; OR=45, 95% CI=18.7-134) (**Table 4**), and that *HLA-B*38:02* had a stronger association with co-trimoxazole-induced SJS/TEN, with *HLA-B*38:02* being observed in 24% of SJS/TEN patients ($P=5.0\times 10^{-4}$, $P_c=0.013$; OR=3.7, 95% CI=1.7-7.3) (**Table 4**). In addition, we found *HLA-B*39:01* ($P=0.010$; OR=3.7) and *HLA-B*15:02* ($P=0.008$; OR=2.7) were weakly associated with co-trimoxazole-induced DRESS and SJS/TEN, respectively, with neither association reaching significance after Bonferroni adjustment.

We next examined the association between *HLA-B* and co-trimoxazole-induced SCAR 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 ($P_c=8.0\times 10^{-4}$; OR=3.6, $P=3.2\times 10^{-5}$; Table E13 in the Online Repository) and Malaysian populations ($P=0.002$; $P_c=0.04$; OR=12.8; Table E14 in the Online Repository). We further analyzed the associations of other *HLA-B* alleles, and found that *HLA-B*38:02* and *B*15:02* showed weak associations with co-trimoxazole-induced SJS/TEN in Thai population (Table E13 and Table E14 in the Online Repository), with neither association reaching significance after Bonferroni adjustment. As a higher prevalence of HIV/AIDS patients was observed in Thailand, we then analyzed the HIV/AIDS patients among the 39 cases and 61 controls of the Thai population (Table E15 in the Online Repository). The results showed that *HLA-B*13:01* was also associated with co-trimoxazole-induced SCAR among the patients who were HIV infected ($P=0.004$; 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 cases and controls according to their phenotype (SJS/TEN or DRESS) and area (Taiwan, Thailand, or Malaysia) (**Figure 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 ($z=3.08$; $P=0.001$) for the *HLA-B*13:01* association with co-trimoxazole-induced SJS/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 (**Figure 2**).

The positive and negative predictive values 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 DRESS and 20 SJS/TEN) among 5005 new users from 2017-2019 at CGMH 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, one patient could be prevented from experiencing SCAR if at least 211 individuals were tested (Table E16 in the Online Repository). And, one 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 SWISS-MODEL. Since 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, shown in Table E17 in the Online Repository), as a template. Docking was automatically performed after uploading 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 **Figure 3**. In the HLA-B*13:01-sulfamethoxazole complex, residues R121 and Y123 were found to be crucial for forming hydrogen bonds and 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, residues T48, S91, and N94 were hydrogen bonded to 4-nitro sulfamethoxazole. The two additional oxygen atoms in 4-nitro sulfamethoxazole, as compared with sulfamethoxazole, contributed all three hydrogen bonds to HLA-B*13:01. In addition, only two residues (I90 and Y123) were suggested by the program of the protein-ligand interaction profiler³⁸ 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 (Figure E5 in the Online Repository).

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

in the Online Repository), and the proposed sulfamethoxazole interaction modes between HLA-B*13:01 and HLA-B*38:02 are 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 (Figure E7 in the Online Repository), suggesting that sulfamethoxazole was the major causative drug for the enrolled SCAR patients. We then investigated the cross-reactivity of co-trimoxazole and dapsone in another 6 co-trimoxazole-induced SCAR patients who carried *HLA-B*13:01* (these patients were different from those in Figure E7). The results showed that the co-trimoxazole-induced SCAR patients who carried *HLA-B*13:01* had a high cross-reactivity to dapsone (sensitivity=83.3%; Figure E8 in the Online Repository). This result was consistent with the genetic finding.

DISCUSSION

SCAR (including SJS/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 0.4~6 cases per million persons per year, it may cause high mortality and permanent disabling complications^{39, 40}. 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 SCAR⁹. 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, 34, 41-44}. 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/TEN (94 cases) and DRESS (57 cases), from multiple centers in Asian countries, and used a whole-genome sequencing approach to determine a novel genetic marker, the SNP *rs41554616*, which is located between the *HLA-B* and *MICA* loci of exonic variants, showed the strongest association with co-trimoxazole-induced SCAR. The significance of the genome-wide analysis may have been affected by the sample size due to the paucity of co-trimoxazole-induced SCAR cases 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, 45-47}, 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^{48, 49}; however, the sulfonamide moiety of co-trimoxazole-sulfamethoxazole or its reactive metabolite (4-nitro sulfamethoxazole) has been reported as the major drug antigen(s) of hypersensitivity reactions^{41, 50}. 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, 51} 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 (PCA) conducted by 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 China¹⁸. 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 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/TEN⁵². Furthermore, Watkins et al.⁵³ previously used molecular dynamics modeling to demonstrate that TCR containing V β 20-1 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 only showed that sulfamethoxazole and 4-nitroso sulfamethoxazole can activate the T cells of co-trimoxazole-SCAR patients. 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/TEN^{10, 54}, while *HLA-A*31:01* was associated with carbamazepine-induced DRESS⁵⁵. 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/TEN, but not co-trimoxazole-induced DRESS. The docking models also confirmed that sulfamethoxazole could be docked into *HLA-B*38:02*. In addition, Ogees et al.⁵⁶ previously reported that 4-nitroso sulfamethoxazole-specific CD4⁺ T cells were activated in mild maculopapular exanthema patients 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 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. Eukaryotic translation elongation factor 2 (EEF2) is an elongation factor that can control the activation of transcription factor NF-κB and regulate inflammation⁵⁷; SLC9B1 is a transporter of glucose and other sugars, bile salts, and organic acids⁵⁸. 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 hypersensitivity³⁴. 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*, is strongly associated with co-trimoxazole-induced SCAR in Asians, and provided relevant information for the potential implementation of preemptive multiple genetic tests to identify patients who are at risk

of suffering from these life-threatening severe hypersensitivity reaction conditions caused by sulfonamide antibiotics.

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Figure legends**Figure 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 $-\log_{10} P$ value derived with a logistic regression model adjusted by sex, age, and principal components (PCs) in 43 cases and 507 general population controls. The red horizontal solid line represents $P=6.99 \times 10^{-7}$, indicating $P = 0.05$ by Bonferroni correction for the multiple comparisons (0.05/71440).

Figure 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 τ^2 and I^2 values represent measures of heterogeneity. The diamonds represent pooled odds ratios (Mantel-Haenszel method, random effects), and the error bars indicate 95% CIs. Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; SCAR, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

Figure 3. The docking modeling of *HLA-B*13:01* binding with sulfamethoxazole and its reactive metabolite (4-nitro sulfamethoxazole).

A, molecular structures of sulfamethoxazole (SMX), 4-nitro sulfamethoxazole (4-nitro SMX; the

779 reactive metabolite of SMX), trimethoprim (TMP), and dapsons. Residues within a contact
780 distance ($< 4 \text{ \AA}$) with the sulfonamide functional group of SMX/4-nitro SMX are labeled. **B**,
781 R121 and Y123 of HLA-B*13:01 were found to be crucial for forming hydrogen bonds to SMX.
782 **C**, T48, S91, and N94 of HLA-B*13:01 are hydrogen bonded to 4-nitro SMX.

Table 1. Demographic and baseline clinical characteristics of co-trimoxazole-induced severe hypersensitivity reaction patients and co-trimoxazole-tolerant controls from Taiwan, Thailand, and Malaysia.

Characteristics	Co-trimoxazole-induced SCAR						Total N=151	Co- trimoxazole- tolerant controls* N=138
	Taiwan		Thailand		Malaysia			
	SJS/TEN N=50	DRESS N=41	SJS/TEN N=41	DRESS N=11	SJS/TEN N=3	DRESS N=5		
Age, years, 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, n (%)								
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 cases, 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%)	N.A
>3 fold	6 (12%)	24 (59%)	9 (22%)	6 (55%)	0 (0%)	2 (40%)	47 (31%)	N.A
Acute renal failure ^a	6 (12%)	4 (10%)	7 (17%)	2 (18%)	0 (0%)	3 (60%)	22 (15%)	N.A
Hematologic abnormalities								
Eosinophilia, eosinophil (%)								
<5%	45 (90%)	10 (24%)	33 (80%)	2 (18%)	1 (33%)	1 (20%)	92 (61%)	N.A
≥5%	5 (10%)	31 (76%)	8 (20%)	9 (82%)	2 (67%)	4 (80%)	59 (39%)	N.A
Atypical lymphocytosis	8 (16%)	10 (24%)	NA	NA	0 (0%)	1 (20%)	-	N.A
Skin detachment, No. (%)								
BSA≥10%	15 (30%)	0 (0%)	13 (32%)	0 (0%)	1 (33%)	0 (0%)	29 (19%)	N.A
BSA<10%	35 (70%)	41(100%)	28 (68%)	11(100%)	2 (67%)	5 (100%)	122 (81%)	N.A
Mucosal involvement, No. (%)								
Oral	48 (96%)	3 (7%)	33 (89%)	1 (9%)	2 (67%)	0 (0%)	87 (59%)	N.A
Ocular	32 (64%)	2 (5%)	21 (57%)	1 (9%)	2 (67%)	0 (0%)	58 (39%)	N.A
Genital	23 (46%)	2 (5%)	10 (27%)	0 (0%)	1 (33%)	0 (0%)	36 (24%)	N.A
Underlying diseases, No. (%)								
Opportunistic infections in HIV patients	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, mean ± SD	1446±313	1429±414	1217±1185	1018±374	1600±0	1600±566	1375±603	1575±284
[range], mg/d	[400-1600]	[200-2400]	[800-4800]	[800-1600]	[1600]	[800-2400]	[240-4800]	[400-2400]
Duration, mean ± SD	12.7±9.0	14.3±10.9	15.9±12.4	28.6±15.	39±27	18.4±7.6	15.9±12.3	145±208
[range], d	[3-47]	[2-41]	[1-48]	[14-65]	[8-60]	[10-30]	[1-65]	[75-1095]

Abbreviations: BSA, body surface area; d, days; DRESS, drug reaction with eosinophilia and systemic symptoms; GPT, glutamic pyruvic transaminase; HIV, human immunodeficiency virus; NA, not applicable; SCAR, severe cutaneous adverse reactions; SD, standard deviation; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

^aThe creatinine value was 1.5-fold higher than the normal value range (0.4-1.5 mg/dL) after drug intake.

*138 Taiwanese patients who had received co-trimoxazole for more than 2.5 months without evidence of adverse reactions were enrolled as tolerant controls.

Table 2. The deleterious variants associated with co-trimoxazole-induced severe cutaneous adverse reactions in WGS discovery cohort of patients from Taiwan.

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

Abbreviations: 95% CI, 95% Confidence Interval; Chr, chromosome; Ctrl, population controls; Fun, functional variant type; HWE, Hardy-Weinberg equilibrium *P* values for 507 controls from the Taiwan general population; MAF, minor allele frequency; OR, odds ratio; NSV, nonsynonymous; SNP,

single-nucleotide polymorphism; WGS, whole-genome sequencing. *P* values were calculated with the use of logistic-regression model adjusted by sex, age, and principal components (PCs).

Gene name: CFL1, Cofilin 1; CLCA2, Chloride Channel Accessory 2; EEF2, Eukaryotic Translation Elongation Factor 2; HLA-A, HLA Class I Histocompatibility Antigen-A; HLA-B, HLA Class I Histocompatibility Antigen-B; 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; 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.

Table 3. The significant HLA alleles associated with co-trimoxazole-induced severe cutaneous adverse reactions in WGS discovery, replication, and combined samples from Taiwan.

HLA	WGS Discovery Cohort				Replication Cohort				Combination				
	Individual, %		OR (95% CI)	P value	Individual, %		OR (95% CI)	P value	Individual, %		OR (95% CI)	P value	P _c value
	SCAR N=43	Ctrls N=507			SCAR N=48	Ctrls N=2038			SCAR N=91	Ctrls N=2545			
HLA-B* :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* 8:02	8 (18.6%)	48 (9.5%)	2.2 (0.8~5.2)	0.066	8 (16.7%)	154 (7.6%)	2.4 (1.1~5.3)	0.029	16 (17.6%)	202 (7.9%)	2.5 (1.4~4.3)	0.003	-
HLA-C* 3:04	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 of the replication cohort were obtained by high resolution sequencing-based typing (SBT) or HLA NGS genotypes which were carried out using HOLOTYPE HLATM X2-96/7.

The main ethnicity of the enrolled SCAR cases and controls from Taiwan was Chinese.

P values were calculated by Fisher exact test.

Table 4. Associations of *HLA-B* alleles with phenotypes of co-trimoxazole-induced severe cutaneous adverse reactions from Taiwan.

Taiwan Subgroup	Case N (%)	General population Ctrl, N (%)	Tolerant Ctrl, N (%)	OR* (95% CI)*	P value*	Pc value*	OR [#] (95% CI) [#]	P value [#]	Pc value [#]
<i>HLA-B*13:01</i>									
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)	0.006	-	3.7 (1.6-8.8)	0.003	-
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
<i>HLA-B*38:02</i>									
SCAR	16/91 (17.6%)	202/2545 (7.9%)	8/138 (5.8%)	2.5 (1.4-4.3)	0.003	-	3.5 (1.4-8.5)	0.007	-
SJS/TEN	12/50 (24.0%)			3.7 (1.7-7.3)	5.0×10⁻⁴	0.013	5.1 (2.0-13.5)	8.9×10⁻⁴	0.02
DRESS	4/41 (9.8%)			1.3 (0.3-3.5)	0.564	-	1.5 (0.5-5.1)	0.475	-
<i>HLA-B*39:01</i>									
SCAR	8/91 (8.8%)	114/2545 (4.5%)	5/138 (3.6%)	2.1 (0.8-4.4)	0.069	-	2.6 (0.8-8.1)	0.143	-
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)	0.010	-	4.6 (1.3-15.8)	0.019	-
<i>HLA-B*15:02</i>									
SCAR	12/91 (13.2%)	212/2545 (8.3%)	10/138 (7.2%)	1.7 (0.8-3.1)	0.122	-	1.9 (0.8-4.7)	0.170	-
SJS/TEN	10/50 (20.0%)			2.7 (1.2-5.7)	0.008	-	3.2 (1.2-8.2)	0.017	-
DRESS	2/41 (4.9%)			0.7 (0.1-2.6)	0.766	-	0.7 (0.1-3.1)	0.736	-

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

[#]Data obtained from comparison of cases with relevant tolerant controls.

The main ethnicity of the enrolled cases and controls from Taiwan was Chinese.

P values were calculated by Fisher exact test.

Figure 1

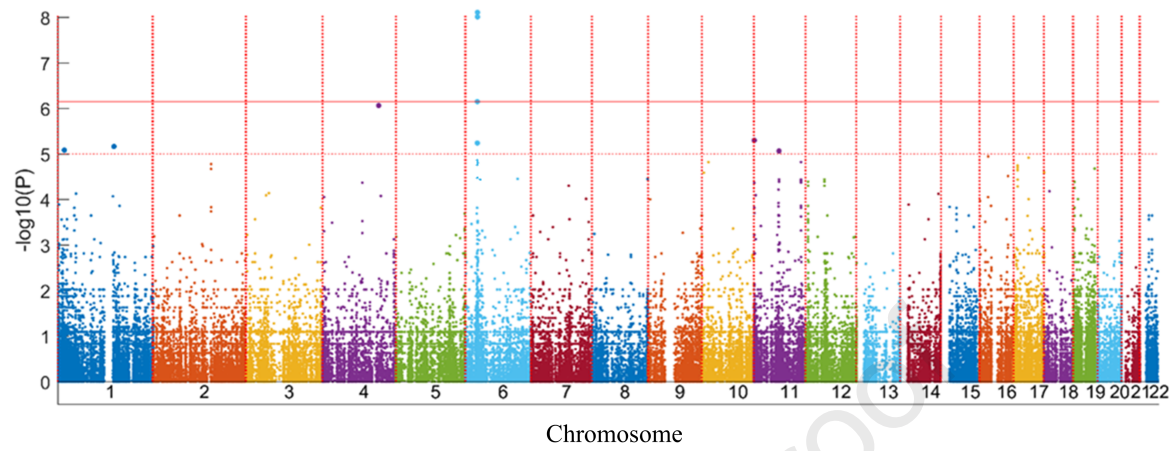


Figure 2

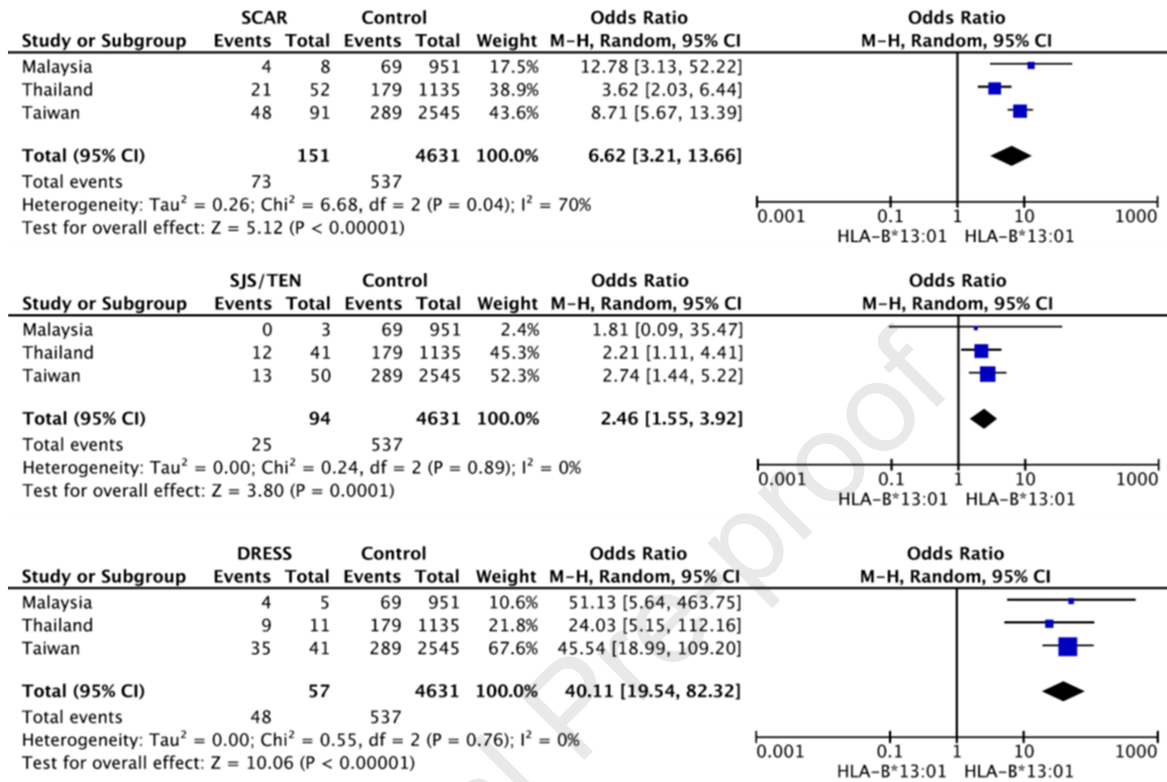
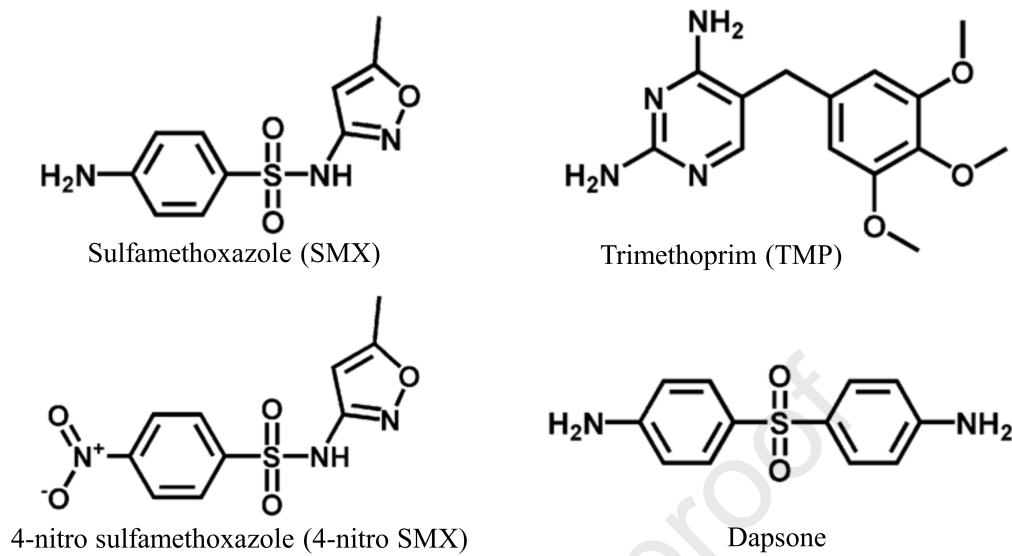
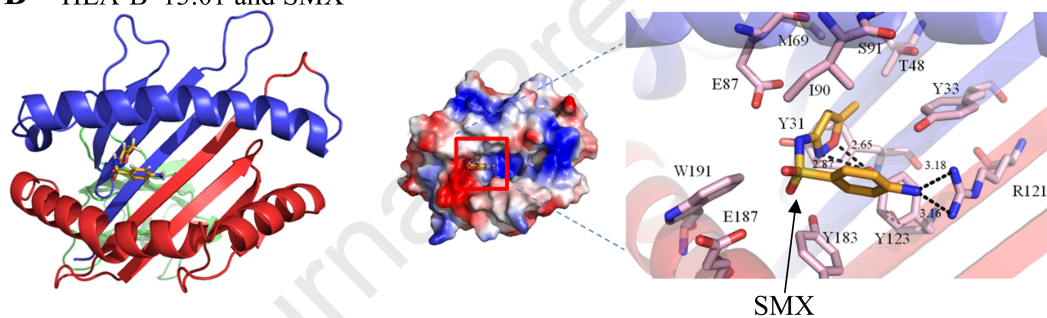


Figure 3

A**B** HLA-B*13:01 and SMX**C** HLA-B*13:01 and 4-nitro SMX