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Pharmacogenomics

*HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* haplotype associated with lamotrigine-induced maculopapular exanthema in Mexican Mestizo patients

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Aim: Several *HLA* alleles have been associated with antiepileptic drugs (AEDs)-induced cutaneous adverse drug reactions (cADRs) in different populations; however, this has not been investigated in Mexican Mestizos (MM). Thus, the purpose of this preliminary study was to determine the association of *HLA* class I alleles with AED-induced cADRs in MM patients. **Materials & methods:** This case–control association study included 21 MM patients with phenytoin (PHT)-, carbamazepine (CBZ)-, or lamotrigine (LTG)-induced maculopapular exanthema (MPE) or Stevens–Johnson syndrome (SJS); 31 MM patients tolerant to the same AEDs; and 225 unrelated, healthy MM volunteers. *HLA* class I genotyping was performed. Differences in *HLA* allele frequencies between AED-induced cADR patients and AED-tolerant patients were assessed. Frequencies of alleles possibly associated with AED-induced cADRs in MM patients were compared with those in MM population. **Results**: The frequency of *HLA-C*08:01* allele in PHT-induced MPE was higher than that in the PHT-tolerant group ($p_c = 0.0179$) or in the MM population ($p_c < 0.0001$). For the first time, *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* haplotype was associated with LTG-induced MPE ($p_c = 0.0048$ for LTG-tolerant groups and $p_c < 0.0001$ for MM population). **Conclusion:** Our data suggest the *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* haplotype may be a biomarker for LTG-induced MPE and the *HLA-C*08:01* allele for PHT-induced MPE. We also identified *HLA-A*01:01:01* and *-A*31:01:02* as candidates alleles associated with CBZ-induced MPE in MM patients. However, further investigations are necessary to confirm these findings.

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Keywords: antiepileptic drug • carbamazepine • cutaneous adverse drug reaction • exanthema maculopapular • human leukocyte antigen • lamotrigine • Mexican Mestizo • pharmacogenetics • phenytoin • Stevens–Johnson syndrome

Background

Cutaneous adverse drug reactions (cADRs) can be caused by different drugs, including antiepileptic drugs (AEDs) [1]. The clinical manifestations of cADRs vary from maculopapular exanthema (MPE) of variable severity, to life-threatening severe cutaneous reactions that include Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-hypersensitivity syndrome (HSS) [2]. The highest rates of AED-related cADRs occur with carbamazepine (CBZ), lamotrigine (LTG) and phenytoin (PHT), which are structurally related through an aromatic ring [3].

cADRs are considered a serious problem for patients and for the healthcare system, as they are unpredictable and are limiting or potentially life-threatening during antiepileptic therapy [4]. The studies on prevention of cADRs have been focused on the *HLA* locus. In Han Chinese, *HLA-B*15:02* was the first allele reported to be associated with AED-induced cADRs, it was present in 100% of

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CBZ-SJS patients, but only in 3% of CBZ-tolerant patients and in 8.6% of the general population [5]. In 2007, the US FDA published a safety alert informing healthcare professionals about the presence of *HLA-B*15:02* allele in patients, especially in Asian patients and patients with Asian ancestry who, when treated with CBZ, experienced skin reactions; subsequently, a recommendation to screen for this *HLA* allele before starting treatment with CBZ was added to the drug label [6]. A similar recommendation was later included for abacavir with the *HLA-B*57:01* allele, considered as a risk factor for serious and fatal hypersensitivity reactions induced by this antiretroviral drug [7].

The *HLA-B*15:02* allele study was extended for other AEDs-induced cADRs; it was found that carriers of this allele showed susceptibility to CBZ, PHT, oxcarbazepine (OXC) and, probably LTG [8]. This conclusion was supported by the observation that carriers of *HLA-B*15:02* allele in Han Chinese population may have genetic susceptibility to OXC-induced MPE [4]. However, for LTG-induced MPE a significant association with *HLA-B*15:02* allele was not found in Han Chinese patients [9].

In agreement with this latter report, two studies have shown a higher frequency of *HLA-B*15:11* in patients with CBZ-induced SJS compared with that in CBZ-tolerant patients in Japanese [10] and Korean patients [11].

A genome-wide association study (GWAS) found that, for Northern European populations, the *HLA-A*31:01* allele was associated with HSS, MPE, and SJS-TEN induced by CBZ [12], but not with LTG or PHT-induced cADRs [13]. *HLA-A*31:01* was first reported by Hung *et al.* to be associated with CBZ-induced MPE for Han Chinese population [14], and was later found as a genetic risk factor for CBZ-induced cADRs in Japanese patients [15]. In contrast, another study did not find this allele implicated in the development of cADRs in Han Chinese; however, these authors found higher frequencies of *HLA-A*02:01* and *HLA-DRB1*14:05* alleles in CBZ-induced MPE patients than in the CBZ-tolerant group for the same population [16]. For a Japanese population, the *HLA-B*59:01* allele was reported as a candidate marker for CBZ-induced SJS [17].

Current evidence shows that the *HLA* allele associated with cADRs is subtype specific and depends on the AED inducer of the cADR and on the ethnic origin of the population studied [1,18–20]. To date, there has been no report of an *HLA* allele associated with AED-cADR susceptibility, which could predict this kind of adverse reactions in Mexican Mestizo (MM) patients. It is well known that, when cADRs are developed, the abrupt termination of treatment with an AED may increase the frequency of seizures and may even require that the patient be hospitalized. Because the identification of pharmacogenetic biomarkers that could help avoid cADRs is important for the care of patients, the aim of the present study was to determine, for the first time, the possible association between *HLA* class I alleles and AED- induced cADRs in MM patients.

Materials & methods

Patients & healthy volunteers

Patients with reported AED-induced cADRs were recruited from the epilepsy clinic at the National Institute of Neurology and Neurosurgery 'Manuel Velasco Suárez' of Mexico. This study was approved by the local Ethics Committee. Written informed consent was obtained from all the participants. The AED-induced cADR group consisted of 21 MM patients (14 females and seven males), ranging in age from 17 to 69 years (mean age: 35 ± 12 years). The diagnoses of SJS/TEN and MPE were based on clinical manifestations, as previously described by Roujeau and Stern [21] and by McCormack *et al.* [12]. In 17 patients (81%) the cADR subtype identified was MPE, while SJS was diagnosed in four cases (19%; Table 1). The cADR severity was classified according to the Drug and Therapeutics Committees from the WHO [22]. The AED-tolerant group consisted of 31 patients (14 females; 17 males), ranging in age 17 to 70 years (mean: 34 ± 13 years), who had been on AEDs treatment for more than 1 year without experiencing cADRs (mean doses: PHT 270 ± 109 mg, CBZ 681 ± 283 mg, and LTG 213 ± 101 mg).

A group of 225 healthy, unrelated volunteers from the MM population (mean age: 42 ± 12 years; 53.3% females and 46.7% males) was incorporated in the investigation. All the subjects included in the study were MM by ethnicity, with at least the two previous generations having been born and brought up in Mexico, and with a Spanish surname.

HLA class I genotyping

For all participants, DNA was isolated from peripheral blood samples by conventional methods. Micro SSP™ HLA DNA typing trays (SSP1L) and HLA Fusion™ v.2.0 software, both from One Lambda Inc. (CA, USA), were used for HLA genotyping. To confirm the identified alleles, high-resolution typing of *HLA* class I was performed by a sequence-based typing (SBT) method, using the AlleleSEQR® HLA-A, AlleleSEQR® HLA-B, and AlleleSEQR® HLA-C plus sequencing kits respectively to confirm the identified alleles (Atria Genetics Inc., CA, USA). The data were analyzed by using Assign™ SBT v.3.5 software (Conexio Genomics, Fremantle, Western Australia) from IMGT data-

Table 1. Cutaneous adverse drug reactions clinical characteristics, and *HLA* class I genotype of antiepileptic drugs-induced cutaneous adverse drug reactions group.

Patient ID No.	Age/ gender	cADR clinical characteristics								*HLA* genotype		
		AED inducer	AED dosage (mg/day)	Days of AED exposure	Subtype	Cutaneous symptoms (affected sites)	Mucosal involvement	Systemic symptoms	Severity†	*-A**	*-B**	*-C**
1	33/female	PHT	400	7	MPE	Exanthem (arms, hands)	No	No	Mild	*02:01:01*	*14:02:01*	*08:01*
										03:01:01	*49:01:01*	*07:02:01*
2	44/male	PHT/CBZ	300/<400	7/<1	MPE	Exanthem (arms)	Genital	No	Moderate	*02:01:01*	*51:01*	*08:01*
										68:01:02	*48:01:01*	*08:01*
3	46/female	CBZ	200	<7	MPE	Exanthem (general)	No	No	Mild	*02:01:01*	*35:01:01*	*04:01:01*
										31:01:02	*40:02:01*	*03:02:01*
4	36/female	CBZ	200	<1	MPE	Exanthem (general)	No	No	Moderate	*01:01:01*	*57:01:01*	*06:02:01*
										01:01:01	*58:01:01*	*07:02:01*
5	23/male	CBZ	200	7	MPE	Exanthem (general)	No	No	Mild	*31:01:02*	*39:06*	*07:02:01*
										24:02:01	*50:01:01*	*06:02:01*
6	69/female	CBZ	200	2	MPE	NR	Oral	No	Moderate	*02:01:01*	*35:02:01*	*04:01:01*
										01:01:01	*37:01:01*	*12:03:01*
7	17/male	LTG	25	3	MPE	Exanthem (arms, legs)	No	No	Moderate	*02:01:01*	*35:01:01*	*04:01:01*
										24:02:01	*18:01:01*	*05:01:01*
8	32/female	LTG	50	20	SJS	Erythema, edema	Oral and ophthalmic	F, E, L	Severe	*24:02:01*	*39:01:01*	*06:02:01*
										01:01:01	*57:01:01*	*07:02:01*
9	33/female	LTG	25	<7	SJS	Erythema, edema	NR	F	Severe	*68:01:02*	*39:01:01*	*03:02:01*
										02:01:01	*40:02:01*	*07:02:01*
10	43/female	LTG	25	<1	MPE	Erythema (face)	No	No	Mild	*03:01:01*	*58:01:01*	*06:02:01*
										31:01:02	*15:05:01*	*01:02:01*
11	45/female	LTG	25	<1	MPE	Exanthem (general)	Oral	No	Moderate	*02:01:01*	*35:01:01*	*04:01:01*

†ADR severity classification according to WHO [22].
AED: Antiepileptic drug; cADR: Cutaneous adverse drug reaction; CBZ: Carbamazepine; E: Eosinophilia, F: Fever; LEV: Levetiracetam; L: Leukocytosis; LTG: Lamotrigine; MPE: Maculopapular exanthema; NR: Not reported; Pg: Pemphigus; PHT: Phenytoin; SJS: Stevens–Johnson syndrome.

Table 1. Cutaneous adverse drug reactions clinical characteristics, and *HLA* class I genotype of antiepileptic drugs-induced cutaneous adverse drug reactions group (cont.).

Patient ID No.	Age/ gender	cADR clinical characteristics								*HLA* genotype		
		AED inducer	AED dosage (mg/day)	Days of AED exposure	Subtype	Cutaneous symptoms (affected sites)	Mucosal involvement	Systemic symptoms	Severity†	*-A**	*-B**	*-C**
11 (cont.)										*02:01:01*	*35:01:01*	*04:01:01*
12	26/ female	LTG	200	60	MPE	NR	Oral	No	Moderate	*02:01:01*	*35:01:01*	*04:01:01*
										02:01:01	*35:02:01*	*04:01:01*
13	39/male	LTG	25	<7	MPE	Exanthem (general)	No	No	Mild	*68:01:02*	*27:05*	*02:02:02*
										29:01:01	*44:02:01*	*16:01:01*
14	37/female	LTG	25	<7	MPE	Exanthem (legs)	No	No	Mild	*24:02:01*	*39:01:01*	*06:02:01*
										30:02:01	*50:01:01*	*07:02:01*
15	36/female	LTG	100	15	MPE	Exanthem (general)	No	No	Mild	*02:01:01*	*35:01:01*	*04:01:01*
										68:01:02	*44:02:01*	*18:01:01*
16	19/male	LTG	50	15	MPE	Exanthem (general)	Oral	No	Moderate	*68:01:02*	*39:01:01*	*03:02:01*
										68:01:02	*40:02:01*	*07:02:01*
17	31/female	LTG	75	45	MPE	Exanthem (face, legs)	No	No	Mild	*02:01:01*	*15:01:01*	*03:03:01*
										02:01:01	*52:01:02*	*03:02:01*
18	27/female	LTG	25	7	SJS	Erythema (general), edema	Oral and ophthalmic	F, E, L	Severe	*68:01:02*	*39:01:01*	*07:02:01*
										23:01:01	*58:01:01*	*05:01:01*
19	27/female	LTG	100	30	SJS	Erythema (legs, back)	NR	F	Severe	*02:01:01*	*40:02:01*	*03:02:01*
										68:01:02	*15:08*	*01:02:01*
20	36/male	LTG	200	60	MPE	Exanthem (arms, legs)	No	No	Moderate	*24:02:01*	*35:01:01*	*04:01:01*
										01:01:01	*35:02:01*	*03:02:01*
21	49/male	LEV	1500	14	Pg	Blistering (general)	No	No	Severe	*02:01:01*	*35:02:01*	*04:01:01*
										02:01:01	*14:01:01*	*12:03:01*

†ADR severity classification according to WHO [22].
AED: Antiepileptic drug; cADR: Cutaneous adverse drug reaction; CBZ: Carbamazepine; E: Eosinophilia, F: Fever; LEV: Levetiracetam; L: Leukocytosis; LTG: Lamotrigine; MPE: Maculopapular exanthema; NR: Not reported; Pg: Pemphigus; PHT: Phenytoin; SJS: Stevens–Johnson syndrome.

base. Haplotypes were determined by using Arlequin v.3.5.1.3 [23] and the Allele Frequency Net Database [24,25]. The relevant haplotype was confirmed by trio analysis.

Statistical analysis

The frequencies of observed *HLA* alleles in the AEDs-induced cADRs group were compared with those in the AEDs-tolerant group. According to the AED administered, patients in both groups were further sub-classified according to both the AED inducer and to the cADR-subtype. In the AED-induced cADR group, 14 patients were in the LTG-induced cADRs group (ten with LTG-induced MPE and four with LTG-induced SJS); four in the CBZ-induced MPE group; one in the PHT-induced MPE group; one patient, who reported MPE induced by CBZ and PHT on separate occasions, was therefore included in both PHT- and the CBZ-induced groups; and one patient exhibited pemphigus induced by levetiracetam (LEV). Due to the use of combined therapy with several AEDs, patients in the AEDs-tolerant group (n = 31) were also included in more than one comparison analysis; accordingly, 28 patients were in the LTG-tolerant group; 18, in the CBZ-tolerant group; and five, in the PHT-tolerant group (Supplementary Table 1; see online at: www.futuremedicine.com/doi/suppl/10.2217/pgs.14.135). Subsequently, *HLA* allele frequencies associated with AEDs-induced cADRs were compared with the allele frequencies in the MM population. Software Epi Info™ 7.0 was used to perform uncorrected and corrected χ^2 test. Significance was defined as $p < 0.05$. Odds ratio (OR) and 95% confidence interval (CI) were also calculated.

Results

AEDs-induced cADRs

The most frequent AED inducer of cADRs in the MM patients included in this study was LTG (14 patients; 66.7%), followed by CBZ (four patients; 19.0%), PHT (one patient; 4.8%), and CBZ and PHT (one patient; 4.8%), with one patient presenting with a severe case of pemphigus while taking LEV (Table 1). No significant gender difference was found between AED-induced cADRs and AED-tolerant groups ($p_c = 0.2139$).

The comparison between *HLA-A* and *-C* alleles frequencies of AED-induced cADR group (n = 21) versus AED-tolerant group (n = 31) did not show any statistically significant difference (Supplementary Tables 2 & 3). However, the *HLA-B*58:01:01* allele was found in a higher frequency in patients with cADRs ($p = 0.0327$; Supplementary Table 4). Three patients with cADR carried this allele: one with CBZ-induced MPE and one each with LTG-induced MPE and SJS (patients #4, #10, and #18, respectively; Table 1).

PHT-induced cADRs

Three *HLA-A* alleles were identified in patients with PHT-induced cADRs (n = 2), as well as four *HLA-B* and two *HLA-C* alleles (Table 1). For the PHT-tolerant group (n = 5), four, seven and five *HLA-A*, *-B* and *-C* alleles were identified, respectively (Supplementary Tables 5–7). The frequency of *HLA-C*08:01* in the PHT-induced cADR group was higher not only than that in the PHT-tolerant group ($p = 0.0020$; $p_c = 0.0179$; Table 2), but also than that in the general MM population ($p < 0.0001$; $p_c < 0.0001$; Table 2); OR: 48.92; 95% CI: 4.92–486.80.

CBZ-induced cADRs

In patients with CBZ-induced cADRs (n = 5), five *HLA-A*, ten *HLA-B* and six *HLA-C* alleles were identified (Table 1). Nine *HLA-A*, 18 *HLA-B* and ten *HLA-C* alleles were identified in the CBZ-tolerant group (n = 18; Supplementary Tables 8–10). The frequencies of *HLA-A*01:01:01* ($p = 0.0280$; OR: 7.29; 95% CI: 1.02–52.01) and *HLA-A*31:01:02* ($p = 0.0061$) alleles were found to be statistically significantly different between CBZ-induced cADR and CBZ-tolerant groups (Table 2). The difference remained when both allele frequencies were compared with those of the MM population: *HLA-A*01:01:01* ($p = 0.0007$; $p_c = 0.0074$; OR: 7.96; 95% CI: 1.93–32.79) and for *HLA-A*31:01:02* ($p = 0.04$; OR: 4.64; 95% CI: 0.93–23.11).

LTG-induced cADRs

Nine *HLA-A*, 14 *HLA-B*, and ten *HLA-C* alleles were identified in 14 patients with LTG-induced cADRs, whereas in the LTG-tolerant group (n = 28), there were 13 *HLA-A* and *HLA-C* alleles, and 23 alleles for *HLA-B* (Supplementary Tables 11–16). Patients with LTG-induced cADRs (n = 14) were classified in two sub-groups according to the cADR's subtype (MPE or SJS). When the allele frequencies of each sub-group were compared with those of LTG-tolerant group and MM population, for the LTG-induced MPE group (n = 10), the frequency of *HLA-B*35:01:01* allele was higher than that in the LTG-tolerant group ($p = 0.0215$, $p_c = 0.0537$ [Table 2]; OR: 4.37; 95% CI: 1.16–16.46), but no difference was found when compared with the MM population. In addition, we identified a higher frequency of a haplotype that include this allele (*-A*02:01:01/-B*35:01:01/-C*04:01:01*) in the LTG-induced MPE group than in the LTG-tolerant group ($p = 0.0009$, $p_c = 0.0048$ [Table 2]; OR: 18.33; 95% CI: 1.99–169.08; Supplementary Table 17). Comparison of the frequency of the haplotype *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* of the patients with that of the MM population also showed a statis-

Table 2. Comparison of *HLA* class I allele frequencies between antiepileptic drug-induced cutaneous adverse drug reactions group, antiepileptic drug-tolerant group, and sample (n = 225) of the general Mexican Mestizo population.

AED inducer	*HLA* allele/ haplotype	AEDs-cADR/ total alleles (2n)	AEDs-tolerant/total alleles (2n)	p*	p_c*	MM population/ total alleles (2n)	p**	p_c**
PHT-MPE	*C*08:01*	3/4	0/10	0.0020	**0.0179**	26/450	<0.0001	**<0.0001**
CBZ-MPE	*A*01:01:01*	3/10	2/36	0.0280	0.1046	23/450	0.0007	**0.0074**
	*A*31:01:02*	2/10	0/36	0.0061	0.0619	23/450	0.0400	0.1773
	*B*35:01:01*	6/20	5/56	0.0215	0.0537	66/450	0.0625	0.1222
LTG-MPE	*A*02:01:01/ B*35:01:01/ C*04:01:01*	5/20	1/56	0.0009	**0.0048**	13/450	<0.0001	**<0.0001**
	*A*23:01:01*	1/8	0/56	0.0077	0.2531	14/450	0.1392	0.6334
LTG-SJS	*B*40:02:01*	2/8	2/56	0.0192	0.1184	26/450	0.0245	0.1323
	*B*57:01:01*	1/8	0/56	0.0077	0.2531	6/450	0.0107	0.2721
	*B*58:01:01*	1/8	0/56	0.0077	0.2531	3/450	0.0004	0.0992

Bold typeface refers to the p-values from corrected χ^2 test that showed statistically significant differences in alleles frequencies between groups ($p < 0.05$).
*p-value from comparison of allele frequencies in AED-induced cADR group versus those in AED-tolerant group.
**p-value from comparison of allele frequencies in AED-induced cADR group versus those in sample MM population.
AED: Antiepileptic drug; cADR: Cutaneous adverse drug reaction; CBZ: Carbamazepine; LTG: Lamotrigine; MM: Mexican Mestizo; MPE: Maculopapular exanthema; n: Sample size; p: Value from uncorrected χ^2 test; p_c: Value from corrected χ^2 test; PHT: Phenytoin; SJS: Stevens–Johnson syndrome.

tically significant difference ($p < 0.0001$, $p_c < 0.0001$ [Table 2]; OR: 11.20; 95% CI: 3.54–35.48). Of the cADR patients (#7, #11, and #12; Table 1) with this haplotype, analyses of their progenitors confirmed the identified haplotype.

In the LTG-induced SJS patients, the frequencies of four *HLA* alleles were higher than those in the LTG-tolerant group (Table 2): *HLA-A*23:01:01* ($p = 0.0077$); *HLA-B*40:02:01* ($p = 0.0192$; OR: 9; 95% CI: 1.07–76.02); and, *HLA-B*57:01:01* and *HLA-B*58:01:01* ($p = 0.0077$ for both cases). The frequency of *HLA-A*23:01:01* was not statistically different from that in the MM population (Table 2). For the other three alleles, the statistically significant difference in the frequencies remained: *HLA-B*40:02:01* ($p = 0.0245$; OR: 5.44; 95% CI: 1.04–28.26), *HLA-B*57:01:01* ($p = 0.0107$; OR: 10.57; 95% CI: 1.12–99.78), and *HLA-B*58:01:01* ($p = 0.0004$; OR: 21.29; 95% CI: 1.96–230.72).

HLA class I allele frequencies in the general MM population

In a sample (n = 225) of the general MM population, 37 *HLA-A* alleles, 55 *HLA-B* alleles, and 22 *HLA-C* alleles were identified. Table 3 shows the five most frequent *HLA* class I A, B, and C alleles and class I haplotypes found in the sample of the MM population. The frequency of *HLA-B*15:02* allele in this sample was 0.889% (Supplementary Tables 18 & 19).

Discussion

In Mexico, epilepsy affects approximately 3.9 per 1000 inhabitants who are ≤15 years old and 4.7 per 1000 inhabitants who are 15–40 years old [26]. Importantly, epilepsy is the primary cause of outpatient consultation at the National Institute of Neurology and Neurosurgery of Mexico, and 56% of these patients have drug-resistant epilepsy [27]. The occurrence of cADRs further complicates the drug therapy of these patients.

In this study with MM patients, cADRs were more frequently induced by LTG than by other AEDs. This result contrasts with investigations in other populations that have reported that PHT [28] and CBZ [29,30] induce cADRs more frequently than does LTG.

Here, one MM patient had a cADR induced by LEV, a drug that has been considered a safe alternative for those patients who are hypersensitive to aromatic-AEDs. Cases of LEV-induced SJS have been reported [31,32]. With only one patient with LEV-induced cADR in the current study, no association analysis was performed.

None of the AED-induced cADR or AED-tolerant patients were carriers of the *HLA-B*15:02* allele. This result may be explained by our finding of a low frequency (0.889%) of this allele in the MM population studied. This finding contrasts with the strong association reported for CBZ-induced SJS/TEN in Han Chinese population in which this allele is prevalent [5,18].

The *HLA-C*08:01* allele was present in the two patients with PHT-induced cADRs, but was not

Table 3. Frequencies of *HLA-A, -B* and *-C* alleles and haplotypes in sample (n = 225) of the general Mexican Mestizo population.

HLA- allele or haplotype	Allele frequency	%
*A*02:01:01*	0.2689	26.89
*A*24:02:01*	0.1800	18.00
*A*68:01:02*	0.0911	9.11
*A*03:01:01*	0.0600	6.00
*A*31:01:02*	0.0511	5.11
*B*35:01:01*	0.1467	14.67
*B*39:01:01*	0.0800	8.00
*B*15:01:01*	0.0733	7.33
*B*51:01:01*	0.0711	7.11
*B*40:02:01*	0.0578	5.78
*C*04:01:01*	0.1644	16.44
*C*07:02:01*	0.1333	13.33
*C*03:04:01*	0.1067	10.67
*C*07:01:01*	0.0933	9.33
*C*01:02:01*	0.0689	6.89
*A*02:01:01/B*35:01:01/C*04:01:01*	0.0294	2.94
*A*24:02:01/B*35:01:01/C*04:01:01*	0.0254	2.54
*A*02:01:01/B*39:01:01/C*07:02:01*	0.0244	2.44
*A*24:02:01/B*35:01:01/C*03:04:01*	0.0199	1.99
*A*02:01:01/B*15:01:01/C*01:02:01*	0.0151	1.51

observed in any PHT-tolerant patient. The patient with more severe cADR was homozygous for *HLA-C*08:01*. Despite the small sample size, our study permitted the identification of a statistically significant difference in *HLA-C*08:01* allele frequency between the PHT-induced cADR and PHT-tolerant groups and between PHT-induced cADR and the MM population; however, further studies are required to confirm this allele as a predictor of PHT-induced cADR in MM patients. This allele had been previously reported to have a higher frequency in PHT-induced cADR than in PHT-tolerant controls [8].

*HLA-A*01:01:01* has been reported as part of the ancestral haplotype 8.1 that will be mentioned ahead. We identified two patients with CBZ-induced MPE that were carriers of *HLA-A*01:01:01*, one was homozygous for this allele and the other was heterozygous; however, no difference was observed in the severity of clinical manifestations between them.

In this investigation, *HLA-A*31:01:02* was observed more frequently in the CBZ-induced MPE group than in CBZ-tolerant group. The two patients heterozygous for *HLA-A*31:01:02* presented mild cADRs. This allele was first reported by Hung *et al.* to have a higher frequency in Han Chinese patients with CBZ-induced MPE than in the CBZ-tolerant group [14]. Kim *et al.* reported a positive association between CBZ-induced HSS and *HLA-A*31:01* allele, as this allele was present in 58.8% of HSS patients, but only in 14% of CBZ-tolerant controls [11]. The GWAS performed by McCormack *et al.* also showed that the presence of the *HLA-A*31:01* allele was associated with CBZ-induced HSS, MPE and SJS/TEN [12]. Herein, the differences in *HLA-A*01:01:01* and *-A*31:01:02* alleles frequencies, found between the CBZ-induced MPE and CBZ-tolerant groups, was not significant when χ^2 test was corrected, therefore the study of these variants needs to be enlarged in order to confirm if there is an association with CBZ-induced MPE.

*HLA-B*35:01* allele has been reported as being associated with nevirapine-induced rash in Australian patients [1], but not for AEDs-induced cADRs. In the current study, the *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* haplotype that was associated with LTG-induced MPE in MM patients was distinct from those previously reported [17,33,34]. The presence of a haplotype found in a high frequency in the LTG-induced cADR group could indicate the presence of another gene in linkage disequilibrium with *HLA* class I genes in the MM population. Further studies are required for the identification of this gene

and to complete the *HLA* haplotype with class II genes. Interestingly, the relatives of the two patients (#7 and #11; Table 1) carrying this haplotype had a medical history of hypersensitivity to antibiotics, analgesics and antiseptics. The progenitors of patient #7 were heterozygous for this haplotype and have had cADR induced by penicillin and trimethoprim, whereas brothers and sisters of patient #11, who was homozygous for the reported haplotype, have experienced cADR induced by penicillin, sulfamethoxazole, clavulanic acid and paracetamol, the father had cADR to iodine solution and two nieces have even developed food allergies.

*HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* was the most common haplotype found in MM population with a frequency of 2.9%; it is considered a conserved extended HLA haplotype, with a possible Amerindian origin and with a high frequency in indigenous Mexican populations (1.5% in Amerindians, 6.1% in Nahuas) [35,36]. Three haplotypes have been previously reported with CBZ-induced cADRs. *HLA-A*01:01/-Cw*07:01/-B*08:01/-DRB1*03:01/-DQA1*05:01/-DQB1*02:01* was associated with CBZ-induced HSS in Caucasians and it was considered that a predisposing locus for this cADR is contained in this ancestral haplotype 8.1 [33,34]. The two remaining haplotypes (*HLA-A*24:01/-B*59:01/-C*01:02* and *HLA-A*02:01/-B*15:18/-C*07:04*) were reported for Japanese patients, the first one was found in two patients, while only one patient was carrier of the last haplotype [17].

The *HLA* alleles more frequently observed in LTG-induced SJS group than in LTG-tolerant group were different from those found in the LTG-induced MPE group. For LTG-induced SJS, *HLA-A*23:01:01* and *-B*40:02:01* alleles have not been previously associated with cADRs. *HLA-B*58:01:01* and *HLA-B*57:01:01* alleles have been reported as genetic markers for allopurinol- and abacavir-induced severe cADRs [37,38]. In our study, the differences observed in the frequencies of these four alleles between LTG-induced SJS and LTG-tolerant patients could be due to the small sample size (n = 4 and n = 28, respectively) especially since the statistical difference did not remain after Yates correction (Table 2). The possibility remains that these alleles have something in common to induce cADRs by several types of drugs. This is especially true for the *HLA-B*58:01:01* allele which showed a statistically significant difference in frequency in the AED-induced cADRs group as compared with that of the AED-tolerant patients, regardless of the AED inducer.

The major limitation in our study is the small sample size, which is partly explained by the low frequency of cADR compared with other types of ADRs. Despite this limitation, we found statistically significant differences in the *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* haplotype and *HLA-C*08:01* allele frequencies between the studied groups. Additionally, some potential alleles associated with several CBZ-induced MPE were also observed in the studied sample. Further research is necessary to confirm these results.

Conclusion & future perspective

To the best of our knowledge, this is the first investigation of *HLA* class I alleles associated with AEDs-induced cADRs in MM patients. The haplotype *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* appears to be a biomarker for LTG-induced MPE in MM patients. This is also the first report of this haplotype being associated with an AED-induced cADR in any population. Additionally, a possible association of *HLA-C*08:01* allele with PHT-induced MPE was found. Future studies need to evaluate the potential influence of *HLA-A*01:01:01* and *HLA-A*31:01:02* alleles on CBZ-induced MPE. The sub-classification of MM patients with cADR according to both the AED inducer and the cADR subtype was important for the correct identification of the biomarkers for AED-induced cADR.

Due to the preliminary nature of the present study, larger, prospective and multi-center studies are required in order to elucidate the participation of the *HLA* locus gene in the cADRs development. Such information will be helpful in improving these patients' health and in preventing cADRs induced by antiepileptic therapy in MM patients.

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Ethical conduct of research

The authors obtained appropriate approval for this study from the institutional review board, and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. In addition, for this investigation involving human subjects, informed consent was obtained from all the participants involved.

Executive summary

Background

- Phenytoin (PHT), carbamazepine (CBZ), lamotrigine (LTG) and other antiepileptic drugs (AEDs) are associated with cutaneous adverse drug reactions (cADRs) of diverse severity; these comprise, among others, maculopapular exanthema (MPE), Stevens–Johnson syndrome (SJS), and/or toxic epidermal necrolysis (TEN).
- *HLA-B*15:02* has been strongly associated with CBZ-induced SJS/TEN in individuals of Han Chinese ancestry. Other *HLA* alleles have been associated with AED-induced cADRs in different populations; however, such associations have not been investigated in Mexican Mestizos (MM). The aim of this study was to determine the association of *HLA* class I alleles with AED-induced cADRs in MM patients.

Results

- In this case–control association study, 21 MM patients with PHT-, CBZ- or LTG-induced MPE or SJS, 31 MM patients tolerant to the same AEDs, and 225 unrelated, healthy MM volunteers, were enrolled.
- LTG was the most frequent AED inducer of cADRs (66.7%) in the MM patients included in this study.
- None of the AED-induced cADR patients or AED-tolerant patients were carriers of the *HLA-B*15:02* allele. We observed a low frequency (0.889%) of this allele in the MM population studied.
- *HLA-C*08:01* allele was related to PHT-induced MPE patients ($p_c = 0.0179$).
- These preliminary results suggest that further investigations of *HLA-A*01:01:01* and *HLA-A*31:01:02* alleles are needed to confirm their impact on CBZ-induced MPE.
- The haplotype *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* was associated for the first time with LTG-induced MPE ($p_c = 0.0048$; OR: 18.33; 95% CI: 1.99–169.08).

Conclusion

- This study represents the first investigation of *HLA* class I alleles associated with AED-induced cADRs in MM patients.
- The haplotype *HLA-A*02:01:01/-B*35:01:01/-C*04:01:01* appears to be a biomarker for LTG-induced MPE in MM patients. This is the first report of this haplotype's being associated with an AED-induced cADR in any population.
- The sub-classification of cADR-patients according to both the AED inducer and the cADR subtype was important for the correct identification of the biomarkers for AED-induced cADR.

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