HLA-B*57:01 confers genetic susceptibility to carbamazepine-induced SJS/TEN in Europeans

To the Editor,

Carbamazepine (CBZ) is a widely used antiepileptic drug for the treatment of epilepsy, as well as bipolar disorder, trigeminal neuralgia, etc. Although effective for treating neurological diseases, CBZ may cause cutaneous adverse reactions ranging from mild maculopapular exanthema (MPE) to life-threatening severe cutaneous adverse reactions (SCAR), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS). CBZ is known as one of the most common causative drugs for SCAR in different populations.1

Stevens-Johnson syndrome/toxic epidermal necrolysis is characterized as epidermal detachment with high mortality rate ranging from 10%–50%.1 HLA-B*15:02 was found to be strongly associated with CBZ-induced SJS/TEN in Han Chinese2 and further validated in many populations, especially in countries of Southeast Asia, such as Thailand, Malaysia, Vietnam, India. Genetic screening of HLA-B*15:02 prior to the use of CBZ for patients with Southeast Asian ancestry is recommended by US FDA and related health administrations in many countries of the world. Furthermore, HLA-A*31:01 was reported to be associated with CBZ hypersensitivity reactions in Europeans.3 Our study revealed that HLA-A*31:01 is much more related to CBZ-induced MPE/DRESS than CBZ-SJS/TEN,4 which was confirmed by other studies.5 However, the genetic susceptibility of CBZ-SJS/TEN in Europeans remains unclear. Therefore, we conducted an international study to enroll patients with CBZ-SJS/TEN. We aimed to investigate the genetic predisposition of CBZ-SJS/TEN and further evaluate the strength of association for CBZ-SCAR in Europeans.

In total, we enrolled 31 patients with CBZ-SJS/TEN, including 17 patients with SJS, 10 with SJS/TEN overlap, and four with TEN from Europe. The phenotypes of SJS/TEN were clinically assessed using the diagnosis criteria for SJS/TEN of the RegiSCAR group6. In a second independent step, causality assessment was performed in each case using the algorithm for assessment of drug causality in epidermal necrolysis, that is SJS/TEN (ALDEN).7 Only probable and definite cases of SJS/TEN and causality for CBZ (ALDEN score >4) were included for this study. Written informed consent was obtained from each participant. Among them, there are two Vietnamese from France and one Thai patient from Germany. The HLA-A and HLA-B data of patients with CBZ-SJS/TEN were determined by SeCore HLA sequence-based typing (Table S1).

To identify the HLA association of CBZ-SJS/TEN among people with European ancestry, we included Europeans (N = 28) for analysis (Table 1). Our result revealed that HLA-B*57:01 was strongly associated with CBZ-SJS/TEN in Europeans using either the dataset of “Central Europe general population controls”8 (Pc = 1.83 × 10−5) (Table 1 and Table S2), or “1000G EUR controls”9 (Pc = 4.58 × 10−4) (Table S3). HLA-B*57:01 allele was observed in 39.29% (11/28) of patients with CBZ-SJS/TEN, but only in 6.69% (593/8862) of European general population controls (OR = 9.0, 95% CI = 4.2–19.4; P = 9.62 × 10−5; Pc = 1.83 × 10−5) (Table 1 and Table S2). Other HLA alleles showed no association as their Pc > 0.05 (Table S2). By comparison, the two Vietnamese patients with CBZ-induced SJS/TEN carried the HLA-B*15:02 allele (Table S1).

We further analyzed the HLA associations in CBZ-SCAR by combining our previous data of 10 patients with CBZ-DRESS4 (Table 1, Table S4). HLA-A*31:01 shows significant association with CBZ-DRESS (present in 70% of patients, P = 4.0 × 10−8), but not with CBZ-SJS/TEN (Table 1). Furthermore, HLA-B*57:01 was absent in any European patient with CBZ-DRESS HLA-B*57:01 or HLA-A*31:01 was present in 55.26% (21/38) of CBZ-SCAR patients, and only in 10.96% (971/8862) of European general population controls (OR = 10.0, 95% CI = 5.3–19.1; P = 3.58 × 10−11) (Table 1). To verify whether the HLA-B*57:01 protein interacts with CBZ, we generated the recombinant protein HLA-B/β2-microglobulin complex and performed Biacore T200 surface plasmon resonance (SPR) assay (see the methods described in Supporting Information). Our results revealed that CBZ interacts with HLA-B*57:01 in a concentration-dependent manner (Figure 1A). Furthermore, CBZ shows affinity to HLA-B*57:01 and HLA-B*15:02 proteins only, but not HLA-B*15:01 or HLA-B*51:01 proteins (Figure 1B).

Severe cutaneous adverse reactions are rare but life-threatening adverse reactions. The incidence of SJS/TEN is low, with 1–2 cases per million persons per year.2 This report provides the evidence that HLA-B*57:01 is strongly associated with CBZ-SJS/TEN in Europeans (Pc = 1.83 × 10−5), but not with CBZ-DRESS in Europeans. We did not find a patient of Han Chinese origin with CBZ-SJS/TEN, who carried HLA-B*57:01 (data not shown). The frequencies of HLA-B*57:01 alleles are different among various ethnic groups. Current information from Allele Frequency Net Database (http://www.allelefrequencies.net/) revealed that HLA-B*57:01 is present in about 2.5%–8.9% of Europeans, 3.7%–6.2% of persons with European ancestry in the USA, 3%–5.5% of persons with European ancestry in Australia, and 2%–8% of Israelis, but is largely absent in Northeast and Southeast Asia.

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Asians (<0.5%). The European population is complicated in terms of genetic ancestry, and the population stratification of European populations is significant. In this study, our association study was not controlled for stratification effects, and our models are not appropriately controlled for these differences. Therefore, we used two datasets of European population controls, and both confirm that HLA-B*57:01 is significantly associated with CBZ-SJS/TEN in Europeans. The HLA-B*57:01 allele is also known to be strongly associated with abacavir hypersensitivity. The abacavir-HLA-B*57:01 interaction has been suggested as an altered peptide repertoire model, in which abacavir causes the conformational changes of endogenous peptides presented by HLA-B*57:01.9 We previously reported that CBZ can directly interact with HLA-B*15:02 protein without the involvement of antigen-processing pathways. CBZ-HLA-B*15:02 interaction is considered as the "pharmacological interaction with immune receptors (p-i)" model.10 Interestingly, in this study, we found that CBZ could also interact with HLA-B*57:01 protein by SPR assay. However, whether HLA-B*57:01 protein binds to CBZ and presents the drug epitope to activate immune response in CBZ-SJS/TEN needs further exploration.

The strong association between HLA-A*31:01 and CBZ-DRESS was observed in our previous study.4 HLA-B*57:01 and HLA-A*31:01 alleles were present in 55.26% (21/38) of patients with CBZ-SCAR (P = 3.58 × 10⁻¹¹). Thus, our findings suggest that the combination of HLA-B*57:01 and HLA-A*31:01 screening as a preemptive test may be able to reduce CBZ-SCAR in Europeans.

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CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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REFERENCES
Obesity causes pulmonary dysbiosis affecting innate immune response in murine asthma model

To the Editor,

The recent efforts towards the comprehension of asthma pathogenesis have been strongly associated with reduction of exposure to microorganisms, frequent antibiotic treatment, birth delivery mode, diet and obesity. In fact, high-fat diet (HFD) and obesity are known to affect pulmonary immunity and hyperresponsiveness; it also causes gut dysbiosis contributing to the observed changes in the lungs. In addition, clinical and experimental studies have demonstrated different immune phenotype in obese asthmatic subjects, characterized by neutrophilia and higher IL-17A levels in the lungs. In the present study, HFD efficiently induced obesity in female Balb/c mice (Figure S1B). After ovalbumin (OVA) sensitizations and challenges (Figure S1A), obese mice displayed reduced IL-4 (Figure S1C) and increased IL-17A levels (Figure S1D) in lung homogenates, associated with diminished eosinophil counts (Figure S1E) and increased MPO−cell, indicating neutrophil presence (Figure S1F), compared to lean-allergic mice, at 48 hours, in accordance with Silva et al. Importantly, obese mice presented higher IL-17A levels (Figure S1D) when compared to control group, suggesting a homoeostasis disruption, regardless of the allergy induction. In addition, goblet cells, hyperplasia and mucus production, were more intense in the OB/PA group compared to those of the PA group at both studied time-points (Figure S1I).

Regarding the peculiar phenotype observed in the lungs of OB/PA group, we investigated the activation of DCs by flow cytometry (Figure S3), through the evaluation of the expression of the activation marker, CD80; the receptors of innate immune, Toll-like receptor 2/4; and the inhibitory ligand, PDL-1 (Figure 1A-D). In OB group, expression of TLR-2 (Figure 1A) and CD80 (Figure 1C) was smaller than in CN group, while the expression of TLR-4 (Figure 1B) and PDL-1 (Figure 1D) was increased. Moreover, obese mice subjected to pulmonary allergy protocol showed reduction of TLR-2 (Figure 1A), TLR-4 (Figure 1B), CD80 (Figure 1C) and PDL-1 (Figure 1D) in comparison with the nonobese allergic group (PA), at the 24-hour time-point. Nevertheless, 48 hours after the last challenge all of these markers presented a greater expression than in 24 hours, in the OB/PA group, while this effect was not noticed in the PA group.

It is known that obesity can be caused by dysbiosis of intestinal microbiota and that diet can affect mainly Firmicutes and Bacteroidetes. Along these lines, qPCR showed an increased percentage of Firmicutes and reduction of Bacteroidetes in OB group, in comparison with CN group (Figure S2B), and augment of Firmicutes/Bacteroidetes ratio in the gut of OB group, in comparison with CN group (Figure S2A-B). Furthermore, pulmonary allergy induction increased Firmicutes/Bacteroidetes ratio in PA and OB/PA groups, when compared to CN group (Figure S2A-B).

Recent evidence indicates that lungs are not sterile. In the work, all the main phyla were detected through fluorescent in situ hybridization (FISH), and specific probes (Data S1) allowed the counting of Bacteroidetes (Figure S5A), Fusobacteria (Figure S5B), Firmicutes (Figure 1E), Proteobacteria (Figure 1F) and Actinobacteria (Figure 1G), in the lungs. Interestingly, obesity reduced the number of Firmicutes (Figure 1E) and increased Proteobacteria (Figure 1F), when compared to nonallergic lean mice. After the induction of allergy, the number of Bacteroidetes (Figure S5A) and Firmicutes (Figure 1E) was significantly higher, in the allergic groups (PA) and (OB/PA) in comparison with the nonallergic mice (CN and OB). In addition, the Proteobacteria phylum (Figure 1B) was more abundant in nonobese allergic (PA) animals, but it did not show any significant growth in obese allergic (OB/PA) mice. Regardless of the reduction in the Proteobacteria phylum, the qPCR relative quantification has shown the Moraxella catarrhalis/Proteobacteria proportion to be increased in obese asthmatic (Figure 1D). This bacterial persistence possibly contributed to the immune profile shift, as this species is possibly contributed to the immune profile shift, as this species is

Abbreviations: BMDC, bone marrow-derived dendritic cell; CN, control negative; DC, dendritic cell; EUB, Eubacteria; FISH, fluorescent in situ hybridization; HFD, high-fat diet; IL, interleukin; MPO, myeloperoxidase; OB, obese; OB/PA, obese/pulmonary allergy; OVA, ovalbumin; PA, pulmonary allergy; PDL-1, programmed death ligand – 1; qPCR, quantitative polymerase chain reaction; Th2, T helper 2; TLR, Toll-like receptor.

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