Thai J. Genet. 2011, 4(2) : 139-154

Relationship between the *3435C>t*Tand *2677G>T/A* polymorphysms and *ABCB1* expression in peripheral blood mononuclear cells extracted from Thai volunteers

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ABSTRACT

Limited data of *ABCB1* 3435C>T and 2677G>T/A SNPs and its expression in peripheral mononuclear cells (PBMCs) with proper normalization of endogenous controls used is available in Thai population. The primary objective was to investigate the relationship of these SNPs and its expression in PBMCs extracted from Thai healthy individuals. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was utilized to determine the SNPs in 30 females and 26 males. Total RNA was extracted from PBMCs followed by reverse transcription. *Actin-β*, *β-2M* and *GAPDH* were internal controls. Real-time quantitative PCR was performed to determine the expression levels. We found that TT genotype of 3435C>T SNP is correlated with higher of *ABCB1* expression levels with a significant covariate of gender. 3435C>T genotypes show a strong relationship to 2677G>T genotypes. Most subjects who carry TT genotype at position 3435 will also carry TT genotype at position 2677. No gender difference was noted in the distributions of these SNPs. The results show that genotypes of SNP 3435C>T and gender have, in part, influenced on the expression levels. Therefore, one who carries TT genotype of 3435C>T SNP may have lower response to drugs which target sites are PBMCs.

Keywords: *ABCB1* expression, 3435C>T, 2677G>T/A, peripheral blood mononuclear cells (PBMCs)

INTRODUCTION

The sequence variation of *ABCB1* gene has been identified as a determinant of its protein expression and function (Schinkel and Jonker, 2003; Hoffmann and Kroemer, 2004; Pauli-Magnus and Kroetz, 2004). Over 50 single nucleotide polymorphisms (SNPs) in *ABCB1* have been reported, and many of them are silent polymorphisms which do not produce a change in P-glycoprotein (P-gp) sequence. Among various populations, a synonymous SNP, 3435C>T (amino acid position 1145) and a non-synonymous SNP, 2677G>T/A (amino acid position 893) are the most common polymorphisms and found to be

in linkage disequilibrium (Kim *et al*., 2001; Ozawa *et al*., 2004; Pauli-Magnus and Kroetz, 2004; Yi *et al*., 2004; Kimchi-Sarfaty *et al*., 2007). In addition, these polymorphisms have shown high inter-ethnic variability (Hoffmeyer *et al*., 2000; Ameyaw *et al*., 2001; Cascorbi *et al*., 2001; Ito *et al*., 2001; Schaeffeler *et al*., 2001). Therefore, the functional changes of P-gp resulted from their single nucleotide polymorphisms (SNPs) may, in part, play roles in inter-individual differences in susceptibility to differences in drug responses as well as pharmacokinetics of numerous drugs (Schinkel and Jonker, 2003; Hoffmann and Kroemer., 2004).

Since P-gp acts as a drug efflux pump and subsequently causes decrease in cellular accumulation of various drug, including some anticancer drugs, immunosuppressant and protease inhibitors (Schinkel and Jonker, 2003; Hoffmann and Kroemer, 2004; Pauli-Magnus and Kroetz, 2004), the expression of *ABCB1* in peripheral blood mononuclear cells (PBMCs) is, therefore, very crucial in predicting cellular drug deposition and response as well as progression of diseases, where PBMCs are the targets of these drugs or diseases. However, the results from the studies of SNP 3435C>T and 2677G>T/A were found to be contradictorily associated with different changes in *ABCB1* expression and function in PBMCs among various ethnic populations (Hitzl *et al*., 2001.; Calado *et al*., 2002; Fellay *et al*., 2002; Illmer *et al*., 2002; Oselin *et al*., 2003; Owen *et al*., 2004).

In Caucasians, Fellay and colleagues found an association of the T allele at SNP 3435C>T with lower *ABCB1* expression in PBMC, and better response to anti-HIV1 drugs as determined by increased CD4+ counts. They also reported that the 3435T allele in exon 26 is associated with a lower plasma concentration of nelfinavir (Fellay *et al*., 2002). These findings were confirmed by few studies (Hitzl *et al*., 2001; Owen *et al*., 2004). On the other hand, there have been found no association of this SNP and ABCB1 expression in lymphocytes (Calado *et al*., 2002; Oselin *et al*., 2003). Nevertheless, the correlation of 2677G>T/A SNP and *ABCB1* expression in lymphocytes was not established (Calado *et al*., 2002; Oselin *et al*., 2003).

In addition, 3435C>T SNP, a silent polymorphism that dose not cause an amino acid change, has been found to be in strong linkage disequilibrium with 2677G>T/A SNP (Kim *et al*., 2001; Furuno *et al*., 2002; Goto *et al*., 2002; Illmer *et al*., 2002; Gaikovitch *et al*., 2003). Notably, there are limited data of *ABCB1* polymorphism distribution and its expression in PBMCs in Thai population. Therefore, the objectives of the present study were 1) to observe the prevalence of these two SNPs in healthy Thai individuals comparing to other populations 2) to determine the correlation between the two SNPS, 3435C>T and 2677G>T/A, and the expression of *ABCB1* gene in PBMCs. The obtained information will be useful for further pharmacogenomic education and research in Thailand, as well as

for the prediction of the association between individuals' genetic variability and their response to specific drugs.

MATERIALS AND METHODS

Studied population

The medical history and blood test of the sixty apparently healthy Thai individuals (30 males and 30 females), aged between 18 and 25 years old, were screened. Subjects meeting any of the following exclusion criteria were not eligible for the study enrollment: 1) administrating of any medications withi4 weeks before screening or plan to use it during the study; 2) having an acute or chronic infection; 3) having history of hypertension, diabetes, renal failure, vascular diseases, stroke or cardiomyopathy; 4) taking any chronic medications or any supplements; 5 having history of drug, alcohol or nicotine abuse. Fifty six subjects (26 males and 30 females), whose blood tests and vital signs were within normal range, were then enrolled into the study, while the four male subjects failed the screening due to their abnormal laboratory tests. This study was approved by the Institutional Review Boards of Naresuan University, Phitsanulok, Thailand. Individual written informed consent was obtained from all subjects.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis

Venous blood samples (9 ml) from 58 individuals, mentioned above, were collected, and genomic DNA was extracted using QIAamp DNA Mini Blood kits (Qiagen®, Canada). The SNPs 3435C>T (rs1045642) and 2677G>T/A (rs2032582) were genotyped by standard PCR-RFLP method previously described (Cascorbi *et al*., 2001) with little modification.

DNA fragments generated after completion of restriction enzyme digestion were separated by 3% agarose gel electrophoresis. Some random specimens were sent for DNA sequencing at Bio-Design Co., Ltd., Thailand for the confirmation of PCR-RFLP results.

Analysis of *ABCB1* expression in PBMCs

Total RNA was isolated from 800 µl of PBMCs using QIAamp® RNA Blood Mini kits (QIAGEN, Canada). All RNA samples were eluted in a final volume of 50 µl RNase-Free water. Quantification of total RNA concentrations and the purity were determined using NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop® Technologies, USA. Isolated RNA samples were stored at -70^{o}C until analysis. Reverse transcription was performed from 100 ng of total RNA using cDNA synthesis kits (Stratagene®, Germany). First strand cDNA was synthesized in a total volume of 50 µl reaction containing 10 µl of 2X cDNA synthesis master mix, 3 µl of oligo (dT) primer, and 1 µl of AffinityScript RT/RNase block enzyme mixture.

The reaction was, then, incubated at 25 ºC for 5 minutes, 42 ºC for 45 and 95 ºC for 5 minutes. Finally, the completed reactions were stored at -20 ºC until analysis. Primers used in the real-time QPCR are shown in Table 1.

Table 1 Primers used in the real-time QPCR

Gene	Accession Number	Sequence (5'-3')	Amplicon length (bp)
Act-β	NM001101	Forward CTGGAACGGTGAAGGTGACA	140
		Reverse AAGGGACTTCCTGTAACAATGCA	
GAPDH	NM002046	Forward TGCACCACCAACTGCTTAGC	87
		Reverse GGCATGGACTGTGGTCATGAG	
β2M	NM004048	Forward CTCCGTGGCCTTAGCTGTG	69
		Reverse TTTGGAGTACGCTGGATAGCCT	
ABCB1	NM000927.3	Commercial product form Supper array®	105

Real-time quantitative polymerase chain reaction (real-time QPCR) of *ABCB1* expression in PBMCs

Real-time QPCR reactions were carried out with Rotor-Gene 6000 (Corbette Life Science, Germany). The expressions of each target gene including *ABCB1*, *GAPDH*, *actin-β* and *β2-M* in PBMCs were determined using a total of 10 µl reaction containing 5 µl of 2X Brilliant II SYBR Green QPCR master mix (Stratagene®, Canada), 0.4 µl of *ABCB1* specific primers, or 0.2 µl of each forward and reverse primers for each housekeeping gene and 1 µl of the cDNA template reaction and 3.6 µl of DNase free water. The PCR reactions were initiated at 95 ºC for 10 minutes, followed by 40 cycles of 95 ºC for 30 seconds, 60 ºC for 60 seconds and 72 ºC for 30 seconds. The PCR cycles were followed by a dissociation curve analysis at 65-95 ºC. Each PCR reaction was repeated in triplicate. PCR products were determined by 3% agarose gel electrophoresis.

Analysis of the distribution of *ABCB1* SNPs 3435C>T and 2677G>T/A

The deviation of all SNPs from Hardy-Weinberg equilibrium, using Chi-squares test, was performed. Allele or genotype frequency differences between all populations were determined using Chi-square test. A pairwise comparison of allele frequencies between ethnic groups was conducted.

Analysis of *ABCB1* expression in PBMCs

The data was generated Threshold values (Ct) were automatically calculated for all samples using Rotor-Gene 6000 Series Software 1.7 (Cybeles®, Germany). Relative gene expression method was utilized to

compare the changes in RNA transcription of *ABCB1*, the reference genes, and the nontemplate control (NTC). The relative target gene quantity in relation to an endogenous control was calculated by using mean Ct value with triplicate runs of each gene. All of three housekeeping genes were used to normalize the *ABCB1* expression levels in PBMCs relative to NTC.

The results were expressed as the fold difference of *ABCB1* expression compared to NTC. If the value of the *ABCB1* relative expression is > 1, it means the number of folds of the *ABCB1* expression levels that higher than the expression levels of endogenous control used. The interpretation is *vice versa* when the *ABCB1* expression level is < 1. The value was, then, corrected as 1/fold difference of *ABCB1* relative expression**.**

Analysis of the relationship between *ABCB1* SNPs 3435C>T and 2677G>T/A and its expression in PBMCs

At least 10 subjects for each SNP were needed to provide a reliable statistical analysis, with at least 10-12 samples per group to provide the power of 70-80% for determining the gene expression levels (Hitzl *et al*., 2001; Calado *et al*., 2002; Fellay *et al*., 2002; Illmer *et al*., 2002; Gaikovitch *et al*., 2003; Owen *et al*., 2004). Log transformed data was used. The univariate analysis (analysis of co-variance; ANCOVA) was performed with covariates of gender and number of white blood cells and lymphocytes. The correlations of *ABCB1* SNPs 3435C>T and 2677G>T/A and *ABCB1* expression in PBMCs were assessed by Pearson's correlation coefficient. A p-value < 0.05 was considered statistical significant.

RESULTS

Distributions of *ABCB1* single nucleotide polymorphisms (3435C>T and 2677G>T/A) in healthy Thai individuals

All enrolled subjects were healthy and eligible for participating into the study and the data of subjects' characteristics was previously published (Sudchada *et al*., 2010). The observed genotype frequency distributions of both genotypes (3435C>T, 2677G>T/A) in 56 healthy volunteers did not show a significant deviation from Hardy-Weinberg equilibrium. The χ^2 was 3.78 for 3435C>T genotype and 0.98 for 2677G>T genotypes. Both values were less than the critical value of 3.84, therefore the null hypothesis that the population is in Hardy–Weinberg frequencies was not rejected for these SNPs. Additionally, no statistically significant differences were observed in allelic distribution between males and females.

For 3435C>T polymorphisms in exon 26, 14% (8/56) CC homozygotes, 63% (35/56) CT heterozygotes, and 23% (13/56) TT homozygotes were found. For 2677G>T/A polymorphisms in exon 21, 53% (30/56) GT heterozygotes, 23% (13/56) TT homozygotes, and 18% (10/56) GG homozygotes, as well as

5% (3/56) of GA, AT or AA genotypes were observed (Table 2). Both SNPs genotypes were in Hardy-Weinberg equilibrium. Considering haplotypes distributions of 3435C>T and 2677G>T/A, the haplotype CT-GT (48.2%) was the most frequently observed, followed by the variant homozygotes TT-TT (21.4%) and CC-GG (10.7%) haplotypes (Table 3). In addition, no gender difference of these haplotype distributions was noted ($p > 0.05$).

Table 2 Distribution of *ABCB1* single nucleotide polymorphisms (3435C>T and 2677G>T/A) in the Thai

SNP	**Allele frequency**			**Genotypes frequency (N= 56)**					
3435C>T	**C**	**T**		**CC**		**CT**		**TT**	
Females (N=30)	0.48	0.52		4		21		5	
Males (N=26)	0.42	0.58		4		14		8	
Overall (N=56)	0.46	0.54		8 (14.3%)		35 (62.5%)		13 (23.2%)	
2677G>T	**G**	**T**	**A**	**GG**	**GT**	**TT**	**GA**	**AT**	**AA**
Females (N=30)	0.45	0.48	0.07	5	16	6	1	1	1
Males (N=26)	0.47	0.53	0.00	5	14	7	0	0	0
Overall (N=56)	0.46	0.51	0.03	10 (17.9%)	30 (53.5%)	13 (23.2%)	1 (1.8%)	1 (1.8%)	1 (1.8%)

Table 3 Haplotypes of 3435C>T and 2677G>T

	Numbers of subjects in each haplotype (N = 56)									
	CC GG	**CC GT**	**CT GG**	**CT GT**	**CT TT**	**TT GT**	**TT TT**	**CC AA**	**CT GA**	**CT AT**
Females (N=30)	3	1	2	15	1	0	5	1	1	1
Males (N=26)	3	1	2	12	0	1	7	0	0	0
Total (%)	6 (10.7)	2 (3.5)	4 (7.1)	27 (48.2)	1 (1.8)	1 (1.8)	12 (21.4)	1 (1.8)	1 (1.8)	1 (1.8)

Comparison of 3435C>T and 2677G>T/A alleles and genotypes frequencies between Thai and other populations

Genotypes of both SNPs were compared between the Thai in present study and other populations from previously published studies, using Chi-square test under the assumption that the frequency of SNPs' alleles or genotypes are not different (ratio 1:1). We found that there were considerably ethnic differences for these two SNPs (Table 4, 5). The frequency of heterozygotes GT for SNP 2677G>T/A appeared to be the highest, comparing to the other populations, while the frequency of homozygotes TT was significantly lower than one of the Indian groups, but significantly higher than one of the Japanese groups and the Korean populations (Table 4). For SNP 3435C>T, it was observed that our subjects had lower frequency of homozygous CC than almost all other populations. On the other hand, the frequency of the homozygous TT was significantly higher in Thai subjects compared to the African American, Kenyan and Sudanese, but significantly lower than the southwest Asians. (Table 5). Moreover, most of subjects who carry TT genotype at position 3435 also carry homozygous TT genotype at position 2677.

Table 4 2677G>T/A allele and genotype frequencies in various populations

Population	N	Allele frequency			Genotype frequency						Ref.
		G	T	A	GG	GT	TT	GA	TA	AA	
This study	56	0.45	0.48	0.07	0.18	0.53	0.23	0.02	0.02	0.02	-
Japanese	48	0.36	0.42	0.22	0.19	0.16*	0.21	0.19	0.25	0	Tanabe *et al*., 2001
Japanese	117	0.44	0.36	0.20	0.13	0.38	0.09*	0.24	0.14	0.02	Horinouchi *et al*., 2002
Japanese	13	0.39	0.46	0.15	0.23	0.23*	0.23	0.08	0.23	0	Moriya *et al*.
Chinese	96	0.38	0.50	0.12	0.17	0.33*	0.26	0.08	0.15	0.01	Chowbay *et al*., 2003
Chinese	104	0.50	0.44	0.06	-	-	-	-	-	-	Tang *et al*., 2002
Korean	232	0.44	0.37	0.19	0.20	0.35*	0.11*	0.13	0.18	0.03	Yi *et al*., 2004
Malaysian	92	0.53	0.44	0.03	0.28	0.47	0.19	0.02	0.04	0	Chowbay *et al*., 2003
Malaysian	93	0.58	0.36	0.06	-	-	-	-	-	-	Tang *et al*., 2002
Indian	87	0.33	0.60	0.07	0.14	0.31*	0.41*	0.08	0.06	0	Chowbay *et al*., 2003
Indian	68	0.34	0.62	0.04	-	-	-	-	-	-	Tang *et al*., 2002
Caucasian (German)	461	0.565	0.416	0.19	0.31	0.49	0.16	0.02	0.02	0.00	Cascorbi *et al*., 2001
Turkish	62	0.81*	0.19*		0.77	0.08*	0.15	-	-	-	Kaya *et al*., 2005

* $p < 0.05$; frequencies of SNPs or genotypes compared between the Thai and other populations using Chi-square test.

Table 5 3435C>T allele and genotype frequencies in various populations

Population	N	Allele frequency		Genotype frequency			Ref.
		C	T	CC	CT	TT	
This study	56	0.46	0.54	0.14	0.63	0.23	-
Japanese	114	0.61	0.39	0.35*	0.53	0.12	Sakaeda *et al*., 2001
Japanese	48	0.51	0.49	0.29*	0.44	0.27	Tanabe *et al*., 2001
Japanese	117	0.62	0.38	0.35*	0.53	0.12	Horinouchi *et al*., 2002
Japanese	13	0.54	0.46	0.38*	0.31*	0.31	Moriya *et al*., 2002
Chinese	265	0.56	0.44	0.32*	0.48	0.20	Li *et al*., 2006 30
Chinese	96	0.47	0.53	0.31*	0.44	0.25	Chowbay *et al*., 2003
Chinese	104	0.60	0.40	-	-	-	Tang *et al*., 2002
Chinese	132	0.53	0.47	0.32*	0.42*	0.26	Ameyaw *et al*., 2001
Korean	232	0.63	0.37	0.38*	0.50	0.12	Yi *et al*., 2004
Filipino	60	0.59	0.41	0.38*	0.42*	0.20	Ameyaw *et al*., 2001
Malaysian	92	0.49	0.51	0.29*	0.44	0.27	Chowbay *et al*., 2003
Indian	87	0.37	0.63	0.45*	0.37*	0.18	Chowbay *et al*., 2003
South-west Asians	89	0.34	0.66	0.15	0.38*	0.47*	Ameyaw *et al*., 2001
Saudi	96	0.55	0.45	0.37*	0.38*	0.26	Ameyaw *et al*., 2001
Ghanaian	206	0.83*	0.17*	0.67*	0.34*	0.00	Ameyaw *et al*., 2001
Kenyan	80	0.83*	0.17*	0.70*	0.26*	0.04*	Ameyaw *et al*., 2001
African American	88	0.84*	0.16*	0.68*	0.31*	0.01*	Ameyaw *et al*., 2001
Sudanese	51	0.73*	0.27*	0.52*	0.43	0.06*	Ameyaw *et al*., 2001
Caucasian, UK	190	0.48	0.52	0.24	0.48	0.28	Ameyaw *et al*., 2001
Caucasian, Germany	188	0.52	0.48	0.28*	0.48	0.24	Ameyaw *et al*., 2001
Caucasian in Germany	461	0.46	0.54	0.21	0.51	0.29	Cascorbi *et al*., 2001
Portuguese	100	0.43	0.57	0.22	0.42*	0.36	Ameyaw *et al*., 2001
Turkish	62	0.40	0.60	0.14	0.52	0.34	Kaya *et al*., 2005

* $p < 0.05$; frequencies of SNPs or genotypes compared between the Thai and other populations using Chi-square test.

Relationship of single nucleotide polymorphisms 3435C>T and 2677G>T/A to *ABCB1* expression in PBMCs

It was found that 3435C>T genotypes were significantly correlated with *ABCB1* expression (Pearson's correlation, r = 0.324;, p = 0.01). SNPs 3435C>T and 2677G>T showed a strong relationship (r = 0.623, $p < 0.001$), and together as a haplotype, they were also significantly correlated with the expression (r = 0.285, p = 0.037)**.** In contrast, 2677G>T/A genotypes were not correlated with *ABCB1* expression in PBMCs (r = 0.232, p >0.05).

Univariate analysis revealed that 3435C>T genotypes influenced the *ABCB1* expression in PBMCs (p = 0.038) with a significant covariate of gender (p = 0.035). TT genotypes had higher *ABCB1* expression levels in PBMCs compared to CT and CC, p < 0.020 (Figure 1), while 2677G>T/A genotypes had no influence on the expression (Figure 2). When multiple comparisons were stratified by genotypes and genders, individuals who carry TT genotype of 3435C>T SNP had the expression levels greater than individuals carry CC and CT, in both genders. Males with TT genotype had higher expression, compared to the others without. In addition, poshoc analysis showed a significant difference of the mean of the expression level between female CT (-6.7 $\pm$ 4.3) and male TT (-2.7 $\pm$2.3) groups, p = 0.027.

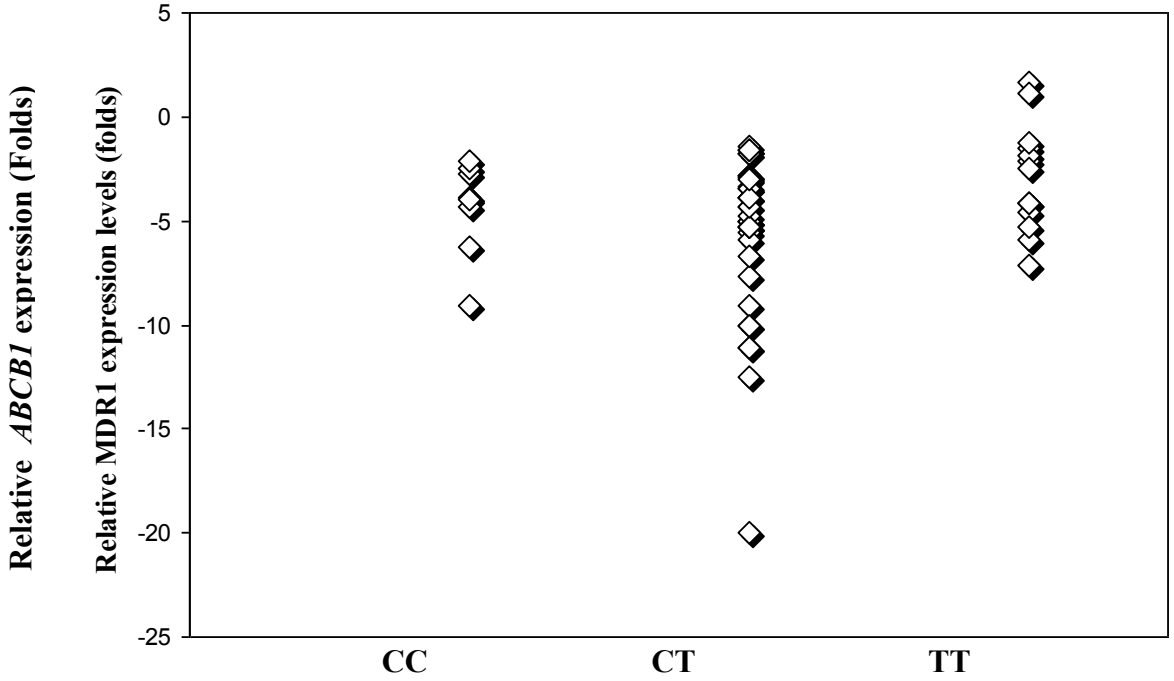

Figure 1 Relative *ABCB1* expression normalized with *GAPDH*, *actin-β* and *β2-M* in PBMCs regarding to 3435C>T genotype groups. TT group is correlated with higher of relative *ABCB1* expression, compared to CT and CC groups, p < 0.020.

Although a strong relationship between 3435C>T and 2677G>T SNPs was observed, we did not find anyone who carries either CC-TT or TT-GG haplotypes in our studied population. Most of our subjects carry CT-GT haplotype (48%) and TT-TT haplotype (21%). Interestingly, although multiple comparisons among theses haplotypes did not show difference in the expression level (p> 0.05), the mean of relative *ABCB1* expression in subjects who carry TT-TT haplotype was higher compared to those carrying other haplotypes (data not shown).

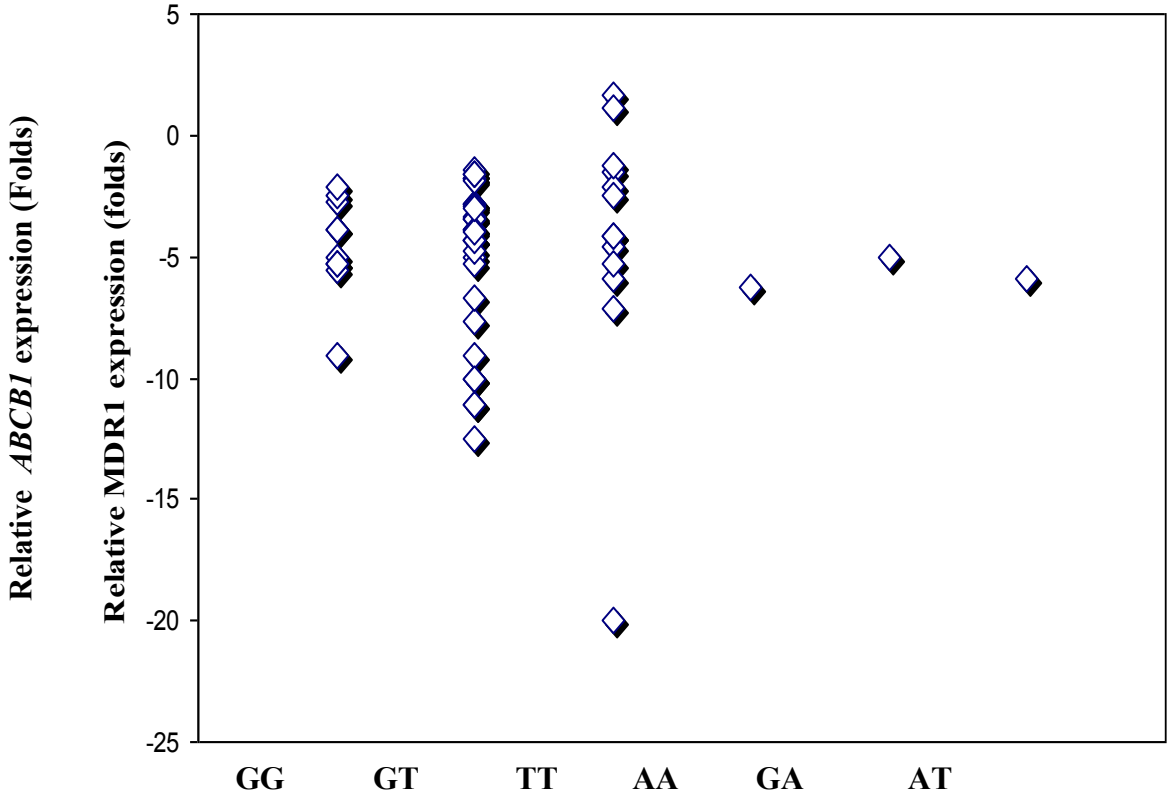

Figure 2 Relative *ABCB1* expression normalized with *GAPDH*, *actin-β* and *β2-M* in PBMCs regarding to 2677G>T/A genotype groups. No statistical difference of relative *ABCB1* expression level was noted between these genotypes.

DISCUSSION

This study aimed to find out the prevalence of two SNPs, 3435C>T and 2677G>T/A, in healthy Thai individuals comparing to other populations, and to determine the correlation between these two SNPs and the expression of *ABCB1* gene in PBMCs. The results in Table 4 and 5 showed that there were considerably ethnic differences for these two SNPs comparing between the Thai and other populations, which confirm the variability in allele and genotype distributions among different populations. Even though, this study may not have enough power to definitely identify the SNPs frequency in Thai population, the study shows the preliminary results of the SNPs' distribution in Thai population

In addition, frequencies of SNPs 3435C>T and 2677G>T/A and relationship with *ABCB1* gene expression in PBMCs were also determined in healthy Thai subjects. Previous studies observed that the TT genotype of 3435C>T SNPs is associated with a decrease of mRNA expression and activity in the PBMCs (Hitzl *et al*., 2001; Fellay *et al*., 2002; Owen *et al*., 2004). The studies by Fellay *et al*. (2002) and Owen *et al*. (2004)

found the homozygous CC had higher *ABCB1* mRNA expression levels and protein compared to the homozygous TT which consistent with the other study (Hitzl *et al*., 2001). There was a trend toward lower *ABCB1* mRNA levels in subjects with TT genotype in comparison to the CC group. Besides, the study in healthy volunteers by Oselin *et al*. (2003) observed no difference amount of *ABCB1* mRNA expression in lymphocytes among these genotypes. Besides, the study in healthy Caucasians investigated the activity of P-gp and observed a slower Rh123 efflux (lower P-gp function) in CD56+ with TT genotypes compared to CC and CT genotypes (Hitzl *et al*., 2001). On the other hand, we found, in this study, that TT genotype of 3435 position was correlated to higher expression of *ABCB1* levels in PBMCs in comparison to CC (trend; $p > 0.05$) and CT ($p < 0.05$) genotypes. Therefore, subjects with TT genotype may have less accumulation of *ABCB1* substrates in PBMCs compared with the CC and CT genotypes in Thai population. In other words, our Thai subjects who carry TT genotype may be more susceptible to drug resistance than those who carry CC or CT genotypes. The results were supported by another study (Illmer *et al*., 2002). The genotype TT of the SNP 3435C>T was correlated with higher *ABCB1* mRNA levels in mononuclear blood cells from bone marrow compared to the CC genotype (Illmer *et al*., 2002). However, some studies found no effect of the 3435C>T polymorphisms on the *ABCB1* expression or activity of P-gp (Calado *et al*., 2002; Oselin *et al*., 2003). The study in healthy subjects observed no differences in the mRNA expression in lymphocytes in relation to the 3435C>T genotypes (Oselin *et al*., 2003) and this was supported by Calado *et al*. (2002) who demonstrated no difference in P-gp function determined by Rh123 efflux assay among 3435C>T genotypes in CD34+ cells.

In agreement with other studies (Calado *et al*., 2002; Oselin *et al*., 2003), the 2677G>T polymorphism were not found, in this study, to be correlated with the expression of *ABCB1* in PBMCs. Therefore, the substantial inter-individual variability in *ABCB1* expression in PBMCs could be accounted for the 3435C>T SNPs while 2677G>T SNPs has no influence on the expression of *ABCB1* in our Thai subjects. In addition, the results of the present study reveals a strong relationship of 3435C>T and 2677G>T/A SNPs ($r = 0.623$, $p = 0.001$) as described in previous literature (Kim *et al*., 2001; Furuno *et al*., 2002; Goto *et al*., 2002; Gaikovitch *et al*., 2003; Yi *et al*., 2004). The correlation of 2677G>T and 3435C>T haplotypes and the expression of *ABCB1* in PBMCs was observed ($r = 0.285$, $p = 0.037$). Subjects who carry TT-TT hapoltype of 2677G>T and 3435C>T SNPs have a trend of higher *ABCB1* expression levels in PBMCs than other haplotypes.

A trend of gender difference of *ABCB1* expression levels in PBMCs in our Thai population was observed in our previous

report. Males have higher levels of the expression than Females about 2 folds (Sudchada, 2010). In addition, this present study shows that gender and TT genotype at position 3435 have influenced on the expression levels. From the univariate analysis, males and TT genotypes are correlated with higher expression of *ABCB1* in PBMCs. Therefore, males with TT genotype of 3435C>T SNPs are likely to be the most susceptible to drug resistance since they have the expression levels higher compared with others. This is supported by haplotype analysis suggests that males and females who carry a haplotype TT-TT of 3435C>T and 2677G>T SNPs is associated with higher *ABCB1* expression level in PBMCs. Subsequently, they are more likely to develop drug resistance.

Limitation of the study was that the subjects were not randomed around the country and they were mostly originally from lower north part of Thailand. Besides, family history and racial origins of the subjects were not obtained. Therefore the genotypes frequency found of these SNPs may not be generalized for the genotype frequency for Thai population. We observed only one subject for each AA, GA, and TA genotypes for 2677G>T/A SNPs. Therefore, we could not determine the relationship of these genotypes with the *ABCB1* expression in PBMCs. In addition this study did not look into the protein expression and activity so further research need to be determined. In addition, we determined the expression levels of *ABCB1* in total peripheral blood mononuclear cells and it would have a great impact to confirm protein expression in the cells. Further more, our study subjects were a group of young healthy volunteers and any age related differences could not be determined.

In conclusion, our study suggested that the genotypes of SNP 3435C>T and gender have, in part, influenced on the expression levels of *ABCB1* gene in PBMCs. Therefore, males with TT genotype of 3435C>T SNPs are likely to be the most susceptible to drug resistance since they have higher *ABCB1* expression levels in PBMCs. These results also confirm the inter-ethnic variability in *ABCB1* SNPs of 3435C>T and 2677G>T/A. Subsequently, these SNPs may contribute to inter-racial variability in pharmacokinetics and pharmacodynamics of drugs that are P-gp substrates. Further studies are warranted to explore the roles of these SNPs and physiological function and pharmacological effects of P-gp. These findings provide a new insight of the genetic information in Thai population

ACKNOWLEDGMENTS

Research supported by the Office of the National Research Council of Thailand (NRTC).

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