

ORIGINAL ARTICLE

Phenobarbital-induced severe cutaneous adverse drug reactions are associated with CYP2C19*2 in Thai children

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Keywords

children; CYP2C19*2; drug rash with eosinophilia and systemic symptoms (DRESS); phenobarbital; severe cutaneous adverse drug reactions; Stevens–Johnson syndrome (SJS); Thai; toxic epidermal necrosis (TEN)

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Abstract

Background: Aromatic anticonvulsant-induced severe cutaneous adverse drug reactions (SCARs), including Stevens–Johnson syndrome (SJS), toxic epidermal necrosis (TEN), and drug rash with eosinophilia and systemic symptoms (DRESS), are fatal immune-mediated adverse drug reactions. CYP2C19, a cytochrome P450 isoform, plays a role in metabolic rate of aromatic anticonvulsant. HLA-B*1502 has also been demonstrated to be associated with carbamazepine-induced SJS-TEN.

Methods: Forty case patients who were diagnosed with SCARs after initiation of phenobarbital (PB), phenytoin (PHT), or carbamazepine (CBZ) for 1–8 wk and forty control patients who received PB, PHT, or CBZ at least 2 months with no adverse drug reactions were enrolled in the study. The genotypes of CYP2C19*1, CYP2C19*2, and HLA-B*1502 were analyzed using allele-specific polymerase chain reaction technique. Clinical characteristics of SCARs patients who used different drugs were also analyzed.

Results: There was no significant difference in sex, onset of symptoms, laboratory results, treatment, and length of stay among patients with SCARs due to PB, PHT, or CBZ. The patients with CYP2C19*2 variant had a trend to have a likelihood to develop SCARs more than the patients with CYP2C19 wild type (OR = 2.5, 95% CI (0.96–67.3) $p = 0.06$). In subgroup analysis, the patients with CYP2C19*2 variant were at four times increased risk of SCARs from phenobarbital more than the patients with CYP2C19 wild type (OR = 4.5, 95% CI (1.17–17.37) $p < 0.03$). There was no association between the HLA-B*1502 and aromatic anticonvulsant-induced severe cutaneous adverse reactions (SCARs).

Conclusion: CYP2C19*2 variant may play a role in the genetic predisposition of SCARs from phenobarbital.

Aromatic anticonvulsant-induced severe cutaneous adverse drug reactions (SCARs), such as Stevens–Johnson syndrome (SJS), toxic epidermal necrosis (TEN), and drug rash with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome, are rare, but potentially fatal immune-mediated adverse drug reaction (ADR). SCARs typically appear 1–8 wk after receiving anticonvulsant drug (1). DRESS are characterized by fever, cutaneous rash, lymphadenopathy, eosinophilia, and internal organ involvement. The incidence varies from 1:1000 to 1:10,000 exposures (2).

SCARs are frequently associated with aromatic anticonvulsant drugs such as phenobarbital (PB), phenytoin (PHT), and carbamazepine (CBZ), which are the first-line drugs used in seizure disorder in children (3).

The factors predisposing individuals to SJS-TEN or DRESS are, in most cases, unknown, but genetic factors have long been postulated to play a role. The mechanisms of aromatic anticonvulsant-induced SCARs, especially DRESS, are not fully understood, but it was thought to be caused by a toxic metabolite, arene oxide, an intermediate substrate from the metabolic pathway of aromatic anticonvulsant (4–6). The

major aromatic anticonvulsant metabolism pathways are catalyzed by cytochrome P450 enzymes (7). It was also found that cytochrome P450 (CYP)-catalyzed metabolism of anticonvulsant is increased in children (8). CYP2C19, a member of cytochrome P450 enzymes, plays an essential role in bioactivating and detoxifying pathways of aromatic anticonvulsants. The imbalance between these pathways resulting in increased hapten formation, which functions as immunogen, potentially initiates immune responses (4). Genetic polymorphisms of CYP2C19 have an impact on metabolic rate of aromatic anticonvulsants (9), and CYP2C19 is highly present in Asian population (10, 11). The most common variant resulting in poor metabolizer are CYP2C19*2 and CYP19*3. A recent study has shown that the frequency of CYP2C19*3 in Thai population was 0.02, which was significantly lower than the frequency previously reported (12). In contrast, CYP2C19*2 has a significantly higher frequency (0.27) (12).

However, the genetic polymorphisms of CYP2C19 have not been studied in the role for aromatic anticonvulsant-induced SCARs in children before. In addition, a recent study has demonstrated HLA-B*1502 as a risk factor for developing SJS-TEN in Thai patients receiving CBZ and PHT (13). Therefore, the aim of the present study was to explore the association of genetic analysis of CYP2C19*1, CYP2C19*2 and HLA-B*1502 with aromatic anticonvulsant-induced SCARs in Thai children. Clinical and laboratory characteristics of patients with SCARs due to PB, PHT, and CBZ were also investigated.

Materials and methods

Subjects

We retrospectively reviewed the medical records from 1987 to 2007 at Ramathibodi Hospital, Bangkok, Thailand. A total of 80 subjects (40 case and 40 control subjects) were recruited. Case subjects were children aged 0–18 yr with diagnosis of severe cutaneous adverse drug reactions (SCARs) (1), including Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS-TEN), drug reaction with eosinophilia and systemic symptoms (DRESS) from phenobarbital, phenytoin, or carbamazepine within 1–8 wk after taking drugs. Control subjects were patients with the same age range taking the same drugs (phenobarbital, phenytoin, or carbamazepine) for at least 8 wk without any adverse drug reaction. We excluded the non-Asian patients from this study due to the difference in allele frequency in different ethnicities. All participating subjects or parents were informed about the contents and the aims of the study and gave their written consent. After written informed consent was obtained, genomic DNA was extracted from peripheral blood, according to the established protocols. The CYP2C19*1, CYP2C19*2, and HLA-B*1502 alleles were determined using allele-specific polymerase chain reactions, and the sequences were analyzed using a DNA sequencer (model 3100 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). Cases with SCARs were also analyzed for demographic characteristics, causative AEDs, length of hospitalization, organ involvement, and laboratory investigation.

The study was reviewed and approved by the human rights and ethic committee of Faculty of medicine, Ramathibodi Hospital, Mahidol University.

Statistical analysis

Sample size was calculated by assuming the type I error (α) = 0.05 and type II error (β) = 0.20, power 80%. Statistical analysis was performed using SPSS software, version 16. Descriptive statistical methods (mean $\pm$ s.d., median, and frequency) were applied to analyze the demographic data. Analyses of the difference in frequencies across groups (sex and clinical manifestation) were performed with the chi-squared test. The continuous variables (age, laboratory parameter, and length of hospitalization) were compared using one-way ANOVA with multiple comparisons. Conditional logistic regression analysis was performed to compare genotype and allele frequency between SCARs and control patients. The strength of association was estimated by calculating the odds ratio. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Clinical characteristics of aromatic anticonvulsant-induced SCARs patients and control patients

There was no significant difference in sex between SCARs and control subjects. Twenty-three subjects (57.5%) in SCARs group and 27 subjects (67.5%) in control group were male. Among 40 patients diagnosed with SCARs and 40 control patients, each group consisted of 20 subjects (50%) prescribed phenobarbital (PB), 17 subjects (42.5%) prescribed phenytoin (PHT), and three patients (7.5%) prescribed carbamazepine (CBZ). The clinical characteristics of SCARs subjects were not significantly different between the three groups in terms of sex, onset of the symptoms, clinical manifestations, the laboratory parameters, treatment, and outcome. The median age of PB group was significantly lower than those of PHT and CBZ groups (2.8 vs. 8.6 vs. 10.6 yr, respectively, $p < 0.002$). The mean onset of symptoms was 14 days. Systemic corticosteroids were prescribed in 25% of SCARs cases, and the mean length of hospitalization was 7 days (Table 1). The severities of cutaneous manifestation of SCARs varied from maculopapular rash to exfoliative dermatitis. Thirty-three cases (82.5%) were diagnosed with drug rash with eosinophilia and systemic symptoms (DRESS). 6 cases (15%) were diagnosed with Stevens–Johnson syndrome (SJS). One case (2.5%) was diagnosed with toxic epidermal necrolysis (TEN).

CYP2C19 genotype, HLA-B*1502, and SCARs

The CYP2C19*2 allele frequency in this study was 32.5%. Patients with CYP2C19*2 allele had a trend to be associated with SCARs but failed to reach the statistical significance (odds ratio = 2.54; 95% CI = 0.96–6.72, p value = 0.055) (Table 2). Genotype frequency of homozygous CYP2C19*1, heterozygous

Table 1 Clinical characteristics of SCARs patients

Factor	PB (n = 20)	PHT (n = 17)	CBZ (n = 3)	Total (n = 40)
Age (years), median (range)	2.8* (0.5–10.5)	8.6 (2.2–14.6)	10.6 (8–13.3)	5.8 (0.5–14.58)
Sex (Male/Female)	14/6	8/9	1/2	23/17 (57.5/42, 5%)
Onset of symptoms (days), mean (s.d.)	13.9 (4.8)	15 (5.1)	14 (4)	14.4 (4.7)
Clinical manifestations				
Cutaneous symptoms	20	17	3	40 (100%)
Gastrointestinal symptoms	4	5	3	12 (30%)
Respiratory symptoms	2	0	0	2 (5%)
Diagnosis				
DRESS	18	15	0	33 (82.5%)
SJS-TEN	2	2	3	7 (17.5%)
Initial laboratory parameters				
Absolute eosinophil (cell/mcl), median (range)	304 (0–1392)	126 (0–949)	104 (81–306)	128 (0–1392)
AST(U/l), median (range)	62 (39–4472)	58.5 (35–3081)	45 (16–152)	59.5 (16–4472)
ALT(U/l), median (range)	71 (26–4243)	56 (29–3104)	55 (21–350)	59.5 (21–4243)
GGT(U/l), median (range)	163 (12–760)	196 (26–812)	114.5 (50–179)	171 (12–812)
Medications				
Antihistamine	16	12	3	31 (77.5%)
Systemic steroid	6	2	2	10 (25%)
Length of hospitalization (days), median (range)	7 (4–17)	8 (3–31)	11 (4–18)	7 (3–31)

PB, phenobarbital; PHT, phenytoin; CBZ, carbamazepine.

*p value < 0.0002.

Table 2 CYP2C19 and HLA-B*2 allele frequency in the SCARs and control subjects

Gene	Variant	SCARs n (%)	No SCARs n (%)	Odds ratio (95% CI)	p value
CYP2C19	CYP2C19*1, positive	39 (97.5)	38 (95)	2.05 (0.18–23.59)	0.5
	CYP2C19*1, negative	1 (2.5)	2 (5)	1	
	CYP2C19*2, positive	17 (42.5)	9 (22.5)	2.54 (0.96–6.7)	0.055
	CYP2C19*2, negative	23 (57.5)	31 (77.5)	1	
HLA-B	HLA-B*1502, positive	7 (17.5)	5 (12.5)	1.4 (0.43–5.1)	0.53
	HLA-B*1502, negative	33 (82.5)	35 (87.5)	1	

CYP2C19*1/*2, and homozygous CYP2C19*2 were 67.5%, 28.8%, and 3.8%, respectively. There was also a trend of association between the genotypes and aromatic anticonvulsant–induced SCARs (p value = 0.054) (Table 3). Due to the low genotype frequency of homozygous CYP2C19*2, there was a trend in significant association between CYP2C19*2 alleles and aromatic anticonvulsant–induced SCARs. Further subgroup analyses were carried out using the combination of patients with heterozygous CYP2C19*1/*2 and those with homozygous CYP2C19*2. It was found that only in phenobarbital group, patients with CYP2C19*2 variant were significantly associated with SCARs (odds ratio = 4.5; 95% CI = 1.17–17.37, p value = 0.03), but there was no significant association in phenytoin group (Table 4). In contrast, there was no association between HLA-B*1502 and aromatic anticonvulsant–induced SCARs either in all groups or in subgroup analysis (p value = 0.5). 17.5% and 12.5% of SCARs and control patients were positive for HLA-B*1502, respectively. Additionally, there was no correlation between CYP2C19*2 and HLA-B*1502 alleles.

Table 3 CYP2C19 genotype frequency in SCARs and control subjects

Genotype	SCARs n (%)	No SCARs n (%)	p value
CYP2C19*1/*1	23 (57.5)	31 (77.5)	0.054
CYP2C19*1/*2	16 (40)	7 (17.5)	
CYP2C19*2/*2	1 (2.5)	2 (5)	

Discussion

Aromatic anticonvulsant drugs share common aromatic ring, which was metabolized by the same bioactivation and detoxifying pathways. Genetic variation in gene involving in their metabolism such as CYP2C19 may result in the increase in arene oxide, which functions as immunogen and potentially initiates immune responses. On the basis of drug metabolism, individuals are classified as extensive metabolizers (EM), intermediate metabolizers (IM), and poor metabolizers (PM).

Table 4 Subgroup analysis of CYP2C19 variants and HLA-B*1502 allele frequency in SCARs and control subjects and adjusted odds ratio with 95% CI

Drug	Variant	SCARs n (%)	No SCARs n (%)	Odds ratio (95% CI)	p value
PB	CYP2C19*2 variant	12 (60)	5 (25)	4.97 (1.22–20.31)	0.025
	Homozygous CYP2C19*1	8 (40)	15 (75)	1	
	HLA-B*1502 positive	5 (25)	2 (10)	3.734 (0.5–25.7)	0.18
	HLA-B*1502 negative	15 (75)	18 (90)	1	
PHT	CYP2C19*2 variant	5 (29.4)	3 (17.6)	1.68 (0.3–8.8)	0.52
	Homozygous CYP2C19*1	12 (70.6)	14 (82.4)	1	
	HLA-B*1502 positive	1 (5.9)	3 (17.6)	0.35 (0.03–3.9)	0.35
	HLA-B*1502 negative	16 (94.1)	14 (82.4)	1	

PB, phenobarbital; PHT, phenytoin.

The most common PM allele for CYP2C is CYP2C19*2. Individuals with CYP2C19*2 variant will be PMs and have a much lower ability than average to oxidize these drugs, resulting in adverse drug reactions (14). In addition, a recent study has demonstrated HLA-B*1502 as a risk factor for developing SJS-TEN in Thai patients receiving CBZ and PHT. As a result, we examined the association of CYP2C19*2 and HLA-B*1502 alleles with aromatic anticonvulsant-induced severe cutaneous adverse drug reactions (SCARs) in children. We found that in patients with CYP2C19*2 variant who have taken phenobarbital, the risk of developing SCARs was found to be significantly increased 4.5 times when compared with the drug-tolerant control group. CYP2C19 genotype has also been demonstrated to be important in PHT and PB metabolism and elimination (7). We did not find the association between CYP2C19*2 variant and SCARs either in the phenytoin group or in the combined group (PB, PHT, and CBZ). These results may be explained from the lower number of studied subjects or the difference in ethnicities. In addition, CYP2C9, other cytochrome P450 superfamily, has been shown to be associated with PHT metabolism and elimination, but not with PB or CBZ⁷. CYP2C9 polymorphism has also been demonstrated to be associated with PHT-induced cutaneous ADR (15). This may imply that the association between CYP2C gene polymorphism and cutaneous drug reactions is subfamily and drug specific. CYP2C19 may be the major metabolic pathway of PB, but not for PHT (16–18). However, the effect of CYP2C19 variant on pharmacokinetics of PB has conflicting results depending on ethnicity. Lee and colleagues did not find the effect of CYP2C19 variant on pharmacokinetics of PB in Korean infants and neonates with seizure (19), while the opposite result was demonstrated in Japanese epileptic patients (20). As a result, more SCARs patients need to be evaluated for CYP2C gene polymorphism to confirm this hypothesis.

This study is the first to show the association between CYP2C19*2 and aromatic anticonvulsant-induced SCARs in children. CYP2C19*2 may have a potential role in future screening before prescribing aromatic anticonvulsant, especially PB in children. However, the limitation in the present study is the small sample size, and CYP2C19*3, another PM variant, was not evaluated due to the much lower reported

allele frequency in Thai population (12) and budget limitation. These indicate that further studies are required which should be performed in an unbiased assessment of predisposition through whole-genome scanning instead of a candidate gene approach. Since most drug hypersensitivity reactions are rare, there are variations in severity and clinical manifestations. Therefore, multicenter multinational collaborations and accurate phenotypic characterization of patients are essential. Besides, the identification of a genetic predisposing factor may only be relevant for one ethnic group and it is likely to be multigenic, so the cost-effectiveness in screening patients prior to starting the drug should be considered.

HLA-B*1502 allele has been demonstrated to be associated with carbamazepine- and phenytoin-induced SJS-TEN in Thai adults (13). However, in the present study, we did not demonstrate the association between HLA-B*1502 and aromatic anticonvulsant-induced SCARs in Thai children. This may be explained from the lower percentage of patients diagnosed with SJS-TEN in the present study.

We also found that the age of patients diagnosed with SCARs from PB was significantly lower than those with SCARs from PHT and CBZ. This can be explained by the fact that PB is the first-line and commonly used anticonvulsive drug in Thai children. As the cutaneous presentations of SCARs vary from maculopapular rash, exfoliative dermatitis to Stevens–Johnson syndrome (SJS), the onset of symptoms could be as early as 9 days as shown in the present study or as late as 3 months (21). Consequently, careful monitoring and follow up is needed in SCARs patients. If clinical course run down, systemic corticosteroids may play a role. The overall prognosis was good as shown in terms of the length of hospitalization and the use of steroid in only one-fourth of cases, and there was no fatality in the present study.

Conclusions

We have demonstrated the association between CYP2C19*2 and phenobarbital-induced SCARs in Thai children. This study shows that genetic predisposing factors play a role in adverse drug reactions.

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