

HLA-A*02 alleles are associated with tetanus antitoxin-induced exanthematous drug eruptions in Chinese patients

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Objective Tetanus antitoxin (TAT) is an effective antitetanus medicine, but may sometimes cause adverse drug reactions such as rapid-onset anaphylactic shock and late-onset cutaneous adverse drug reactions, including exanthematous drug eruptions (EDE). Human leukocyte antigen (HLA) class I alleles are strongly associated with different types of cutaneous adverse drug reactions. This study aimed to assess whether there is an association between TAT-induced EDE and HLA-A, HLA-B, and HLA-C alleles in the Chinese Han population.

Patients and methods We carried out an association study in 15 patients with TAT-induced EDE and two groups of general Han Chinese patients. Allele-level genotypes of the HLA-A, HLA-B, and HLA-C genes of each patient were determined using the PCR-sequence-specific oligonucleotides method.

Results The carrier frequency of HLA serotype A2 was significantly higher in the TAT-induced EDE patients than in the general Han Chinese study participants from the human major histocompatibility complex database [$n = 283$, odds ratio (OR) = 6.93; $P = 0.0061$]. Particularly, the carrier frequency of three A2 alleles, including HLA-A*02:01, HLA-A*02:06, and HLA-A*02:07, is significantly higher than that of the control group (OR = 14.40; $P = 2.4 \times 10^{-5}$). Furthermore, HLA-B*39:01 was in complete linkage

disequilibrium with HLA-A*02:06 in the case patients. Consequently, the distribution of the HLA-A*02:06/-B*39:01 haplotype was also significantly different in the cases and the controls (OR = 105.00; $P = 0.0024$).

Conclusion The HLA-A*02:06/-B*39:01 haplotype is a potential genetic marker for the TAT-induced EDE. Furthermore, the HLA-A2 serotype, especially three alleles A*02:01, A*02:06, and A*02:07, was identified to be associated with the TAT-induced EDE in the Han Chinese population for the first time. *Pharmacogenetics and Genomics* 26:538–546 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

Pharmacogenetics and Genomics 2016, 26:538–546

Keywords: cutaneous adverse drug reactions, human leukocyte antigen, pharmacogenetics, tetanus antitoxin

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Received 6 April 2016 Accepted 8 September 2016

Introduction

Tetanus occurs worldwide, but most frequently in hot-wet climates. It caused about 58 900 deaths in 2013 alone [1]. Tetanus antitoxin (TAT) purified from the horse serum is a commonly used antitoxin for treating and preventing tetanus in patients who have a deep wound [2]. However, it was found to cause severe hypersensitivity reactions, such as life-threatening anaphylactic shock [3] and several other types of serum reactions in some patients [4]. Although homologous tetanus vaccine of human origin [tetanus immune globulin (human), TIG (H)] is a safer substitute [5], TAT is still used widely in

developing countries because of its advantages such as low price and convenient regimen.

In domestic journals of China, TAT-induced adverse drug reactions have been reported sometimes [6,7]. Skin tests are required before the TAT injection to avoid anaphylactic shocks. However, delayed cutaneous adverse drug reactions (cADRs) such as exanthematous drug eruptions (EDE) cannot be predicted by the skin test [8]. EDE are the most common late-onset allergic reactions induced by drugs and vaccines [9–11]. These occur as a widespread rash with lesions and serious itching, and are sometimes accompanied by respiratory digestive symptoms, often resulting in hospitalization [12]. EDE may also be an earlier clinical presentation of the severe cutaneous adverse reactions such as drug

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reaction with eosinophilia and systemic symptoms in some cases [13]. Treatment of EDE with antihistamines and corticoid may carry health risks. Therefore, it is important to identify biomarkers for the prediction of TAT-induced EDE and use alternative medicines, such as TIG (H) instead of TAT, to avoid or reduce EDE [14].

The human leukocyte antigen (*HLA*) system is comprised of a large number of genes that can regulate specific immune responses and account for the interindividual differences in susceptibility to diseases or allergens. Previous studies [15–18] have suggested that *HLA* class I alleles are highly associated with many allergic reactions induced by different drugs. However, whether *HLA* is associated with allergic reactions induced by horse serum-derived product containing immunoglobulin G antibodies against venom and toxin is relatively unexplored.

The identification of genetic variants that contribute toward adverse drug reactions is the first step toward the development of predictive diagnostic markers and exploration of the pharmacogenetics mechanism of cADRs. Therefore, the aim of the present study was to determine, for the first time, the possible association between *HLA* class I alleles and TAT-induced EDE in Han Chinese patients.

Patients and methods

Patients

From 2009 to 2014, 15 patients who fulfilled the diagnostic criteria for TAT-induced EDE were recruited from the dermatology ward of Huashan Hospital, Shanghai, China. Diagnostic criteria for EDE were based on the clinical morphology described by Stern [12] EDE are characterized by a diffuse and symmetric eruption of pink-to-red macules or small papules that may coalesce to form plaques. Two dermatologists confirmed the diagnosis independently by assessment of photographs, pathological slides, and medical records. Clinical documents were reviewed to rule out cases of viral eruptions and toxic eruptions. In all cases, TAT was considered the causative agent if the onset of EDE symptoms occurred within 4 weeks after the injection of TAT. Concomitant medications were used in three of the 15 patients. However, no adverse drug reactions related to the prescribed drugs other than TAT were found in the medical records of the patients.

All patients in the case group were unrelated Han Chinese from the South-east of China. Two control groups were enrolled in the study. The first one included 283 healthy southern Han Chinese from the human major histocompatibility complex database (dbMHC) [19,20]. Moreover, 118 general Han Chinese patients from the similar regions of China were recruited as an additional control group. Only Han Chinese patients were recruited in anticipation that there would be no

major ethnic differences between the case and the control groups.

This study was approved by the ethics committee of the Huashan Hospital, Fudan University. Informed consents were obtained from all participants. The methods were carried out in accordance with the approved guidelines in the Helsinki Declaration.

Genotype analyses

Genomic DNA was extracted from peripheral blood of the participants using an AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biotechnology, California, USA). Allele-level genotypes of the *HLA-A*, *HLA-B*, and *HLA-C* genes from each participant were determined by the PCR-sequence-specific oligonucleotide method using the LABT SSO Kit (One Lambda Inc., Los Angeles, California, USA). This genotyping was reanalyzed using *HLA* Fusion software, version 3.0 (One Lambda Inc.). Genotyping was performed by Mentality Bio-Tech Co., Ltd (Beijing, China).

Statistical analyses

The allele-carrying frequency was calculated by dividing the number of patients carrying the allele by the total number of group members. Allele frequency was obtained by dividing the number of identified alleles by the total number of all alleles on the same locus. Fisher's exact test was performed to compare allele-carrying frequencies of *HLA* alleles carried by more than one patient. Odds ratios (ORs) were calculated with Haldane's modification, adding 0.5 to all cells to accommodate the possible zero counts [21]. The two tailed *P*-values and 95% confidence intervals (CI) were calculated using R software, version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria). *P_c* values (*P*-values corrected) were obtained with the Bonferroni correction for multiple comparisons. Statistical significance level was set at a *P_c* value less than 0.05.

Computational analyses

Among all the *HLA-A*02* alleles, only *HLA-A*02:01* has a corresponding crystal structure (PDB ID: 1AKJ) [22] in the RCSB Protein Data Bank [23] (RCSB PDB: <http://www.rcsb.org>). To analyze the shape and chemistry of *A*02:03*, its homolog structure model was constructed on the basis of the known structure of *A*02:01* by mutating the known variations (residue Ala 149 Thr, Val 152 Glu, Leu 156 Trp) between them in the Crystallographic Object-Oriented Toolkit modeling program [24]. The outline of the binding pocket was generated from the peptide surface in the *HLA-A*02:01* structure as reported by Saper *et al.* [25] The structure cartoon and surface were generated by PyMOL 1.8 (DeLano Scientific LLC, San Carlos, California, USA) [26]. The electrostatic surface was generated in PyMOL using application plug-in

PDB2PQR and APBS [27]. The hydrophobic surface was also generated by PyMOL with a plug-in script.

Results

Characteristics of patients

From 2009 to 2014, 15 TAT-induced EDE patients were enrolled. Their clinical characteristics and *HLA* genotypes are listed in Table 1. Eleven (73.3%) patients were women. The mean age of the patients was 43.6 ± 13.5 (range: 24–61 years) years. The mean latency time was 6.8 (range: 1–9 days) days. Five patients had a history of previous penicillin or sulfonamide antibiotic drug allergy. Three patients used concomitant medications. All EDE patients had symmetrical lesions consisting of erythematous macules or small papules, which may coalesce, and serious itching in the area where the rash developed. Some patients also had respiratory digestive symptoms including chest distress, throat obstruction, and diarrhea.

Screening for association of candidate human leukocyte antigen class I alleles

To assess whether there were any *HLA* alleles that were enriched in the TAT-EDE patients, the allele-carrying frequencies were compared between the TAT-EDE patients and 283 Chinese Han population study participants from dbMHC. Serologic specificities of identified alleles (e.g. *A2* associated with *A*02* alleles) were obtained from the *HLA* nomenclature report [28,29].

As shown in Table 2, serotype *A2* was present in 86.7% (13/15) of the patients with TAT-induced EDE and 48.4% (137/283) of the general population ($n=283$; OR = 6.93; 95% CI: 1.54–31.26; $P=0.0061$; $P_c=0.024$). Although serotype *A11* was associated weakly with resistance to the TAT-induced EDE (OR = 0.24; 95% CI: 0.07–0.89; $P=0.03$; $P_c=0.12$), the association was not significant after Bonferroni correction.

Four *HLA-A*02* subtypes (*A*0201*, *0203*, *0206*, and *0207*) were common in the 283 general Han Chinese. The most frequent allele was *A*02:03* (19.8%), followed by *A*02:07*

(15.5%), *A*02:01* (9.9%), and *A*02:06* (6.7%). In contrast to the other three alleles (*A*02:01*, *A*02:06*, and *A*02:07*), the frequency of *A*02:03* was higher in the general population than in the TAT-induced EDE patients (1/15; 6.6%). The only patient who carried *A*02:03* was also a carrier of *A*02:06*. Notably, the frequency of three *HLA-A*02* alleles increased in the patients including *HLA-A*02:01*, *HLA-A*02:06*, and *HLA-A*02:07*. These three alleles presented in 86.7% (13/15) of the TAT-induced EDE patients, which was significantly higher than that found in 31.1% (88/283) of the general population (OR = 14.40; 95% CI: 3.18–65.19; $P=2.4 \times 10^{-5}$; $P_c=0.00017$). Furthermore, *HLA-A*02:06* and *HLA-A*02:07* were associated weakly with TAT-induced EDE (OR = 5.05; 95% CI: 1.47–17.38; $P=0.02$; $P_c=0.14$; OR = 3.62; 95% CI: 1.23–10.68; $P=0.02$; $P_c=0.14$, respectively), whereas *HLA-A*11:01* was associated weakly with resistance to the TAT-induced EDE (OR = 0.26; 95% CI: 0.07–0.95; $P=0.03$; $P_c=0.24$). However, these associations were no longer significant after Bonferroni correction.

Among the *HLA-B* alleles, *HLA-B*39:01* was associated with TAT-induced EDE (OR = 43.38; 95% CI: 3.69–509.85; $P=0.0069$; $P_c=0.048$). *HLA-B*39:01* was in full linkage disequilibrium with *HLA-A*02:06* in the patients. Moreover, a significant association was found between the occurrence of TAT-induced EDE and the *HLA-A*02:06-B*39:01* haplotype, which was present in 13.3% of the TAT-induced EDE patients, but in none of the healthy Han Chinese population (OR = 105.00; 95% CI: 4.80–2296.22; $P=0.0024$; $P_c=0.0048$). A common haplotype *HLA-A*02:07-B*46:01* in Han Chinese was associated weakly with TAT-EDE (OR: 3.543; 95% CI: 1.144–10.971; $P=0.037$; $P_c=0.074$). However, the association was not significant after Bonferroni correction.

Because all of the significantly enriched alleles were *HLA-A* and *HLA-B* allotypes, we performed *HLA-A* and *HLA-B* genotyping in a control group of 118 general Han Chinese. As shown in Table 3, serotype *A2* showed an

Table 1 Clinical characteristics and human leukocyte antigen genotypes of tetanus antitoxin-induced exanthematous drug eruption patients

Sex/age (years)	Diagnosis	Concomitant drug	History of drug allergy	Latency	<i>HLA-A</i>		<i>HLA-B</i>		<i>HLA-C</i>	
Female/60	EDE	No	Penicillin	8	<i>A*24:02</i>	<i>A*24:02</i>	<i>B*13:01</i>	<i>B*35:01</i>	<i>C*03:04</i>	<i>C*03:03</i>
Female/56	EDE	No	No	7	<i>A*02:03</i>	<i>A*02:06</i>	<i>B*38:02</i>	<i>B*51:01</i>	<i>C*07:02</i>	<i>C*14:02</i>
Female/39	EDE	No	Penicillin; sulfanilamide	6	<i>A*01:01</i>	<i>A*02:01</i>	<i>B*46:01</i>	<i>B*57:01</i>	<i>C*01:02</i>	<i>C*06:02</i>
Male/28	EDE	No	No	6	<i>A*02:07</i>	<i>A*33:03</i>	<i>B*46:01</i>	<i>B*58:01</i>	<i>C*01:02</i>	<i>C*03:02</i>
Female/48	EDE	No	Penicillin	5	<i>A*02:06</i>	<i>A*33:03</i>	<i>B*40:06</i>	<i>B*54:01</i>	<i>C*01:02</i>	<i>C*08:01</i>
Male/28	EDE	No	No	5	<i>A*02:01</i>	<i>A*33:03</i>	<i>B*15:11</i>	<i>B*44:03</i>	<i>C*03:03</i>	<i>C*14:03</i>
Female/26	EDE	No	No	7	<i>A*02:07</i>	<i>A*11:01</i>	<i>B*46:01</i>	<i>B*40:01</i>	<i>C*01:02</i>	<i>C*07:02</i>
Male/41	EDE	Cefotiam; ibuprofen	No	9	<i>A*02:07</i>	<i>A*11:01</i>	<i>B*46:01</i>	<i>B*38:02</i>	<i>C*01:02</i>	<i>C*07:02</i>
Female/37	EDE	No	Penicillin	6	<i>A*02:06</i>	<i>A*26:01</i>	<i>B*39:01</i>	<i>B*58:01</i>	<i>C*03:02</i>	<i>C*03:04</i>
Female/61	EDE	No	No	7	<i>A*02:01</i>	<i>A*24:02</i>	<i>B*46:01</i>	<i>B*58:01</i>	<i>C*01:02</i>	<i>C*03:02</i>
Female/24	EDE	Azithromycin	No	8	<i>A*02:07</i>	<i>A*24:02</i>	<i>B*35:01</i>	<i>B*46:01</i>	<i>C*01:02</i>	<i>C*03:04</i>
Male/33	EDE	No	No	6	<i>A*02:07</i>	<i>A*33:03</i>	<i>B*46:01</i>	<i>B*51:01</i>	<i>C*01:02</i>	<i>C*03:02</i>
Female/60	EDE	Metronidazole; erythromycin	No	7	<i>A*02:06</i>	<i>A*24:02</i>	<i>B*15:27</i>	<i>B*39:01</i>	<i>C*04:01</i>	<i>C*07:02</i>
Female/58	EDE	No	No	8	<i>A*02:07</i>	<i>A*11:01</i>	<i>B*15:02</i>	<i>B*48:01</i>	<i>C*08:01</i>	<i>C*08:01</i>
Female/55	EDE	No	Sulfanilamide	7	<i>A*11:02</i>	<i>A*68:01</i>	<i>B*27:04</i>	<i>B*44:03</i>	<i>C*04:01</i>	<i>C*12:02</i>

EDE, exanthematous drug eruptions; HLA, human leukocyte antigen.

Table 2 Comparison of human leukocyte antigen class I allele frequencies between the tetanus antitoxin-induced exanthematous drug eruptions group and the sample ($n = 283$) of the general Han Chinese population from the human major histocompatibility complex database

HLA genotypes	Carriers and carrier frequency [n (%)]		Dominant model analysis		
	Case ($n = 15$)	Control ($n = 283$)	P	P_c	Odds ratio (95% CI)
HLA-A					
A*02:01	3 (20.0)	28 (9.9)	0.20		
A*02:06	4 (26.7)	19 (6.7)	0.02	0.14	5.05 (1.47–17.38)
A*02:07	6 (40.0)	44 (15.5)	0.02	0.14	3.62 (1.23–10.68)
A*24:02	4 (26.7)	89 (31.4)	0.78		
A*33:03	4 (26.7)	64 (22.6)	0.75		
A*11:01	3 (20.0)	138 (48.8)	0.03	0.21	0.26 (0.07–0.95)
A*02:01/02:06/02:07	13 (86.7)	88 (31.1)	2.4×10^{-5}	0.00017	14.40 (3.18–65.19)
HLA-B					
B*38:02	2 (13.3)	40 (14.1)	1.00		
B*51:01	2 (13.3)	26 (9.2)	0.64		
B*46:01	7 (46.7)	60 (21.2)	0.05		
B*58:01	3 (20.0)	49 (17.3)	0.73		
B*44:03	2 (13.3)	9 (3.2)	0.10		
B*39:01	2 (13.3)	1 (0.4)	0.0069	0.048	43.38 (3.69–509.85)
B*35:01	2 (13.3)	10 (3.5)	0.12		
HLA haplotype					
HLA-A*02:07-B*46:01	5 (33.3)	35 (12.4)	0.037	0.074	3.54 (1.14–10.97)
HLA-A*02:06-B*39:01	2 (13.3)	0 (0.0)	0.0024	0.0048	105.00 (4.80–2296.22)
HLA-A serotype					
A2	13 (86.7)	137 (48.4)	0.0061	0.024	6.93 (1.54–31.26)
A24	4 (26.7)	95 (33.6)	0.78		
A33	4 (26.7)	64 (22.6)	0.75		
A11	3 (20.0)	143 (50.5)	0.03	0.12	0.24 (0.07–0.89)
HLA-C					
C*03:04	3 (20.0)	65 (23.0)	1.00		
C*03:03	2 (13.3)	23 (8.1)	0.36		
C*07:02	4 (26.7)	108 (38.2)	0.43		
C*01:02	8 (53.3)	88 (31.1)	0.09		
C*03:02	4 (26.7)	48 (17.0)	0.31		
C*08:01	2 (13.3)	69 (24.4)	0.53		
C*04:01	2 (13.3)	23 (8.1)	0.36		

Bold typeface refers to the P -values from Fisher's exact test that showed statistically significant differences in alleles frequencies between groups ($P < 0.05$, before or after correction).

CI, confidence interval; EDE, exanthematous drug eruption; HLA, human leukocyte antigen; TAT, tetanus antitoxin.

P : P -values obtained by Fisher's exact tests are shown.

P_c : P -values corrected for the multiplicity of testing by the number of comparison: 7, 7, 2, 4 for HLA-A, HLA-B, HLA haplotype, and HLA serotype, respectively. Those alleles only detected in one case were excluded from the analysis.

Table 3 Comparison of HLA-A, HLA-B allele frequencies between the tetanus antitoxin-induced exanthematous drug eruptions group and Han Chinese patients from Huashan Hospital ($n = 118$)

HLA alleles	Carriers and carrier frequency [n (%)]		Dominant model analysis		
	Case ($n = 15$)	Control ($n = 118$)	P	P_c	Odds ratio (95% CI)
HLA-A					
A*02:01/02:06/ 02:07	13 (86.7)	51 (43.2)	0.0018	0.013	8.54 (1.84–39.54)
HLA-B					
B*46:01	7 (46.7)	22 (18.6)	0.02	0.14	3.82 (1.25–11.65)
HLA haplotype					
A*02:06-B*39:01	2 (13.3)	0 (0.0)	0.012	0.024	43.89 (2.00–962.74)
HLA-A serotype					
A2	13 (86.7)	58 (49.2)	0.0061	0.024	6.72 (1.45–31.11)

Bold typeface refers to the P -values from Fisher's exact test that showed statistically significant differences in allele frequencies between groups ($P < 0.05$, before or after correction).

CI, confidence interval; EDE, exanthematous drug eruption; HLA, human leukocyte antigen; TAT, tetanus antitoxin.

P : P -values obtained by Fisher's exact tests are shown.

P_c : P -values corrected for the multiplicity of testing by the number of comparison: 7, 7, 2, 4 for HLA-A, HLA-B, HLA haplotype, and HLA serotype, respectively. Those alleles only detected in one case were excluded from the analysis.

association with TAT-induced EDE ($n = 118$; OR = 6.72; 95% CI: 1.45–31.11; $P = 0.0061$; $P_c = 0.024$). The frequency of three alleles HLA-A*02:01, HLA-A*02:06, and HLA-A*02:07 in the TAT-induced EDE patients was

significantly higher than that in the control participants (OR = 8.54; 95% CI: 1.84–39.54; $P = 0.0018$; $P_c = 0.013$). HLA-B*46:01 was associated weakly with the risk of TAT-induced EDE (OR = 3.82; 95% CI: 1.25–11.65;

$P=0.02$; $P_c=0.14$). The association was not significant after Bonferroni correction. In addition, the distribution of *HLA* haplotypes in the case and control groups was analyzed. A significant association was found between the occurrence of TAT-induced EDE and the *HLA-A*02:06-B*39:01* haplotype (OR: 43.89; 95% CI: 2.00–962.74; $P=0.012$; $P_c=0.024$).

Feature analysis of the risk human leukocyte antigen alleles for tetanus antitoxin-induced exanthematous drug eruptions

Amino acid sequence analysis indicated that there were only three residue variations between the *HLA-A*02:03* and the other three *HLA-A*02* subtypes (see Supplementary Table, Supplemental digital content 1, <http://links.lww.com/FPC/B75>). Residue 149 may interact with the T cell receptor (TCR), whereas residues 152 and 156 sit in the center of the binding groove [25]. The peptide-binding groove of *HLA-A*02* is shown in Fig. 1. We compared the features of alleles with polymorphism. It was found that the binding groove of *HLA-A*02:01* is mainly positively charged and the residues Ala at position 149 and Val at position 152 are hydrophobic [Fig. 2 (a, c)]. In contrast, *HLA-A*02:03* has two hydrophilic residues: Thr at position 149 and Glu at position 152. These two residues are negatively charged [Fig. 2 (b, d)]. It was found that the differences in hydrophobicity did not alter the overall potential distribution of the pockets significantly. However, they may alter the specificity of ligand binding. Moreover, the variation (Trp instead of Leu at position 156) would alter the shape and hydrophilicity–hydrophobicity distribution of the pockets. Models of hydrophobic differences of residues and surfaces between the structure of *A*02:01* and *A*02:03* were prepared and compared. The residue color corresponds to its hydrophobicity scale (red fading to white

indicates scale values changing from high to low) (see Supplementary Video, Supplemental digital content 2, <http://links.lww.com/FPC/B76>).

Discussion

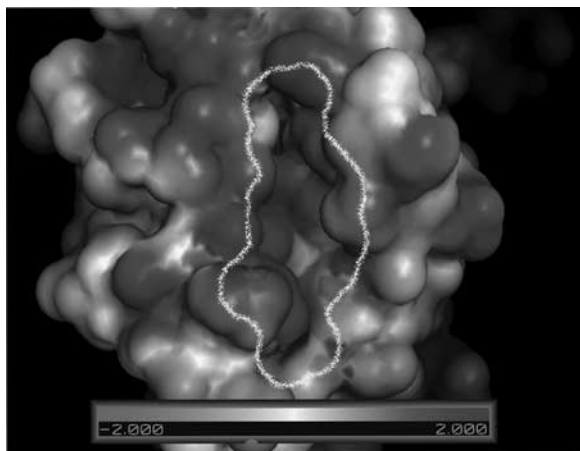
In this study, possible *HLA* alleles as risk factors for TAT-induced EDE were examined. The carrier frequency of serotype *HLA-A2*, especially the set of three alleles *HLA-A*02:01*, *HLA-A*02:06*, and *HLA-A*02:07*, was found to be significantly higher in the patients with TAT-induced EDE than in the controls. In addition, our results suggested the haplotype *HLA-A*02:06-B*39:01* as a potential marker for the prediction of TAT-induced EDE.

Nowadays, the mechanism of TAT hypersensitivity is still elusive. The TAT-induced EDE are pharmacologically classified into type B adverse drug reactions that are less dependent on dose, unpredictable, and primarily determined by host genetics [31]. The Gell and Coombs classification divides drug hypersensitivity into four categories, known as type I–IV allergies [32]. As horse serum is a strong allergen for xenogeneic animals, it can stimulate interleukin-4 and total immunoglobulin E (IgE) secretion [33]. Both of these play a role in type I allergies, such as acute shock and urticarial [34–36]. Antigen-specific IgE binds to high-affinity Fc-IgE receptors on mast cells and basophils. The cross-linking of these receptors leads to the release of various mediators, eliciting several symptoms [37]. In the delayed drug hypersensitivity reactions, drug-specific CD4+ and CD8+ T cells recognize antigen through the TCR in an MHC-dependent manner [38]. It is still unknown whether peptides of the horse serum or their metabolites bind to the TCR directly. However, it has been suggested that an additional interaction of the TCR with the MHC molecules (*HLA* in human) is needed.

Several studies have shown that there are strong associations between *HLA* allotypes and adverse drug events [39–41]. *HLA-B*15:02* and *HLA-B*57:01* were recommended by the US Food and Drug Administration (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>) as risk factors for carbamazepine-induced Stevens–Johnson syndrome (SJS)/TEN and abacavir-induced hypersensitivity, respectively. During the past decade, some *HLA*–drug associations have been reported in Han Chinese. For example, associations between *HLA-B*58:01* and allopurinol induced cADRs, including SJS/TEN and drug-induced hypersensitivity/drug reaction with eosinophilia and systemic symptoms [42], *HLA-B*13:01* and salazosulfapyridine induced drug rash [43], and *HLA-B*59:01* and methazolamide induced hypersensitivity syndrome [44].

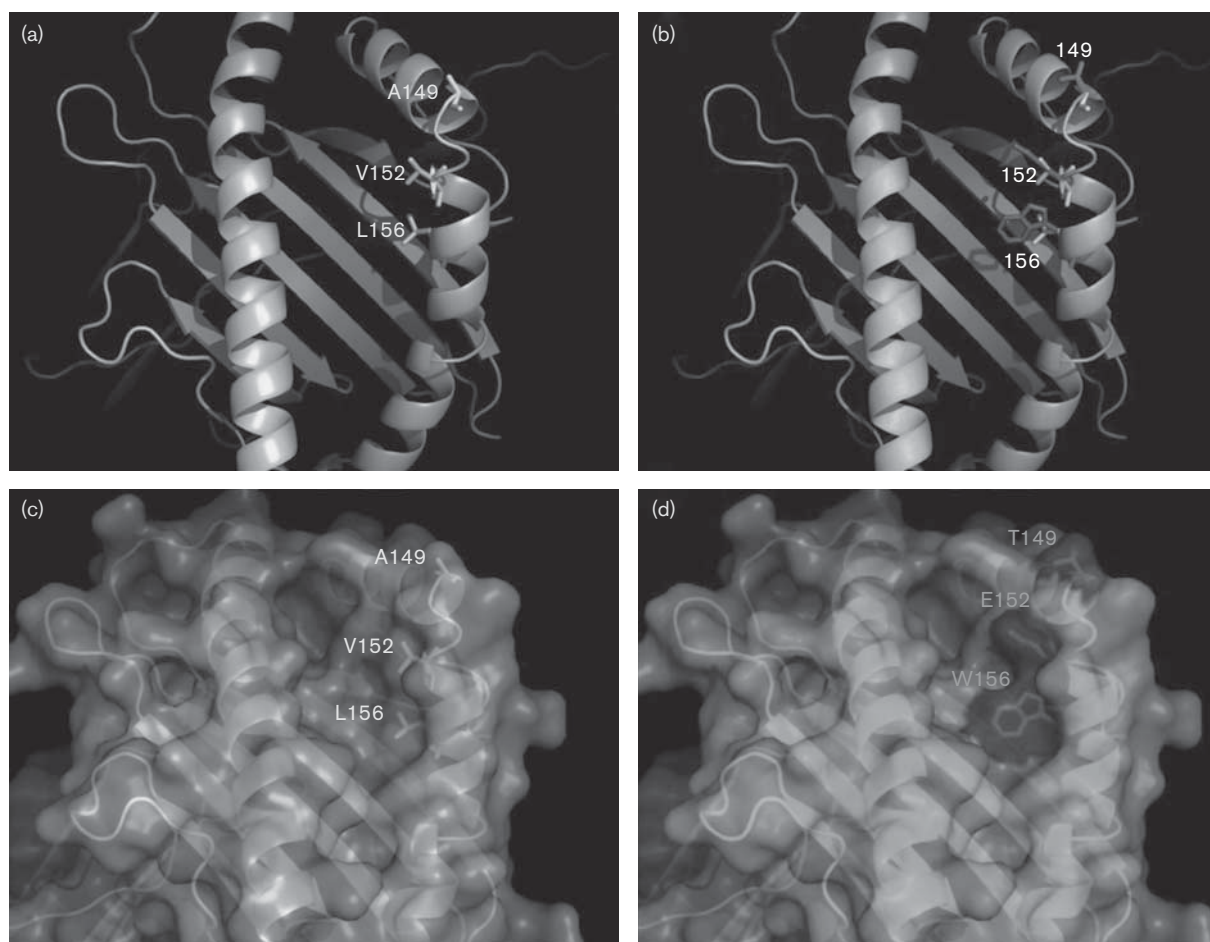
Although many cases of TAT or other serum-derived products-induced cADRs have been reported, the role of *HLA* was not assessed in these reports. To the best of our knowledge, this study carried out high-resolution *HLA* genotyping in the TAT-induced EDE patients for the first time.

Fig. 1



The pocket model of *HLA-A*02*. The pocket of *HLA-A*02* is shown as a white outline. *HLA*, human leukocyte antigen.

Fig. 2



The comparison of binding sites between *HLA-A*02:03* and *A*02:01*. *HLA-A*02:01* residues A149, V152, and L156 (stick) are located on the side of the binding cleft [structure cartoon (a)]; V152 and L156 are sitting in the center of the binding groove; refer to Chelvanayagam *et al.* [30] [shown in the surface map (c)]. The *HLA-A*02:03* residue variations T149, E152, and W156 (stick) with the same side-chain orientation of *HLA-A*02:01* residues are shown in the binding pocket [structure cartoon (b), surface map (d)]. *HLA*, human leukocyte antigen.

We found that three alleles of *HLA-A*02*, including *HLA-A*02:01*, *HLA-A*02:06*, and *HLA-A*02:07*, were significantly enriched in the TAT-induced EDE patients. However, the most common allele *HLA-A*02:03* was rare in the TAT-induced EDE patients. These allelic variants were detected by sequencing exons 2 and 3 of the *A*02* genes, which contain the majority of genetic polymorphism. As these exons encode the α -1 and α -2 domains of the MHC class I molecules, which form the α -helices and β -pleated sheet around the peptide-binding groove [45], the variations within the genes may influence the antigen presentation and peptide-binding specificity of the gene products of each allele [46]. It has been reported previously that different *A*02* variants elicited different T cell response [47–50].

The mechanism of how TAT interacts with *HLA-A*02* alleles remains unknown. A previous report showed that abacavir binds with specificity to the C–F pockets of

*HLA-B*57:01* as an altered repertoire of peptides that can bind *HLA-B*57:01* [51]. Further characterization of *HLA-A*02* alleles identified six pockets (A–F) in the groove. Residue 152 sits on the surface of pocket D, whereas residue 156 is located on the surface of pocket E, which is adjacent to pocket D and between the side-chain of residue 152. The conformational binding of peptides was provided by A and F pockets [52], whereas the specificity of peptide-binding was imparted by pockets B–E and to some extent F [53]. *A*02:03* contains two amino acid variations compared with *A*02:01* in the pocket D and pocket E (Glu instead of Val at position 152, Trp instead of Leu at position 156). These variations, especially the latter one, might alter the space and physical characteristics of pockets D and E, and affect the amino acid residues to which they may effectively bind. The same difference was found between *HLA-B*15:02* (Leu at position 156), which is strongly associated with carbamazepine-induced SJS/TEN

in the Asian populations [40], and the closely related allele *HLA-B*15:01* (Trp at position 156) (see Supplementary Table, Supplemental digital content 3, <http://links.lww.com/FPC/B77>). It has been reported that the presence of Trp at position 156 closes the D pocket that could impede the docked conformation of carbamazepine sterically [51,54] (see figure S4, which refers to Illing *et al.* [51]). This substitution has a significant influence on the peptide-binding ability of different *HLA* molecules because Trp has a much larger side chain than Leu, which would take up more space in the pocket, and thus the space left would only accommodate small residues of the bound peptide (see Fig. 1, which refers to Bade-Doeding *et al.* [55]). Further studies should be designed to determine whether TAT interacts directly with *HLA-A*02:01/02:06/02:07* to trigger immunological reactions and whether any specific peptides are involved in the interaction.

A significant association was also found between *HLA-A*02:06/-B*39:01* and the TAT-induced EDE. *HLA-A*02:06/-B*39:01* was a rare haplotype and observed in only 32 patients out of all the 12 212 patients from dbMHC. Most of the carriers were from South-East Asia. Several haplotypes have been reported to be associated with drug-induced cADRs in previous studies. *HLA-A*24:01/-B*59:01/-C*01:02* and *HLA-A*02:01/-B*15:18/-C*07:04* haplotypes were reported with carbamazepine-induced cADRs in Japanese patients; the former was found in two patients, whereas the latter was found in only one patient [56]. Moreover, *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* was reported to be associated with lamotrigine-induced maculopapular exanthema in Mexican Mestizo patients [57].

The pathogenesis of the TAT-induced EDE should be multifactorial. The EDE are caused by vascular dilatation and leakage of fluid into the skin in response to several molecules. In addition to the immunological mediators of EDE including antibodies and T cells that result in mast cell activation, other risk factors including membrane receptors involved in the innate immunity, such as toll-like receptors and cytokine/chemokine, or direct toxicity of xenobiotics are also possibly associated with EDE [58,59].

As reported previously, ethnicity might play an important role in the associations between *HLA* allotypes and adverse drug reactions. For example, *HLA-B*15:02* is associated strongly with the carbamazepine-induced SJS in Southeast Asian patients, but not in Caucasian and Japanese patients [40,60–62]. Therefore, a study with a larger sample size is required to clarify whether the findings of this study can be applied to other ethnic groups.

There are several limitations to our study. One limitation is that most patients in our case group were women. If the *HLA-A*02* frequencies of female and male patients are different, it may be possible that *HLA-A*02* is associated with female sex rather than TAT-induced EDE. Nevertheless, we would not

expect *HLA* differences between women and men. However, all the male patients in our study carried *HLA-A*02*. Another major limitation in our study is the small sample size. We could not eliminate the possibility that the association observed is a false-positive result.

Despite these limitations, we found statistically significant differences in the frequencies of the *HLA-A*02* alleles and the *HLA-A*02:06/-B*39:01* haplotype between the patients and the general population. It is noteworthy that these alleles are sufficient but not necessary to trigger TAT-induced EDE because there were also two case patients who did not carry these alleles, which indicates that those patients can develop TAT-induced EDE through other factors. However, these results may be combined with additional data to account for ancestry and other polymorphic genes involved in the immune response of TAT, possibly providing a more predictive model in the future.

Conclusion

To the best of our knowledge, this is the first report specifically testing the associations between *HLA* alleles and the TAT-induced EDE in Han Chinese population. Carriers of the *HLA-A*02* serotype (especially for alleles *HLA-A*02:01*, *HLA-A*02:06*, and *HLA-A*02:07*) were found to be the high-risk groups for the TAT-induced EDE. This is also the first report of this serotype being associated with a horse serum-derived-product-induced cADRs in any population. Furthermore, the haplotype *HLA-A*02:06/-B*39:01* appears to be a genetic risk factor for the TAT-induced EDE in Han Chinese patients.

To an extent, these alleles might serve as markers for the prediction of the TAT-induced EDE in Han Chinese individuals before the initiation of TAT to reduce adverse reaction risks. Our study suggests that susceptible populations who carry these alleles should use alternative therapies such as TIG (H) or take anti-histamines as a preventive measure. Notably, studies with larger sample sizes or patients of different ethnicities are required in the future.

Acknowledgements

The authors thank Mentality Bio-Tech Co., Ltd (China) for the determination of genotypes. They also thank the Department of Dermatology and Rheumatology [Huashan Hospital, Fudan University (Shanghai, China)] for providing clinical data. They would also like to thank Editage (<http://www.editage.cn>) for English language editing.

This work was supported by the National Natural Science Foundation of China (grants 11179012, 81472873, 81071287, 30971582, and 31371274), the 973 Projects (2011CB710801), the Shanghai Municipal Commission of Science and Technology Program (14DJ1400100) and Scientific and Technological Research in Open Collaborative Projects of Henan Province (152106000044).

Conflicts of interest

There are no conflicts of interest.

Reference

- Naghavi M, Wang HD, Lozano R, Davis A, Liang XF, Zhou MG, *et al.* Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; **385**:117–171.
- [No authors listed]. Tetanus antitoxin. *Lancet* 1939; **234**:609.
- Buff B. Fatal anaphylactic shock: following intradermal skin test with dilute horse serum tetanus antitoxin. *JAMA* 1960; **174**:1200–1201.
- Arbesman CE, Kantor SZ, Rose NR, Witebsky E. Serum sickness: serologic studies following prophylactic tetanus antitoxin. *J Allergy* 1960; **31**:257–272.
- Kabura L, Ilibagiza D, Menten J, Van den Ende J. Intrathecal vs. intramuscular administration of human antitetanus immunoglobulin or equine tetanus antitoxin in the treatment of tetanus: a meta-analysis. *Trop Med Int Health* 2006; **11**:1075–1081.
- Huang QX, Huang SJ. Analysis of patients with late phase allergic reaction caused by TAT injection and nursing. *J Nursing* 2010; **17**:67–69.
- Li Q, Lei ZB. One case of delay cnidosis induced by tetanus antitoxin injection. *Chin J Pharmacovigilance* 2010; **7**:252–252.
- Freeman AG. Dangers of tetanus antitoxin. *Br Med J* 1964; **2**:247.
- Stern RS. Utilization of hospital and outpatient care for adverse cutaneous reactions to medications. *Pharmacoepidemiol Drug Saf* 2005; **14**:677–684.
- Shehab N, Patel PR, Srinivasan A, Budnitz DS. Emergency department visits for antibiotic-associated adverse events. *Clin Infect Dis* 2008; **47**:735–743.
- Rosenblatt AE, Stein SL. Cutaneous reactions to vaccinations. *Clin Dermatol* 2015; **33**:327–332.
- Stern RS. Exanthematous drug eruptions. *N Engl J Med* 2012; **366**:2492–2501.
- Chen YC, Chiu HC, Chu CY. Drug reaction with eosinophilia and systemic symptoms: a retrospective study of 60 cases. *Arch Dermatol* 2010; **146**:1373–1379.
- Shirkey HC. Tetanus immune globulin (human) in prophylaxis against tetanus. *J Pediatr* 1965; **67**:643–646.
- Bharadwaj M, Illing P, Theodossis A, Purcell AW, Rossjohn J, McCluskey J. Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. *Annu Rev Pharmacol Toxicol* 2012; **52**:401–431.
- Roujeau JC, Huynh TN, Bracq C, Guillaume JC, Revuz J, Touraine R. Genetic susceptibility to toxic epidermal necrolysis. *Arch Dermatol* 1987; **123**:1171–1173.
- Goncalo M, Coutinho I, Teixeira V, Gameiro AR, Brites MM, Nunes R, *et al.* HLA-B*58:01 is a risk factor for allopurinol-induced DRESS and Stevens–Johnson syndrome/toxic epidermal necrolysis in a Portuguese population. *Br J Dermatol* 2013; **169**:660–665.
- Zhang Y, Wang J, Zhao LM, Peng W, Shen GQ, Xue L, *et al.* Strong association between HLA-B*1502 and carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in mainland Han Chinese patients. *Eur J Clin Pharmacol* 2011; **67**:885–887.
- Kitts A, Feolo M, Helmberg W. The major histocompatibility complex database, dbMHC – the NCBI handbook – NCBI bookshelf. *National Center for Biotechnology Information* 2003; **11**:1–22.
- Trachtenberg E, Vinson M, Hayes E, Hsu YM, Houtchens K, Erlich H, *et al.* HLA class I (A, B, C) and class II (DRB1, DQA1, DQB1, DPB1) alleles and haplotypes in the Han from southern China. *Tissue Antigens* 2007; **70**:455–463.
- Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956; **20**:309–311.
- Gao GF, Tormo J, Gerth UC, Wyer JR, McMichael AJ, Stuart DI, *et al.* Crystal structure of the complex between human CD8 alpha (alpha) and HLA-A2. *Nature* 1997; **387**:630–634.
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al.* The protein data bank. *Nucleic Acids Res* 2000; **28**:235–242.
- Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of COOT. *Acta Crystallogr D Biol Crystallogr* 2010; **66**:486–501.
- Saper MA, Bjorkman PJ, Wiley DC. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J Mol Biol* 1991; **219**:277–319.
- Schrodinger LLC. The PyMOL molecular graphics system, version 1.3r1: 2010.
- Unni S, Huang Y, Hanson RM, Tobias M, Krishnan S, Li WW, *et al.* Web servers and services for electrostatics calculations with APBS and PDB2PQR. *J Comput Chem* 2011; **32**:1488–1491.
- Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, *et al.* Nomenclature for factors of the HLA system, 2010. *Tissue Antigens* 2010; **75**:291–455.
- Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Dupont B, Erlich HA, *et al.* Nomenclature for factors of the HLA system, 1994. *Tissue Antigens* 1994; **44**:1–18.
- Chelvanayagam G. A roadmap for HLA-A, HLA-B, and HLA-C peptide binding specificities. *Immunogenetics* 1996; **45**:15–26.
- Pirmohamed M. Pharmacogenetics of idiosyncratic adverse drug reactions. In: Uetrecht J, editor. *Adverse drug reactions*. Berlin, Heidelberg: Springer; 2010. pp. 477–491.
- Descotes J, Choquet-Kastylevsky G. Gell and Coombs's classification: is it still valid? *Toxicology* 2001; **158**:43–49.
- Xiao GN, Sun QP. Contribution of serum IL-4 and IgE to the early prediction of horse serum allergies in guinea pigs. *Asia Pac Allergy* 2012; **2**:264–268.
- Bettiol J, Sele J, Henket M, Louis E, Malaise M, Bartsch P, *et al.* Cytokine production from sputum cells after allergenic challenge in IgE-mediated asthma. *Allergy* 2002; **57**:1145–1150.
- Schmidt-Weber CB. Anti-IL-4 as a new strategy in allergy. *Chem Immunol Allergy* 2012; **96**:120–125.
- Jahn-Schmid B, Pickl WF, Bohle B. Interaction of allergens, major histocompatibility complex molecules, and T cell receptors: a 'ménage à trois' that opens new avenues for therapeutic intervention in type I allergy. *Int Arch Allergy Immunol* 2011; **156**:27–42.
- Pichler WJ. Immune mechanism of drug hypersensitivity. *Immunol Allergy Clin North Am* 2004; **24**:373–397.
- Posadas SJ, Pichler WJ. Delayed drug hypersensitivity reactions – new concepts. *Clin Exp Allergy* 2007; **37**:989–999.
- Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, *et al.* Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002; **359**:727–732.
- Chung WH, Hung SI, Hong HS, Hsieh MS, Yang LC, Ho HC, *et al.* Medical genetics: a marker for Stevens–Johnson syndrome. *Nature* 2004; **428**:486.
- Cao ZH, Wei ZY, Zhu QY, Zhang JY, Yang L, Qin SY, *et al.* HLA-B*58:01 allele is associated with augmented risk for both mild and severe cutaneous adverse reactions induced by allopurinol in Han Chinese. *Pharmacogenomics* 2012; **13**:1193–1201.
- Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, *et al.* HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci USA* 2005; **102**:4134–4139.
- Yang F, Gu B, Zhang L, Xuan J, Luo H, Zhou P, *et al.* HLA-B*13:01 is associated with salazosulfapyridine-induced drug rash with eosinophilia and systemic symptoms in Chinese Han population. *Pharmacogenomics* 2014; **15**:1461–1469.
- Yang F, Xuan J, Chen J, Zhong H, Luo H, Zhou P, *et al.* HLA-B*59:01: a marker for Stevens–Johnson syndrome/toxic epidermal necrolysis caused by methazolamide in Han Chinese. *Pharmacogenomics J* 2015; **16**:83–87.
- Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987; **329**:506–512.
- Barouch D, Friede T, Stevanović S, Tussey L, Smith K, Rowland-Jones S, *et al.* HLA-A2 subtypes are functionally distinct in peptide binding and presentation. *J Exp Med* 1995; **182**:1847–1856.
- Rötzschke O, Falk K, Stevanović S, Jung G, Rammensee HG. Peptide motifs of closely related HLA class I molecules encompass substantial differences. *Eur J Immunol* 1992; **22**:2453–2456.
- Latron F, Moots R, Rothbard JB, Garrett TP, Strominger JL, McMichael A. Positioning of a peptide in the cleft of HLA-A2 by complementing amino acid changes. *Proc Natl Acad Sci USA* 1991; **88**:11325–11329.
- Utz U, Koenig S, Coligan JE, Biddison WE. Presentation of three different viral peptides, HTLV-1 Tax, HCMV gB, and influenza virus M1, is determined by common structural features of the HLA-A2.1 molecule. *J Immunol* 1992; **149**:214–221.
- Tussey LG, Matsui M, Rowland-Jones S, Warburton R, Frelinger JA, McMichael A. Analysis of mutant HLA-A2 molecules. Differential effects on peptide binding and CTL recognition. *J Immunol* 1994; **152**:1213–1221.
- 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.
- Tanigaki N, Fruci D, Chersi A, Falasca G, Tosi R, Butler RH. HLA-A2-binding peptides cross-react not only within the A2 subgroup but also with other HLA-A-locus allelic products. *Hum Immunol* 1994; **39**:155–162.
- Kubo RT, Sette A, Grey HM, Appella E, Sakaguchi K, Zhu NZ, *et al.* Definition of specific peptide motifs for four major HLA-A alleles. *J Immunol* 1994; **152**:3913–3924.

- 54 Roder G, Blicher T, Justesen S, Johannesen B, Kristensen O, Kastrup J, *et al.* Crystal structures of two peptide-HLA-B*1501 complexes; structural characterization of the HLA-B62 supertype. *Acta Crystallogr D Biol Crystallogr* 2006; **62**:1300–1310.
- 55 Bade-Doeding C, DeLuca DS, Seltsam A, Blasczyk R, Eiz-Vesper B. Amino acid 95 causes strong alteration of peptide position Pomega in HLA-B*41 variants. *Immunogenetics* 2007; **59**:253–259.
- 56 Ikeda H, Takahashi Y, Yamazaki E, Fujiwara T, Kaniwa N, Saito Y, *et al.* HLA class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions. *Epilepsia* 2010; **51**:297–300.
- 57 Fricke-Galindo I, Martinez-Juarez IE, Monroy-Jaramillo N, Jung-Cook H, Falfan-Valencia R, Ortega-Vazquez A, *et al.* HLA-A*02:01:01/-B*35:01:01/-C*04:01:01 haplotype associated with lamotrigine-induced maculopapular exanthema in Mexican Mestizo patients. *Pharmacogenomics* 2014; **15**:1881–1891.
- 58 Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med* 2000; **343**:338–344.
- 59 Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001; **13**:114–119.
- 60 Tassaneeyakul W, Tiamkao S, Jantararungtong T, Chen P, Lin S-Y, Chen W-H, *et al.* Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. *Epilepsia* 2010; **51**:926–930.
- 61 Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, *et al.* A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 2008; **18**:99–107.
- 62 Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K, *et al.* HLA-B*1511 is a risk factor for carbamazepine-induced Stevens–Johnson syndrome and toxic epidermal necrolysis in Japanese patients. *Epilepsia* 2010; **51**:2461–2465.