



Lack of association of ABCB1 haplotypes on five loci with response to treatment in epilepsy

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ABSTRACT

Approximately one third of newly treated epilepsy patients do not respond to antiepileptic drugs (AEDs). Overexpression of P-glycoprotein (P-gp) efflux transporter has been proposed to have a critical role in causing resistance to AEDs. P-gp is a product of the ATP-binding cassette subfamily B member 1 (ABCB1) gene. The purpose of this study was to investigate a possible link between ABCB1 rs3789243 C > T, C1236T, G2677T/A, rs6949448 C > T, and C3435T haplotypes with response to carbamazepine (CBZ) or sodium valproate (VPA) monotherapy in Malaysian epilepsy patients. No ABCB1 haplotype association was found with response to either CBZ or VPA monotherapy in the Chinese, Indian, and Malay patients. C3435 allele carriers of the Indian males with cryptogenic epilepsy were more prone to resistance to either CBZ or VPA than carriers of T allele. Moreover, rs3789243T allele carriers of Malay females with symptomatic epilepsy were more resistant to either CBZ or VPA than C allele carriers. Our findings suggest that the ABCB1 rs3789243 C > T, C1236T, G2677T/A, rs6949448 C > T, and C3435T haplotypes do not contribute to response to AED treatment in epilepsy.

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1. Introduction

Approximately, one third of patients with epilepsy do not respond to antiepileptic drugs (AEDs) and it has been hypothesized that this is due to over-expression of the multidrug-transporter. Overexpression of P-glycoprotein (P-gp) has been reported to play an important role in refractoriness to AEDs.^{1,2} This protein functions as a transmembrane efflux pump, by moving drugs from the intracellular to the extracellular domain. It is a product of the ATP-binding cassette subfamily B member 1 (ABCB1) gene.³ It has been hypothesized that genetic variation may be involved in resistance to treatment. The most common polymorphisms of ABCB1 gene are C1236T (rs1128503), G2677T/A (rs2032582), and C3435T (rs1045642), and from these three, the C3435T polymorphism has received the most attention as a critical variant in resistance to AEDs. Several pharmacogenomics studies have demonstrated an association between ABCB1 C3435T polymorphism and resistance to AEDs treatment.⁴ However, our meta-analysis involving 6864 subjects comprising Asian and Caucasian patients, and controls, did not verify this hypothesis.⁵

Some studies have indicated that the C3435T locus may have an effect on response to treatment through linkage disequilibrium (LD) with other ABCB1 variants in a haplotype block. The authors assessed the association of ABCB1 haplotypes derived from C1236T, G2677T/A, and C3435T loci in response to AEDs in different epilepsy populations. However, our haplotype meta-analysis did not confirm these results.⁶ Two studies examined the possible linkage between C3435T locus and some other loci of ABCB1 gene such as rs6949448 C > T and rs3789243 C > T, and one of these studies reported positive results.^{7,8} Here, we addressed the question of whether ABCB1 rs3789243 C > T, C1236T, G2677T/A, rs6949448 C > T, and C3435T haplotypes are associated with response to treatment in the Malaysian Chinese, Indian, and Malay populations with epilepsy. We also meta-analyzed the pooled studies including our data.

2. Methodology

2.1. Subjects

The present retrospective study is part of an ongoing multi-centre cooperation between the University of Malaya Medical Centre and the Universiti Kebangsaan Malaysia Medical Centre. The study protocol was approved by the ethics committees of the

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Table 1

Genotypes and allele frequencies of ABCB1 polymorphisms of drug-resistant ($n=323$) and drug-responsive ($n=362$) epilepsy patients in three ethnic subgroups of the Malaysian population.

Allele/genotype	Chinese ($n=277$)		Indian ($n=160$)		Malay ($n=248$)		Total (685)	
	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)	NR (%)	R (%)
rs3789243 C > T								
C	163 (62)	179 (61)	94 (70)	120 (64)	142 (57)	157 (64)	399 (62)	456 (63)
T	99 (38)	113 (39)	40 (30)	66 (36)	108 (43)	89 (36)	247 (38)	268 (37)
CC	53 (40)	57 (39)	34 (51)	38 (41)	40 (32)	51 (41)	127 (39)	146 (40)
CT	57 (44)	65 (45)	26 (39)	44 (47)	62 (50)	55 (45)	145 (45)	164 (45)
TT	21 (16)	24 (16)	7 (10)	11 (12)	23 (18)	17 (14)	51 (16)	52 (14)
1236 C > T								
C	113 (43)	122 (42)	59 (44)	84 (45)	111 (44)	95 (39)	283 (44)	301 (42)
T	149 (57)	170 (58)	75 (56)	102 (55)	139 (56)	151 (61)	363 (56)	423 (58)
CC	27 (21)	28 (19)	14 (21)	20 (22)	24 (18)	22 (18)	65 (20)	70 (19)
CT	59 (45)	66 (45)	31 (46)	44 (47)	63 (46)	51 (41)	153 (47)	161 (45)
TT	45 (34)	52 (36)	22 (33)	29 (31)	38 (36)	50 (41)	105 (33)	131 (36)
2677 G > T								
G	144 (55)	144 (49)	49 (37)	84 (45)	138 (55)	125 (51)	331 (51)	353 (49)
T	118 (45)	148 (51)	85 (63)	102 (55)	112 (45)	121 (49)	315 (49)	371 (51)
GG	36 (27)	35 (24)	8 (12)	18 (19)	39 (31)	25 (20)	83 (26)	78 (22)
GT	72 (55)	74 (51)	33 (49)	48 (52)	60 (48)	75 (61)	165 (51)	197 (54)
TT	23 (18)	37 (25)	26 (39)	27 (29)	26 (21)	23 (19)	75 (23)	87 (24)
rs6949448 C > T								
C	190 (68)	166 (57)	60 (45)	97 (51)	158 (63)	146 (59)	389 (60)	409 (56)
T	91 (32)	126 (43)	74 (55)	89 (49)	92 (37)	100 (41)	257 (40)	315 (44)
CC	52 (40)	50 (34)	10 (15)	27 (29)	54 (43)	42 (34)	116 (36)	119 (33)
CT	67 (51)	66 (45)	40 (60)	43 (46)	50 (40)	62 (50)	157 (49)	171 (47)
TT	12 (9)	30 (21)	17 (25)	23 (25)	21 (17)	19 (15)	50 (15)	72 (20)
3435 C > T								
C	147 (56)	167 (57)	78 (58)	89 (48)	151 (60)	144 (58)	376 (58)	400 (55)
T	115 (44)	125 (43)	56 (42)	97 (52)	99 (40)	102 (42)	270 (42)	324 (45)
CC	39 (30)	49 (34)	23 (34)	17 (18)	47 (38)	44 (36)	109 (34)	110 (30)
CT	69 (53)	69 (47)	32 (48)	55 (59)	57 (46)	56 (45)	158 (49)	180 (50)
TT	23 (18)	28 (19)	12 (18)	21 (23)	21 (17)	23 (19)	56 (17)	72 (20)

Abbreviations: NR, drug-resistant; R, drug-responsive.

two centres. In the previous study we reported the inclusion and exclusion criteria and definition of drug-resistance and drug-responsiveness for 685 recruited patients from the epilepsy clinics.⁹ The previous data of ABCB1 C1236T, G2677T/A, and C3435T polymorphisms was used for the current haplotype analysis and adding on to the data from the rs3789243 C > T and rs6949448 C > T loci. Real-time PCR analysis of rs3789243 C > T and rs6949448 C > T loci was carried out using the Applied Biosystem Assay on Demand reagents (Applied Biosystem, CA, USA).

2.2. Statistical methods

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. Association of alleles and genotypes with response to CBZ or VPA monotherapy was calculated with binary logistic regression. The odds ratios (ORs) with 95% confidence intervals (CIs), adjusted for confounders (ethnicity, drug type, gender, age at recruitment, onset age of epilepsy, seizure type, and etiology of epilepsy).

The alternative genetic models for the ABCB1 C1236T, G2677T/A, and C3435T polymorphisms were previously reported, but the present meta-analysis includes rs3789243 C > T and rs6949448 C > T loci for both alleles (C vs. T) and genotypes, for codominant (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. Haplotype and LD analysis for the five ABCB1 polymorphisms was performed with the Haploview 4.2 program and corrected for multiple testing using 100,000 permutations for individual locus 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 (ver. 15.0; SPSS, Chicago, IL, USA).

2.3. Meta-analysis

Published studies until November 2010 that determined the distribution of the ABCB1 rs3789243 C > T and rs6949448 C > T genotype in epilepsy patients and healthy people were included in this meta-analysis with no language limitation. Pooled ORs with 95% CIs and p values were calculated for the ABCB1 rs3789243 C > T and rs6949448 C > T polymorphisms under alternative genetic models. To measure the strength of genetic association, we used the I^2 test to assess the proportion of statistical heterogeneity and the Q -statistic test with $p \leq 0.10$ to define a significant degree of heterogeneity. Fixed effects were calculated as the inverse variance weighted average of the log OR if there was no heterogeneity and random effects were calculated when there was substantial heterogeneity. Statistical analyses were performed using validated Meta-analysis Made Easy (MIX) version 1.7.¹⁰

3. Results

The patients' demographic features were previously reported.⁹ Table 1 lists the allele and genotype percentages of the ABCB1 frequencies of rs3789243 C > T, 1236 C > T, 2677G > T, rs6949448 C > T, and 3435 C > T polymorphisms from 685 epilepsy patients. Crs3789243 allele was more frequent than the T-allele in the Chinese (62%), Indians (67%), and Malays (61%). However, C1236 allele was less common than T-allele in the Chinese (43%), Indians (45%), and Malays (42%). Both G2677 and Crs6949448 alleles were more frequent in the Chinese (52 and 63%, respectively) and Malay (53 and 61%, respectively) patients than minor alleles compared with Indians (48 and 41%, respectively). Finally, C3435 allele was more common than T allele in the Chinese (57%), Indian (53%), and Malay (59%) patients.

Table 2
Genotypes and allele frequencies of ABCB1 polymorphisms in drug-resistant ($n = 323$) and drug-responsive ($n = 362$) epilepsy patients in the pooled population studied and in the three ethnic subgroups of the Malaysian population under alternative genetic models.

Genotype	Ethnicity															
	Chinese ($n = 277$)				Indian ($n = 160$)				Malay ($n = 248$)				Total (685)			
	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 vs. T	262	292	0.56	0.89 (0.61–1.30)	134	186	0.86	1.04 (0.64–1.70)	250	246	0.12	0.72 (0.48–1.09)	646	724	0.29	0.88 (0.69–1.11)
C/C vs. T/T	72	80	0.63	0.82 (0.37–1.83)	36	49	0.89	1.07 (0.41–2.81)	62	72	0.19	0.57 (0.25–1.32)	170	201	0.30	0.77 (0.48–1.23)
C/T vs. T/T	104	118	0.76	0.91 (0.50–1.66)	53	73	0.97	0.99 (0.44–2.20)	101	101	0.09	0.57 (0.29–1.09)	258	292	0.30	0.82 (0.56–1.19)
C/C + C/T vs. T/T	131	146	0.61	0.87 (0.50–1.51)	67	93	0.95	1.02 (0.49–2.13)	125	123	0.08	0.59 (0.32–1.07)	323	362	0.25	0.81 (0.57–1.16)
C/C vs. C/T + T/T	131	146	0.66	0.86 (0.44–1.69)	67	93	0.82	1.11 (0.47–2.59)	125	123	0.54	0.79 (0.37–1.68)	323	362	0.61	0.90 (0.59–1.37)
rs6949448 C > T																
C vs. T	262	292	0.09	0.72 (0.49–1.05)	134	186	0.25	1.33 (0.82–2.18)	250	246	0.59	0.89 (0.59–1.34)	646	724	0.47	0.91 (0.72–1.16)
C/C vs. T/T	64	80	0.11	2.12 (0.85–5.33)	27	50	0.34	1.67 (0.58–4.81)	75	61	0.98	1.01 (0.42–2.45)	166	191	0.72	1.10 (0.66–1.83)
C/T vs. T/T	79	96	0.08	0.47 (0.20–1.10)	57	66	0.48	1.36 (0.58–3.15)	71	81	0.13	1.89 (0.83–4.31)	207	243	0.66	0.90 (0.57–1.43)
C/C + C/T vs. T/T	131	146	0.06	0.46 (0.21–1.02)	67	93	0.96	0.98 (0.45–2.14)	125	123	0.34	1.45 (0.67–3.15)	323	362	0.62	0.89 (0.58–1.38)
C/C vs. C/T + T/T	131	146	0.30	1.34 (0.77–2.31)	67	93	0.05	2.49 (1.01–6.12)	125	123	0.12	0.62 (0.34–1.14)	323	362	0.50	1.13 (0.79–1.62)
G2677T																
G vs. T	262	292	0.11	0.73 (0.50–1.07)	134	186	0.07	1.60 (0.97–2.65)	250	246	0.51	0.87 (0.58–1.31)	646	724	0.79	0.97 (0.76–1.23)
G/G vs. T/T	59	72	0.41	0.71 (0.32–1.61)	34	45	0.10	2.53 (0.82–7.78)	65	48	0.38	0.66 (0.26–1.68)	158	165	0.85	0.95 (0.58–1.57)
G/T vs. T/T	95	111	0.09	0.55 (0.27–1.10)	59	75	0.16	1.82 (0.79–4.19)	86	98	0.29	1.50 (0.71–3.21)	240	284	0.55	1.13 (0.75–1.72)
G/G vs. (G/T + T/T)	131	146	0.06	0.54 (0.28–1.04)	67	93	0.08	1.98 (0.91–4.30)	125	123	0.58	1.23 (0.59–2.55)	323	362	0.71	1.08 (0.73–1.59)
(G/G + G/T) vs. T/T	131	146	0.39	0.77 (0.42–1.40)	67	93	0.18	1.95 (0.73–5.19)	125	123	0.11	0.57 (0.28–1.13)	323	362	0.41	0.85 (0.57–1.26)
C3435T																
C vs. T	262	292	0.33	1.02 (0.83–1.75)	134	186	0.13	0.69 (0.42–1.12)	250	246	0.74	0.93 (0.62–1.40)	646	724	0.71	0.96 (0.75–1.21)
C/C vs. T/T	62	77	0.36	0.69 (0.31–1.53)	35	38	0.08	0.37 (0.12–1.14)	68	67	0.72	0.86 (0.37–2.01)	165	182	0.62	1.13 (0.69–1.85)
C/T vs. T/T	92	97	0.93	0.97 (0.46–2.05)	44	76	0.49	1.40 (0.54–3.60)	78	79	0.59	0.80 (0.36–1.77)	214	252	0.97	0.99 (0.62–1.58)
C/C + C/T vs. T/T	131	146	0.64	0.85 (0.42–1.70)	67	93	0.85	0.92 (0.38–2.20)	125	123	0.60	0.82 (0.39–1.72)	323	362	0.93	1.02 (0.66–1.57)
C/C vs. C/T + T/T	131	146	0.29	0.74 (0.42–1.29)	67	93	0.02*	0.38 (0.16–0.86)	125	123	0.95	1.02 (0.55–1.87)	323	362	0.63	1.09 (0.76–1.57)
rs3789243 C > T																
C vs. T	262	292	0.66	0.92 (0.63–1.34)	134	186	0.36	0.78 (0.46–1.33)	250	246	0.10	1.42 (0.93–2.15)	646	724	1.00	1.00 (0.78–1.28)
C/C vs. T/T	74	81	0.80	0.91 (0.42–1.96)	41	49	0.69	1.29 (0.37–4.50)	63	68	0.16	0.53 (0.22–1.29)	178	198	0.88	1.04 (0.63–1.72)
C/T vs. T/T	78	89	0.91	0.96 (0.43–2.11)	33	55	0.84	0.86 (0.21–3.47)	85	72	0.55	0.77 (0.32–1.82)	196	216	0.82	0.94 (0.57–1.57)
C/C + C/T vs. T/T	131	146	0.93	0.97 (0.47–1.98)	67	93	0.68	0.78 (0.24–2.51)	125	123	0.30	0.66 (0.30–1.46)	323	362	0.86	1.04 (0.65–1.66)
C/C vs. C/T + T/T	131	146	0.57	0.86 (0.50–1.47)	67	93	0.33	1.42 (0.70–2.91)	125	123	0.11	0.61 (0.33–1.12)	323	362	0.91	0.98 (0.70–1.38)

Abbreviations: NR, drug-resistant; R, drug-responsive.

^a OR estimated odds ratio by binary logistic regression analysis adjusted for age at study, onset age at epilepsy, etiology, ethnicity, gender, drug type, and seizure type.

* After correction of multiple comparisons with Bonferroni's correction for the Indians, this significant association was lost ($p = 0.02 > 0.05/5$).

The ORs adjusted for ethnicity, gender, age at recruitment, age at onset of epilepsy, seizure type, drug type, and epilepsy syndromes. No allelic association of ABCB1 variants was observed with response to treatment in each ethnicity (Table 2). Stratified analysis by ethnicity, gender, and etiology demonstrated a significant association between C3435 allele in the Indian males with cryptogenic epilepsy and response to treatment (adjusted OR 0.17, CI 95% 0.037–0.80, $p = 0.02$). C3435 allele was more common in the drug-resistant patients (75%) than drug-responsive subjects (46%). Similarly, T-allele of rs3789243 locus was associated with response to treatment in the Malay females with symptomatic epilepsy (adjusted OR 0.25, 0.078–0.83, $p = 0.02$). T-allele was more frequent in the drug-resistant patients (50%) compared with drug-responsive subjects (19%). We also divided the etiology into idiopathic generalized in 150 patients (23.2%) and localization-related epilepsy in 497 subjects (all non-idiopathic generalized epilepsy). Stratified analysis by ethnicity and etiology was performed for all polymorphisms. No significant allelic association of every locus was observed with response to CBZ or VPA in each ethnicity.

The genotype distribution of the ABCB1 polymorphisms in both drug-resistant and drug-responsive patients in the total group or in each ethnic subgroup was consistent with HWE. No significant association of ABCB1 polymorphisms was observed with response to treatment in the Chinese and Malays under alternative genetic models. However, the C3435T variant in the Indian subgroup showed a significant association with response to treatment (adjusted OR 0.38, 95% CI 0.16–0.86, $p = 0.02$) as well as for rs6949448 C > T at the marginal level (adjusted OR 2.49 95% CI 1.01–6.12, $p = 0.05$) under autosomal recessive model. After correction of multiple comparisons with Bonferroni's method, we did not find any significant association between ABCB1 polymorphisms and response to treatment (0.05/5).

Table 3 lists the haplotypes of ABCB1 polymorphisms in the drug-resistant and drug-responsive patients, encountered with frequencies of above 3%. Overall, the CCGCC (19%) haplotype was more frequent than other ABCB1 gene haplotypes. The ratio of frequency of this haplotype to the mutant haplotype frequency was 1.41. There was no significant association between ABCB1 haplotypes and response to treatment in each ethnicity. Moreover, no significant association of rs3789243–1236–2677 (T–C–G), rs3789243–2677–3435 (T–G–C), rs3789243–1236 (C–T), rs3789243–2677 (T–G and T–G/A), and rs3789243–3435 (T–C) haplotypes was observed with response to CBZ or VPA in each ethnicity (Table 4).

Among diplotypes, CCGCC–TTTTT was more frequent in the Indians and Malays but not in the Chinese. CCGCC–TTTTT frequency in the Chinese patients was higher than other diplotypes. No significant association was observed between ABCB1 diplotypes and response to treatment in each ethnicity. The pairwise LD coefficients D' between ABCB1 polymorphisms in the Chinese, Indian, and Malay drug-resistant and drug-responsive patients have been shown in Fig. 1. There was diversity of LD pattern between ethnicities as well as drug-resistant and drug-responsive patients. A strong LD was observed between C3435T/rs5949448 C > T in the Indian drug-resistant patients. Mostly, a low LD was seen between G2677T–C1236T and C1236T/rs3789243 C > T polymorphisms but a moderate LD between rs5949448 C > T/G2677T in the Chinese and Malay drug-resistant and drug-responsive patients.

Table 5 lists the results of meta-analysis of allele and genotype of the ABCB1 frequencies of rs3789243 C > T and rs6949448 C > T polymorphisms for epilepsy patients. There were 2 reports of the rs3789243 C > T locus in the Asians and Caucasians and one report from rs5949448 locus in the Asian epilepsy population. Meta-analysis of the pooled data from previous reports and from this

Table 3 Haplotype frequency of ABCB1 polymorphisms within drug-resistant ($n = 323$) and drug-responsive ($n = 362$) epilepsy patients in the pooled population studied and in the three ethnic subgroups of the Malaysian population.

Haplotypes	Ethnicity															
	Chinese ($n = 277$)				Indian ($n = 160$)				Malay ($n = 248$)				Total (685)			
	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%)
CGCC	19	18	–	Referent	16	16	–	Referent	22	18	–	Referent	20	18	–	Referent
TTTCT	13	14	0.67	1.15 (0.59–2.25)	17	14	0.99	1.00 (0.42–2.34)	10	13	0.46	0.76 (0.37–1.58)	13	14	0.95	1.01 (0.67–1.54)
TTTTT	11	14	0.26	0.68 (0.34–1.34)	12	16	0.57	0.77 (0.32–1.85)	12	14	0.61	0.84 (0.42–1.68)	12	15	0.20	0.76 (0.50–1.15)
TTTTT	9	10	0.36	0.72 (0.35–1.47)	12	7	0.21	1.89 (0.69–5.15)	12	9	0.62	1.21 (0.57–2.54)	11	9	0.70	1.09 (0.70–1.72)
TGCGC	6	7	0.50	0.75 (0.32–1.74)	5	5	0.85	0.89 (0.26–3.01)	4	9	0.09	0.44 (0.17–1.13)	5	7	0.12	0.65 (0.38–1.12)
CCGTC	3	3	0.43	1.66 (0.47–5.79)	4	4	0.53	0.65 (0.17–2.46)	6	3	0.20	2.16 (0.66–7.01)	4	3	0.43	1.33 (0.66–2.66)
CCTTC	3	5	0.17	0.52 (0.20–1.33)	7	4	0.24	2.17 (0.60–7.88)	3	4	0.88	0.91 (0.26–3.11)	4	5	0.73	0.89 (0.48–1.67)
CCGCT	4	3	0.22	2.00 (0.66–6.10)	1	3	0.25	0.27 (0.03–2.60)	2	2	0.62	1.50 (0.30–7.56)	3	2	0.42	1.39 (0.63–3.06)
CTTTC	2	4	0.25	0.47 (0.13–1.68)	4	2	0.42	1.80 (0.43–7.60)	2	4	0.19	0.44 (0.13–1.50)	2	3	0.37	0.72 (0.35–1.48)
TTGTT	3	3	0.99	0.99 (0.34–2.93)	1	4	0.13	0.18 (0.02–1.64)	3	2	0.66	1.31 (0.40–4.29)	3	3	0.61	0.83 (0.41–1.69)
Others ^b	27	19	0.46	1.24 (0.70–2.17)	21	25	0.84	0.93 (0.43–2.01)	24	22	0.79	0.92 (0.50–1.69)	23	21	0.76	1.06 (0.74–1.52)
Total	100	100	–	–	100	100	–	–	100	100	–	–	100	100	–	–

Abbreviations: NR, drug-resistant; R, drug-responsive.
^a Haplotypes with total frequencies over total patients below 3%.

Table 4

Haplotype frequency of ABCB1 polymorphisms within drug-resistant ($n = 323$) and drug-responsive ($n = 362$) epilepsy patients in the pooled population studied and in the three ethnic subgroups.

Haplotypes	Haplotypes	Kwan et al.			This study								
		NR	R	p^*	Chinese			Indian			Malay		
					NR	R	p^*	NR	R	p^*	NR	R	p^*
Rs3789243–1236–2677	T–C–G	7.9	13.4	0.02	10.9	9.4	NS	14.4	17.2	NS	16.7	10.2	NS
Rs3789243–2677–3435	T–G–C	27	35.5	0.02	17.4	14.5	NS	18.9	16.2	NS	21.2	16	NS
Rs3789243–1236	C–T	42.3	33.8	0.01	33.4	34.4	NS	45.1	42.6	NS	32.2	41.1	NS
Rs3789243–2677	T–G	27.4	37.4	<0.01	25.7	21.2	NS	20.8	24.2	NS	28.6	21.5	NS
Rs3789243–2677	T–G/A	29.4	38.5	0.01	26.2	24.3	NS	25.2	27.8	NS	31.1	22.4	NS
Rs3789243–3435	T–C	28.7	36.3	0.04	25.9	25.6	NS	23.2	23.7	NS	32.5	21.1	NS

Abbreviations: NR, drug-resistant; R, drug-responsive; NS, not significant.

* After 100,000 permutations.

Table 5

Allele and genotype meta-analysis of ABCB1 rs3789243 C > T and rs6949448 C > T polymorphisms among previous studies and the present study under alternative genetic models.

Allele/genotype	rs3789243 C > T ($n = 1497$)				rs6949448 C > T ($n = 1132$)					
	NR ($n = 634$) vs. R ($n = 863$)			I^2 (%)	p	NR ($n = 513$) vs. R ($n = 619$)			I^2 (%)	p
	OR	CI	p			OR	CI	p		
G vs. A	1.20	0.93–1.54	0.16	70	<0.01	1.00	0.74–1.36	0.98	67	0.03
GG vs. AA	1.68	0.81–3.52	0.17	83	<0.01	0.90	0.62–1.31	0.58	77	<0.01
GA vs. AA	1.69	0.85–3.36	0.50	82	<0.01	0.99	0.47–2.10	0.98	78	<0.01
GG + GA vs. AA	1.69	0.84–3.41	0.14	84	<0.01	0.99	0.49–2.01	0.97	77	<0.01
GG vs. GA + AA	1.15	0.95–1.39	0.14	33	0.14	1.00	0.66–1.50	0.98	59	0.06

Abbreviations: NR, drug-resistant; R, drug-responsive.

study of rs3789243 C > T ($n = 2114$) and rs5949448 ($n = 1132$) loci showed no significant association between alleles and alternative genotype models with response to treatment. Therefore, these two polymorphisms of ABCB1 do not contribute to response to AEDs. A wide heterogeneity (33–84%) was observed among the studies of drug-resistant patients versus drug-responsive group. Unlike the recessive genotype model for rs3789243 C > T locus, heterogeneity was significant for allele and other genotype patterns.

4. Discussion

To identify whether ABCB1 rs3789243 C > T, C1236T, G2677T/A, rs6949448 C > T, and C3435T haplotypes polymorphisms and their haplotypes contribute to response to treatment, we carried out a study in Malaysian tri-ethnic patients with epilepsy as well as performed a meta-analysis of the pooled data of rs3789243 C > T and rs6949448 C > T loci. The results of this study showed no haplotype association of these ABCB1 loci with response to CBZ or VPA monotherapy. Meta-analysis data also showed no association of rs6949448 C > T and rs3789243 C > T loci with response to AEDs. Stratified analysis showed that C3435 allele carriers among the Indian males with cryptogenic epilepsy were more prone to resistance to either CBZ or VPA than the T allele carriers. Moreover, rs3789243T allele carriers of the Malay females with symptomatic epilepsy were more resistant to CBZ or VPA than C allele carriers.

Stratified analysis by ethnicity and etiology (idiopathic generalized and localization-related epilepsy) showed no association of ABCB1 polymorphisms with response to treatment. Furthermore, we analyzed the ABCB1 rs3789243 C > T gene polymorphism in LD with 1236 C > T, 2677 G > T/A, 2677 G/A > T and 3435 C > T loci in the epilepsy patients. We did not find any association between rs3789243–1236–2677, rs3789243–2677–3435, rs3789243–1236, rs3789243–2677, and rs3789243–3435 haplotypes and response to either CBZ or VPA in the Malaysian Chinese, Indian, and Malays. Our negative finding of both allele and haplotype analysis did not verify the previous report from China.⁸ The plausible explanation for this inconsistency between

the data is polytherapy, sample size, and Chinese subethnicities. Drug interaction or their competition for interaction with common transporters, targets and metabolic mediators may have an effect on the results. For example, VPA interacts with CBZ, lamotrigine, and phenytoin.¹¹ Therefore, we stringently selected 42% of all epilepsy patients who were on monotherapy to have a homogenous population and obtain more reliable data. We focused more on VPA and CBZ monotherapy as an inclusion criterion, since these drugs are not only widely used in epilepsy but also has a broad spectrum of activity.¹² However, inclusion of this criterion in the study caused smaller sample size especially in the case of the Chinese patients (277) compared to a previous study in which the sample size was 464 subjects who received a wide variety of AEDs in a polytherapy regimen among which VPA (25%), CBZ (23%), and PHT (16%) were the most commonly used AEDs. It seems that our finding gives a clearer picture of the kind of association that is being studied. Furthermore we applied adjusted ORs strategy for various confounders including drug type in this study to minimize the effects of these covariates on the association study.

There is a debate in the literature that some AEDs such as CBZ and VPA may not be substrates of P-gp.^{13,14} Our negative findings in the Chinese and Malay patients who were on CBZ and VPA monotherapy are in agreement with the main hypothesis and suggest that these ABCB1 polymorphisms may not significantly contribute to response to CBZ and VPA in epilepsy. However, positive allelic results in the C3435 allele carriers of Indians males with cryptogenic and rs3789243T allele carriers of the Malay females with symptomatic epilepsy are inconsistent with this hypothesis. This finding suggests that although CBZ or VPA may induce P-gp expression and function, this effect may indirectly contribute through other mediators or in connection of these genes with other genes and loci.^{15–17} Moreover, epigenetic factors can also contribute to pharmacotherapy outcomes through histone modification and DNA methylation.¹⁸ Further studies are necessary to find the probable link between these factors with response to CBZ or VPA.



Fig. 1. Illustration of the ABCB1 LD (D') polymorphisms in the Chinese, Indian, and Malay drug-resistant (NR) and drug-responsive (R) patients. Indicating rs1128503 (C1236T), rs2032582 (G2677T), rs1045642 (C3435T).

Treatment outcome may be influenced by a complex multi-variant interaction via LD rather than at a single locus. It therefore follows that analysis of LD between a locus with causal loci and haplotype analysis would provide more useful information rather than testing each polymorphic locus separately.¹⁶ In this study, LD patterns of the ABCB1 loci varied among the ethnicities as well as among drug-resistant and drug-responsive patients. There was a strong LD between C3435T-rs5949448 in the Indian drug-

resistant patients. Evidence has shown that despite of the emergence of Asians from Europeans and Europeans from Africans, frequency of genetic polymorphisms varies between these populations. Since, prevalence of diseases varies among populations, so it is expected that the frequencies of genetic variants that contribute to their causation also vary. Geographic and cultural factors can cause genetic variation in different populations. This could be contributed by natural selection in a specific local

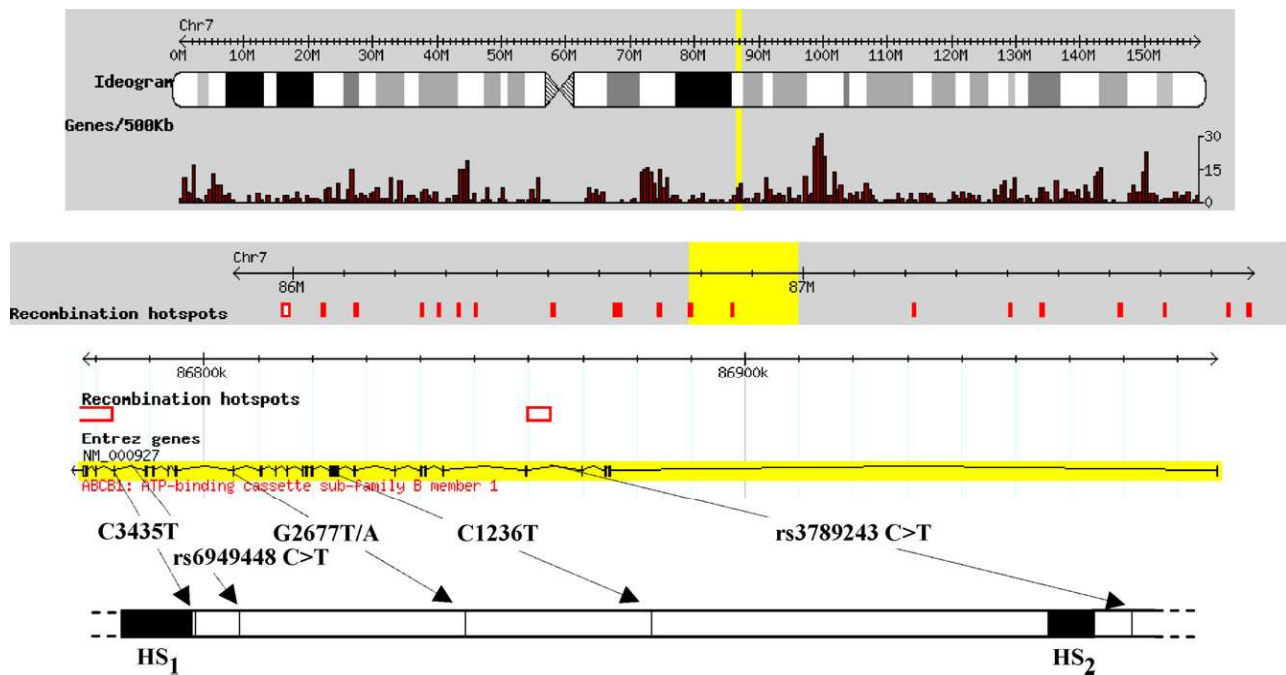


Fig. 2. The 2 recombination hotspots of the ABCB1 gene: HS1 and HS2 (black bars). The ABCB1 C3435T locus is very close to HS1, the rs4949448 C > T and the G2677T/A polymorphisms are farther away, while the C1236T locus lies midway between HS1 and HS2 and the rs3789243 C > T locus is located after HS2.

environment. When two geographically separate populations are subject to distinct environmental or cultural pressure, positive selection may change the allele frequency in one population but not in another.^{19–23} It is plausible therefore, that high variation of these polymorphisms among different epilepsy patient populations is caused by these factors.

Other than drug transporters, drug response in epilepsy may also depend on the multiple genes involved in the metabolism of AEDs. Many AEDs are first activated by phase I drug-metabolizing enzymes such as cytochrome P450 (CYP), then conjugated with ligands such as glucuronidases, and glutathione synthetase by phase II drug-metabolizing enzymes. For example, CYP and UDP-glucuronyltransferase (UGT) are involved in the metabolism of CBZ and VPA. There is an interindividual variation in the enzyme activity of these enzymes that can lead to variability in drug responsiveness. Genetic polymorphisms of drug metabolizing enzymes can influence the optimum dosage requirement for each individual thereby resulting in treatment failure due to inadequate drug levels affecting the metabolic pathways of AEDs thereby affecting drug levels.^{24–27} The frequency of variant alleles for drug metabolizing enzymes differs among ethnic groups. For example, CYP2D6 metabolizes approximately 25% of drugs such as antidepressants. There is an interethnic variability in the CYP2D6 allele, resulting in expression and activity difference of this gene.^{28,29} Taking into account of the functional variants in the relevant genes encoding the metabolizing enzymes may be important for predicting metabolism of the AEDs concerned thereby improving responsiveness to AED treatment.

Finally, another cause of difference of our results with the previous data may be due to the proximity of some variants such as C3435T to the first hotspot and rs3789243 C > T to the second hotspot of ABCB1 gene (http://snp.cshl.org/cgi-perl/gbrowse/hapmap21_B35/, Fig. 2) where crossing-over happens. Accordingly, crossing-over events in this hotspot may not only be involved with high variation of C3435T locus but also that the location of this hotspot may vary among the patient populations of the studies in epilepsy.⁶

In conclusion, this study failed to show any association between the ABCB1 rs3789243 C > T, C1236T, G2677T/A, rs6949448 C > T, and C3435T haplotypes with response to CBZ or VPA monotherapy in the Malaysian tri-ethnic groups. However, C3435 allele carriers of the Indian males with cryptogenic epilepsy were more prone to resistance to CBZ or VPA than T allele. Moreover, rs3789243T allele carriers of the Malay females with symptomatic epilepsy were more resistant to either CBZ or VPA than C allele carriers. Our findings suggest that ABCB1 haplotypes of these loci may not contribute to response to drug treatment in epilepsy.

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