Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population

Takeshi Ozeki¹, Taisei Mushiroda¹, Amara Yowang¹, Atsushi Takahashi², Michiaki Kubo³, Yuji Shirakata⁴, Zenro Ikezawa⁵, Masafumi Iijima⁶, Tetsuo Shiohara⁷, Koji Hashimoto⁴, Naoyuki Kamatani² and Yusuke Nakamura¹,8,∗

¹Research Group for Pharmacogenomics, ²Research Group for Medical Informatics and ³Research Group for Genotyping, RIKEN Center for Genomic Medicine, Yokohama 230-0045, Japan, ⁴Department of Dermatology, Ehime University Graduate School of Medicine, Ehime 791-0295, Japan, ⁵Department of Dermatology, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan, ⁶Department of Dermatology, Showa University School of Medicine, Tokyo 142-8555, Japan, ⁷Department of Dermatology, Kyorin University School of Medicine, Tokyo 181-8611, Japan and ⁸Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

Received September 12, 2010; Revised November 28, 2010; Accepted December 6, 2010

An anticonvulsant, carbamazepine (CBZ), is known to show incidences of cutaneous adverse drug reactions (cADRs) including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). To identify a gene(s) susceptible to CBZ-induced cADRs, we conducted a genome-wide association study (GWAS) in 53 subjects with the CBZ-induced cADRs, including SJS, TEN and DIHS, and 882 subjects of a general population in Japan. Among the single nucleotide polymorphisms (SNPs) analyzed in the GWAS, 12 SNPs showed significant association with CBZ-induced cADRs, and rs1633021 showed the smallest P-value for association with CBZ-induced cADRs (P = 1.18 × 10⁻¹³). These SNPs were located within a 430 kb linkage disequilibrium block on chromosome 6p21.33, including the HLA-A locus. Thus, we genotyped the individual HLA-A alleles in 61 cases and 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant controls) and found that HLA-A*3101 was present in 60.7% (37/61) of the patients with CBZ-induced cADRs, but in only 12.5% (47/376) of the CBZ-tolerant controls (odds ratio = 10.8, 95% confidence interval 5.9–19.6, P = 3.64 × 10⁻¹⁵), implying that this allele has the 60.7% sensitivity and 87.5% specificity when we apply HLA-A*3101 as a risk predictor for CBZ-induced cADRs. Although DIHS is clinically distinguished from SJS and TEN, our data presented here have indicated that they share a common genetic factor as well as a common pathophysiological mechanism. Our findings should provide useful information for making a decision of individualized medication of anticonvulsants.

INTRODUCTION

Cutaneous adverse drug reactions (cADRs) characterized by acute inflammatory reaction of skin and mucous membranes are dose-independent, unpredictable and sometimes life-threatening. Manifestations range from a mild erythematous maculopapular rash [maculopapular eruption (MPE)], a self-limited, exanthematous, cutaneous variant with minimal oral
involvement [erythema multiforme (EM)] to progressive, fulminating, severe variant with extensive mucocutaneous epithelial necrosis [Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)]. Drug-induced hypersensitivity syndrome (DIHS) is also described as severe cADR, characterized by rash, fever and multiorgan systemic reactions such as lymphadenopathy, hepatitis and leukocytosis with eosinophilia (1,2). High fever, no mucocutaneous involvement and reactivation of human herpesvirus 6 (HHV-6) are important features of DIHS that can be distinguished from SJS and TEN (2,3).

Almost all drugs have been reported to have a risk to cause cADRs. Some drugs, such as anticonvulsants, antibiotics and non-steroidal anti-inflammatory drugs, are known to show higher incidences of cADRs, including SJS and TEN (2,3), while the culprit drugs for DIHS are limited to several drugs, including carbamazepine (CBZ), phenytoin (PHT), phenobarbital, dapsone, mexiletine, salazosulfapyridine, allopurinol and minocycline (4). Although several studies have indicated that T-cell-mediated allergic reactions might be related to pathogenesis of cADRs (5), the detailed mechanisms are not yet understood. Similarly, the underlying mechanism for DIHS also remains unknown, although the HHV-6 reactivation is suggested to associate with symptoms of DIHS, such as fever and hepatitis (6). Hence, there is no clinical test available to predict a risk of DIHS.

In case of CBZ, Taiwanese study demonstrated that HLA-B*1502 was associated with SJS/TEN induced by CBZ (7). This strong genetic association could be applied for the prediction and prevention of cADRs. However, the allelic frequencies of the HLA loci differ significantly among different ethnic groups. For example, the HLA-B*1502 allele is present at high frequency in south-eastern Asians (8.6%) (7), but it was only 0.1% in Japanese and Caucasian populations (http://www.allelefrequencies.net/). Thus, HLA-B*1502 is not so useful as genetic predictors for the CBZ-induced cutaneous reactions in Japanese and Caucasian populations.

Single nucleotide polymorphisms (SNPs) are the most abundant DNA sequence variations, and a large body of SNP information was already constructed through the International HapMap project (8). In addition, the rapid technological development enabled us to perform genome-wide association study (GWAS) (9) routinely for identifying genetic risk factors for many complex diseases and traits (10). In the present study, we aimed to identify novel susceptibility loci associated with cADRs induced by CBZ in the Japanese population through case–control GWAS with the high-throughput SNP genotyping technology.

RESULTS

Genome-wide association study

We first genotyped 55 cases and 898 subjects of a general population in Japanese with Illumina HumanHap550v3 Genotyping
Since the most significant association was observed with the HLA-B locus, we further genotyped the individual HLA-B alleles for 61 cases and 376 CBZ-tolerant subjects. The HLA-B*1502 allele was absent in any of our cases. In addition, no HLA-B allele showed significant association with CBZ-induced cADRs (Supplementary Material, Table S1). Our LD analysis indicated that HLA-G was also located in the LD block including the landmark SNP, rs1633021 (Fig. 2). Thus, we also genotyped the HLA-G alleles for 61 cases and 376 CBZ-tolerant subjects and found that HLA-G*010102 showed the most significant association with CBZ-induced cADRs (P = 1.31 × 10−7, OR = 4.8, 95% CI 2.6–8.9) (Supplementary Material, Table S1).
Table S2). However, multiple logistic regression analysis revealed that the HLA-A*3101 genotype had the significant effect on risk of CBZ-induced cADRs (OR = 7.9, 95% CI 3.9–16.2, \( P = 1.64 \times 10^{-8} \)), but HLA-G*010102 did not (OR = 1.7, 95% CI 0.8–3.7, \( P = 0.16 \)) (Supplementary Material, Table S3).

**DISCUSSION**

This study is the first GWAS to investigate genetic factors associated with cADRs induced by CBZ. We demonstrated that the 11 SNPs showing the significant association were located within a 430 kb LD block, including the HLA-A locus. HLA-A belongs to the HLA class I heavy chain paralogues, which play a central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen. We thus considered that the association of these SNPs with CBZ-induced cADRs should reflect variations in antigen-binding affinities of HLA-A that might affect the immune response in the pathogenesis of the cADRs. All of the HLA-A, B and G belong to the HLA class I molecules, while HLA-B*1502 has been reported to be associated with SJS/TEN induced by CBZ in Han-Chinese population, and the HLA-G locus was located in the LD block including the landmark SNP, rs1633021, in our LD analysis. However, in our study, no HLA-B allele showed significant association with CBZ-induced cADRs in the Japanese population. Furthermore, multiple logistic regression analysis suggested that the association of HLA-G*010102 was confounding of the association between HLA-A*3101 and CBZ-induced cADRs.

In the present study, the general population subjects were used for the case–control association study. The use of general population has a disadvantage, a reduced statistical power, because part of the general population had a potential to show CBZ-induced cADRs. However, the reduction of power is dependent on prevalence of ADRs and can be compensated by increasing the number of subjects. In the case of the CBZ-induced cADRs, the prevalence is generally low. For a prevalence of 2.9%, which was reported for Japanese population (http://www.info.pmda.go.jp/), statistical power estimates were 0.980 and 0.992 for the use of general...
and trigeminal neuralgia such as PHT and valproic acid, there are several alternative drugs to CBZ for epilepsy typing to predict the risk of CBZ-induced cADRs since

apply

the 376 CBZ-tolerant controls, implying that this allele

confident about the clinical benefit of the

HLA-A

rs1633021 in 376 CBZ-tolerant controls revealed that the

population and CBZ-tolerant controls, respectively. These results indicate that the use of the general population, in place of the CBZ-tolerant controls, can yield sufficient power to permit the identification of strong genetic factors, such as our landmark SNP, rs1633021. Besides, since type I error rate will not be affected by using the general population, the possibility of false-positive results should be similar to that in the use of CBZ-tolerant controls. Consequently, we concluded that the use of the general population for our GWAS would be suitable.

We genotyped HLA-A alleles and identified a strong association of the HLA-A*3101 allele with the risk of the CBZ-induced cADRs in Japanese population. Comparison of genotypes of the HLA-A*3101 and the marker SNP rs1633021 in 376 CBZ-tolerant controls revealed that the G allele of rs1633021 was in strong LD with the HLA-A*3101 (R^2 = 0.79, D’ = 0.95). HLA-A*3101 was present in 37 (60.7%) of the 61 subjects with cADRs induced by CBZ and was present in only 47 (12.5%) of the 376 CBZ-tolerant controls, implying that this allele has the 60.7% sensitivity and 87.5% specificity when we apply HLA-A*3101 as a risk predictor for CBZ-induced cADRs in the Japanese population. If a prevalence of CBZ-induced cADRs was 2.9% (http://www.info.pmda.go.jp/), the positive and negative predictive values would be estimated to be 12.7 and 98.7%, respectively. That is, it might become possible to lower the frequency of CBZ-induced cADR from 2.9 to 1.1% by excluding the patient judged to be HLA-A*3101 positive by the genetic diagnosis from the CBZ treatment. We are confident about the clinical benefit of the HLA-A*3101 typing to predict the risk of CBZ-induced cADRs since there are several alternative drugs to CBZ for epilepsy and trigeminal neuralgia such as PHT and valproic acid, which induce cADRs with low prevalence. Although the efficacy of these alternative drugs might be inferior to CBZ for treating trigeminal neuralgia, a prevention of CBZ-induced cADRs, which are sometimes life-threatening, must be more important for patients. Our findings should provide useful information for making a decision of individualized medication for these diseases.

Recently, Kashiwagi et al. (11) performed HLA genotyping in Japanese subjects with CBZ-induced cADR and found an association with HLA-A*3101 (P = 4.0 × 10^-4 in the allele frequency, OR = 4.3, 95% CI 2.1–9.1). In the study, 22 cases (6 MPE/EM, 3 erythroderma, 4 DIHS, 2 SJS and 7 other drug eruptions) were included. Our results demonstrated that HLA-A*3101 showed significant associations with CBZ-induced DIHS/SJS/TEN and other cADRs.

In the Han-Chinese population, the HLA-A*3101 was reported to be associated with MPE induced by CBZ, but not with SJS/TEN (12). However, we demonstrated that HLA-A*3101 was present in four (80.0%) of the five subjects with SJS/TEN induced by CBZ in the combined cohort. Thus, all of CBZ-induced cADRs including SJS/TEN and MPE are likely to be associated with the same HLA-A allele, HLA-A*3101, in Japanese population on the basis of the present study. The controversial results of the association of HLA-A*3101 with SJS/TEN between Japanese and Chinese studies might be due to ethnic differences in allele frequencies of HLA-B*1502 and HLA-A*3101. It has been found that the HLA-B*1502 allele is extremely rare in Japanese (allele frequency: 0.1%) compared with Han-Chinese (allele frequency: 8.6%) (7). In contrast, the HLA-A*3101 allele is present at a higher allelic frequency in Japanese (9.1%), but only 1.8% in Han-Chinese (http://www.allelefrequencies.net/).

MHC-dependent presentation of drugs and/or the metabolites on HLA class II molecules to CD4^+ helper T cells and on class I molecules to CD8^+ cytotoxic T cells are considered to be critical for the severe cADRs (13,14). Both of HLA-A and -B belongs to MHC class I molecules. Thus, there might be common underlying mechanisms involved in the CBZ-induced cADRs, although HLA-B*1502 seemed to be specifically involved in the CBZ-induced SJS/TEN.

To date, DIHS has been considered to be a different clinical entity from SJS and TEN, because of its delayed onset of symptoms from the beginning of the drug therapy as well as high fever, no mucocutaneous involvement and HHV-6 reactivation (2.3). However, the present study suggests that DIHS and other cADRs, including SJS/TEN, which were induced by CBZ, might share the common pathogenesis from the fact that they were associated with the same HLA-A allele, HLA-A*3101. Although further studies using a large sample size will be necessary to confirm this observation, the HLA-A*3101 could be directly involved in the pathogenesis of all types of the CBZ-induced cADRs in the Japanese population, in view of the fact that HLA-B*5801 has been reported to be a genetic factor associated with both SJS/TEN and DIHS induced by allopurinol in Taiwanese, Japanese and Europeans (15–17).

In conclusion, we have demonstrated that HLA-A*3101 was significantly associated with susceptibility to cADRs induced by CBZ in Japanese population. Unfortunately, because of limitation of the subject information from BioBank Japan,

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Number of carriers (Case %)</th>
<th>CBZ-tolerant control (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*0101</td>
<td>0 (0.0)</td>
<td>6 (1.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*0201</td>
<td>5 (8.2)</td>
<td>93 (24.7)</td>
<td>2.74 × 10^-3</td>
</tr>
<tr>
<td>A*0206</td>
<td>1 (1.6)</td>
<td>68 (18.1)</td>
<td>2.46 × 10^-4</td>
</tr>
<tr>
<td>A*0207</td>
<td>3 (4.9)</td>
<td>23 (6.1)</td>
<td>0.10</td>
</tr>
<tr>
<td>A*0210</td>
<td>2 (3.3)</td>
<td>2 (0.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>A*0301</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*1101</td>
<td>7 (11.5)</td>
<td>68 (18.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>A*1110</td>
<td>0 (0.0)</td>
<td>1 (0.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*2402</td>
<td>37 (60.7)</td>
<td>211 (56.1)</td>
<td>0.58</td>
</tr>
<tr>
<td>A*2405</td>
<td>0 (0.0)</td>
<td>1 (0.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*2420</td>
<td>0 (0.0)</td>
<td>4 (1.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*2601</td>
<td>2 (3.3)</td>
<td>65 (17.3)</td>
<td>3.36 × 10^-3</td>
</tr>
<tr>
<td>A*2602</td>
<td>2 (3.3)</td>
<td>15 (4.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*2603</td>
<td>11 (18.0)</td>
<td>22 (5.9)</td>
<td>2.61 × 10^-3</td>
</tr>
<tr>
<td>A*2605</td>
<td>1 (1.6)</td>
<td>1 (0.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>A*2901</td>
<td>0 (0.0)</td>
<td>1 (0.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*3001</td>
<td>0 (0.0)</td>
<td>2 (0.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>A*3101</td>
<td>37 (60.7)</td>
<td>47 (12.5)</td>
<td>6.40 × 10^-15</td>
</tr>
<tr>
<td>A*3303</td>
<td>5 (8.2)</td>
<td>59 (15.7)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

61 cases and 376 CBZ-tolerant controls.

**Note:** CBZ, carbamazepine.

*aSignificant after Bonferroni’s correction.

Table 2. Frequencies of HLA-A alleles in patients with carbamazepine-induced cutaneous adverse drug reactions and carbamazepine-tolerant controls.
the timings of the onset of reaction in relation to drug use, and CBZ doses were not available. Thus, although a prospective study of CBZ-induced cADRs with detailed clinical information and further investigations will be required to determine the clinical utility and to clarify the molecular mechanisms responsible for the risk of these cADRs, respectively, our findings should shed light on its pathogenesis and facilitate development of genetic test to identify individuals at risk for this potentially life-threatening condition caused by CBZ in the Japanese population.

MATERIALS AND METHODS

Participants

We obtained 62 patients with cADRs induced by CBZ (Supplementary Material, Table S4). The BioBank Japan Project started in 2003 with the aim of collecting genomic DNA and serum samples as well as clinical information from 300,000 patients diagnosed with any of 47 different diseases by collaboration with 66 hospitals in Japan (18). The biological materials and the clinical information were obtained with a written informed consent for participation in this project from the registered samples in BioBank Japan from June 2003 to March 2008, we obtained 33 patients with non-DIHS cutaneous reactions induced by CBZ. The subjects included four patients with SJS/TEN, 16 EM, 4 MPE, 2 erythema, 1 erythroderma, 1 fixed drug eruption and 5 unclassified drug rashes. SJS and TEN were diagnosed as mucocutaneous disorders characterized by wide-spread erythema, blisters, detachment, erosions and fever. SJS was defined by identification of skin detachment of less than 10% of the body-surface area, whereas TEN was diagnosed by finding skin detachment of more than 10%, and excluding staphylococcal scalded skin syndromes (19). We obtained 29 patients with typical DIHS induced by CBZ who were recruited at Yokohama City University Hospital, Showa University Hospital, Kyorin University Hospital and Ehime University Hospital from October 2005 to October 2009. The diagnosis criteria of the typical DIHS were maculopapular rash (developing it more than 2 weeks after the beginning of the therapy with a limited number of drugs) as well as all of the following symptoms: fever (>38°C), hepatitis, hematological disorder [leukocytosis (>11,000 per mm³), atypical lymphocytosis (>5%) or eosinophilia (>1500 per mm³)], lymphadenopathy and HHV-6 reactivation. All the DIHS cases had all the manifestations listed.

Two control groups were used in this study. We used 898 volunteers recruited at the Midosuji and other related Rotary Clubs as the population of general Japanese individuals (general population) for GWAS (20). The 898 volunteers did not have any clinical histories of epilepsy, cranial nerve disorder, cancer and treatment of CBZ. As the second control group for the further detailed HLA genotyping, we selected 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant controls) from the BioBank Japan. The median ages of cases, the subjects of general population and CBZ-tolerant controls were 54 years (range 12–82), 55 years (18–93) and 52 years (1–88), respectively.

For a replication study, we enrolled 16 patients with CBZ-induced cADRs and 44 CBZ-tolerant controls, who were recruited at Yokohama City University Hospital, Showa University Hospital, Kyorin University Hospital and Ehime University Hospital, as the second cohort

<table>
<thead>
<tr>
<th>Population</th>
<th>HLA-A*0206</th>
<th>CBZ-tolerant controls (%)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
<th>HLA-A*3101</th>
<th>CBZ-tolerant controls (%)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First study</td>
<td>1/61 (1.6)</td>
<td>68/376 (18.1)</td>
<td>2.46 × 10⁻⁴</td>
<td>0.1 (0.0–0.6)</td>
<td>37/61 (60.7)</td>
<td>47/376 (12.5)</td>
<td>3.64 × 10⁻¹⁵</td>
<td>10.8 (5.9–19.6)</td>
</tr>
<tr>
<td>Replication study</td>
<td>2/16 (12.5)</td>
<td>8/44 (18.2)</td>
<td>0.72</td>
<td>0.6 (0.1–3.4)</td>
<td>8/16 (50.0)</td>
<td>7/44 (15.9)</td>
<td>1.53 × 10⁻²</td>
<td>5.3 (1.5–24.5)</td>
</tr>
<tr>
<td>Combined analysis</td>
<td>3/77 (3.9)</td>
<td>76/420 (18.1)</td>
<td>1.02 × 10⁻³</td>
<td>0.2 (0.1–0.6)</td>
<td>45/77 (58.4)</td>
<td>54/420 (12.9)</td>
<td>1.09 × 10⁻¹⁶</td>
<td>9.5 (5.6–16.3)</td>
</tr>
</tbody>
</table>

CBZ, carbamazepine; CI, confidence interval.

Table 3. Association of HLA-A*0206 and A*3101 alleles with carbamazepine-induced cutaneous adverse drug reactions

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Number of patients</th>
<th>Positive for HLA-A*3101</th>
<th>Negative for HLA-A*3101</th>
<th>Total</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CBZ-induced cADRs</td>
<td>45</td>
<td>32</td>
<td>77</td>
<td>1.09 × 10⁻¹⁶</td>
<td>9.5 (5.6–16.3)</td>
<td></td>
</tr>
<tr>
<td>DIHS</td>
<td>21</td>
<td>15</td>
<td>36</td>
<td>2.06 × 10⁻⁸</td>
<td>9.5 (4.6–19.5)</td>
<td></td>
</tr>
<tr>
<td>SJS/TEN</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>2.35 × 10⁻⁴</td>
<td>33.9 (3.9–295.6)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>19</td>
<td>16</td>
<td>35</td>
<td>4.74 × 10⁻⁸</td>
<td>8.0 (3.9–16.6)</td>
<td></td>
</tr>
<tr>
<td>CBZ-tolerant controls</td>
<td>54</td>
<td>366</td>
<td>420</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

cADRs, cutaneous adverse drug reactions; CBZ, carbamazepine; CI, confidence interval; DIHS, drug-induced hypersensitivity syndrome; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis.

Table 4. Subgroup analysis of association of the HLA-A*3101 allele with carbamazepine-induced cutaneous adverse drug reactions

aSignificant after Bonferroni’s correction.
(Supplementary Material, Table S5). The median ages of cases and CBZ-tolerant controls in the second cohort were 61 years (range 24–74) and 55 years (16–83), respectively.

Collection of blood samples and clinico-pathological information from patients and volunteers was undertaken with informed consent and was approved by the Ethical Committees at The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, and their use for this study was approved in The Institutes of Physical and Chemical Research (RIKEN), Yokohama, Japan.

**Genome-wide association study**

A genome-wide analysis for 55 cases and 898 subjects of the general population was conducted using Illumina Human-Hap550v3 Genotyping BeadChip according to the manufacturer’s protocols (San Diego, CA, USA). Of 62 cases mentioned above, 7 subjects were not included in the GWAS because these subjects were obtained after the GWAS. We did not include the SNPs of X-chromosome in our GWAS. In case of the X-chromosome, male and female subjects must be separately analyzed, leading to the decrease in sample size. Furthermore, in female, either one of the two X-chromosomes might be inactivated, which can impair genotype–phenotype correlations. A PCA was performed via an ‘Eigen analysis’ in the computer program smartpca, from the EIGENSOFT package (21). Genotype data for the cases and general population subjects and those for 89 East-Asian individuals (44 Japanese and 45 Han Chinese) from the International HapMap project (8) were analyzed for the PCA. PCA plots were obtained using the first two components (Eigenvectors 1 and 2). To validate the genotyping results, we performed genotyping by means of multiplex PCR-based Invader assays (Third Wave Technologies, Madison, WI, USA) (22) and compared the data obtained by the two platforms. To draw an LD map including SNPs which showed significant associations with CBZ-induced cADRs, we applied Haploview software (23).

**HLA genotyping**

HLA-A and -B genotyping was carried out using a WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan), which is based on PCR-sequence-specific oligonucleotide probes coupled with multiple analyte profiling (xMAP) technology (Luminex System; Luminex Corporation, Austin, TX, USA). The data analysis was performed using the WAKFLOW TYPING software (Wakunaga). HLA-G was genotyped by a sequence-based method reported previously (24).

**Statistical analyses**

A statistical significance of the association with each SNP or HLA allele was assessed using Fisher’s exact test. For the GWAS, we carried out the statistical analysis for association by comparing the case and control groups using the allele-frequency model, dominant-inheritance model and recessive-inheritance model. SNPs were rank-ordered according to the lowest $P$-value in these models. Significance levels after Bonferroni’s correction for multiple testing were $1.12 \times 10^{-7}$ (0.05/444 823), $2.63 \times 10^{-3}$ (0.05/19) and $2.50 \times 10^{-2}$ (0.05/2) in the GWAS, HLA-A genotyping and the replication study, respectively. For power estimation, we used QUANTO (http://hydra.usc.edu/GxE/) (25), under the following conditions, assuming a dominant inheritance model: 53 cases, 881 controls; prevalence of CBZ-induced cADRs, 0.029; significance level, $1.0 \times 10^{-2}$; risk allele frequency, 0.08 for general population; ORs, 9.08 and 10.15 for general population and CBZ-tolerant controls, respectively. The Breslow–Day test (26) was used to evaluate the heterogeneity of the ORs between association studies of two cohorts.

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

**ACKNOWLEDGEMENTS**

We thank all of the patients who participated in this study and the Midosuji and other related Rotary Clubs for cooperation in this study. We thank all members of BioBank Japan, Institute of Medical Science, The University of Tokyo, RIKEN Center for Genomic Medicine and of Japanese Research Committee on Severe Cutaneous Adverse Reaction (J-SCAR) for their contribution to the completion of our study.

**Conflict of Interest statement.** None declared.

**FUNDING**

This work was partly supported by Health and Labour Sciences Research Grants (Research on Intractable Diseases) from the Ministry of Health, Labour and Welfare of Japan (H19-nanchi-ippan-004).

**REFERENCES**


