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Association between *ABCB1* polymorphism and response to sodium valproate treatment in Malaysian epilepsy patients

Batoul Sadat Haerian [1], Kheng Seang Lim [2], Hui Jan Tan [3], Elsa Hanifa Mejia Mohamed [1], Chong Tin Tan [2], Azman Ali Raymond [3], Chee Piau Wong [4], Sau Wei Wong [5], Haslyna Omar [1], Harun Roslan [6], Zahurin Mohamed [1]

[1] Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur
[2] Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur
[3] Division of Neurology, Department of Medicine, Faculty of Medicine, National University of Malaysia, Kuala Lumpur
[4] Department of Pediatrics, Faculty of Medicine, University of Monash, Kuala Lumpur
[5] Department of Pediatrics, Faculty of Medicine, National University of Malaysia, Kuala Lumpur
[6] Medical Molecular Biology Institute, National University of Malaysia, Kuala Lumpur, Malaysia

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ABSTRACT – Over-expression of P-glycoprotein, encoded by the *ABCB1* gene, is proposed to be involved in resistance to antiepileptic drugs in about 30% of patients with epilepsy. Here, we investigated the possible association between *ABCB1* polymorphisms and sodium valproate (VPA) treatment in Malaysian epilepsy patients. Genotypes were assessed in 249 drug-resistant and 256 drug-responsive Malaysian patients for C1236T, G2677T/A, and C 5T polymorphisms in the *ABCB1* gene. No genotypes, alleles, or haplotypes were associated with the response to VPA in either the overall group or Chinese, Indian, and Malay subgroups. Our data suggest that C1236T, G2677T/A, and C3435T polymorphisms in the *ABCB1* gene do not contribute to the response to VPA in patients with epilepsy.

Key words: epilepsy, *ABCB1*, polymorphism, P-glycoprotein, antiepileptic drug, sodium valproate

Correspondence:
B.S. Haerian
Department of Pharmacology,
Faculty of Medicine,
University of Malaya,
60302, Kuala Lumpur,
Malaysia
<batoolsadat@yahoo.com>

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Although a variety of antiepileptic drugs (AEDs) are useful to control seizures, about one third of epilepsy patients do not respond to medication (Kwan and Brodie, 2000). Epilepsy is a complex disease in which environmental factors, genetic factors, or both are believed to contribute to resistance to AEDs (Bell and Taylor, 1997). The drug transporter hypothesis is a dominant theory and is based on the

suggestion that the dysfunction of multidrug transporters at the blood-brain barrier influences the brain uptake of a variety of drugs, including many AEDs (Löscher, 2005). Over-expression of P-glycoprotein (P-gp), an efflux transporter in brain tissue from drug-resistant patients, is proposed to be linked to resistance to AEDs in epilepsy (Tishler *et al.*, 1995).

P-gp is encoded by the *ABCB1* gene which is located at chromosome 7p21 in humans and is composed of 29 exons (http://www.ncbi.nlm.nih.gov). The C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642) loci are the most commonly studied genetic variants in the *ABCB1* gene, of which the synonymous C3435T polymorphism in exon 27 has received more attention as a critical variant in AED resistance (Hoffmeyer *et al.*, 2000; Löscher *et al.*, 2009). An initial study demonstrated that the frequency of the C/C genotype among drug-resistant patients was significantly higher than that in drug-responsive patients Siddiqui *et al.* (2003). Several studies have attempted to examine this association, but the results have been inconsistent. Moreover, our previous meta-analysis involving 6,755 Asian and Caucasian patients did not confirm this association based on either the total study population or ethnic subgroups (Haerian *et al.*, 2010).

Linkage disequilibrium (LD) of the silent C3435T polymorphism in a haplotype block with other variants in the *ABCB1* gene has also been proposed. Several studies have assessed the association between *ABCB1* haplotypes, derived from C1236T, G2677T/A, and C3435T, and response to AEDs in different populations, but reported contradictory results (Zimprich *et al.*, 2004; Hung *et al.*, 2005; Seo *et al.*, 2006; Kim *et al.*, 2006; Shahwan *et al.*, 2007; Kwan *et al.*, 2009a; Lakhan *et al.*, 2009; Ufer *et al.*, 2009; Vahab *et al.*, 2009; Grover *et al.*, 2010; Alpman *et al.*, 2010). We speculate that the effect of common confounders, such as ethnicity, and polypharmacy contribute to the discrepancy of results regarding the C3435T polymorphism and linkage with other *ABCB1* loci. In order to eliminate the confounding effect of drug interaction, in this study we focused on patients receiving sodium valproate (VPA) monotherapy. This drug is extensively used as first-line therapy for different epilepsy syndromes. However, administration of this drug depends on the type of epileptic syndrome, tolerability, gender, pharmacokinetics, current or likely future need for concomitant medication for comorbid conditions, and cost (Elger and Schmidt, 2008). In the current study, we addressed the question of whether *ABCB1* C1236T, G2677T, and C3435T polymorphisms and haplotypes are associated with the response to VPA in a Malaysian epilepsy population, as well as in Chinese, Indian, and Malay ethnic subgroups.

Methods

Subjects

The present retrospective study is part of an ongoing multicentre cooperation between the University of Malaya Medical Centre and the National University of Malaysia Medical Centre. The study protocol was approved by the ethics committees of both centres. A total of 505 patients with epilepsy who were receiving VPA monotherapy were recruited from the epilepsy clinics and diagnosed by neurologists who were blind to the genotype data. Seizures and epilepsy syndromes were classified according to the International League Against Epilepsy guidelines (Commission on Classification Terminology of the International League Against Epilepsy, 1981). On the basis of more recent definitions of treatment outcome (Kwan *et al.*, 2009b), drug responsiveness was defined as being completely seizure-free for at least one year during treatment with VPA, and drug resistance was defined as the occurrence of seizures over a period of one year during treatment with VPA at maximally tolerated therapeutic doses.

Epilepsy patients of Chinese, Indian, and Malay ethnicities were eligible for inclusion if they had received VPA monotherapy and were followed for at least one year. Exclusion criteria included severe adverse drug reactions, poor compliance with VPA therapy, unreliable record of seizure frequency, significant psychiatric comorbidity, history of pseudoseizures, alcohol or drug abuse, and presence of progressive or degenerative neurological or systemic disorders. Written informed consent was obtained from all patients or guardians of those younger than 18 years old. A standardized extraction template was provided to collect demographic details and information on seizure type and frequency, medical history, concomitant drug history, and relevant family history from the records.

Genotyping

Genomic DNA was extracted from either whole blood or buccal swabs by using standard methods. The *ABCB1* C1236T, G2677T/A, and C3435T polymorphisms were genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). Amplification of the polymorphisms was performed by PCR with the following forward and reverse primers: C1236T: 5′-TCT TTG TCA C TT TAT CCA GC-3′ and 5′-TCTCACCATC CCCTCTGT-3′; G2677T/A: 5′-TGC AGG CTA TAG GTT CCA GG-3′ and 5′-TTT AGT TTG A CT CAC CTT CCC G-3′; C3435T: 5′-TGC TGG TCC TGA AGT TGA TCT GTG AAC-3′ and 5′-ACA TTA GGC AGT GAC TCG ATG AAG GCA-3′. PCR-amplified products with C1236T,

G2677T, G2677A, and C3435T were digested with *Hae*III (modified from *Eco*0109I), *Ban*I, *Rsa*I, and *Mbo*I (Tang *et al*., 2002; Cascorbi *et al*., 2001). PCR and RFLP products were analyzed by electrophoresis on 2% agarose gels. The results of PCR-RFLP were confirmed through DNA sequencing of a random selection of samples (5%) by using the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, USA) and ABI Prism Big Dye Terminator v3.1 cycle sequencing kit.

Statistical methods

All values were presented either as the mean ± standard deviation for continuous data or as frequency for categorical data. The age at time of study and onset of epilepsy were not normally distributed, according to the Kolmogorov-Smirnov test. Therefore, the frequency of these two variables was compared for VPA-resistant and VPA-responsive patients by the non-parametric Mann-Whitney U test or the Kruskal-Wallis rank sum test. Comparison of distribution of ethnicity, epilepsy syndrome, seizure type, alleles, and genotypes in overall patients, irrespective of response to VPA, was performed by the χ^2 test. Binary logistic regression was used to adjust for the potential effect of clinical factors on response to VPA (ethnicity, gender, age at recruitment, onset age of epilepsy, seizure type, and epilepsy syndrome). The odds ratios (ORs) with 95% confidence intervals (CIs), adjusted and unadjusted for confounders, were obtained through binomial logistic regression analysis.

The alternative genetic models for C1236 and C3435T polymorphisms included alleles (C *vs* T) and genotypes for co-dominant (C/C *vs* T/T and C/T *vs* T/T), dominant (C/C+C/T *vs* T/T), and recessive (C/C *vs* C/T+T/T) models. For simplification of the analysis of genetic models of the triallelic G2677T/A variant, the A and T alleles were not distinguished. The alternative genetic models for the G2677T polymorphism included alleles (G *vs* T) and genotypes for co-dominant (G/G *vs*. T/T and G/T *vs*. T/T), dominant (G/G+G/T *vs* T/T), and recessive (G/G *vs* G/T+T/T) models. A goodness-of-fit χ^2 test with one degree of freedom was applied to test Hardy-Weinberg equilibrium (HWE) of the three polymorphisms; $p<0.05$ indicated a lack of agreement with HWE. Haplotype and LD analysis for the three single nucleotide polymorphisms (SNPs) was performed with the Haploview 4.2 program and corrected for multiple testing by using 100,000 permutations for individual SNPs and haplotypes. Bonferroni's method was used for correction of multiple comparisons. Two-sided tests of statistical significance were used to determine statistically significant *p* values ($p<0.05$) with the SPSS software package (version 15.0; SPSS, Chicago, IL, USA).

Results

Patient characteristics

A total of 505 epilepsy patients consisting of 197 (39%) Chinese, 136 (27%) Indians, and 172 (34%) Malays were enrolled in this case-control study. The demographic characteristics of 249 (49%) VPA-resistant and 256 (51%) VPA-responsive patients are shown in *table 1*. The distribution of ages at time of study and onset of epilepsy did not significantly differ between VPA-resistant and VPA-responsive patients in total or among the Chinese, Indian, and Malay patients. Males were over-represented ($p=0.003$), but this was not significantly different between VPA-resistant and VPA-responsive patients or ethnic subgroups. Partial seizures were significantly more common among drug-resistant patients than drug-responsive patients in the overall ($p=0.003$) and Malay subgroup ($p<0.0001$). Moreover, symptomatic (36%) and cryptogenic (33%) epilepsy were also more often diagnosed in patients than idiopathic epilepsy. Symptomatic and cryptogenic epilepsy were significantly more common in drug-resistant than drug-responsive patients in the overall ($p<0.0001$ and $p<0.0001$, respectively) and Malay subgroup ($p<0.0001$ and $p=0.01$, respectively) and cryptogenic epilepsy was significantly more common in drug-resistant than drug-responsive patients in the Indian subgroup ($p=0.03$).

Allelic and genotypic association of polymorphisms with resistance to AEDs

The allele and genotype frequencies of the *ABCB1* polymorphisms in VPA-resistant and VPA-responsive patients are listed in *table 2*. The frequency of *ABCB1* 1236T, 2677T, and 3435T alleles in the total patient population was 58, 51, and 44%, respectively. There was no significant association between these three alleles and response to VPA in the overall group or in the Chinese, Indian, and Malay subgroups. The genotype distribution of the *ABCB1* 1236T, 2677T, and 3435T polymorphisms, in both VPA-resistant and VPA-responsive patients in the total group or in each ethnic subgroup, was consistent with HWE. The distribution of *ABCB1* 3435TT, 2677TT, and 3435TT genotypes in the total patient population was 35, 20, and 19%, respectively. There was no significant association between the C1236T, G2677T, and C3435T genotypes and resistance to VPA in the total sample or in the Indian and Malay subgroups using alternative genetic models *(table 3)*. However, the G2677T variant in the Chinese subgroup showed a significant association with response to VPA only using the co-dominant model (G/G *vs* T/T; adjusted OR 0.38, 95% CI 0.16-0.90, $p=0.03$). The type of epilepsy syndrome had a confounding effect

Table 1. Demographic characteristics of the patients.

Characteristics	Chinese (n=197)				Indian (n=136)				Malay (n=172)				Total (n=505)			
	NR (100)	R (97)	p	OR (CI 95%)	NR (57)	R (79)	p	OR (CI 95%)	NR (92)	R (80)	p	OR (CI 95%)	NR (249)	R (256)	p	OR (CI 95%)
Age (years), mean (SD)	29 (18)	30 (19)	0.74	1.00 (0.99-1.02)	29 (16)	26 (16)	0.46	0.99 (0.97-1.01)	27 (15)	23 (15)	0.19	0.99 (0.97-1.01)	28 (16)	27 (17)	0.44	1.00 (0.99-1.01)
Onset age of epilepsy (years), mean (SD)	17 (18)	19 (17)	0.34	1.00 (0.99-1.02)	15 (15)	15 (12)	0.93	1.00 (0.97-1.03)	11 (12)	12 ((13)	0.56	1.01 (0.98-1.03)	14 (16)	16 (15)	0.31	1.01 (0.99-1.02)
Sex, *N* (%)																
Females	44 (43)	41 (42)	-	Referent	24 (42)	36 (46)	-	Referent	41 (45)	34 (42)	-	Referent	109 (44)	111 (43)	-	Referent
Males	56 (57)	56 (58)	0.92	1.07 (0.61-1.89)	33 (58)	43 (54)	0.82	0.87 (0.44-1.73)	51 (55)	46 (58)	0.91	1.09 (0.59-1.99)	140 (56)	145 (57)	1.00	1.02 (0.72-1.45)
Seizure type, *N* (%)																
Partial	55 (55)	47 (48)	-	Referent	22 (39)	30 (38)	-	Referent	56 (61)	23 (29)	-	Referent	133 (53)	100 (39)	-	Referent
Generalized	41 (41)	41 (42)	0.70	1.17 (0.65-2.09)	31 (54)	45 (57)	0.99	1.06 (0.52-2.18)	32 (35)	51 (64)	0.0001	3.88 (2.01-7.48)	104 (42)	137 (53)	0.003	1.75 (1.22-2.52)
Unspecified	4 (4)	9 (9)	-	-	4 (7)	4 (5)	-	-	4 (4)	6 (7)	-	-	12 (5)	19 (7)	-	-
Aetiology, N (%)			-													
Idiopathic	14 (14)	23 (24)	-	Referent	15 (26)	36 (46)	-	Referent	14 (15)	31 (39)	-	Referent	43 (17)	90 (35)	-	Referent
Symptomatic	43 (43)	37 (38)	0.16	0.52 (0.24-1.16)	20 (35)	22 (28)	0.11	0.46 (0.19-1.08)	42 (46)	20 (25)	0.0001	0.21 (0.09-0.49)	105 (42)	79 (31)	0.0001	0.36 (0.23-0.57)
Cryptogenic	39 (39)	29 (30)	0.09	0.45 (0.20-1.03)	21 (37)	18 (23)	0.03	0.36 (0.15-0.85)	33 (36)	24 (30)	0.01	0.33 (0.14-0.75)	93 (37)	71 (28)	0.0001	0.37 (0.23-0.59)
Unspecified	4 (4)	8 (8)	-	-	1 (2)	3 (4)	-	-	3 (3)	5 (6)	-	-	8 (3)	16 (6)	-	-

NR: VPA-resistance; R: VPA-responsive; OR: odds ratio; CI: confidence interval; SD: standard deviation.

Table 2. Genotypes and allele frequencies of *ABCB1* C1236T, G2677T and C3435T polymorphisms in VPA-resistance (n=249) and VPA-responsive (n=256) epilepsy patients and ethnic subgroups.

Allele/genotype	Ethnicity												Total (505)			
	Chinese (*n*=197)				Indian (*n*=136)				Malay (*n*=172)							
	NR (100)	R (97)	*p*	OR[a] (CI 95%)	NR (57)	R (79)	*p*	OR[a] (CI 95%)	NR (92)	R (80)	*p*	OR[a] (CI 95%)	NR (249)	R (256)	*p*	OR[a] (CI 95%)
C1236T																
C	87 (43)	78 (40)	-	Referent	47 (41)	71 (45)	-	Referent	80 (43)	63 (39)	-	Referent	214 (43)	212 (41)	-	Referent
T	113 (57)	116 (60)	0.60	0.89 (0.57-1.39)	67 (59)	87 (55)	0.63	1.14 (0.67-1.95)	104 (57)	97 (61)	0.44	0.81 (0.49-1.37)	284 (57)	300 (59)	0.61	0.93 (0.70-1.23)
CC	23 (23)	15 (15)	-	Referent	11 (19)	16 (20)	-	Referent	18 (20)	13 (16)	-	Referent	52 (21)	44 (17)	-	Referent
CT	41 (41)	48 (50)	0.13	0.51 (0.21-1.22)	25 (44)	39 (49)	0.70	0.82 (0.30-2.27)	44 (48)	37 (46)	0.43	0.66 (0.23-1.85)	110 (44)	124 (48)	0.11	0.64 (0.37-1.10)
TT	36 (36)	34 (35)	0.43	0.70 (0.29-1.70)	21 (37)	24 (30)	0.73	1.20 (0.42-3.49)	30 (33)	30 (38)	0.39	0.63 (0.22-1.82)	87 (35)	88 (34)	0.42	0.79 (0.45-1.38)
G2677T																
G	109 (55)	95(49)	-	Referent	38(33)	68(43)	-	Referent	101(55)	82 (52)	-	Referent	248(50)	245(48)	-	Referent
T	91 (45)	99 (51)	0.18	0.51 (0.19-1.36)	76 (67)	90 (57)	0.79	0.85 (0.26-2.82)	83 (45)	78 (48)	0.61	1.36 (0.42-4.34)	250 (50)	267 (52)	0.35	0.74 (0.40-1.38)
GG	25 (25)	23 (24)	-	Referent	6 (10)	13 (17)	-	Referent	29 (31)	17 (21)	-	Referent	60 (24)	53 (21)	-	Referent
GT	59 (59)	49 (50)	0.73	1.14 (0.53-2.48)	26 (46)	42 (53)	0.70	1.26 (0.38-4.21)	43 (47)	48 (60)	0.56	0.76 (0.31-1.89)	128 (51)	139 (54)	0.84	0.95 (0.58-1.56)
TT	16 (16)	25 (26)	0.45	0.50 (0.19-1.28)	25 (44)	24 (30)	0.10	2.88 (0.82-10.06)	20 (22)	15 (19)	0.79	1.16 (0.38-3.60)	61 (25)	64 (25)	0.75	1.10 (0.62-1.96)
C3435T																
C	114 (57)	105 (54)	-	Referent	60 (53)	75 (47)	-	Referent	110 (60)	96 (60)	-	Referent	284 (57)	276 (54)	-	Referent
T	86 (43)	89 (46)	0.95	1.02 (0.65-1.58)	54 (47)	83 (53)	0.70	0.90 (0.53-1.53)	74 (40)	64 (40)	0.90	1.03 (0.62-1.72)	214 (43)	236 (46)	0.91	0.98 (0.74-1.30)
CC	30 (30)	30 (31)	-	Referent	18 (32)	14 (18)	-	Referent	35 (38)	31 (39)	-	Referent	83 (33)	75 (29)	-	Referent
CT	54 (54)	45 (46)	0.54	1.25 (0.61-2.55)	24 (42)	47 (59)	0.05	0.38 (0.15-1.00)	40 (44)	34 (42)	0.56	1.28 (0.56-2.92)	118 (47)	126 (49)	0.66	0.90 (0.57-1.42)
TT	16 (16)	22 (23)	0.95	0.97 (0.39-2.42)	15 (26)	18 (23)	0.65	0.77 (0.26-2.33)	17 (18)	15 (19)	0.99	0.99 (0.36-2.74)	48 (19)	55 (22)	0.96	0.98 (0.56-1.73)

NR: VPA-resistance; R: VPA-responsive; OR: odds ratio; CI: confidence interval [a]OR estimated odds ratio by binary logistic regression analysis adjusted for age at study, age at epilepsy onset, epilepsy syndrome, ethnicity, gender, and seizure type.

on this association (symptomatic epilepsy: OR 5.25, 95% CI 1.55-17.76, p=0.008 and cryptogenic epilepsy: OR 4.92, 95% CI 1.46-16.56, p=0.019). The frequency of the 2677TT genotype in drug-resistant patients was less than that in drug-responsive patients with symptomatic epilepsy (29% *vs* 71%) or idiopathic epilepsy (33% *vs* 67%), but not with cryptogenic epilepsy (58% *vs* 41%). There was a marginal association of this variant in the Chinese subgroup using the dominant model (adjusted OR 0.46, 95% CI 0.21-1.00, p=0.05) as well as in the Indian subgroup using the co-dominant model (G/G *vs* T/T; adjusted OR 2.39, 95% CI 0.99-5.76, p=0.05). After correction of multiple comparisons with Bonferroni's method, we did not find any significant association between *ABCB1* polymorphisms and response to VPA (0.05/3).

Haplotype and diplotype association of *ABCB1* polymorphisms with resistance to AEDs

For all patients, all eight possible haplotypes were encountered with frequencies of above 5% *(table 4)*. Overall, the TTT (21.5%) and CGC haplotypes (18.5%) were more frequent than other *ABCB1* gene haplotypes. Similar results for the TTT and CGC haplotypes were observed in the Chinese (21 and 19%, respectively) and Malay subgroups (20.5 and 19.5%, respectively), but for only the TTT haplotype in the Indian subgroup (24%). The association between *ABCB1* haplotypes and response to VPA was not significant in patients overall or in the Chinese and Malay subgroups, but was significant for the CTC haplotype in the Indian subgroup (adjusted OR 3.23, 95% CI 1.04-10.03, p=0.04). The type of epilepsy syndrome had a strong confounding effect on this association (symptomatic epilepsy: OR 2.80, 95% CI 1.29-6.09, p=0.01 and cryptogenic epilepsy: OR 3.67, 95% CI 1.72-7.83, p=0.001). The CTC haplotype was more frequent in VPA-resistant patients with symptomatic and cryptogenic epilepsy (25% and 42%, respectively) than in VPA-responsive patients (11% and 22%, respectively). However, this haplotype was less frequent in VPA-resistant patients (33%) than VPA-responsive patients (67%) with idiopathic epilepsy. After using Bonferroni's correction for multiple comparisons, the association was lost (0.05/8). Of the 27 possible diplotypes in all patients, only six (CGC-TTT, TGC-TTT, CGC-TTC, CGC-TGC, TTT-TTT, and TGC-TTC) were present at frequencies above 5% *(table 4)*. Overall, CGC-TTT and TGC-TTT were the most frequent diplotypes, although the frequency of TGC-TTT was greatest (14%) in the Chinese patients. There were no CGC-TGC or TGC-TTC diplotypes in the Indian VPA-responsive patients. No significant association was observed between *ABCB1* diplotypes and response to VPA in patients overall or in each ethnic subgroup. There was no strong LD between C1236T/G2677T, G2677T/C3435T, and C1236T/C3435T polymorphisms in the overall group or in each ethnic subgroup.

Discussion

In the present study, we found no association between *ABCB1* C1236T, G2677T/A, and C3435T polymorphisms and haplotypes with response to VPA in Malaysian epilepsy patients. Overall, the results of these three polymorphisms are consistent with our previous meta-analysis (Haerian *et al*., 2010) but inconsistent with the original report of Siddiqui *et al*. (2003)

Unlike Siddiqui *et al*. (2003) who demonstrated an increased prevalence of the 3435CC genotype in drug-resistant epilepsy, we did not find a similar result in the Malaysian patients receiving VPA monotherapy. Similar results were obtained using subsidiary analysis of ethnicity in the Chinese, Indian, and Malay patients. The results of the Chinese subgroup in the current study are consistent with two studies reporting no association, but inconsistent with four studies of Chinese epilepsy patients (Haerian *et al*., 2010). Our data of the Indian subgroup is consistent with all three previous studies of epilepsy patients in India.

In the current study, in the overall group, the 3435T allele was less frequent in VPA-resistant patients than in VPA-responsive patients, consistent with some Asian and Caucasian studies. A similar result was obtained in the Chinese subgroup, consistent with two studies reporting an association with Chinese patients, but inconsistent with four other Chinese studies. However, the result of the Indian subgroup was inconsistent with the findings from all three studies previously performed in India (Grover *et al*., 2010; Haerian *et al*., 2010). As an alternative method, we applied the adjusted ORs for ethnicity, gender, age at recruitment, age at onset of epilepsy, seizure type, and cause of epilepsy to minimize their effects on the association study.

One plausible explanation for the inconsistency of results of all loci between our study and previous reports is that false-positive results, due to type I errors in the studies reporting positive results, may be caused by the effect of sample size and confounders such as ethnicity, definition of drug-resistance and drug-responsiveness, and polytherapy, (Ameyaw *et al*., 2001; Otto, 2004; Tan *et al*., 2004; Löscher *et al*., 2009). Sample size limitation is a common problem in association studies, including most previous epilepsy association studies, as well as our own. This leads to underpowered allelic and genotypic data. This limitation could be resolved by worldwide collaboration between different centres. A recent genome-wide

Table 3. Genotypes and allele frequencies of *ABCB1* C1236T, G2677T/A, and C3435T polymorphisms in VPA-resistance (n=249) and VPA-responsive (n=256) epilepsy patients and ethnic subgroups using alternative genetic models.

Genotype	Ethnicity												Total (505)			
	Chinese (n = 197)				Indian (n = 136)				Malay (n = 172)							
	NR	R	P	OR[a] (CI 95%)	NR	R	p	OR[a] (CI 95%)	NR	R	p	OR[a] (CI 95%)	NR	R	p	OR[a] (CI 95%)
C1236T																
C/C *vs* T/T	61	49	0.51	0.73 (0.29-1.86)	32	40	0.73	1.21 (0.42-3.50)	48	43	0.30	0.66 (0.30-1.45)	141	132	0.41	0.79 (0.45-1.39)
C/T *vs* T/T	77	82	0.38	1.37 (0.67-2.82)	46	63	0.38	1.48 (0.62-3.55)	74	67	0.78	0.89 (0.39-2.02)	197	212	0.40	1.21 (0.78-1.89)
C/C+C/T *vs* T/T	100	97	0.76	1.11 (0.57-2.13)	57	79	0.43	1.38 (0.62-3.07)	92	80	0.67	0.85 (0.40-1.81)	249	256	0.72	1.08 (0.72-1.63)
C/C *vs* C/T+T/T	100	97	0.20	0.59 (0.26-1.32)	57	79	0.94	0.97 (0.38-2.48)	92	80	0.37	0.65 (0.25-1.70)	249	256	0.18	0.71 (0.43-1.17)
G2677T																
G/G *vs* T/T	40	48	0.59	0.76 (0.28-2.04)	31	37	0.13	2.74 (0.75-10.06)	49	31	0.88	0.91 (0.26-3.22)	120	116	0.89	1.04 (0.57-1.90)
G/T *vs* T/T	75	74	0.03	0.38 (0.16-0.90)	51	66	0.05	2.39 (0.99-5.76)	63	63	0.42	1.50 (0.56-3.98)	177	203	0.57	1.15 (0.71-1.87)
G/G *vs* (G/T + T/T)	100	97	0.77	1.12 (0.54-2.31)	57	79	0.32	0.56 (0.18-1.75)	92	80	0.72	1.17 (0.49-2.82)	249	256	0.98	1.00 (0.62-1.61)
(G/G + G/T) *vs* T/T	100	97	0.05	0.46 (0.21-1.00)	57	79	0.04	2.40 (1.04-5.51)	92	80	0.46	1.41 (0.56-3.56)	249	256	0.57	1.14 (0.72-1.81)
C3435T																
C/C *vs* T/T	46	52	0.97	0.98 (0.39-2.50)	33	27	0.24	0.47 (0.13-1.67)	52	46	0.97	0.98 (0.32-3.04)	131	125	0.85	0.95 (0.53-1.68)
C/T *vs* T/T	70	67	0.54	0.76 (0.32-1.79)	39	69	0.06	2.60 (0.97-7.02)	57	49	0.72	0.83 (0.30-2.30)	166	185	0.63	1.14 (0.67-1.91)
C/C+C/T *vs* T/T	100	97	0.68	0.85 (0.38-1.89)	57	79	0.36	1.50 (0.62-3.63)	92	80	0.77	0.87 (0.35-2.19)	249	256	0.85	1.05 (0.64-1.71)
C/C *vs* C/T+T/T	100	97	0.65	1.17 (0.59-2.29)	57	79	0.11	0.48 (0.19-0.19)	92	80	0.67	1.18 (0.55-2.51)	249	256	0.73	0.93 (0.60-1.42)

NR: VPA-resistance; R: VPA-responsive; OR: odds ratio; CI: confidence interval; [a]OR estimated odds ratio by binary logistic regression analysis adjusted for age at study, age at epilepsy onset, epilepsy syndrome, ethnicity, gender, and seizure type.

Table 4. Haplotype and diplotype frequencies of *ABCB1* C1236T, G2677T/A, and C3435T polymorphisms in VPA-resistance (n=249) and VPA-responsive (n=256) epilepsy patients and ethnic subgroups.

Haplotypes/	Ethnicity												Total (505)			
	Chinese (n=197)				Indian (n=136)				Malay (n=172)							
	NR (%)	R (%)	*p*	OR[a] (CI 95%)	NR (%)	R (%)	*p*	OR[a] (CI 95%)	NR (%)	R (%)	*p*	OR[a] (CI 95%)	NR (%)	R (%)	*p*	OR[a] (CI 95%)
Haplotype CGC	20	18	-	Referent	11	16	-	Referent	15	24	-	Referent	18	19	-	Referent
TTT	14	28	0.45	0.79 (0.42-1.47)	25	23	0.30	1.50 (0.70-3.23)	20	21	0.62	0.83 (0.41-1.70)	18	25	0.74	0.94 (0.64-1.38)
TTC	18	10	0.70	0.86 (0.39-1.89)	16	12	0.13	2.12 (0.81-5.56)	20	9	0.52	1.33 (0.56-3.19)	17	11	0.21	1.37 (0.84-2.22)
TGC	13	16	0.78	1.11 (0.53-2.36)	14	14	0.39	1.61 (0.54-4.78)	13	19	0.22	0.59 (0.25-1.38)	14	16	0.84	0.95 (0.58-1.55)
CGT	11	10	0.38	1.55 (0.59-4.06)	5	8	0.73	0.79 (0.20-3.11)	13	3	0.30	1.99 (0.54-7.37)	10	6	0.23	1.49 (0.78-2.82)
CTC	6	11	0.31	0.62 (0.24-1.59)	12	6	0.04	3.23 (1.04-10.03)	7	11	0.60	0.74 (0.25-2.26)	8	9	0.90	1.04 (0.58-1.87)
TGT	11	6	0.97	0.98 (0.39-2.49)	4	6	0.84	0.86 (0.21-3.48)	8	8	0.65	0.78 (0.27-2.23)	8	7	0.79	0.92 (0.51-1.68)
CTT	7	2	0.76	1.21 (0.36-4.10)	14	16	0.27	1.82 (0.63-5.22)	3	5	0.88	0.89 (0.20-3.98)	7	7	0.50	1.26 (0.64-2.49)
Total	100	100	-	-	100	100	-	-	100	100	-	-	100	100	-	-
Diplotype																
CGC-TTT	14	11	0.47	1.61 (0.45-5.80)	19	14	0.87	1.15 (0.20-6.81)	20	13	0.56	1.54 (0.36-6.50)	16	14	0.25	1.60 (0.71-3.59)
TGC-TTT	16	12	0.57	1.54 (0.35-6.85)	6	5	0.52	1.69 (0.34-8.36)	11	5	0.32	2.16 (0.47-9.94)	15	10	0.09	2.09 (0.89-4.89)
CGC-TTC	7	3	0.20	0.30 (0.05-1.91)	8	11	0.07	4.51 (0.87-23.48)	6	8	0.52	1.79 (0.31-10.42)	10	8	0.37	1.53 (0.60-3.91)
CGC-TGC	4	7	0.60	0.61 (0.09-4.01)	-	4	1.00	-	8	10	0.76	1.30 (0.25-6.83)	7	6	0.32	1.72 (0.60-4.92)
TTT-TTT	1	9	0.63	0.67 (0.13-3.37)	5	12	1.00	-	6	5	0.68	0.70 (0.13-3.78)	6	5	0.92	1.05 (0.38-2.94)
TGC-TTC	4	3	0.83	1.12 (0.40-3.10)	-	7	0.52	1.43 (0.48-4.26)	8	7	0.22	1.94 (0.67-5.63)	6	6	0.16	1.52 (0.84-2.75)
Others[b]	54	55	-	-	62	47	-	-	41	52	-	-	40	52	-	-
Total	100	100	-	-	100	100	-	-	100	100	-	-	100	100	-	-

NR: VPA-resistance; R: VPA-responsive; OR: odds ratio; CI: confidence interval; [a]OR estimated odds ratio by binary logistic regression analysis adjusted for age at study, age at epilepsy onset, epilepsy syndrome, ethnicity, gender, and seizure type; [b]diplotypes with total frequencies below 5% in all patients.

association study between common variants and risk of partial epilepsy was performed in 10,380 participants of European ancestry (3,445 patients and 6,935 controls), the largest sample size reported for an epilepsy study (Kasperavičiūtė *et al*., 2010). Ethnicity is another confounder which may affect the results. A comparison of 3.4 million loci from 270 individuals of African, American, European, Japanese, and Chinese ethnicity has shown obvious differences in patterns of LD, haplotype diversity, and haplotype frequency among populations. This variation has been shaped by evolutionary forces, most likely natural selection in ancestral populations. Such haplotype pattern diversity is greatest in European and East Asian populations (Tishkoff and Verrelli, 2003; Hutchison *et al*., 2004). This factor may affect the population structure in various geographical regions and thus lead to contradictory results. For example, of five Caucasian studies using the same definition of drug resistance and drug responsiveness, *ABCB1* C3435T was shown to be associated with resistance to antiepileptic drugs in four studies; from the United Kingdom, Croatia, and Spain, in agreement with the initial study of Siddiqui *et al*., (2003) from the United Kingdom, in contrast to two other studies from Australia and Scotland (Haerian *et al*., 2010; Sánchez *et al*., 2010). The lack of association in the Indian subgroup is consistent with all three previous studies in India (Haerian *et al*., 2010; Grover *et al*., 2010) and the lack of association in the Chinese subgroup is consistent with one previous study but inconsistent with four studies performed in China (Haerian *et al*., 2010).

Variation in the definition of drug resistance and drug responsiveness in the different studies as well as duration of follow-up may also lead to contradictory results. Of 25 studies, the same definition of treatment outcome was used in 10 (40%); in five Asian and five Caucasian studies. Of the Caucasian studies, 80% of the studies which used the same definition as that of Siddiqui *et al*., (2003) and 20% which used alternative definitions reported an association. In the Asian reports, no association was observed in any study which used the same definition as that of (Siddiqui *et al*., 2003)Siddiqui *et al*., (2003), but 50% of studies which used alternative definitions of drug resistance and drug responsiveness reported associations (Grover *et al*., 2010; Haerian *et al*., 2010; Sánchez *et al*., 2010). When we used the alternative definition of treatment outcome in our study, the results were consistent with 50% of studies from Asia which reported no association.

In this study, we considered VPA monotherapy as an inclusion criterion. VPA is a widely used AED with a broad spectrum of activity. It is effective for the treatment of various epileptic seizures such as absence, myoclonic, and generalised tonic-clonic seizures, as well as partial seizures with or without secondary generalisation. It is also effective for the acute treatment of status epilepticus. In polytherapy, VPA interacts with other AEDs such as carbamazepine, lamotrigine, and phenytoin (Patsalos *et al*., 2002). The majority of previous *ABCB1* polymorphism studies have consisted of patients on polytherapy, however, AEDs including VPA were also considered individually. Despite some studies suggesting that VPA is not a substrate for P-gp (Baltes *et al*., 2007; Rivers *et al*., 2008), VPA was the most commonly used first-line AED in some reports which demonstrated a negative (43.4%; Ufer *et al*., [2009] and 35.8%; Szoeke *et al*., [2009]) and positive (80.2%; Seo *et al*., [2006]) association between polymorphisms and drug efficacy. To further clarify this association, we stringently selected patients who were receiving VPA monotherapy in order to study a more homogenous population. Despite the inclusion of this criterion, our sample size was still much larger than that of previous studies of subjects receiving monotherapy. Consistent with some previous studies, we found no association between polymorphisms and response to VPA overall in patients or in any ethnic subgroup. These findings support the observation that although VPA induces P-gp expression and function (Tang *et al*., 2004; Eyal *et al*., 2006), this may be influenced by other mediators such as the nuclear pregnane X receptor (NR1I2), a regulator of *ABCB1* gene expression (Cerveny *et al*., 2007). Further studies are necessary to identify a possible link between the loci of these mediators and response to VPA.

In the current study, no association was found between the *ABCB1* haplotypes or diplotypes, derived from C1236T, G2677T, and C3435T polymorphisms, and the response to VPA in the total group or in each ethnicity. These results are inconsistent with three studies previously performed in Austria (Zimprich *et al*., 2004), China (Hung *et al*., 2005), and Japan (Seo *et al*., 2006) *(table 5)*. In addition, in our study, the 1236/2677/3435 TTT haplotype was more common than the other haplotypes in the total group and in each ethnic subgroup, consistent with four previous reports; one from Japan (Seo *et al*., 2006) and three from India (Lakhan *et al*., 2009; Vahab *et al*., 2009; Grover *et al*., 2010). Some studies have also reported a high frequency of the CGC haplotype and diplotype in Caucasian (Zimprich *et al*., 2004) and Korean (Kim *et al*., 2006) populations, and a high frequency of CGC, TGC, and TTT haplotypes in a Chinese population (Hung *et al*., 2005). Some of these studies have reported a strong LD between C1236T/G2677TA/C3435T loci (Hung *et al*., 2005; Seo *et al*., 2006) and C1236T/G2677TA (Grover *et al*., 2010). However, we did not observe any strong LD between C1236T/G2677TA/C3435T, C1236T/G2677T (exon 12/26), G2677T/C3435T, and C1236T/C3435T polymorphisms in the total group or in each ethnic group. Among the

Table 5. Characteristics of the analyzed studies of *ABCB1* C1236/G2677T/C3435T haplotypes in drug-resistant (n=1,133) and drug-responsive (n=1,412) epilepsy patients.

Haplotype	Previous studies												This study									
	(Zimprich *et al.*, 2004)Zimprich *et al.*, (2004)		(Hung *et al.*, 2005)Hung *et al.*, (2005)		(Kim *et al.*, 2006)Kim *et al.*, (2006)		(Seo *et al.*, 2006)Seo *et al.*, (2006)		(Lakhan *et al.*, 2009)Lakhan *et al.*, (2009)		(Vahab *et al.*, 2009)Vahab *et al.*, (2009)		(Grover *et al.*, 2010)Grover *et al.*, (2010)		Chinese		Indian		Malay		Total Malaysian	
	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)
Association	+		+		-		+		-		-		-		-		-		-		-	
CGC	46.9	45.6	35.2	4	19.91	17.24	15.1	19.6	22.9	19.3	8.2	13.6	29.5	22.2	20	18	11	16	15	24	18	19
TTT	39.5	39.5	30.6	6.9	17.12	15.74	42.1	30.4	44.7	40.9	25.6	25.2	51.6	52.6	14	28	25	23	20	21	18	25
CGT	8.3	9.9	1.4	28.5	-	-	4	3	4.3	4.8	7.9	9.9	3.2	8.3	11	10	5	8	13	3	10	6
TTC	2.1	2.2	5.1	34.1	10.40	9.13	6.7	10.7	5.9	7.4	2.4	6.2	5.8	7.5	18	10	16	12	20	9	17	11
CTT	1.2	1.5	0.9	1.6	0.79	1.44	0.8	1.8	7.4	5.4	26.9	22.8	4.2	5.3	7	2	14	16	3	5	7	7
Others	1.9	1.3	26.8	24.9	51.79	56.45	31.4	34.5	14.9	22.2	29	22.3	5.8	5.8	30	33	30	26	28	38	30	32
D´ exon 12/21	0.95		0.29*		-		-		0.59		-		95	92	35		14		35		27	
D´ exon 21/26	0.89		0.62*		-		-		0.54		-		55	73	22		35		18		26	
D´ exon 12/26	0.84		0.23*		-		-		0.63		-		53	66	20		8		24		16	

NR: drug-resistance: R, drug-responsive; *r^2 value.

diplotypes, CGC/TTT and TGC/TTT were more common in the total group, consistent with an Indian study of the CGC/TTT diplotype (Grover *et al*., 2010). However, the TGC/TTT diplotype was more common than other diplotypes in Chinese patients, consistent with a Chinese report (Hung *et al*., 2005). Finally, the frequency of the CGC/CGC diplotype was less than 5%, which is inconsistent with two previous studies (Zimprich *et al*., 2004; Seo *et al*., 2006). A study of Japanese epilepsy patients demonstrated that this diplotype was associated with a good response to AEDs (Seo *et al*., 2006), but another study in Caucasians reported the frequency of this diplotype to be six times greater in drug-resistant patients compared to drug-responsive patients (Zimprich *et al*., 2004).

In conclusion, our results have failed to identify any association between *ABCB1* polymorphisms or related haplotypes and response to VPA, suggesting that these polymorphisms may not contribute to the response to VPA in patients with epilepsy. □

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