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

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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 ($P = 1.18 \times 10^{-13}$). 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, $P = 3.64 \times 10^{-15}$), 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

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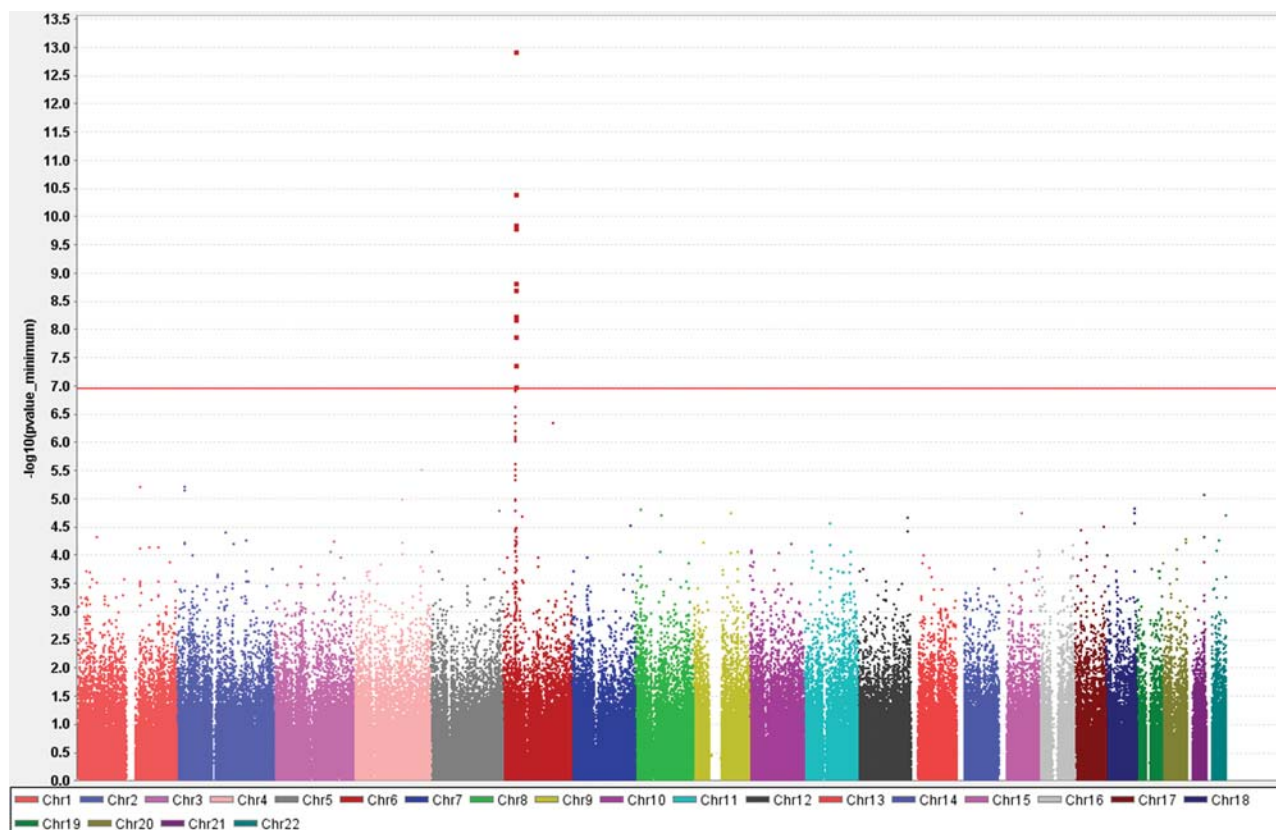
Figure 1. $-\text{Log}_{10}$ *P*-value plots at the GWAS. Each dot represents *P*-value obtained from GWAS using 53 patients with carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population in Japanese. The *Y*-axis represents the $-\log_{10}$ of the minimal *P*-values calculated by Fisher's exact tests for three models: dominant, recessive and allele frequency models in the case–control association study.

involvement [erythema multiforne (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

Table 1. Association of 12 SNPs showing *P*-value less than 1.12×10^{-7} in the GWAS with cutaneous adverse drug reactions induced by carbamazepine in Japanese population

| SNP | Chr | Allele (1/2) | Case | | | | General population | | | | *P*-value | | |
			11	12	22	MAF	11	12	22	MAF	11 versus 12 + 22	11 + 12 versus 22	1 versus 2
rs1633021	6	A/G	19	32	2	0.340	736	142	3	0.084	1.18×10^{-13}	2.83×10^{-2}	1.58×10^{-12}
rs2571375	6	T/C	23	26	4	0.321	736	143	3	0.084	2.44×10^{-10}	2.85×10^{-4}	3.82×10^{-11}
rs1116221	6	T/C	4	26	23	0.321	4	149	729	0.089	5.46×10^{-4}	7.12×10^{-10}	1.35×10^{-10}
rs2844796	6	T/C	4	26	23	0.321	4	150	728	0.090	5.46×10^{-4}	8.26×10^{-10}	1.58×10^{-10}
rs1736971	6	A/C	6	35	12	0.443	27	282	573	0.190	8.43×10^{-3}	1.49×10^{-9}	1.46×10^{-8}
rs1611133	6	T/C	6	35	12	0.443	27	285	570	0.192	8.43×10^{-3}	1.90×10^{-9}	1.63×10^{-8}
rs2074475	6	A/G	28	21	4	0.274	750	129	3	0.077	9.99×10^{-8}	2.85×10^{-4}	5.60×10^{-9}
rs7760172	6	T/C	16	31	6	0.406	622	236	24	0.161	6.07×10^{-9}	5.16×10^{-3}	7.05×10^{-9}
rs2517673	6	T/C	6	31	16	0.406	24	237	621	0.162	5.16×10^{-3}	6.61×10^{-9}	7.67×10^{-9}
rs2524005	6	T/C	6	31	16	0.406	24	244	614	0.166	5.16×10^{-3}	1.28×10^{-8}	1.43×10^{-8}
rs12665039	6	T/C	17	30	6	0.396	621	236	25	0.162	4.96×10^{-8}	6.12×10^{-3}	4.19×10^{-8}
rs1362088	6	A/G	29	20	4	0.264	742	134	6	0.083	1.19×10^{-6}	1.50×10^{-3}	9.86×10^{-8}

53 cases of carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population.
Chr, chromosome; MAF, minor allele frequency.

BeadChip. After excluding one case and 16 subjects of the general population which were judged to be outliers in a principal component analysis (PCA), we applied SNP quality control [call rate of ≥ 0.99 in both cases and subjects of the general population, and *P*-value of the Hardy−Weinberg equilibrium test of $\geq 1.0 \times 10^{-6}$ in subjects of the general population] and excluded one case with the call rate of <0.98. Of 554 496 SNPs genotyped, 444 823 SNPs on autosomal chromosomes passed the quality control and were further analyzed.

Among the SNPs analyzed in the GWAS, 12 SNPs showed significant association with CBZ-induced cADRs after the correction of multiple testing, and rs1633021 revealed the lowest *P*-value for association ($P = 1.18 \times 10^{-13}$) (Fig. 1, Table 1). To validate the first genotyping data, we additionally performed genotyping by means of multiplex PCR-based Invader assays for the rs1633021 and confirmed the accuracy of the data obtained by the two platforms with the concordance rate of 100%.

Interestingly, all of these 12 SNPs were located within a 463 kb region on chromosome 6p21.33 (Table 1). Thus, we plotted linkage disequilibrium (LD) blocks using 882 subjects of the general population and found that 11 of these 12 SNPs were included in an LD block of 29.84−30.27 Mb and the remaining SNP rs1362088 was located closely to the particular LD block (Fig. 2). This region corresponded to the MHC I region containing the *HLA-A* locus.

HLA allele frequency

Since the most significant association was observed with the SNPs near the *HLA-A* locus, we further genotyped the individual *HLA-A* alleles for 61 cases including 7 additional cases and 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant subjects). As shown in Table 2, the frequency of *A*3101* was significantly higher in CBZ-induced cADR cases than CBZ-tolerant controls; *HLA-A*3101* was present in 37 (60.7%) of the 61 patients with cADRs induced by CBZ, while in only 47 (12.5%) of the 376 CBZ-tolerant controls [$P = 3.64 \times 10^{-15}$, odds ratio (OR) = 10.8, 95% confidence interval (CI) of 5.9−19.6]. In addition, the frequency of

HLA-A*2603 was nearly 3-fold higher (18.0 versus 5.9%) in the cADR cases with a suggestive *P*-value. However, this association should be validated by a larger number of cases. On the other hand, the allele frequency of *HLA-A*0206* was significantly lower in CBZ-induced cADR cases than CBZ-tolerant controls ($P = 2.74 \times 10^{-4}$, OR = 0.1, 95% CI 0.0−0.6).

To validate the significant associations of *HLA-A*3101* and *HLA-A*0206*, we performed a replication study using an independent Japanese case−control cohort which consisted of 16 CBZ-induced cADR cases and 44 CBZ-tolerant controls (Table 3). The association of *HLA-A*3101* was replicated in the second cohort ($P = 1.53 \times 10^{-2}$, OR = 5.3, 95% CI 1.5−24.5; combined-analysis $P = 1.09 \times 10^{-16}$, OR = 9.5, 95% CI 5.6−16.3), whereas that of *HLA-A*0206* was not replicated. In the case of *HLA-A*3101*, the *P*-value for the Breslow−Day test was not significant ($P = 0.32$), indicating the absence of significant heterogeneity in these two cohorts.

We further analyzed the association of *HLA-A*3101* according to the type of cADR using the combined cohorts (Table 4). *HLA-A*3101* showed significant associations with DIHS ($P = 2.06 \times 10^{-9}$, OR = 9.5, 95% CI 4.6−19.5) and SJS/TEN ($P = 2.35 \times 10^{-4}$, OR = 33.9, 95% CI 3.9−295.6) as well as other cADRs ($P = 4.74 \times 10^{-8}$, OR = 8.0, 95% CI 3.9−16.6), respectively.

Although our GWAS analysis did not find any significant association with the *HLA-B* locus, *HLA-B*1502* has been reported its strong association with SJS/TEN induced by CBZ in Han-Chinese population (7). In order to confirm that the *HLA-B* locus was not associated with CBZ-induced cADRs, we carried out genotyping of the *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 ($P = 1.31 \times 10^{-7}$, OR = 4.8, 95% CI 2.6−8.9) (Supplementary Material,
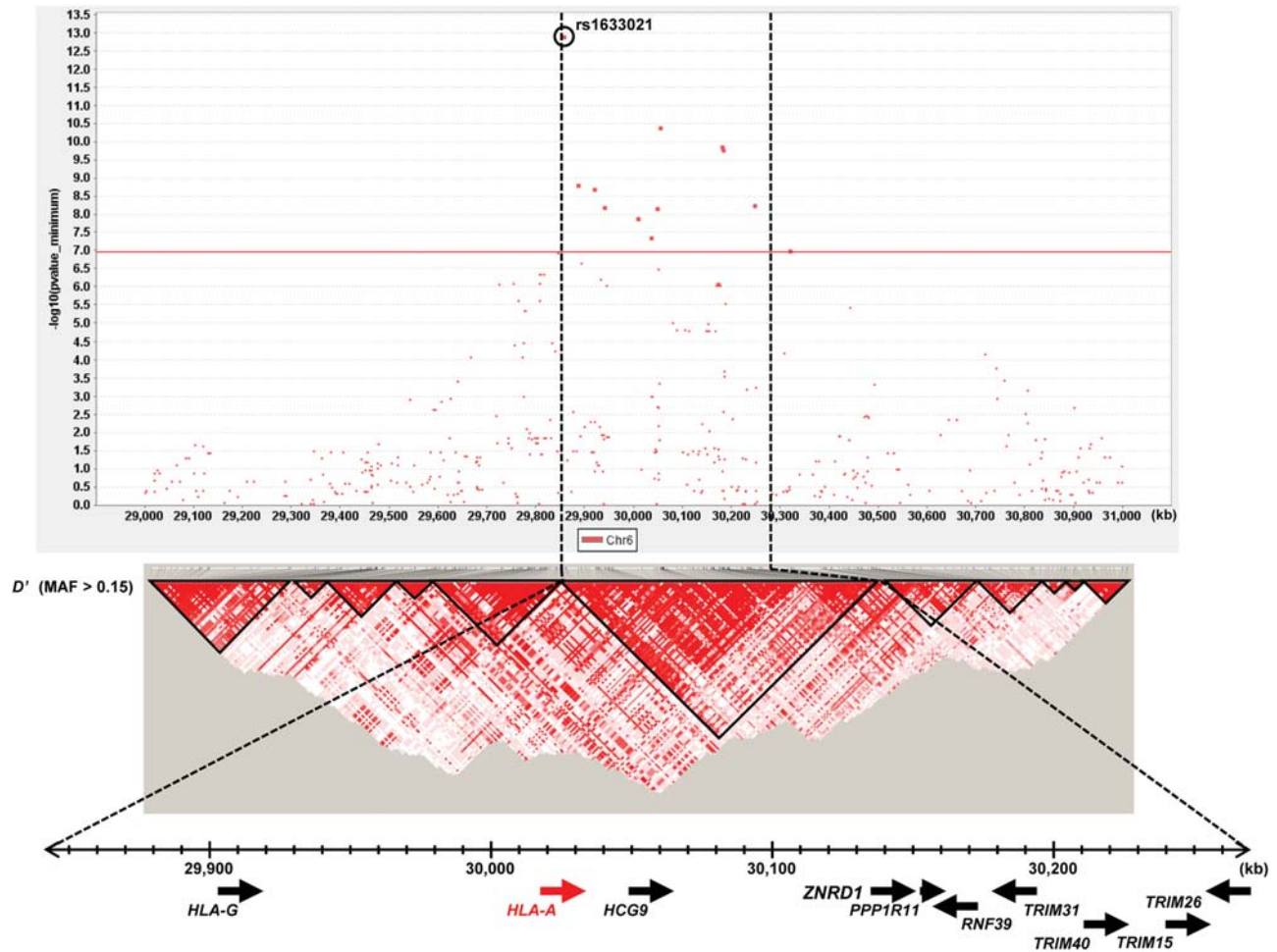
Figure 2. $-\text{Log}_{10}$ *P*-value plots, LD map and genomic structure of an *HLA-A* region. Each dot represents *P*-value obtained from GWAS using 53 patients with carbamazepine-induced cutaneous adverse drug reactions and 882 subjects of a general population in Japanese. The *Y*-axis represents the $-\log_{10}$ of the minimal *P*-values calculated by Fisher's exact tests for three models: dominant, recessive and allele frequency models in the case–control association study. Pairwise *D'* was based on the genotype data of the 882 subjects of the general population. MAF, minor allele frequency.

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

Table 2. Frequencies of HLA-A alleles in patients with carbamazepine-induced cutaneous adverse drug reactions and carbamazepine-tolerant controls

HLA allele	Number of carriers		*P*-value
	Case (%)	CBZ-tolerant control (%)	
*A*0101*	0 (0.0)	6 (1.6)	1.00
*A*0201*	5 (8.2)	93 (24.7)	2.74×10^{-3}
*A*0206*	1 (1.6)	68 (18.1)	[a]2.46×10^{-4}
*A*0207*	3 (4.9)	23 (6.1)	0.10
*A*0210*	2 (3.3)	2 (0.5)	0.03
*A*0301*	0 (0.0)	2 (0.5)	1.00
*A*1101*	7 (11.5)	68 (18.1)	0.27
*A*1110*	0 (0.0)	1 (0.3)	1.00
*A*2402*	37 (60.7)	211 (56.1)	0.58
*A*2405*	0 (0.0)	1 (0.3)	1.00
*A*2420*	0 (0.0)	4 (1.1)	1.00
*A*2601*	2 (3.3)	65 (17.3)	3.36×10^{-3}
*A*2602*	2 (3.3)	15 (4.0)	1.00
*A*2603*	11 (18.0)	22 (5.9)	2.61×10^{-3}
*A*2605*	1 (1.6)	1 (0.3)	0.26
*A*2901*	0 (0.0)	1 (0.3)	1.00
*A*3001*	0 (0.0)	2 (0.5)	1.00
*A*3101*	37 (60.7)	47 (12.5)	[a]3.64×10^{-15}
*A*3303*	5 (8.2)	59 (15.7)	0.17

61 cases and 376 CBZ-tolerant controls.
CBZ, carbamazepine.
[a]Significant after Bonferroni's correction.

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* ($P = 4.0 \times 10^{-4}$ 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* 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 3. Association of *HLA-A*0206* and *A*3101* alleles with carbamazepine-induced cutaneous adverse drug reactions

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

CBZ, carbamazepine; CI, confidence interval.
[a]Significant after Bonferroni's correction.

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

Subgroup	Number of patients Positive for *HLA-A*3101*	Negative for *HLA-A*3101*	Total	*P*-value	OR (95% CI)
All CBZ-induced cADRs	45	32	77	[a]1.09×10^{-16}	9.5 (5.6–16.3)
DIHS	21	15	36	[a]2.06×10^{-9}	9.5 (4.6–19.5)
SJS/TEN	5	1	6	[a]2.35×10^{-4}	33.9 (3.9–295.6)
Others	19	16	35	[a]4.74×10^{-8}	8.0 (3.9–16.6)
CBZ-tolerant controls	54	366	420	–	–

cADRs, cutaneous adverse drug reactions; CBZ, carbamazepine; CI, confidence interval; DIHS, drug-induced hypersensitivity syndrome; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis.
[a]Significant after Bonferroni's correction.

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 dug 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 mm^3), atypical lymphocytosis ($>5\%$) or eosinophilia (>1500 per mm^3)], 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

(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 of 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), 2.63×10^{-3} (0.05/19) and 2.50×10^{-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, 1.0×10^{-7}; 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.

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