The Pharmacogenomics Journal (2016) 16, 83–87

www.nature.com/tpj

ORIGINAL ARTICLE

*HLA-B*59:01*: a marker for Stevens–Johnson syndrome/toxic epidermal necrolysis caused by methazolamide in Han Chinese

F Yang[1,6], J Xuan[2,6,7], J Chen[3,6], H Zhong[2], H Luo[4], P Zhou[5], X Sun[3], L He[2], S Chen[1], Z Cao[1], X Luo[1] and Q Xing[2]

Methazolamide is an intraocular pressure-lowering drug that is used in the treatment of glaucoma and other ophthalmologic abnormalities. The use of methazolamide has been shown to cause Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in patients of Asian ancestry. Methazolamide-induced SJS/TEN is associated with the presence of HLA-B59 serotype/*HLA-B*59:01* in Korean and Japanese populations. To better understand the genetic risk factors for these adverse reactions in the Han Chinese population, we characterized the *HLA* class I genotypes of eight Chinese patients with methazolamide-induced SJS/TEN from 2008 to 2014. The frequency of *HLA-B*59:01* was 87.5% (7/8) in the case patients, which was significantly different from 0% (0/30) in the methazolamide-tolerant patients (odds ratio (OR) = 305.0; $P = 6.3 \times 10^{-7}$) and 0.35% (1/283) in healthy subjects from the human major histocompatibility complex database (OR = 1974.0; $P = 2.0 \times 10^{-12}$). *HLA-C*01:02*, which is closely linked to *HLA-B*59:01*, had a weaker but notable association with methazolamide-induced SJS/TEN compared with the tolerant controls (OR = 12.1; $P = 0.016$) and general population (OR = 15.5; $P = 2.0 \times 10^{-3}$). The distribution of the *HLA-B*59:01-C*01:02* haplotype was also significantly different in cases and controls. This study demonstrated a strong association between *HLA-B*59:01* and methazolamide-induced SJS/TEN in the Han Chinese population for the first time. Pretherapy screening for *HLA-B*59:01* would be useful to reduce the risk of methazolamide-induced SJS/TEN.

The Pharmacogenomics Journal (2016) **16,** 83–87; doi:10.1038/tpj.2015.25; published online 28 April 2015

INTRODUCTION

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse reactions (SCARs) characterized by extensive necrosis and detachment of full-thickness epidermis and erosions of the skin and mucous membranes.[1] Although these adverse reactions are rare, they are life-threatening conditions with high morbidity and mortality. The incidence rates of TEN and SJS are generally low (0.4–1.2 and 1.2–6 cases per million population per year, respectively),[2] but mortality is as high as 1–5% for SJS and 20–30% for TEN.[2,3]

Methazolamide is a sulfonamide derivative used as a carbonic anhydrase inhibitor to reduce intraocular pressure elevations associated with glaucoma and other ocular disorders. Sulfonamides are a class of drugs with a high risk of inducing SCARs.[4,5] Sulfonamide derivatives that are potent inhibitors of carbonic anhydrase have been reported to cause SJS and TEN,[6,7] albeit less frequently than antimicrobial sulfonamides.

The first cases of methazolamide-induced SJS were reported in two Japanese-American women.[7] Five more cases were subsequently reported in Japan.[8,9] Shirato *et al.*[8] identified three of four patients as *HLA-B*59* carriers. A later series of reports highlighted the risk of SJS/TEN induced by methazolamide treatment and provided further evidence for the association of *HLA-B*59* with adverse reactions in the Korean population.[10–13] Kim *et al.*[14] recently performed allele-level genotyping of *HLA-A*, *-B* and *-C* genes and observed a strong association between methazolamide-induced SJS/TEN and *HLA-B*59:01* in five Korean patients.

Case reports of methazolamide-induced SJS/TEN have primarily been limited to patients of Japanese and Korean descent. To date, only two cases of SJS in glaucoma patients following methazolamide treatment have been reported in domestic journals of China, and no genotyping was performed.[15,16] In the present study, we diagnosed a total of eight Han Chinese patients with methazolamide-induced SJS/TEN and characterized the *HLA* class I genotypes of each patient to identify risk alleles for adverse reactions in the Han Chinese population. To our knowledge, this is the first association study of *HLA* genes with methazolamide-induced SJS/TEN in Han Chinese.

MATERIALS AND METHODS

Subjects

Study subjects were recruited during January 2008 through November 2014 from the dermatology and emergency wards of Huashan Hospital in Shanghai, China. Eight patients who fulfilled the diagnostic criteria for methazolamide-induced SJS or TEN were enrolled in the study. All patients came from the southern region of China. Diagnosis was performed by two independent dermatologists who examined photographs, pathological

[1]Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China; [2]Children's Hospital and Institutes of Biomedical Sciences, Fudan University, Shanghai, China; [3]Department of Ophthalmology and Vision Science, Eye & ENT Hospital, Fudan University, Shanghai, China; [4]University of Arkansas at Little Rock/University of Arkansas for Medical Sciences Joint Bioinformatics Program, Little Rock, AR, USA and [5]Center of Bioinformatics (COBI) and Key Laboratory for Neuroinformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China. Correspondence: Professor X Luo, Huashan Hospital, Fudan University, 12 Middle Wulumuqi Road, Shanghai 200040, China or Professor Q Xing, Children's Hospital and Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China.
E-mail: xiaoqunluoshanghai@gmail.com or xingqinghe@hotmail.com
[6]These authors contributed equally to this work.
[7]Current address: National Center for Toxicological Research, US Food and Drug Administration, 3900 NCTR Road, Jefferson, AR 72079, USA.
Received 14 February 2014; revised 26 February 2015; accepted 2 March 2015; published online 28 April 2015

slides and medical records. All clinical records were reviewed to exclude cases of immunobullous diseases, viral infections and toxic eruptions. In accordance with the clinical morphology described by Roujeau *et al.*,[17] SJS was defined as epidermal detachment involving $<10\%$ of the total body surface area, overlap SJS/TEN as epidermal detachment of 10–30%, and TEN as $>30\%$. Diagnoses of SJS and TEN were confirmed by skin biopsies showing full-thickness necrosis of the epidermis on pathological examination. Staphylococcal-scalded skin syndrome and other less likely differential diagnoses were excluded using these procedures. Methazolamide was considered the offending drug if the onset of SJS or TEN symptoms occurred within the first 2 months of exposure and the symptoms resolved after withdrawal of the drug. Patients with an absence of symptoms after re-exposure to methazolamide were excluded. Concomitant medications were used in five of the eight patients studied, but no adverse drug reactions (ADRs) related to prescribed drugs other than methazolamide were found in the medical records of these patients.

Two control groups were enrolled in the study. The general population control group consisted of 283 healthy southern Han Chinese subjects from the human major histocompatibility complex database.[18] The methazolamide-tolerant group contained 30 patients who did not develop any cutaneous manifestations at least 3 months after the methazolamide treatment. All subjects in case and control groups were unrelated Han Chinese. This study was approved by the ethics committee of Fudan University, and informed consent was obtained from all participants.

HLA genotyping

Peripheral blood samples from all participants were collected using ethylenediaminetetraacetic acid-containing tubes. Genomic DNA was extracted using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biotechnology, Union City, CA, USA). *HLA-A*, *-B* and *-C* alleles were genotyped using the PCR sequencing of specific oligonucleotide (PCR-SSO) method and the LABT SSO Kit (One Lambda, Los Angeles, CA, USA). Genotyping was performed by Mentality Bio-Tech (Beijing, China).

Statistical analysis

Comparisons of the parametric data and categorical data were conducted using Student's two-tailed *t*-test and Fisher's exact test, respectively. Odds ratios (ORs) were calculated using Haldane's modification, which adds 0.5 to all cells to avoid zero counts.[19] The statistical significance level was set at $P<0.05$. All statistical analyses were performed using R software version 3.0.2; The R Foundation for Statistical Computing, Vienna, Austria.

The power for detecting significant differences in *HLA* allele frequencies between patients and controls was estimated using the R-package statmod (version 1.4.20) (http://cran.r-project.org/web/packages/statmod). The proportion of methazolamide–SJS/TEN patients with *HLA* alleles (p1) and that of tolerant or general population controls (p2) were compared. Samples sizes were the numbers of patients (n1) and controls (n2). The significance level was set to 0.05. The alternative hypothesis was a one-tailed test because *HLA* alleles were assumed to be enriched in patients but not controls.

Molecular docking and dynamics simulation

To evaluate the potential interaction pattern between HLA-B*59:01 and methazolamide, molecular docking was performed between the two molecules followed by molecular dynamics simulation. The protein structure of HLA-B*59:01 was modeled based on existing crystal structure 1E27 (ref. 20) with 97% identity using MODELLER 9.11.[21] The entire binding groove of HLA-B*59:01 protein was defined as the binding pocket using AutoDock Tools.[22] Autodock Vina[23] was implemented for *in silico* docking simulation using default parameters. The docked HLA–methazolamide complex structure models were solvated in a rectangular box of ~20 000 TIP3P water molecules,[24] and the boundary of the box was at least 9 Å away from any solute atom. Counter Na^+ ions were placed based on the Columbic potential to keep the entire system neutral. Particle mesh Ewald was employed to consider the long-range electrostatic interactions.[25] Before molecular dynamics (MD) simulation, a series of minimizations was preformed: all hydrogen atoms were first minimized, followed by all water molecules with counter ions, and then a cycle of minimization was performed to relax all atoms without constraints. The maximum number of minimization steps was set to 5000, and the convergence criterion for the energy gradient was 0.1 kcal mol^{-1} $Å^{-1}$. The SHAKE algorithm[26] was employed to constrain all bonds involving hydrogen atoms in MD simulation, and the time step was set to 2 fs. The MD simulation consisted of a gradual temperature increase from 0 to 300 K over 100 ps and a 2-ns simulation for equilibration followed by a 3-ns simulation for data collection. All the above computations were performed using AMBER ff10 force field[27] in AMBER11[28] package.

RESULTS

Of the eight patients enrolled in this study, six were diagnosed with TEN and two with SJS. Table 1 shows the clinical characteristics and *HLA* genotypes of the patients. The mean age of the patients was 50.8 ± 11.1 years (range 33–67 years), and 62.5% of the patients ($n=5$) were male. The mean duration of methazolamide exposure was 22.0 ± 16.9 days and ranged from 2 to 58 days. The latent period before the onset of symptoms varied from 12 to 58 days with a mean of 23.0 ± 15.7 days.

To investigate whether the risk of methazolamide-induced SJS/TEN might be associated with specific *HLA* alleles, patients who were prescribed methazolamide without developing SCARs for more than 3 months after treatment were recruited as tolerant controls. Table 2 shows demographic and clinical variables of patients with methazolamide-induced SJS/TEN and tolerant patients. There were no significant differences in age, gender and methazolamide exposure between the methazolamide–SJS/TEN group and the methazolamide-tolerant group. The case patients received methazolamide for the treatment of post-operative intraocular pressure elevation and glaucoma, and all tolerant patients were prescribed methazolamide for the treatment of glaucoma.

The association of *HLA* alleles with the occurrence of methazolamide-induced SJS/TEN was first assessed between methazolamide–SJS/TEN patients and tolerant controls. Significant associations were subsequently validated in a second, independent cohort of 283 healthy southern Han Chinese from the general population. *HLA* alleles that showed significantly different frequencies between cases and both control groups are listed in Table 3. Notably, *HLA-B*59:01* was present in 87.5% (7/8) of the patients with methazolamide-induced SJS/TEN, 0% (0/30) of the methazolamide-tolerant controls (OR = 305.0; 95% confidence interval (CI), 11.3–8259.9; $P=6.3\times10^{-7}$), and 0.35% (1/283) of the general population (OR = 1974.0; 95% CI, 111.8–34 868.4; $P=2.0\times10^{-12}$). Compared with *HLA-B*59:01*, *HLA-C*01:02* showed a significant but weaker association with methazolamide-induced SJS/TEN. *HLA-C*01:02* is a common *HLA* allele in the Han Chinese population, and it was present in 87.5% (7/8) of the methazolamide–SJS/TEN patients, a significantly higher frequency than the 36.7% (11/30) of the methazolamide-tolerant patients (OR = 12.1; 95% CI, 1.3–111.7; $P=0.016$) and 31.1% (88/283) of the general population (OR = 15.5; 95% CI, 1.9–128.0; $P=2.0\times10^{-3}$). The distribution of HLA haplotypes in case and control groups was also analyzed. *HLA-C*01:02* was closely linked to *HLA-B*59:01*, which formed the *HLA-B*59:01-C*01:02* haplotype. A significant association was found between the occurrence of methazolamide-induced SJS/TEN and the *HLA-B*59:01-C*01:02* haplotype, which was present in 87.5% of methazolamide–SJS/TEN patients but in none of tolerant patients and 0.35% of healthy Han Chinese.

The predictability of *HLA* alleles as markers for methazolamide-induced SJS/TEN was examined. *HLA-B*59:01* exhibited high sensitivity (87.5%, 95% CI, 0.47–0.99) and specificity (100%, 95% CI, 0.86–1.00) in predicting the risk of SJS/TEN caused by methazolamide, whereas *HLA-C*01:02* exhibited high sensitivity (87.5%, 95% CI, 0.67–0.99) but low specificity (63.3%, 95% CI, 0.44–0.79). In addition, the positive predictive value and negative predictive value of *HLA-B*59:01* were 100% (95% CI, 0.56–1.00) and 96.8% (95% CI, 0.56–1.00), respectively.

In silico docking was conducted to characterize the interaction between *HLA*-B*59:01 and methazolamide, and a 5-ns atomistic MD simulation was addressed on the complex structure model of HLA-B*59:01 with methazolamide created by the molecular

Table 1. Clinical characteristics and HLA genotypes of patients with methazolamide-induced SJS/TEN

Case subject	*Age (years)/ gender*	*SCAR*	*Exposure duration/ latency (days)*	*Dose (mg per day)*	*Mucosal involvement*	*Systemic manifestations*	HLA-A *genotype*	HLA-B *genotype*	HLA-C *genotype*
1	51/F	TEN	17/14	50	Oral, eye, genitalia	LFI	*02:01/24:02*	*15:27/**59:01***	***01:02**/04:01*
2	59/F	TEN	32/32	50	Oral, eye, genitalia	RFI	*11:01/11:02*	*27:04/**59:01***	***01:02**/12:02*
3	58/F	TEN	10/23	50	Oral	Leukopenia, thrombocytopenia	*31:01/69:01*	*52:01/55:02*	*01:06/12:02*
4	38/M	TEN	16/12	50	Oral, genitalia	LFI	*03:01/11:01*	*44:02/**59:01***	***01:02**/05:01*
5	51/M	TEN	21/18	50	Oral, eye, genitalia	LFI, RFI	*02:06/24:02*	*48:03/**59:01***	***01:02**/08:01*
6	49/M	TEN	2/12	50	Oral, eye	RFI	*02:06/11:01*	*15:01/**59:01***	***01:02/01:02***
7	33/M	SJS	20/15	50	Oral, eye, genitalia	None	*33:03/11:01*	*58:01/**59:01***	***01:02**/03:02*
8	67/M	SJS	58/58	50	Oral, eye, genitalia	LFI	*11:01/11:01*	*45:01/**59:01***	***01:02**/06:02*

Abbreviations: F, female; LFI, liver function impairment; M, male; RFI, renal function impairment; SCAR, severe cutaneous adverse reaction; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis. The bold entries highlight that *HLA-B*59:01* or *HLA-C*01:02* is positive in these patients.

Table 2. Demographic and clinical variables of patients with methazolamide-induced SJS/TEN and methazolamide-tolerant patients

	Methazolamide–SJS/TEN case (n = *8*)	*Methazolamide–tolerant control* (n = *30*)	P-*value*
Demographic variables			
Age,[a] years	50.8 (33–67)	57.6 (20–80)	0.19
Gender,[b] *n* (%)			0.24
Male	5 (62.5)	11 (36.7)	
Female	3 (37.5)	19 (63.3)	
Methazolamide exposure[a]			
Dosage, mg per day	50	56 (50–100)	0.32
Duration, days	22.0 (2–58)	18.4 (2–180)	0.77
Therapeutic indication[b]			1.1×10^{-4}
Postoperative IOP elevation, *n* (%)	5 (62.5)	0 (0)	
Glaucoma, *n* (%)	3 (37.5)	30 (100)	

Abbreviations: IOP, intraocular pressure; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis. Data are expressed as the means (range). [a]*P*-values were calculated using Student's two-tailed *t*-test. [b]*P*-values were calculated using Fisher's exact test.

docking protocol. The minimum docking score of all eight possible binding poses of methazolamide and HLA-B*59:01 was − 6.2. The receptor HLA and the ligand methazolamide exhibited a slight fluctuation over the simulation during the MD process (Figure 1a). However, the relative movement of methazolamide toward HLA-B*59:01 over the simulation was minor, which suggests a relatively stable binding pose of the HLA-B*59:01–methazolamide complex. Quantitative MM-PB/SA (molecular mechanics Poisson–Boltzmann surface area) analysis of the free-energy changes during HLA–methazolamide interaction showed that the total interaction energies ΔG_{total} of methazolamide with HLA-B*59:01 was $-26.9\,kcal\,mol^{-1}$, which was further decomposed into van der Waals energy ($-27.1\,kcal\,mol^{-1}$), electrostatic potential ($-21.4\,kcal\,mol^{-1}$) and solvent effects ($21.6\,kcal\,mol^{-1}$). Figure 1b shows the binding pose of methazolamide and HLA-B*59:01 following MD simulation. Methazolamide tended to bind close to the A pocket of HLA-B*59:01, and two hydrogen bonds were formed from methazolamide toward residues TYR-9 and TYR-159 on HLA-B*59:01.

DISCUSSION

The highly polymorphic *MHC* genes in humans, also known as the *HLA* genes, encode cell-surface proteins that bind and present antigenic peptides to T cells, which trigger the acquired immune response. Strong associations between serious adverse events induced by certain drugs and specific *HLA* alleles were demonstrated, and these associations led to changes in drug labeling by the U.S. Food and Drug Administration (FDA) (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm). For example, the FDA recommends pretherapy screening for the presence of *HLA-B*15:02* and *HLA-B*57:01* as risk factors for carbamazepine-induced SJS/TEN and abacavir-induced hypersensitivity, respectively.[29,30] Screening patients for risk *HLA* alleles before the initiation of drug therapies may provide an efficient way to reduce the risk of adverse drug events.

The HLA-B59 serotype/*HLA-B*59:01* has been associated with methazolamide-induced SJS/TEN in Japanese and Korean populations.[8,12–14] The HLA-B59 serotype has also been detected in SJS caused by acetazolamide, another carbonic anhydrase inhibitor, in the Korean population.[31,32] Few cases of methazolamide-induced SJS have been reported in China, and the risk factors have not been identified. Our study is the first to perform high-resolution *HLA* genotyping in Han Chinese patients with methazolamide-induced SJS/TEN. We identified *HLA-B*59:01* as a strong genetic risk factor for methazolamide-induced SJS/TEN in the Han Chinese population. The *HLA-C*01:02* allele was also found to be significantly associated with methazolamide-induced SJS/TEN in this study. However, considering that this allele was closely linked with *HLA-B*59:01*, it could be encompassed by the association signal of the causal allele. Kim *et al.*[14] demonstrated that *HLA-C*01:02* and *A*24:02* alleles were closely linked to *HLA-B*59:01*, and the *B*59:01-C*01:02-A*24:02* haplotype had a significantly higher frequency in Korean patients with methazolamide-induced SJS/TEN than in the general population. Inconsistently, our study showed that *HLA- A*24:02* was present in

Table 3. Frequencies of *HLA-B*59:01, C*01:02* and the haplotype and association analyses with methazolamide-induced SJS/TEN

Genotype	*Methazolamide–SJS/TEN (n = 8)*	*Tolerant controls (n = 30)*	*OR (95% CI)*	P-*value*	*General population (n = 283)*	*OR (95% CI)*	P-*value*
*B*59:01*	7 (87.5)	0 (0)	305.0 (11.3–8259.9)	6.3×10^{-7}	1 (0.35)	1974.0 (111.8–34868.4)	2.0×10^{-12}
*Cw*01:02*	7 (87.5)	11 (36.7)	12.1 (1.3–111.7)	0.016	88 (31.1)	15.5 (1.9–128.0)	2.0×10^{-3}
*B*59:01, Cw*01:02*	7 (87.5)	0 (0)	305.0 (11.3–8259.9)	6.3×10^{-7}	1 (0.35)	1974.0 (111.8–34868.4)	2.0×10^{-12}

Abbreviations: CI, confidence interval; OR, odds ratio; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis. *P*-values were calculated using Fisher's exact test.

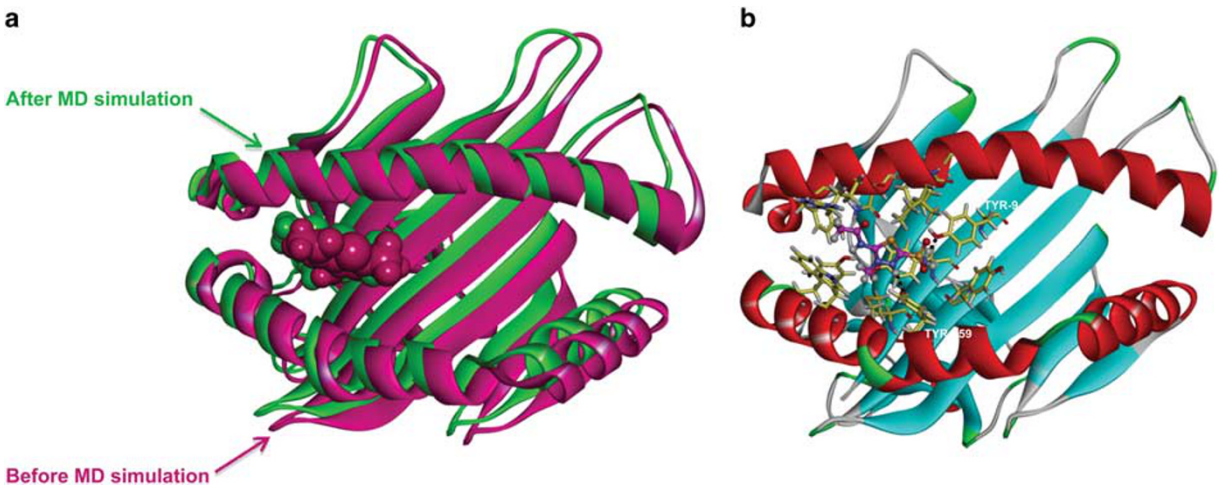

Figure 1. The binding mode of methazolamide to HLA-B*59:01. (**a**) Superposition of HLA-B*59:01–methazolamide complex structures before and after molecular dynamics simulation. (**b**) The interaction details of methazolamide with HLA-B*59:01. Hydrogen bonds between methazolamide and amino acid residues on HLA-B*59:01 are indicated by black dashed lines.

only 25% (2/8) of case patients, but 89 of 283 (31.45%) subjects in the general Han Chinese population carried the *HLA-A*24:02* allele. Therefore, no significant association was found between *HLA-A*24:02* and methazolamide-induced SJS/TEN, which suggests that *HLA-A*24:02* may not contribute to the etiology of SJS and TEN caused by methazolamide treatment.

The racial/ethnic differences in associations between *HLA* genotypes and drug hypersensitivity may depend primarily on the frequencies of risk *HLA* alleles among ethnic groups. The allele frequency of *HLA-B*59:01* (0.1–0.2%) in Han Chinese is ~10-fold lower than the frequency (1–2%) in Korean and Japanese populations.[33] This difference may explain why cases of methazolamide-induced SJS/TEN are rarely reported in the Chinese population. Additionally, *HLA-B*59:01* has rarely been found in other ethnic populations, including people of Caucasian and African descent.[33] Despite the low frequencies of the risk allele *HLA-B*59:01*, there is still an urgent need to develop preventive strategies to minimize the risk of SCARs related to methazolamide, especially in Asian populations, considering their life-threatening potential.

For genetic studies of ADRs, especially rare ADRs, it would be difficult to collect large numbers of cases within a reasonable time frame. However, some ADRs are associated with large-effect genetic risk factors that can be identified using small numbers of affected individuals in large-scale genomic screens.[34–36] For instance, as few as six cases and 200 population controls is sufficient to achieve an 80% power at a 2×10^{-5} significance level to detect the large genetic effect of *HLA-B*15:02* on carbamazepine-induced SJS in genome-wide analyses.[35,37] Additionally, it has been suggested that the use of population controls in the study of rare ADRs (incidence $<1\%$) does not have a significant impact on statistical power.[35] In our study, we used 283 population controls, 30 clinical controls and 8 cases of methazolamide-induced SJS/TEN for the identification of genetic risk factors. Strong associations of methazolamide-induced SJS/TEN with *HLA-B*59:01* were identified, which could be attributed to the large effect size of this rare allele. Our power analysis showed that the power and probability of detecting a true difference in the frequency of HLA-B*59:01 between patients and controls was 100%.

Although the mechanism by which *HLA-B*59:01* contributes to SJS and TEN in patients using methazolamide has not been elucidated, it is noteworthy that the pathogenesis of drug-induced SCARs can be mediated by multiple determinants, including the drug, *HLA* allotypes, T-cell receptors, HLA-bound peptides and other co-stimulatory molecules.[38] In the current study, the positive and negative predictive values of *HLA-B*59:01* were 100% and 96.8%, respectively. These results suggest that *HLA-B*59:01* is sufficient but not necessary to trigger methazolamide-induced SJS and TEN. None of the tolerant patients carried *HLA-B*59:01*, but there was also one case patient who did not carry the allele, which indicates that patients can develop methazolamide-induced SJS/TEN via other HLA factors.

The mechanism of how methazolamide interacts with HLA-B*59:01 remains unknown. It is unclear whether the interaction is mediated via methazolamide or any of its metabolites, and whether any drug-specific peptides are involved in the interaction. Although specific peptides or drug metabolites were not considered in the MD simulation,[39] the results suggest a potential interaction mechanism of HLA-B*59:01 with methazolamide. Previous studies focusing on other drug–HLA interactions demonstrated that abacavir binds closely to the F pocket of HLA-B*57:01 as an altered repertoire,[40,41] and carbamazepine

binds around the A–D pockets of HLA-B*15:02.[40] The binding pattern of HLA-B*59:01 and methazolamide is more similar to that of carbamazepine and HLA-B*15:02. Because carbamazepine may directly interact with HLAs to activate T-cell receptors in accordance with the p-i concept,[42] further studies can be designed to determine whether methazolamide directly interacts with HLA-B*59:01 to trigger immunological reactions using wet-lab approaches, such as binding experiments and *in vitro* immunological assays.[42,43]

To our knowledge, this study is the first report of an association between *HLA-B*59:01* and methazolamide-induced SJS/TEN in Han Chinese patients. The findings of our study and the case reports in Korea and Japan suggest that *HLA-B*59:01* is a strong predictive marker for methazolamide-induced SJS and TEN in patients of Asian ancestry. Screening for *HLA-B*59:01* before initiation of methazolamide therapy may reduce the risk of methazolamide-induced SCARs in Asians.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grants 81472873, 81071287, 30971582 and 31371274) and the Shanghai Municipal Commission of Science and Technology Program (14DJ1400100).

REFERENCES

1 Bastuji-Garin S, Rzany B, Stern RS, Shear NH, Naldi L, Roujeau JC. Clinical classification of cases of toxic epidermal necrolysis, Stevens-Johnson syndrome, and erythema multiforme. *Arch Dermatol* 1993; **129**: 92–96.
2 Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. *N Engl J Med* 1994; **331**: 1272–1285.
3 Rzany B, Mockenhaupt M, Baur S, Schroder W, Stocker U, Mueller J *et al.* Epidemiology of erythema exsudativum multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis in Germany (1990-1992): structure and results of a population-based registry. *J Clin Epidemiol* 1996; **49**: 769–773.
4 Fritsch PO, Sidoroff A. Drug-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. *Am J Clin Dermatol* 2000; **1**: 349–360.
5 Mockenhaupt M, Viboud C, Dunant A, Naldi L, Halevy S, Bouwes Bavinck JN *et al.* Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. *J Invest Dermatol* 2008; **128**: 35–44.
6 Sud RN, Grewal SS. Stevens Johnson syndrome due to Diamox. *Indian J Ophthalmol* 1981; **29**: 101–103.
7 Flach AJ, Smith RE, Fraunfelder FT. Stevens-Johnson syndrome associated with methazolamide treatment reported in two Japanese-American women. *Ophthalmology* 1995; **102**: 1677–1680.
8 Shirato S, Kagaya F, Suzuki Y, Joukou S. Stevens-Johnson syndrome induced by methazolamide treatment. *Arch Ophthalmol* 1997; **115**: 550–553.
9 Cotter JB. Methazolamide-induced Stevens-Johnson syndrome: a warning!. *Arch Ophthalmol* 1998; **116**: 117.
10 Park YJ, Moon JI, Park CK. Three cases of Stevens-Johnson syndrome associated with methazolamide treatment. *J Korean Ophthalmol Soc* 1999; **40**: 613–618.
11 Ahn EY, Lee JB, Lee SC, Won YH. Four cases of Stevens–Johnson syndrome associated with methazolamide treatment. *Korean J Dermatol* 2000; **38**: 798–801.
12 Moon JI, Seo JH, Park CK. Association of HLA type with Stevens–Johnson syndrome induced by methazolamide treatment. *J Korean Ophthalmol Soc* 2000; **41**: 2241–2246.
13 Sung KH, Jwong Y, Choi HU, Lee SK. Two cases of HLA-B59(+) Stevens–Johnson syndrome (SJS)–toxic epidermal necrolysis (TEN) associated with methazolamide treatment. *Korean J Dermatol* 2005; **43**: 561–563.
14 Kim SH, Kim M, Lee KW, Kim SH, Kang HR, Park HW *et al.* HLA-B*59:01 is strongly associated with methazolamide-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. *Pharmacogenomics* 2010; **11**: 879–884.
15 Jin RJ, Zhang XY, Jiang JX, Sun LP. One case of methazolamide-induced Stevens-Johnson syndrome [in Chinese]. *Chin J Derm Venereol* 2012; **26**: 363–364.
16 Yan C, Xue HY, Cai CC. Stevens-Johnson syndrome induced by methazolamide tablet [in Chinese]. *Chin J Drug Appl Monit* 2012; **9**: 179–181.
17 Roujeau JC. Clinical heterogeneity of drug hypersensitivity. *Toxicology* 2005; **209**: 123–129.
18 Kitts A, Feolo M, Helmberg W. The Major Histocompatibility Complex Database, dbMHC In: McEntyre J, Ostell J (eds) *The NCBI Handbook*. National Center for Biotechnology Information NIH: Bethesda, MD, USA, 2003.
19 Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956; **20**: 309–311.
20 Maenaka K, Maenaka T, Tomiyama H, Takiguchi M, Stuart DI, Jones EY. Nonstandard peptide binding revealed by crystal structures of HLA-B*5101 complexed with HIV immunodominant epitopes. *J Immunol* 2000; **165**: 3260–3267.
21 Eswar N, Webb B, Marti-Renom M, Madhusudhan M, Eramian D, Shen M *et al.* Comparative protein structure modeling using Modeller. *Curr Protoc Protein Sci* 2007; **Chapter 2**: Unit 2.9.
22 Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS *et al.* AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 2009; **30**: 2785–2791.
23 Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010; **31**: 455–461.
24 Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. *J Chem Phys* 1983; **79**: 926.
25 Darden T, York D, Pedersen L. Particle Mesh Ewald—an N.Log(N) Method for Ewald Sums in Large Systems. *J Chem Phys* 1993; **98**: 10089–10092.
26 Ryckaert JP, Ciccotti G, Berendsen HJC. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J Comput Phys* 1977; **23**: 327–341.
27 Duan Y, Wu C, Chowdhury S, Lee MC, Xiong G, Zhang W *et al.* A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. *J Comput Chem* 2003; **24**: 1999–2012.
28 Salomon-Ferrer R, Case DA, Walker RC. An overview of the Amber biomolecular simulation package. *Wiley Interdiscip Rev-Comput Mol Sci* 2013; **3**: 198–210.
29 U.S. Food Drug Adm *Information for Healthcare Professionals: Dangerous or Even Fatal Skin Reactions—Carbamazepine (marketed as Carbatrol, Equetro, Tegretol, and generics)*. U.S. Food Drug Adm.: Silver Spring, MD, USA, 2007.
30 U.S. Food Drug Adm *Information for Healthcare Professionals: Abacavir (marketed as Ziagen) and Abacavir-Containing Medications*. U.S. Food Drug Adm.: Silver Spring, MD, USA, 2008.
31 Ha JH, Song JY, Kim HO, Kim JW. A case of Stevens-Johnson syndrome caused by acetazolamide. *Korean J Dermatol* 2003; **41**: 248–250.
32 Her Y, Kil MS, Park JH, Kim CW, Kim SS. Stevens-Johnson syndrome induced by acetazolamide. *J Dermatol* 2011; **38**: 272–275.
33 Gonzalez-Galarza FF, Christmas S, Middleton D, Jones AR. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Res* 2011; **39**: D913–D919.
34 Ross CJ, Katzov-Eckert H, Dube MP, Brooks B, Rassekh SR, Barhdadi A *et al.* Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. *Nat Genet* 2009; **41**: 1345–1349.
35 Nelson MR, Bacanu SA, Mosteller M, Li L, Bowman CE, Roses AD *et al.* Genome-wide approaches to identify pharmacogenetic contributions to adverse drug reactions. *Pharmacogenomics J* 2009; **9**: 23–33.
36 Daly AK. Genome-wide association studies in pharmacogenomics. *Nat Rev Genet* 2010; **11**: 241–246.
37 Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC *et al.* Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* 2004; **428**: 486.
38 Uetrecht J, Naisbitt DJ. Idiosyncratic adverse drug reactions: current concepts. *Pharmacol Rev* 2013; **65**: 779–808.
39 Yun J, Marcaida MJ, Eriksson KK, Jamin H, Fontana S, Pichler WJ *et al.* Oxypurinol directly and immediately activates the drug-specific T cells via the preferential use of HLA-B*58:01. *J Immunol* 2014; **192**: 2984–2993.
40 Illing PT, Vivian JP, Dudek NL, Kostenko L, Chen Z, Bharadwaj M *et al.* Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature* 2012; **486**: 554–558.
41 Ostrov DA, Grant BJ, Pompeu YA, Sidney J, Harndahl M, Southwood S *et al.* Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci USA* 2012; **109**: 9959–9964.
42 Wei CY, Chung WH, Huang HW, Chen YT, Hung SI. Direct interaction between HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson syndrome. *J Allergy Clin Immunol* 2012; **129**: 1562–1569.
43 Chessman D, Kostenko L, Lethborg T, Purcell AW, Williamson NA, Chen Z *et al.* Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity. *Immunity* 2008; **28**: 822–832.