

FULL-LENGTH ORIGINAL RESEARCH

Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with HLA-B*1502 allele in Thai population

*Chaichon Locharernkul, *Jakrin Loplumlert, *Chusak Limotai, *Wiwat Korkij, †Tayard Desudchit, †Siraprapa Tongkobpetch, ‡Oratai Kangwanshiratada, ‡Nattiya Hirankarn, †Kanya Suphapeetiporn, and †Vorasuk Shotelersuk

Departments of *Medicine, †Pediatrics, and ‡Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

SUMMARY

Purpose: Previous studies found a strong association between HLA-B*1502 and carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS) in Han Chinese, but not in Caucasian populations. Even in Han Chinese, the HLA-B*1502 was not associated with CBZ-induced maculopapular eruptions (MPE). This study seeks to identify whether HLA-B*1502 is associated with CBZ- or phenytoin (PHT)-induced SJS or MPE in a Thai population.

Methods: Eighty-one Thai epileptic patients between 1994 and 2007 from the Chulalongkorn Comprehensive Epilepsy Program were recruited. Thirty-one subjects had antiepileptic drug (AED)-induced SJS or MPE (6 CBZ-SJS, 4 PHT-SJS, 9 CBZ-MPE, 12 PHT-MPE), and 50 were AED-tolerant controls.

Results: For the first time, a strong association between HLA-B*1502 and PHT-induced SJS was

found ($p = 0.005$). A strong association was also found between the HLA-B*1502 and CBZ-induced SJS ($p = 0.0005$), making Thai the first non-Chinese population demonstrating such an association. Some patients, who were HLA-B*1502 and suffered from CBZ-induced SJS, could be tolerant to PHT and vice versa. This suggests that HLA-B*1502 may be a common attribute required for a Thai patient to develop SJS from these two AEDs; other different elements, however, are also needed for each AED. In addition, no association between HLA-B alleles and CBZ- or PHT-induced MPE was found.

Conclusions: CBZ- and PHT-induced SJS, but not MPE, is associated with HLA-B*1502 allele in Thai population.

KEY WORDS: HLA-B*1502, Carbamazepine, Phenytoin, Stevens-Johnson syndrome, Maculopapular eruption, Thai.

Carbamazepine (CBZ) and phenytoin (PHT) are among the most common causes of antiepileptic drug (AED)-related cutaneous adverse reactions (Arif et al., 2007). Manifestations range from a mild erythematous maculopapular rash to life-threatening Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Mortality of severe cutaneous reactions can reach up to 30% (Svensson et al., 2001).

Identification of genetic factors predisposing to development of AED-induced severe cutaneous reactions offers

the possibility of avoiding these drugs in individuals with such susceptibility. With advances in genetic techniques, it became possible to implicate HLA-B*1502. The first study was reported by Chung and coworkers (2004), who found a strong association between HLA-B*1502 and CBZ-induced SJS in Han Chinese, with a 100% sensitivity and 97% specificity. Two subsequent studies in Han Chinese observed similar results (Hung et al., 2006; Man et al., 2007). However, studies in Caucasian populations did not observe the same association, suggesting that HLA-B*1502 is not a universal marker for CBZ-induced SJS and that ethnicity could also play a role in the difference of the individual genetic susceptibility (Alfirevic et al., 2006; Lonjou et al., 2006).

Maculopapular eruption (MPE) induced by AED has been known to precede SJS. A study demonstrated that

Accepted May 20, 2008; Early View publication July 14, 2008.

Address correspondence to Vorasuk Shotelersuk, Department of Pediatrics, Sor Kor Building 11th floor, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. E-mail: vorasuk.s@chula.ac.th

Wiley Periodicals, Inc.

© 2008 International League Against Epilepsy

HLA-B*1502 was not associated with CBZ-induced MPE, even in populations with positive association between HLA-B*1502 and CBZ-induced SJS (Hung et al., 2006). A better understanding of genetic susceptibility may lead to safer AED use and avoid life-threatening cutaneous adverse reactions. We studied the association between HLA-B*1502 and AED-induced SJS or MPE in a Thai epilepsy population.

MATERIALS AND METHODS

Subjects of this study were 31 Thai epileptic patients suffering from MPE or SJS. They were recruited between 1994 and 2007 from the epilepsy clinic of the Chulalongkorn Comprehensive Epilepsy Program, a university-based tertiary epilepsy surgery center of King Chulalongkorn Memorial Hospital, Bangkok. They were classified into two groups according to their cutaneous allergic reactions. The type of AED used and its association with MPE or SJS in each individual were critically evaluated. Only reliable temporal relation and clinical manifestation of the drug to the cutaneous reactions were included. Ten patients had AED-induced SJS (Table 1), and 21 had AED-induced MPE. CBZ and PHT were the two most frequent AEDs inducing rashes (Table 2). Fifty AED-tolerant epileptics were also recruited as controls.

SJS was defined when clinical morphology fulfilled Roujeau's diagnostic criteria (Roujeau, 1994). MPE was defined as erythematous exanthem without blistering or pustulation. The diagnosis was confirmed by W.K. on clinical findings or by history review in all cases. Phone calls to the patients for more detailed description of the rash and drug use were made when necessary. For patients with AED-induced MPE, we did not perform further investigations to definitely define hypersensitivity syndrome (HSS). Patients who developed SJS after simultaneous use of more than one drug were excluded. Patients who had been on AEDs for more than 3 months without allergic reactions were classified as "tolerant." As soon as they developed cutaneous adverse reactions, all medications were withdrawn. All 10 patients with AED-induced SJS were admitted to the hospital with complete recovery.

After written informed consent was obtained, genomic DNA was extracted from peripheral blood, according to the established protocols. HLA-B genotypes were determined by polymerase chain reaction using sequence-specific primers (Micro SSP HLA Class IB Locus Specific; OneLambda, Canoga Park, CA, U.S.A.) according to the manufacturer's instructions.

Statistical analysis of the differences in HLA-B*1502 frequencies among patients with AED-SJS, AED-MPE, and those with AED tolerance was performed by Fisher's exact test. The strength of association was estimated by calculating the odds ratio. All reported p-values are

Table 1. Characteristics, ethnicity, and AED cotherapy in 10 patients with AED-induced SJS

Patient	Sex	Age (years)	Seizure onset (years)	Diagnosis of epilepsy	HLA-B*1502	Maternal grandfather	Maternal grandmother	Paternal grandfather	Paternal grandmother	CBZ	PHT	PB	VPA	CZP	LTG	TPM	LVT	CLB
1	M	22	19	TLE, cavernoma	+ve	Thai	Chinese	Thai	Chinese	S	-	-	+	-	-	-	+	+
2	F	17	7	TLE, HS	+ve	Thai	Thai	Thai	Vietnamese	S	+	+	+	-	-	-	-	-
3	F	32	20	TLE, HS	+ve	Thai	Thai	Thai	Thai	S	+	-	+	-	-	-	-	-
4	F	45	18	Nonlesional focal epilepsy	+ve	Thai	Thai	Peguan	Peguan	S	+	-	+	-	+	-	-	+
5	M	17	9	Generalized epilepsy	+ve	Peguan	Thai	Chinese	Chinese	S	-	-	+	-	-	-	+	-
6	M	10	6	Nonlesional focal epilepsy	+ve	Chinese + Peguan	Thai	Thai	Thai	S	-	-	+	-	-	-	-	-
7	M	24	15	Focal epilepsy, hippocampal atrophy	+ve	Thai	Thai	Chinese	Thai	-	S	+	+	-	-	-	-	-
8	M	23	13	OLE, posttraumatic epilepsy	+ve	Thai	Thai	Thai	Thai	+	S	-	+	-	+	+	+	+
9	M	16	12	Nonlesional OLE	+ve	Thai	Thai	Thai	Thai	-	S	-	+	-	-	-	+	-
10	M	12	10	Focal epilepsy, cerebral sinus thrombosis	+ve	Thai	Thai	Chinese	Thai	-	S	-	-	-	-	-	-	-

CBZ, carbamazepine; PHT, phenytoin; PB, phenobarbital; VPA, sodium valproate; CZP, clonazepam; LTG, lamotrigine; TPM, topiramate; LVT, levetiracetam; CLB, clobazam; M, male; F, female; TLE, temporal lobe epilepsy; HS, hippocampal sclerosis; OLE, occipital lobe epilepsy; +ve, positive; -, never been exposed to the medication; +, tolerant to the medication; S, AED-induced SJS.

Table 2. Association of HLA-B*1502 allele with AED-induced cutaneous adverse reactions

Phenotype	N (81)	HLA-B*1502 (N)	Non HLA-B*1502 (N)	p-value ^a	Odds ratio (95% CI) ^a
SJS	10	10	0	—	—
CBZ	6	6	0	0.0005	25.5 (2.68–242.61)
PHT	4	4	0	0.005	18.5 (1.82–188.40)
MPE	21	3	18	—	—
CBZ	5	0	5	1.14	1.21 (0.21–6.99)
PHT	9	1	8	0.84	1.54 (0.34–7.00)
CBZ and PHT	3	2	1	—	—
CBZ and LVT	1	0	1	—	—
Other AEDs ^b	3	0	3	—	—
Tolerant	50	8	42	—	—
CBZ	4	0	4	—	—
PHT	7	0	7	—	—
CBZ and PHT	38	8	30	—	—
Valproic acid	1	0	1	—	—

^aCompared with “tolerant” group. To allow calculation of the odds ratio and 95% CI, we substituted the 0 for 1.

^bOne each for lamotrigine (LTG), oxcarbazepine (OXC), clobazam (CLB).

two-sided. Values of $p < 0.05$ were considered to indicate statistical significance.

Ethnicity of all subjects was elucidated by racial history identification from both parents, two generations back. The individual is considered a “pure” Thai if none of the four biological grandparents came from other races. Individuals having at least one grandparent of non-Thai race were classified as mixed Thai.

RESULTS

Of the 10 patients with SJS induced by either CBZ ($n = 6$) or PHT ($n = 4$), all tested positive for HLA-B*1502 (Tables 1 and 2). In contrast, of the 50 tolerant controls, there were only eight who had HLA-B*1502. This makes the frequency of HLA-B*1502 19% (8 of 42) in the CBZ-tolerant group and 18% (8 of 45) in the PHT-tolerant group. When the frequencies of HLA-B*1502 in the CBZ-SJS subjects (6 of 6) and the CBZ-tolerant groups (8 of 42) were compared, significant associations were found ($p = 0.0005$). When the CBZ-tolerant group was used as a control, the presence of HLA-B*1502 had a 43% [95% confidence interval (CI): 21%–67%] positive predictive value for CBZ-SJS, whereas its absence had a negative predictive value of 100% (95% CI: 90%–100%). The HLA-B*1502 allele, therefore, has a 100% (95% CI: 61%–100%) sensitivity and 75% (95% CI: 67%–90%) specificity for CBZ-SJS.

By the same token, when the frequencies of HLA-B*1502 in the PHT-SJS patients (4 of 4) and the PHT-tolerant groups (8 of 45) were compared, a significant association was found ($p = 0.005$). When the PHT-tolerant group was used as control, the presence of HLA-B*1502 had a 33% (95% CI: 14%–61%) positive predictive value for PHT-SJS, whereas its absence had a

negative predictive value of 100% (95% CI: 91%–100%). In the test for PHT-SJS, the HLA-B*1502 allele has a 100% (95% CI: 51%–100%) sensitivity and 82% (95% CI: 69%–91%) specificity.

All of the 10 epileptic patients with HLA-B*1502 positivity, who had SJS induced by either CBZ or PHT, received other AEDs at different times (Table 1). Three of the six CBZ-induced SJS patients received PHT, to which all three were tolerant (patient 2, 3, 4; Table 1), whereas one of the four PHT-induced SJS patients was tolerant to CBZ (patient 8). The HLA-B*1502 positivity is strongly associated with PHT- or CBZ-induced SJS, but its presence does not exclusively determine that SJS will develop to both drugs in the same person. Moreover, three patients having HLA-B*1502 and PHT-SJS or CBZ-SJS were tolerant to phenobarbital (case 2 and 7) and lamotrigine (case 8), the other two AEDs having been reported to induce SJS.

Of the 21 patients with AED-induced MPE, 9 had CBZ-MPE and 12 had PHT-MPE (3 had MPE from both CBZ and PHT) (Table 2). Of the 9 CBZ-MPE, 2 had HLA-B*1502 (2 of 9). When compared with the frequency of HLA-B*1502 in the CBZ-tolerant group (8 of 42), no significant association was found (p -value = 1.14, odds ratio 1.21, 95% CI: 0.21–6.99). Similarly, there was no difference in frequencies of HLA-B*1502 between the PHT-MPE (3 of 12) and the PHT-tolerant patients (8 of 45) (p -value = 0.84, odds ratio 1.54, 95% CI: 0.34–7.0). It is noteworthy to mention that no significant associations were found between any other HLA-B alleles and CBZ- or PHT-induced MPE (data not shown). Also of note, none of the four patients with other AED-induced MPE, (levetiracetam, lamotrigine, oxcarbazepine, and clobazam; $n = 1$ for each AED) had test positivity for HLA-B*1502.

Of the 21 individuals who had HLA-B*1502 (10 with AED-SJS), 20 were heterozygous for the HLA-B*1502. The only individual who had homozygous HLA-B*1502 was tolerant to both CBZ and PHT.

Of the 10 patients having HLA-B*1502 in association with AED-SJS, three individuals were pure Thai. Of the remaining mixed Thai subgroups, five have at least one Chinese grandparent, three have at least one Peguan (Mon) grandparent, and one had one Vietnamese grandparent.

DISCUSSION

HLA-B*1502 was present in 100% (4 of 4) of the patients with CBZ-SJS and 19% (8 of 42) of the CBZ-tolerant patients in this study. This revealed a 100% (95% CI: 61%–100%) sensitivity and 75% (95% CI: 67%–90%) specificity of HLA-B*1502 as a test for CBZ-SJS in the Thai population. Similar association was reported in Han Chinese residing in either Taiwan (Chung et al., 2004; Hung et al., 2006) or Hong Kong (Man et al., 2007), but never in Thai people. Studies in Caucasians found no associations between HLA-B*1502 and CBZ-SJS (Alfirevic et al., 2006; Lonjou et al., 2006). In the only Caucasian study that showed such an association in three individuals, it revealed that all three cases had some non-Chinese Asian ancestries (Lonjou et al., 2006). The Asian ethnicity preponderance of the HLA-B*1502 in association with CBZ-SJS was therefore confirmed. However, in the European study, there was only one individual with CBZ-SJS having HLA-B*1502 among three different Asian countries, namely Vietnam, Cambodia, and Reunion Island. This small number was unable to come to a conclusion of association concerning non-Chinese ethnicity. The present study is the first to show a strong association between HLA-B*1502 and CBZ-SJS in Thais, another Asian population, who are non-Han Chinese.

HLA-B*1502 was also found to be associated with PHT-induced SJS in this study. All four patients having PHT-induced SJS were tested positive for the HLA-B*1502 (100%). On the contrary, only 8 of the 45 PHT-tolerant patients (18%) had test positivity. The association was statistically significant (*p*-value of 0.005). Although there was a previous study mentioning one patient with PHT-SJS having HLA-B*1502 (Man et al., 2007), statistical significance of the association between HLA-B*1502 and PHT-SJS has, for the first time, been shown in this study.

Three of the six patients having HLA-B*1502 in the CBZ-SJS group were tolerant to PHT, while one of the four PHT-SJS patients having HLA-B*1502 was tolerant to CBZ. The HLA-B*1502 positivity in these four nonoverlapped patients indicates that patients who are HLA-B*1502 and suffer from SJS induced by CBZ or PHT do not necessarily develop SJS from the other medication. This suggests that, although the HLA-B*1502 may be

necessary, it is not sufficient to cause SJS from either PHT or CBZ in our population. In other words, HLA-B*1502 may be a common attribute required for a Thai patient to develop SJS from these two AEDs; other different elements, however, are also needed for each AED. This possibility needs further elucidation.

Unlike AED-induced SJS, there was no significant association between either CBZ- or PHT-induced MPE and HLA-B*1502 in our study. This finding has been previously reported in Han Chinese (Hung et al., 2006) and suggests that different genetic mechanisms may be involved in the pathogenesis of AED-induced SJS and MPE. Although maculopapular rash usually heralds the phase of epidermal necrolysis and bullous formation in SJS, the pathophysiologic relation between MPE and SJS is still unknown. It has been the practice that if a patient develops AED-induced MPE, all possible causative drugs should be discontinued, otherwise SJS may follow if the offending medications are allowed to continue. Our findings may challenge this belief, if different genetic backgrounds of the patients play a role in these two different cutaneous manifestations. Pharmacogenomic studies before using a potential allergenic drug may provide future means to prevent or properly manage severe allergic cutaneous reactions.

Of the eight AED-tolerant controls with HLA-B*1502, one was homozygous for the allele, making the prevalence of HLA-B*1502 in this study accounting for 9% (9 of 100 alleles). This is comparable to 8.2% from previous studies in Thai populations (Pimthanotai et al., 2002). The prevalence is also similar to that in Han Chinese (8.6%), but much more than in Caucasians (1%–2%) (Chung et al., 2004). This may explain the higher incidence of AED-induced SJS in Han Chinese and in the Thai epileptic population. In the current study, 10 out of a total of 3,000 patients in the authors' tertiary epilepsy clinic reported SJS during a period of 14 years. The prevalence of SJS in Thai epileptics is approximately 3.3 in 1000. The incidence of developing SJS in epileptic patients who were started with AEDs at our center (new AED users) is estimated to be 2.7 in 1,000 exposures (three PHT and five CBZ new users). In Western literatures, risk estimates of SJS and TEN among new users for CBZ, PHT, lamotrigine, and phenobarbital are 1 in 1,000–10,000 exposures (Knowles et al., 1999; Mockenhaupt et al., 2005).

In conclusion, the authors found that there was a strong association between HLA-B*1502 and PHT-induced SJS in Thais. The study also demonstrated that, besides Han Chinese, the Thai population also has significant association between HLA-B*1502 and CBZ-induced SJS. Some patients who had HLA-B*1502 and suffered from CBZ- or PHT-induced SJS could be tolerant to the other medications. On the contrary, no association between HLA-B alleles and CBZ- or PHT-induced MPE was observed in this Thai epileptic population.

ACKNOWLEDGMENTS

This study was supported by Light for Life Foundation for Epilepsy, Chulalongkorn Comprehensive Epilepsy program (CCEP), under the patronage of Professor Doctor Her Royal Highness Princess Chulabhorn, the Research Unit Grant from Chulalongkorn University, the National Center for Genetic Engineering and Biotechnology, and the Thailand Research Fund. The authors thank the Unit of Research Affairs of the Faculty of Medicine, Chulalongkorn University for help in editing this manuscript.

Conflict 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. The authors declare no conflicts of interest.

REFERENCES

- Alfirevic A, Jorgensen AL, Williamson PR, Chadwick DW, Park BK, Pirmohamed M. (2006) HLA-B locus in Caucasian patients with carbamazepine hypersensitivity. *Pharmacogenomics* 7:813–818.
- Arif H, Buchsbaum R, Weintraub D, Koyfman S, Salas-Humara C, Bazil CW, Resor SR Jr, Hirsch LJ. (2007) Comparison and predictors of rash associated with 15 antiepileptic drugs. *Neurology* 68:1701–1709.
- Chung WH, Hung SI, Hong HS, Hsieh MS, Yang LC, Ho HC, Wu JY, Chen YT. (2004) Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* 428:486.
- Hung SI, Chung WH, Jee SH, Chen WC, Chang YT, Lee WR, Hu SL, Wu MT, Chen GS, Wong TW, Hsiao PF, Chen WH, Shih HY, Fang WH, Wei CY, Lou YH, Huang YL, Lin JJ, Chen YT. (2006) Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet Genomics* 16:297–306.
- Knowles SR, Shapiro LE, Shear NH. (1999) Anticonvulsant hypersensitivity syndrome: incidence, prevention and management. *Drug Saf* 21:489–501.
- Lonjou C, Thomas L, Borot N, Ledger N, de Toma C, LeLouet H, Graf E, Schumacher M, Hovnanian A, Mockenhaupt M, Roujeau JC. (2006) A marker for Stevens-Johnson syndrome ...: ethnicity matters. *Pharmacogenomics J* 6:265–268.
- Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, Ng MH. (2007) Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48:1015–1018.
- Mockenhaupt M, Messenheimer J, Tennis P, Schlingmann J. (2005) Risk of Stevens-Johnson syndrome and toxic epidermal necrolysis in new users of antiepileptics. *Neurology* 64:1134–1138.
- Pimtanonthai N, Charoenwongse P, Mutirangura A, Hurley CK. (2002) Distribution of HLA-B alleles in nasopharyngeal carcinoma patients and normal controls in Thailand. *Tissue Antigens* 59:223–225.
- Roujeau JD. (1994) The spectrum of Stevens-Johnson syndrome and toxic epidermal necrolysis: a clinical classification. *J Invest Dermatol* 102:28S–30S.
- Svensson CK, Cowen EW, Gaspari AA. (2001) Cutaneous drug reactions. *Pharmacol Rev* 53:357–379.