BRIEF COMMUNICATION

HLA-B*15:02 association with carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in an Indian population: a pooled-data analysis and meta-analysis

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Summary

This study aimed to investigate the prevalence and association of HLA-B*15:02 with carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis (CBZ-SJS/TEN) in the Indian population in Malaysia, which mostly originated from Southern India. HLA-B alleles in five Indian case patients with CBZ-SJS/TEN and 52 CBZ-tolerant controls, and followed by a pooled sample of seven cases from two centers in Malaysia were analyzed. Positive association for HLA-B*15:02 with CBZ-SJS/TEN was detected in Indians (40% [2/5] vs. 3.8% [2/52], odds ratio [OR] 16.7, $p = 0.0349$), of which 80% (4/5) of the Indian patients originated from Southern India. A pooled sample of seven cases showed stronger association between HLA-B*15:02 and CBZ-SJS/TEN (57.1% [4/7] vs. 3.8% [2/52], OR 33.3, 95% confidence interval [CI] 4.25–162.21, $p = 1.05 \times 10^{-3}$). Subsequent meta-analysis on Indians from Malaysia and India further demonstrated a significant and strong association between HLA-B*15:02 and CBZ-SJS/TEN (OR 38.54; 95% CI 6.83–217.34, $p < 1.0 \times 10^{-4}$). Our study is the first on Indians predominantly from Southern India that demonstrated HLA-B*15:02 as a strong risk factor for CBZ-SJS/TEN despite a low population allele frequency. This stressed the importance of testing for HLA-B*15:02, irrespective of the ancestral background, including populations with low allele frequency.

KEY WORDS: Carbamazepine, Steven-Johnson syndrome, Indian, HLA-B*15:02, Malaysia.

Carbamazepine (CBZ), a first-line antiepileptic drug (AED), is one of the main causal drugs for Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).[1] Studies from Malaysia and Singapore have reported CBZ as the main causative drug comprising 17.9–27.7% of all SJS/TEN cases.[2,3]

Studies among Han Chinese populations in Taiwan, China, and Hong Kong identified a strong association between CBZ-induced SJS/TEN and the HLA-B*15:02 allele.[4–6] The associations were also reported later on in Thai and Malay populations but not observed in Europeans, Japanese, and Koreans.[7–9] This may be due in part to HLA allele frequency differences among different ethnic groups.

A review of the Allele Frequencies Database (AFND) showed that the HLA-B*15:02 allele is prevalent in most Southeast Asian populations: 10.2% in Han Chinese, 8.4% in Malay, 8.4% in Thai, and 12.0–16.7% in

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Indonesia Javanese.[10] However, in India, the allele frequency of HLA-B*15:02 is not homogeneously distributed. Indian populations are genetically diverse because of different waves of immigration resulting in subdivided castes and tribes, which are further subdivided by geography, language, and religion, forming a nonpanmictic population in which the mating within the breeding population is not random. This is supported by evidence that groups in India inherited different proportions of ancestry from ancestral Northern Indians and ancestral Southern Indians, two genetically diverse ancient populations.[11] The most recent immigration is of Indo-European speakers who entered India primarily from the northwest, establishing the Hindu caste system, and after initial admixture, the indigenous Dravidian speakers were displaced and migrated southward. Population structure studies showed admixture of Central and West Asians population with Northern Indian population as evidenced by lower genetic differentiation between Central Asian and Northern Indian populations than with Southern Indian populations.[12] Indian populations can be broadly classified into Northern Aryans and Southern Dravidians. HLA-B*15:02 allele frequency in the Indian population ranges from 0% to 6% depending on region, with as low as 0% in west coast Parsi, 1.6% in Northern Indian, to 4% in west Bhil, and 6% in Pawra in Khandesh region, but not available for populations in Southern India and Sri Lanka.[10]

Malaysia has a heterogeneous genetic composition with multiethnic population from Austronesia (Malay), Southern China (Han Chinese), and India (predominantly Southern Indian). Previous studies on the Malaysian population[8] focused mainly on Malay and Chinese. There is only one published report on an Indian population, recruited from Modasa, a city in Northwest India. Thus, we aimed to study the prevalence of HLA-B*15:02 among Malaysian Indians and the association with CBZ-SJS/TEN, using a pooled-data analysis incorporating samples from one previous study in Malaysia and then a meta-analysis with a study from India.[13]

Materials and Methods

Patients

Patients who attended University of Malaya Medical Centre (UMMC) Neurology clinic were reviewed retrospectively from May 2012 to January 2014. Medical records of 310 patients who were taking at least one AED therapy were identified and reviewed. Patients with a diagnosis of SJS or TEN were assessed and confirmed by a dermatologist, according to clinical morphology by Roujeau et al. CBZ was identified as the causal drug for SJS/TEN if the onset of cutaneous symptoms occurred in the first 3 months of exposure and resolved when withdrawn. Patients with SJS/TEN caused by other drugs were excluded from this study. Patients who had taken CBZ for at least 3 months without experiencing cutaneous reactions were recruited as controls from the same clinic as CBZ-SJS/TEN patients.

All patients provided written informed consent. Participants were interviewed for the ethnicity background of their biologic parents and grandparents. Patients with non-Indian ancestry in the previous two generations were excluded. We identified five CBZ-SJS Indian patients, of which four were from Southern India, and 52 CBZ-tolerant Indian controls. This study was approved by the University of Malaya Medical Ethics Committee. Venous blood samples were obtained from each patient. In the event that blood sampling was not feasible, buccal swabs were obtained from three CBZ-tolerant controls.

HLA-B*15:02 and other HLA-B allele genotyping

HLA-B allele genotyping was first performed with WAKFlow HLA typing kit (Wakunaga Pharmaceutical Co. Ltd, Hiroshima, Japan) and analyzed by Luminex 200 (Luminex, Austin, TX, U.S.A.). The HLA-B*15:02 positive samples were later validated with PG1502 FastGel kit (Pharmigene, Inc., Taipei, Taiwan) and DNA sequencing (See Supporting Information for sequencing primers)

Pooled-data analysis

To increase the sample size of cases in our study, we performed a pooled-sample analysis using the results from a previous reported study from Malaysia (Chang et al.[8]). This study is the only Malaysian study that had recruited two Indian patients with CBZ-SJS/TEN; both tested positive for HLA-B*15:02. However, the Indian controls were not recruited, thus the association between Indian with CBZ-SJS/TEN and HLA-B*15:02 was not studied.[8]

Meta-analysis

Meta-analysis was performed for HLA-B*15:02 and Indian with CBZ-SJS/TEN. Case–control studies on the association of HLA-B*15:02 in Indians were identified from MEDLINE, EMBASE, and Cochrane libraries. All databases were searched from their inception to 11 February 2014 using the following terms: HLA-B, carbamazepine, SJS, TEN, and Indian. Reference lists from included studies were reviewed. Only human studies were included. Inclusion criteria were the following: (1) population ethnicity was restricted to Indian and (2) studies investigated the association between HLA-B*15:02 and CBZ-SJS/TEN. Review articles, case reports, conference abstracts, editorial articles, duplicated studies, and studies with insufficient data for meta-analysis were excluded.

Statistical analysis

Based on an estimation of 4% of controls carried the risk allele, in a sample of five cases, and a 10:1 matching control-case ratio, the study gives 86% statistical power. Carrier (n) is individual tested positive for at least one copy of a particular allele. Comparison of carrier

frequencies between groups, odds ratio (OR), and 95% confidence interval (CI) were performed with Fisher's exact test; p-value is two-tailed and $p < 0.05$ indicates statistical significance. Demographic characteristics of case and control was compared with Pearson's chi-square for categorical data and Mann-Whitney test for numerical data. Meta-analysis was performed with Review Manager (version 5.2; The Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark) in a random-effects model with Mantel-Haenszel statistic. Heterogeneity was assessed with Cochran's Q and I^2. Substantial heterogeneity was indicated by $I^2 > 50\%$, and $p < 0.10$ indicated statistical significance.

Results

Characteristics of patients and controls

Five Indian patients with CBZ-SJS/TEN (mean age 39.2 years, range 16–65 years; one female, four male) and 52 Indian CBZ-tolerant controls (mean age 37.8 years, range 14–73 years; 25 female, 27 male) were recruited. Of the five Indian subjects, four were Southern Indian (Dravidian). There was no significant difference in age and gender between the two groups. In the CBZ-tolerant control, all but one had Southern Indian ancestry. The only tolerant control with Northern Indian ancestry had HLA-B*15:168 and B*27:03.

Association between HLA-B*15:02 and CBZ-SJS/TEN

Before sample pooling, HLA-B*15:02 allele was present in two Indian subjects with CBZ-SJS/TEN, and both were Southern Indians. There was significant positive association between HLA-B*15:02 allele and CBZ-SJS/TEN (40% [2/5] vs. 3.8% [2/52]; OR 16.7; 95% CI 1.70–163.0; $p = 0.0349$) with 40% sensitivity and 96.2% specificity. After sample pooling, there was a total of seven Indian subjects with CBZ-SJS/TEN, two cases from Chang's study (Chang et al.[8]). In the pooled sample, HLA-B*15:02 carrier frequency was 57.1% in Indian subjects with CBZ-SJS/TEN. Strong association between HLA-B*15:02 and CBZ-SJS/TEN was identified in the Indian population (57.1% [4/7] vs. 3.8% [2/52]; OR 33.33; 95% CI 4.25–261.21; $p = 0.00105$) with 57.1% sensitivity and 96.2% specificity (Table 1).

Association between other HLA-B alleles and CBZ-SJS/TEN

Three Indian patients (60.0%) with CBZ-SJS/TEN had negative HLA-B*15:02. Other common HLA-B alleles were analyzed for the association with CBZ-SJS/TEN. HLA-B alleles with carrier rate of >10% in either cases or tolerant-controls were summarized (see Table S1). Statistically significant differences were not detected in other HLA-B alleles.

Meta-analysis of association between HLA-B*15:02 and CBZ-SJS/TEN in Indians

In total, 96 articles were identified, of which, 32 duplicates; 46 review articles, editorial articles, and consortium guidelines; 6 case reports, one conference abstracts; and 7 articles did not meet inclusion criteria and were removed. Three conference abstracts on Southern Indian partially met inclusion criteria but lacked key information.[14] One article met the inclusion criteria. No suitable article was found from the reference of the article. Meta-analysis of the study from India[13] and the present study entailed 15 CBZ-SJS/TEN Indian cases and 62 CBZ-tolerant controls. Carrier frequency of HLA-B*15:02 was compared between the two groups demonstrating a significant association in a random-effects model with no evidence of heterogeneity ($p < 1.0 \times 10^{-4}$; OR 38.54; 95% CI 6.83–217.34) (Fig. 1). In combination, this test has 66.7% sensitivity and 96.8% specificity.

Discussion

This study showed a significant association between HLA-B*15:02 and CBZ-SJS/TEN in the Indian ethnic group in Malaysia, despite a very low carrier frequency of HLA-B*15:02 in Indians (3.8%). This is the first reported association between HLA-B*15:02 and CBZ-SJS/TEN in a cohort with predominantly Southern Indians, different from another Indian study from Mehta et al. [13] on Northwest India. However, subsequent meta-analysis of these combined studies showed a strong association suggesting HLA-B*15:02 as a valid pharmacogenetic marker for CBZ-SJS/TEN in the Indian population.

More importantly, this study has demonstrated the association of the HLA-B*15:02 and CBZ-SJS/TEN despite a low

Table 1. Association of HLA-B*15:02 with CBZ-SJS/TEN (before and after sample pooling)

	HLA-B*15:02 carrier frequency, n/total in group (%)			
	CBZ-SJS/TEN (n = 5) (n = 7)[a]	CBZ-tolerant (n = 52)	OR (95% CI)	p-Value
Indian	2/5 (40.0)	2/52 (3.8)	16.67 (1.70–162.96)	3.490×10^{-2}
	4/7 (57.1)[a]	2/52 (3.8)	33.33 (4.25–261.21)	1.05×10^{-3}

CBZ, carbamazepine; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis.
p-Value < 0.05 indicated statistical significance.
[a]After sample pooling with previous reported studies from Malaysia (Chang et al.).

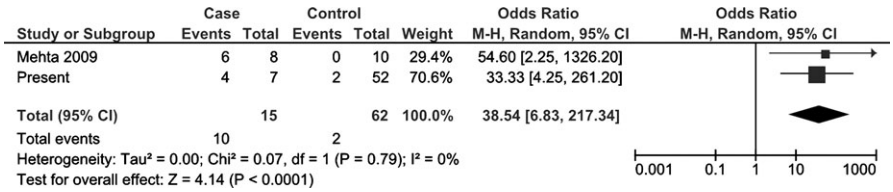

Figure 1.

Analysis of association between HLA-B*15:02 and Indians with CBZ-SJS/TEN from Malaysia and India. Event represented SJS/TEN. Study weight (inversed variance) is shown in the size of the square. Diamond indicated pooled odds ratios. The horizontal lines indicate 95% confidence intervals. χ^2 and I^2 indicate heterogeneity.

population allele frequency. This argued against current regulatory U.S. Food and Drug Administration (FDA) recommendation that this association is only relevant to populations where HLA-B*15:02 is prevalent, and supports more recent recommendation of testing all patients, irrespective of ancestral background.[15,16]

In our study, HLA-B*15:02 was present in only 57% of those with CBZ-SJS/TEN after sample pooling, a frequency lower than that among the Han Chinese, Thai, and Malay populations (75–100%).[4,7,8] This suggested that other than antigen binding and presentation by the HLA-B*15:02 molecules, there are other genetic and nongenetic factors contributing to the pathogenesis of CBZ-SJS/TEN. A full HLA-B genotyping performed did not reveal significant associations between any other HLA-B alleles and CBZ-SJS/TEN perhaps due to the limited number of SJS/TEN cases. In the study by Mehta et al.,[13] HLA-B*15:08 was detected in CBZ-SJS/TEN, and it was also detected in one of our Indian cases, with an OR of 12.75; however, when compared with the tolerant group the association was not statistically significant. Of interest, HLA-B*15:02 and B*15:08 are both members of the HLA-B75 serotype families. Other members of the same serotype family that was found to be associated with CBZ-SJS/TEN were HLA-B*15:11 in a study from Japan,[17] elucidating involvement of subfamilies of this serotype in the pathogenesis of CBZ-SJS/TEN.

Conclusion

In conclusion, our study supports the association between HLA-B*15:02 and CBZ-SJS/TEN in the Indian population in Malaysia. This is the first study on an Indian cohort predominantly from Southern India demonstrating that, despite a low carrier frequency, HLA-B*15:02 remained a highly significant risk predictor of CBZ-SJS/TEN. This stressed the importance of testing for HLA-B*15:02, even in populations with low allele frequency.

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Disclosure

All authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Carrier frequency of HLA-B alleles in CBZ-induced SJS/TEN and CBZ-tolerant control.

Table S2. HLA-B genotype of patients with carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis.

Table S3. HLA-B genotype of carbamazepine-tolerant controls.