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Research Article

The Risk of Recurrence in Breast Cancer Patients Treated with Tamoxifen: Polymorphisms of *CYP2D6* and *ABCB1*

L. K. Teh,[1,8] N. I. Mohamed,[1] M. Z. Salleh,[1] M. Rohaizak,[2] N. S. Shahrun,[2] J. J. Saladina,[2] J. K. S. Shia,[1] H. Roslan,[3] S. Sood,[4] T. S. Rajoo,[5] S. P. Muniandy,[5] G. Henry,[5] H. A. Ngow,[6] K. T. Hla U,[7] and J. Din[7]

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Abstract. *CYP2D6* plays a major role in the metabolism of tamoxifen, and polymorphism of P-glycoprotein has been associated with resistance of many drug therapies. This study investigates the clinical impact of genetic variants of *CYP2D6* and *ABCB1* in breast cancer patients treated with tamoxifen. Blood samples from 95 breast cancer patients treated with tamoxifen were collected and genotyped for *CYP2D6* and *ABCB1* variants using allele-specific PCR method. Recurrence risks were calculated using Kaplan–Meier analysis and compared using the log-rank test. Patients carrying *CYP2D6*10/*10* and heterozygous null allele (IM) showed higher risks of developing recurrence and metastasis (OR 13.14; 95% CI 1.57–109.94; P=0.004) than patients with *CYP2D6*1/*1* and **1/*10* genotypes. Patients with homozygous *CC* genotypes of *ABCB1 C3435T* showed a shorter time to recurrence. Patients who were *CYP2D6* IM and homozygous *CC* genotype of *C3435T* have statistically significant higher risks of recurrence (P=0.002). Similarly, median time to recurrence in these patients was only 12 months (95% CI=0.79–23.2) compared to those without this combination which was 48 months (95% CI=14.7–81.2). Patients with *CYP2D6* IM and homozygous *CC* genotype of *ABCB1 C3435T* have shorter times to recurrence. The results confirmed the findings of previous studies and support FDA recommendation to perform pre-genotyping in patients before the choice of therapy is determined in breast cancer patients.

KEY WORDS: *ABCB1*; breast cancer; *CYP2D6*; recurrence and metastasis; tamoxifen.

INTRODUCTION

Pharmacogenomics is an area with fast-evolving implication in clinical setting, and thus, its incorporation to pharmaceutical care is believed to enhance patient-centred quality care. The US Food Drug and Administration has encouraged the practice of pharmacogenetics testing prior to drug therapy initiation in some clinically important therapeutics, and tamoxifen is one of them. Tamoxifen has been widely used as the standard adjuvant therapy for hormone receptor-positive breast cancer patients, especially in the high-risk pre- and postmenopausal women. However, 30% to 50% of oestrogen receptor (ER)-positive breast cancer patients do not respond to tamoxifen therapy (1–3). Major challenges to effective tamoxifen therapy include issues of drug resistance and tamoxifen-induced adverse events. Tamoxifen is metabolized by a number of cytochrome P-450 enzymes including CYP2D6, CYP3A4 and CYP2C9. Genetic polymorphisms of *CYP2D6* have been reported as the major cause of variation in the metabolism of tamoxifen that leads to adverse effects or lack of therapeutic efficacy (4,5).

Genetic variation in *CYP2D6* results in different metabolic phenotypes including extensive (EM), intermediate (IM), ultrarapid (UM) and poor metabolizer. *CYP2D6*10* is responsible for the reduced enzyme activity in IMs whereas *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*14* are null alleles which encode no enzyme at all. UM (*CYP2D6*xN*) has a duplication or multiduplication of functional alleles and leads to increase enzymes activity (6–8). This variation causes variability in patients' outcomes because CYP2D6 had been reported to biotransform tamoxifen into several active metabolites. Studies on the pharmacokinetics of tamoxifen demonstrated significantly lower steady-state plasma and serum concentrations of 4-hydroxy-*N*-desmethyltamoxifen and 4-hydroxytamoxifen in *CYP2D6*10/*10* than other genotypes (4,9,10).

[1] Pharmacogenomics Centre (PROMISE), Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Campus Puncak Alam, 42300, Puncak Alam, Selangor Darul Ehsan, Malaysia.
[2] Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia.
[3] UKM Medical Biology Institute (UMBI) and Department of Medicine, Universiti Kebangsaan Malaysia Medical Centre, (UKMMC), Kuala Lumpur, Malaysia.
[4] Faculty of Medicine, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor Darul Ehsan, Malaysia.
[5] Department of Surgery, Selayang Hospital, Kuala Lumpur, Malaysia.
[6] Department of Internal Medicine, Kulliyah of Medicine, International Islamic University Malaysia (IIUM)/Tengku Ampuan Afzan Hospital (HTAA), Pahang Darul Makmur, Malaysia.
[7] Department of Surgery, International Islamic University Malaysia (IIUM), Pahang Darul Makmur, Malaysia.
[8] To whom correspondence should be addressed. (e-mail: tehlaykek@puncakalam.uitm.edu.my)

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Active metabolites from primary and secondary metabolism pathways of tamoxifen known as endoxifen (4-hydroxy-*N*-desmethyltamoxifen) and 4-hydroxytamoxifen were found to be 100-fold more potent than tamoxifen (11). A recent study among the breast cancer patients with *CYP2D6*10/*10* in Asians has shown that the steady-state plasma levels of 4-hydroxytamoxifen and endoxifen were significantly lower, and these patients have significant shorter median time to disease progression compared to patients who were wild type or heterozygote of *CYP2D6*1/*10* (4,9,12). Furthermore, co-administration of selective serotonin reuptake inhibitors in breast cancer patients who suffer depressive syndrome was found to reduce the metabolite concentrations and thus affect the outcomes of tamoxifen therapy (13,14).

ABCB1 is responsible for multidrug resistance, the principal mechanism by which many patients with cancer disorders develop resistance to chemotherapeutic drugs. This gene encodes P-glycoprotein (P-gp) and functions as an energy-dependent drug efflux pump and transports a variety of toxins, nutrients, environmental carcinogens and drugs (15). Over-expression of this protein in breast cancer tumours was significantly associated with disease relapse and shorter disease-free survival period (16). This gene was found to be highly polymorphic and causes susceptibility to various disease and therapeutic clinical outcomes. A silent mutation in exon 26 namely *C3435T* has been reported to be associated with therapeutic outcomes in breast cancer treatment and other disease (17–19). Another allele *G2677A/T* in exon 21 had been shown to be associated with an amino acid change in Ala893Thr and Ala893Ser. Substitution of these nucleotides results in change of a lipophilic residue to a hydrophilic one and affects the geometric precision of the interaction site and the secondary structure (20). Tamoxifen, 4-hydroxytamoxifen and endoxifen are known to bind P-gp and are substrates of P-gp which may acts as a barrier and limits the accessibility of active metabolites of tamoxifen to various critical target tissues and the success of tamoxifen therapy (21,22). P-gp expression was also found to increase from 40–50% to 60–70% after chemotherapy in breast cancer patients and resulted in a shorter overall survival in patients (21).

Our earlier studies reported the heterogeneity of *CYP2D6* among the three different ethnic groups in Malaysia (23–26), but no studies have investigated the influence of the polymorphism of *CYP2D6* and *ABCB1* in tamoxifen therapy among the patients in Malaysia. We therefore investigated the impact of *CYP2D6* and *ABCB1* genotypes on the outcomes of tamoxifen therapy in a cohort of breast cancer patients.

MATERIALS AND METHOD

Subjects

The study was approved by the Medical Research and Ethics Committee of Ministry of Health Malaysia, institutional review board of both Universiti Teknologi MARA and Universiti Kebangsaan Malaysia Medical Centre. Five millilitres of blood samples was collected from breast cancer patients at Universiti Kebangsaan Malaysia Medical Centre, Selayang Hospital and Tengku Ampuan Afzan Hospital after a written informed consent. Patients recruited comprised of three ethnic groups predominant in Malaysian, namely Malays, Chinese and Indians. The ethnicities of all subjects were confirmed by individual screening and verified against the new National Registry Identification Cards. Ninety-five breast cancer patients (53 Malays, 36 Chinese and 6 Indian) were successfully recruited. Status of ER and progesterone receptor breast cancer tumour was determined by immunohistochemistry, and patients received tamoxifen 20 mg/day.

Sample Preparation and Genotyping

Genomic DNA was extracted using alkaline lysis method as described previously (10), and DNA was stored at −20°C until analysis. Patients' samples were genotyped for *CYP2D6*xN*, *CYP2D6*4* (rs3892097), *CYP2D6*5*, *CYP2D6*10* (rs1065852), *CYP2D6*14* (rs5030862), *ABCB1 C3435T* (rs1045642) and *G2677A/T* (rs2032582). Nested PCR approach was used to identify variants of *CYP2D6.* In the first PCR, 0.2 μM of each primer, fragment A; forward primer—5′ ACCAGGCCCCTC CACCGG 3′, reverse primer—5′ AATGCGCCACGCT CACCCATTGG 3′; fragment B: forward primer—5′ ATGGGTGAGCGTGGCGCATTTCC 3′, reverse primer—5′ TGCACTGTTTCCCAGATGGGCTC 3′, 1.0 U of BioTools *Taq* (Biotools B&M Labs. S.A) and 100 ng of DNA were used to amplify fragments A and B. Fragment A spanning exon 1 and 2 served as template for *CYP2D6*10* while fragment B spanning exon 3 to 6 served as template for detection of *CYP2D6*4* and *CYP2D6*14*. The thermocycling conditions for this PCR were 95°C for 5 min (initial denaturation), followed by 35 cycles of 94°C for 1 min; 64°C for 30 s; 72°C for 1.5 min and a final extension of 72°C for 5 min. Eight microlitres of the PCR product was checked on 1% agarose gel and appropriately diluted product and was used as the template in the second PCR.

Allele-specific primers were used for detection of variant and wild-type alleles in a multiplex PCR. The final reaction was carried out in a total volume of 25 μl PCR mix containing diluted first PCR product (1:100), 0.06 μM of common primer, forward and reverse primer: *CYP2D6*4*—5′ TTGGAGTGGGTGGTGGATGGT 3′, 5′ AGCCCGACTCCTCCTTCAG 3′; *CYP2D6*10*—5′ ATTTGGTAGTGAGGCAGGTAT 3′, 5′ AACGGCACT CAGGACTAACT 3′; *CYP2D6*14*—5′ CAGAGAC GAGGTGGGGCAA 3′, 5′ TTGCTCACGGCTTTGTCCA 3′, 0.4 μM of wild-type primers, forward primer: *CYP2D6*4*—5′ CGCATCTCCCACCCCCCG 3′, *CYP2D6*10*—5′ GCTGGGCTGCACGCTAAC 3′, *CYP2D6*14*—5′ GCCTTCGCCAACCACTCAG 3′, 0.2 μM of variant primer, reverse primer: *CYP2D6*4*—5′ TGGGGCGAA AGGGGCGTGT 3′, *CYP2D6*10*—5′ TGGCAGGGGGC CTGGTTA 3′, *CYP2D6*14*—5′ CTTCTGCCCATCACC CATT 3′, 1% of dimethyl sulfoxide (Sigma-Aldrich Co., St. Louis USA) and 12.5 μl of 1× of Go*Taq*® Green Master Mix (Promega Corporation, Madison, USA) for amplification of *CYP2D6*4*, *CYP2D6*10* and *CYP2D6*14*. The thermocycling conditions were 94°C for 5 min (initial denaturation), followed by 18 cycles of 94°C for 45 s; 56°C for 45 s; 72°C for 1.0 min and 72°C for 5 min (extra elongation). Eight microlitres of the PCR product was checked on 1.5% agarose gel. The specificity of the PCR amplification was confirmed using a positive control for each allele.

For gene duplication and deletion of *CYP2D6*, previously reported methods were used with some modification (27,28). Amplification reactions were performed using 1 U Biotools® *Taq* DNA Polymerase (Biotools B&M Labs. S.A, Madrid, Spain), 0.2 μM of primers for duplication and 0.4 μM of primers for deletion with 12 s denaturing at 94°C and 5 min annealing/extension at 68°C for 35 cycles and final extension at 72°C for 10 min. All reactions were performed in a Takara PCR machine (TP600, Takara Bio Inc., Japan).

Genotyping for *ABCB1 C3435T* was performed using previously designed primers (13) with modification for common reverse primer: 5′ CAAATAAACAGCATGG GAGCA 3′. Amplification reactions were performed using 12.5 μl of 1× Go*Taq*® Green Master Mix (Promega Corporation, Madison, USA), 0.1 μM of forward primer, 0.2 μM of reverse primer and 0.3 μM of each allele-specific primers were used. The reaction was split into two tubes for parallel amplification of wild-type and variant allele. The conditions for this PCR were 94°C for 5 min (initial denaturation), followed by 10 cycles of 94°C for 30 s; 61°C for 30 s; 72°C for 20 s; followed by 25 cycles of 94°C for 30 s; 55°C for 30 s and 72°C for 30 s.

The variant and wild-type specific primers of *ABCB1 G2667A/T* were split into three tubes, and each reaction was internally controlled for amplification failure. 1× Go*Taq*® Green Master Mix (Promega Corporation, Madison, USA), 0.1 μM of common forward primer: 5′ CTATAG GTTCCAGGCTTGCTG 3′, 0.2 μM of common reverse primer: 5′ GGAAGGAAGAA CAGTGTGAAGAC 3′ and 0.3 μM allele-specific primers: 5′ TGAAAGATAAGAAAGAACTAGAAGGCG 3′, 5′ TGAAAGATAAGAAAGAACTAGAAGGCA 3′, 5′ TGAAAGATAAGAAAGAACTAGAAGGCT 3′ were used for each allele. Specificity of the primer sets was confirmed using a positive control for each allele. The PCR conditions were 80°C for 5 min and 94°C for another 5 min (initial denaturation), followed by 8 cycles of 94°C for 30 s; 61°C for 45 s; 72°C for 20 s; and subsequent 25 cycles of 94°C for 45 s; 58°C for 30 s; 72°C for 1 min and 72°C for 5 min. Ten microlitres of the PCR product was checked on 1.5% agarose gel.

The metabolizer status of CYP2D6 was defined as (1) EM, with absence of variant alleles; (2) heterozygous intermediate metabolizer (Het-IM), with presence of one allele of *CYP2D6*10* and (3) IM with homozygous *CYP2D6*10* or having a combined allele of heterozygous null alleles. For statistical reasons, individuals with duplicated allele without any null alleles were included in the EM group.

Statistical Analysis

The association of the genetic variants of *CYP2D6* and *ABCB1* with recurrence and metastasis of breast cancer was investigated. Allele frequencies of *CYP2D6* and *ABCB1* with their respective Hardy–Weinberg equilibrium were calculated. The differences in allele frequencies with different patients' characteristic were determined using the $\varkappa^2$ test or Fisher's exact test. Odds ratios and corresponding 95% CI were calculated for risk estimation and trend of association between genotypes and recurrence or metastasis. Tukey's test was used to determine the combination effects of *CYP2D6* and *ABCB1* based on the number of risk alleles. Recurrence-free rate was analysed using Kaplan–Meier methods. The recurrence-free period was defined as the period between surgery and initiation of tamoxifen therapy to the recurrence of breast cancer or metastasis. Statistical significance analysis of a relationship between outcomes and genetic polymorphism was assessed by the log-rank test. All *P* values were two sided, and values less than 0.05 were considered statistically significant. Statistical tests were run using SPSS version 18 on an IBM-compatible computer.

RESULT

Allelic Distribution of *CYP2D6* and *ABCB1*

*CYP2D6*4*, *CYP2D6*xN*, *CYP2D6*5*, *CYP2D6*10*, *CYP2D6*14* and *C3435T*, *G2677A/T* alleles of *ABCB1* were successfully amplified in 95 patients (Table I). Their genotype frequencies are as shown in Table II. The genotype and allele frequencies of *CYP2D6* in the breast cancer patients were compared with those among Malaysian healthy populations and Singapore breast cancer patients (7,10–13). The most common allele detected was *CYP2D6*10*; 47.2% patients were heterozygous and 22.6% were homozygous *CYP2D6*10*. The genotypes for both *CYP2D6* and *ABCB1* were in Hardy–Weinberg equilibrium.

Risk Estimation Between Genotypes of *CYP2D6* and *ABCB1* Variants

Patients with *CYP2D6* IM genotype appeared to be at higher risk of developing recurrence and metastasis compared to *CYP2D6* EM genotype (OR 13.14; 95% CI 1.54–109.94; P= 0.004; Table III). Carriers of *CYP2D6* Het-IM genotype also showed an increased risk, but the difference was not statistically significant (OR 2.71; 95% CI 0.28–25.78; P=0.78). On the other hand, patients with combination of *CT* and *TT* genotypes of *ABCB1 C3435T* have lower risk to disease relapse but was not significant (OR 0.46; 95% CI 0.16–1.34; P=0.15). Patients with genotypes of *G/A* or G/*T* and *AT* of *G2677A/T* were found to have higher risk of disease relapse, but no statistically significant association was observed (OR 1.41; 0.35–5.71; P=0.63).

CYP2D6 and *ABCB1* Variants and Onset of Recurrence and Metastasis

Time to develop recurrence and metastasis was evaluated using the Kaplan–Meier analysis. The recurrence-free rate was measured from the date of diagnosis to the date of the first instance of recurrence and metastasis. Patients carrying *CYP2D6* IM genotypes had significantly shorter recurrence-free survival compared to patients with *CYP2D6* EM and Het-IM genotypes (log-rank; P=0.031, Fig. 1a). No statistically significant association was detectable for *ABCB1 C3435T* (log-rank; P=0.22, Fig. 1b), but patients with *CC* genotype have earlier onset of relapse compared to *CT* and *TT* genotypes. Kaplan–Meier analysis showed a different trend for genotypes of *ABCB1 G2677T* compared to *C3435T*, but it was not statistically significant (P=0.97, Fig. 1c).

Table 1. Clinical and Pathological Characteristics of 95 Patients Subgrouped According to *CYP2D6* and *ABCB1* Alleles

Variant	*CYP2D6*			*ABCB1 C3435T*			*ABCB1 G2677 (A/T)*		
	WT	VT	*P* (VT *vs.* WT)	C	T	*P* (C *vs.* T)	G	A/T	*P* (G *vs.* A/T)
Characteristic (*n*=95, alleles=190)			*P* value/OR (95% CI)			*P* value/OR (95% CI)			*P* value/OR (95% CI)
Age at surgery									
Median (range), 51 (33–79)									
Menopausal status (pre- *vs.* post-)									
Pre- (*n*=33, alleles=66)	25	41	0.13[a]	43	23	0.68[a]	38	28	0.37[a]
Post- (*n*=62, alleles=124)	61	63	1.58 (0.86–2.92)	77	47	1.14 (0.61–2.13)	63	61	1.31 (0.71–2.39)
Histology (DIC *vs.* LIC)									
DIC (*n*=82, alleles=164)	77	87	0.29[b]	103	61	0.71[b]	90	74	0.14[b]
LIC (*n*=4, alleles=8)	2	6	0.37(0.07–1.92)	6	2	0.56 (0.11–2.88)	2	6	3.65 (0.72–18.62)
Others (*n*=9, alleles=18)									
Tumour grade (2+3 *vs.* 1)									
1 (*n*=34, alleles=86)	39	47	1.0[a]	52	34	0.008[a, c]	45	41	0.84[b]
2 and 3 (*n*=52, alleles=104)	47	57	1.01 (0.57–1.79)	68	18	2.47 (1.26–4.86)	56	48	1.06 (0.59–1.88)
Tumour stage (III + IV *vs.* 0–II)									
0–II (*n*=70, alleles=140)	69	69	0.05[a]	81	47	0.06[a]	75	65	0.84[a]
III and IV (*n*=25, alleles=50)	17	33	1.94 (0.99–3.81)	39	11	2.05 (0.96–4.39)	26	24	0.94 (0.49–1.79)
ER and/or PR status (Pos *vs.* Neg)									
Pos (*n*=78, alleles=156)	69	87	0.54[a]	97	59	0.55[a]	85	71	0.43[a]
Neg (*n*=17, alleles=34)	17	17	1.26 (0.59–2.65)	23	11	0.78 (0.36–1.73)	16	18	1.34 (0.64–2.03)

Patients with *C* allele of *ABCB1 C3435T* were found to have statistically higher odds ratio for tumour grade 2 and 3 compared to tumour grade 1 (OR 2.47; 95% CI 1.26–4.86; *P*=0.008)

Abbreviations: *WT* wild type, *VT* variant, *DIC* ductal invasive carcinoma, *LIC* lobular invasive carcinoma, *ER* oestrogen receptor, *PR* progesterone receptor, *Pos* positive, *Neg* negative

[a] χ^2 test

[b] Fisher's exact test

[c] A significant association

Association of Genotypes and Patients' Clinical Outcomes

Patients carrying *CC* genotype of *ABCB1 C3435T* were found to have shorter recurrence-free survival. This genotype was combined with *CYP2D6* IM to investigate the combination effect on the onset of relapse. Patients were classified into five groups according to the number of risk alleles (0, 1, 2, 3, 4 risk alleles group; Table III). Patients carrying three and four risk alleles were found to have higher risk in developing disease compared with other alleles (OR 9.9; 95% CI 2.11–46.6; *P*=0.001). Kaplan–Meier analysis revealed that patients have shorter recurrence-free survivals (*P*=0.01, Fig. 2a) if they have a combination of risk alleles. Time to recurrence and metastasis were evaluated among relapsing

Table II. The *CYP2D6* and *ABCB1* Allele Frequencies

		Allele frequencies			*P* value (95% CI)	
		Current study	Malaysian population[a]	Singapore breast cancer patients[b]		
Polymorphism	Allele	A (*n*=95)	B (*n*=429)	C (*n*=165)	A *vs.* B	A *vs.* C
CYP2D6	**1*	41.6	51.5	36.7	Ref[c]	
	**4*	2.1	1.6	0.3	1.0	0.5
	**5*	4.7	1.9	8.0	0.2	0.4
	**10*	48.9	43.1	51.0	0.3	0.4
	**14*	1.1		2.0		
	**xN*	1.6	1.9	2.0	1.0	1.0
ABCB1						
C3435T	*C*	63.2	56.5[d]		0.3	
	T	36.8	43.5			
G2677*A/T*	*G*	53.2				
	A	11.0				
	T	35.8				

Abbreviations: *A vs. B* current study *vs.* Malaysian population, *A vs. C* current study *vs.* Singapore breast cancer patients

[a] (1) Teh *et al.* 2001, (2) Ismail *et al.* 2001 and (3) Ismail *et al.* 2003

[b] Lim *et al.* 2011

[c] *CYP2D6*1* was used as the reference

[d] Teh *et al.* 2007

Table III. Risk Estimation Between Genotype and Recurrences and Metastasis in Breast Cancer Patients

Variant	Overall		No event		Event		Statistic		
	No.	Percentage	No.	Percentage	No.	Percentage	OR	95% CI	*P*
CYP2D6									
Homo-EM	24	25.3	23	29.5	1	5.9	1.0 (ref)[a]		
Het-IM	38	40.0	34	43.6	4	23.5	2.71	0.28–25.78	0.78[b]
IM	33	34.7	21	26.9	12	70.6	13.14	1.54–109.94	0.004[b, c]
ABCB1									
C3435T									
CC	41	43.2	31	39.7	10	58.8	1.0 (ref)[a]		
CT	38	40.0	32	41.0	6	35.3	0.58	0.19–1.79	0.34[d]
TT	16	16.8	15	19.3	1	5.9	0.21	0.02–1.76	0.15[e]
CT and *TT* combined									
CT+TT	54	56.8	47	60.3	7	41.2	0.46	0.16–1.34	0.15[d]
G2677A/T									
GG	32	33.7	29	34.5	3	27.3	1.0 (ref)[a]		
G/(*A* or *T*)	37	38.9	32	38.1	5	45.5	1.51	0.33–6.89	0.72[e]
AT	26	27.4	23	27.4	3	1.2	1.26	0.23–6.84	1.00[e]
G/(*A* or *T*) and *AT* combined									
G/(*A* or *T*)+*AT*	63	66.3	55	65.5	8	72.7	1.41	0.35–5.71	0.63[e]
No. of risk alleles of *CYP2D6* and *ABCB1 C3435T*									
0	6	6.3	6	7.7	0	0			
1	15	15.8	15	19.2	0	0			
2	28	29.5	26	33.3	2	11.8	1.0 (ref)[f]		
3	30	31.6	20	25.6	10	58.8	6.5	1.28–33.05	0.04[b, c]
4	16	16.8	11	14.2	5	29.4	5.9	0.99–35.21	0.15[b]
No. of risk alleles of *CYP2D6* and *ABCB1 C3435T* combined									
0	6	6.3	6	7.7	0	0			
1+2	43	45.3	41	52.6	2	11.8	1.0 (ref)[f]		
3+4	46	48.4	31	39.7	15	88.2	9.9	2.11–46.6	0.001[b, c]

No. of risk alleles—patients were classified into five groups according to combination genotypes of *CYP2D6* and *ABCB1 C3435T* (0—Homo-EM of *CYP2D6* and homozygous mutant of *ABCB1*, 1—Het-IM of *CYP2D6* and homozygous mutant of *ABCB1* or Homo-EM of *CYP2D6* and heterozygous *ABCB1*, 2—Het-IM of*CYP2D6* and heterozygous of *ABCB1*, 3—Het-IM of *CYP2D6* and homozygous wild type of *ABCB1* or IM *CYP2D6* and heterozygous of *ABCB1*, 4—IM of *CYP2D6* and homozygous wild type for *ABCB1*)
Abbreviations: *Homo-EM* homozygous wild-type allele for **xN*, **4*, **5*, **10*, **14*, *HetIM* one allele of **10* and *IM* homozygous of **10* or having a combined allele of heterozygous null alleles; *ABCB1 C3435T* (*CC* homozygous wild type, *CT* heterozygous, *TT* homozygous mutant) and *G2677A/T* (*GG* homozygous wild type, *G/(A or T)* heterozygous, *AT* homozygous mutant)
[a] The reference category included variant without any risk allele
[b] Tukey's test
[c] A significant association
[d] χ^2 test
[e] Fisher exact test
[f] The reference category included ≤2 risk alleles

patients with their genotypes (recurrence and metastasis, $n=11$; metastasis only, $n=6$). Median time to develop recurrence and metastasis in intermediate metabolizers of *CYP2D6* and *CC* group of *ABCB1 C3435T* was only 12 months (95% CI= 0.79–23.2, $n=3$) compared to 48 months (95% CI=14.7–81.2, $n=8$) in patients with genotype of *CYP2D6* IM or *CC* group of *ABCB1 C3435T* or other genotypes. The difference was found to be statistically significant (log-rank; $P=0.002$, Fig. 2b). A similar trend was observed in patients who have both recurrence and metastasis ($n=11$) and those with metastasis only ($n=6$), but no statistical significance was found (Fig. 2c).

DISCUSSION

The clinical benefit of tamoxifen has significantly impacted ER-positive breast cancer patients, but approximately one third of patients have recurrence within 15 years (29). Heterogeneity in patients' responses to tamoxifen among breast cancer patients is consistently observed across patient populations where administration of the same dose of this drug results in a range of outcomes which include adverse events or therapeutic failure. Many clinical variables have been associated with drug response such as age, diet, menopausal status, lifestyle and tumour biology. Important achievements also have been obtained in optimisation of drug therapy based on classification of diseases using protein expression profiles of the breast cancer tumours. However, the genetic background of a patient with respect to metabolizing enzymes, drug transporters and correlation of patients' clinical data that determine efficacy and toxicity of the therapy is still lacking.

Lim *et al.* demonstrated that patients prescribed with tamoxifen and carrying *CYP2D6*10/*10* genotype have slower response towards therapy compared patients with *CYP2D6*1/*1* or *CYP2D6*1/*10* genotypes (4). Furthermore, breast cancer patients who received adjuvant tamoxifen therapy revealed worse therapy outcomes in patients with

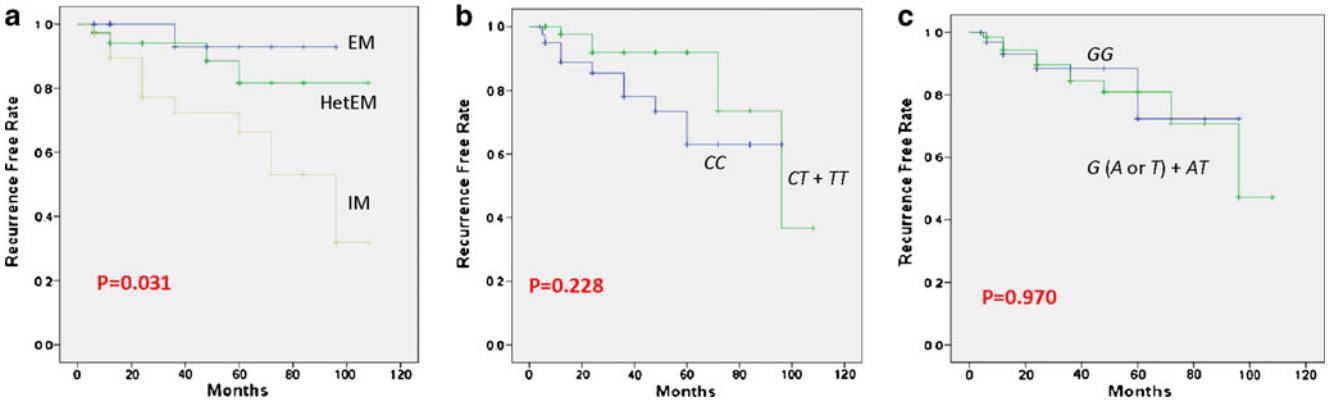

Fig. 1. Kaplan–Meier probabilities of time to develop recurrence and metastasis in patients treated with tamoxifen predicted from *CYP2D6* and *ABCB1* genotypes: **a** carrier of *CYP2D6*, extensive metabolizer (*EM*), heterozygous intermediate metabolizer and intermediate metabolizer (*IM*); **b** carrier of *ABCB1 C3435T*, homozygous wild type (*CC*) *vs.* heterozygous (*CT*) and homozygous mutant (*TT*); **c** carrier of *ABCB1 G2677A/T*, homozygous wild type (*GG*) *vs.* heterozygous {*G(A or T)*} and homozygous mutant (*AT*)

the *CYP2D6*10/*10* genotype and significantly higher incidence of recurrence within 10 years after the operation (10,12). Among Caucasians, tamoxifen-treated patients carrying the heterozygous and homozygous *CYP2D6*4* had a significantly increased risk of recurrence of breast cancer, shorter relapse-free periods and worse event-free survival rates compared with carriers of functional alleles (30,31). In contrast, two studies from Japan showed that *CYP2D6*10/*10* genotype was unlikely to have any clinical significance on breast cancer patients receiving adjuvant tamoxifen (32,33).

Our result are consistent with the hypothesis that the *CYP2D6*10/*10* and heterozygous null alleles metabolize tamoxifen slower and thus produce less pharmacologically active tamoxifen metabolites resulting in a higher risk of recurrence and metastasis. We demonstrated that carrier of *CYP2D6*10/*10* with combination heterozygous null allele showed higher incidence of developing recurrence and metastasis than patients with *CYP2D6*1/*1* and *CYP2D6*1/*10* (log-rank test; P=0.031). In addition, genetic polymorphism of Malays was found to be different from Chinese and Far Eastern races and their CYP2D6 activity was hypothesised to be intermediate between East Asian and Caucasian (23). This could explain the discrepancy results between this study and the study from Japan.

From our study, the *CC* genotype for *ABCB1 C3435T* has a shorter time in developing recurrence and metastasis although the association was not statistically significant (P= 0.22). Previous studies showed that expression of P-gp protein level was twofold higher for homozygous *CC* genotype compared to *TT* genotype (34). Thus, the expression of this genotype would cause lower bioavailability of tamoxifen and its metabolites in patients. Genotypes of *CT*, *TT* and combination of *CT*/*TT* did not show an increased risk in developing relapse. Homozygous *TT* genotype is associated with lower *ABCB1* expression and has been correlated with reduced expression and functional status of P-gp protein compared to *CC* genotypes (35). Transportation of tamoxifen would be slower and benefited patients with *TT* genotype. However, accumulation of tamoxifen or active metabolites after several years would increase chances of cells becoming resistant towards therapy. In addition, *ABCB1* mRNA levels were higher in high-grade tumours compared to low-grade tumours and this relationship might be an indicator of patient's clinical outcomes (36). Recent studies in non-small cell lung cancer showed *GG* and *CC* genotype of *G2677A/T*

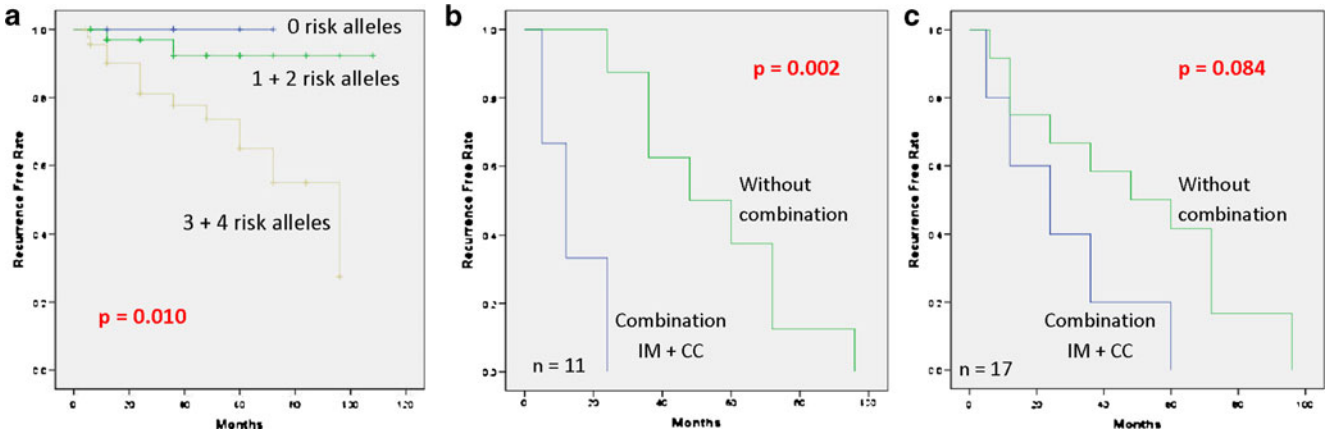

Fig. 2. Kaplan–Meier probabilities of time to develop recurrence and metastasis in patients treated with tamoxifen. Patients were classified according to combined genotypes of *CYP2D6* and *ABCB1*. **a** Patients were classified into five groups (0, 1, 2, 3, 4) based on the number of risk alleles, combined for 1+2 risk alleles and 3+4 risk alleles; **b** combined effect of *CYP2D6* and *ABCB1 C3435T* genotypes with time to develop recurrence + metastasis (combination of *CYP2D6* IM+CC genotypes of *ABCB1 C3435T vs.* without combination—other *CYP2D6* and *ABCB1 C3435T* genotype combination); **c** combined effect of *CYP2D6* and *ABCB1 C3435T* with time to develop recurrence and metastasis + metastasis only

and *C3435T* were associated with a significantly better response to docetaxel–cisplatin compared with the combined of heterozygous and homozygous mutant of both gene, respectively (37).

In this study, we also performed combination analysis of patients having both *CYP2D6* IM and *CC* genotype of *ABCB1* in patients with relapse. This group was compared with similar groups of patients but carrying only either *CYP2D6* IM or homozygous wild-type *CC* or other genotypes of *ABCB1*. Patients with combination of these genotypes have shorter recurrence-free rates (P=0.002). To our knowledge, this is the first study to address the correlation between *CYP2D6* and *ABCB1* variant with clinical outcomes among breast cancer patients treated with tamoxifen.

CONCLUSION

This finding is particularly important in the discovery of the effectiveness and resistance of tamoxifen therapy among breast cancer patients. These lines of evidence imply that pregenotyping of *CYP2D6* intermediate metabolizer and homozygous wild-type *C3435T* is important before prescribing tamoxifen. It is thus recommended that clinical pharmacists should be aware of this and incorporate the practice of "PharmacoDiagnostics" in the relevant pharmacy practice as routine when appropriate.

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