Impact of *CYP2D6*, *CYP3A5*, *CYP2C9* and *CYP2C19* polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tamoxifen is metabolized to active metabolites, 4-hydroxytamoxifen and endoxifen, by multiple cytochrome P450 (CYP) enzymes including *CYP2D6*, *CYP3A4/5*, *CYP2C9/19*, *CYP1A2* and *CYP2B6*.
- The steady-state plasma concentrations of tamoxifen and its metabolites can be affected by variations in the activity of these enzymes.
- Although *CYP2D6**4 and *10 have been shown to influence the plasma concentration of endoxifen in Caucasian and Korean patients respectively, there is still a paucity of data on *CYP2D6* pharmacogenetics in other Orientals such as Chinese, Malays and Indians.

WHAT THIS STUDY ADDS

- Pharmacogenetic analyses of a comprehensive panel of *CYP2D6* polymorphisms (*2, *2A, *3, *4, *5, *6, *7, *8, *9, *10, *12, *14, *17, *29, *41 and *xN) were performed in three distinct Asian ethnic groups and breast cancer patients with *CYP2D6**5 and *10 found to be highly prevalent.
- Both *CYP2D6**5 and *10 were significantly associated with lower endoxifen and higher N-desmethyltamoxifen concentrations as well as a lower rate of metabolic conversion of N-desmethyltamoxifen to endoxifen.
- Polymorphisms present in *CYP3A5*, *CYP2C9* and *CYP2C19* were not found to be significantly associated with plasma concentrations of analytes suggesting that these enzymes may be playing minor roles in the metabolic pathway of tamoxifen compared with *CYP2D6*.

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AIM

To investigate the impact of genetic polymorphisms in *CYP2D6*, *CYP3A5*, *CYP2C9* and *CYP2C19* on the pharmacokinetics of tamoxifen and its metabolites in Asian breast cancer patients.

METHODS

A total of 165 Asian breast cancer patients receiving 20 mg tamoxifen daily and 228 healthy Asian subjects (Chinese, Malay and Indian; $n = 76$ each) were recruited. The steady-state plasma concentrations of tamoxifen and its metabolites were quantified using high-performance liquid chromatography. The *CYP2D6* polymorphisms were genotyped using the INFINITI™ *CYP450* 2D6I assay, while the polymorphisms in *CYP3A5*, *CYP2C9* and *CYP2C19* were determined via direct sequencing.

RESULTS

The polymorphisms, *CYP2D6**5 and *10, were significantly associated with lower endoxifen and higher N-desmethyltamoxifen (NDM) concentrations. Patients who were *1/*1 carriers exhibited 2.4- to 2.6-fold higher endoxifen concentrations and 1.9- to 2.1-fold lower NDM concentrations than either *10/*10 or *5/*10 carriers ($P < 0.001$). Similarly, the endoxifen concentrations were found to be 1.8- to 2.6-times higher in *1/*5 or *1/*10 carriers compared with *10/*10 and *5/*10 carriers ($P \leq 0.001$). Similar relationships were observed between the *CYP2D6* polymorphisms and metabolic ratios of tamoxifen and its metabolites. No significant associations were observed with regards to the polymorphisms in *CYP3A5*, *CYP2C9* and *CYP2C19*.

CONCLUSIONS

The present study in Asian breast cancer patients showed that *CYP2D6**5/*10 and *10/*10 genotypes are associated with significantly lower concentrations of the active metabolite of tamoxifen, endoxifen. Identifying such patients before the start of treatment may be useful in optimizing therapy with tamoxifen. The role of *CYP3A5*, *CYP2C9* and *CYP2C19* seem to be minor.

Introduction

Tamoxifen is widely used in the treatment and prevention of oestrogen receptor (ER) and/or progesterone receptor (PR) positive breast cancers [1]. It undergoes metabolic activation to form two highly potent metabolites, namely 4-hydroxytamoxifen (4-OHT) formed via 4-hydroxylation of tamoxifen and 4-hydroxy-N-desmethyltamoxifen (endoxifen) resulting from the N-demethylation of 4-OHT. Both metabolites exhibit 100-fold greater affinity for ER and 30- to 100-fold greater potency than tamoxifen [2]. Up to 97% of endoxifen is formed via an alternative pathway involving sequential CYP3A4/5 mediated N-demethylation of tamoxifen to form N-desmethyltamoxifen (NDM) and CYP2D6 catalysed 4-hydroxylation of NDM [3]. Two other enzymes, CYP2C9 and CYP2C19, have been found to play minor roles in these metabolic pathways [4], although recent studies have highlighted an important role of CYP2C19 in influencing treatment response to tamoxifen [5]. There is a wide degree of interindividual and interethnic differences in the steady-state plasma concentrations of tamoxifen and its metabolites which have been shown to influence therapeutic outcome [6, 7]. This area of research has generated intense clinical interest because of its potential to select women with postmenopausal hormone receptor positive early stage breast cancer who are poor metabolizers of tamoxifen to receive alternative adjuvant endocrine therapy with aromatase inhibitors [8]. The deviations in plasma concentrations of tamoxifen and its metabolites can be due to drug–drug interactions or genetic variants present in the genes responsible for encoding the various proteins involved in regulating its disposition [3].

Among the various candidate genes, polymorphic variants in the *CYP2D6* gene play an important role in affecting the catalytic activity of the CYP2D6 enzyme and hence the plasma concentrations of 4-OHT and endoxifen [3, 9–12]. Caucasian breast cancer patients homozygous for the *4 (1846G>A; rs3892097) allele are known to display lower concentrations of endoxifen compared with patients who are *1/*1 carriers [3, 9, 13, 14]. The plasma concentration of endoxifen is also affected by the presence of other null alleles such as *3 (2549delA; rs35742686), *5 (*CYP2D6*del) and *6 (1707delT; rs5030655) [9, 10, 13]. However, the distribution of functional *CYP2D6* polymorphisms varies between breast cancer patients from different ethnic backgrounds which may influence therapeutic outcome to tamoxifen treatment [10].

Although *CYP2D6**4 (1846G>A; rs3892097) is the predominant defective allele in the Caucasian population [3, 9, 13, 14], it is rare in the Asian population (1–3%). Instead, *CYP2D6**5 (*CYP2D6*del) and *CYP2D6**10 (100C>T; rs1065852) alleles [15] have been reported to be more prevalent [16] in Asian populations, occurring at frequencies of approximately 5% to 10% and more than 40%, respectively [17, 18]. A recent study in Korean breast cancer

patients has shown that the steady-state plasma concentrations of 4-OHT and endoxifen were significantly lower and the median time to progression significantly shorter in *CYP2D6**10 homozygotes compared with patients who were wild-type or heterozygotes [19]. In view of the greater prevalence of *CYP2D6**5 (*CYP2D6*del) and *10 (100C>T; rs1065852) among Asian populations, these polymorphisms may be more important in influencing the pharmacokinetics of tamoxifen in Asian breast cancer patients. Apart from CYP2D6, other cytochrome P450 enzymes (CYP3A4/5, CYP2C9 and CYP2C19) also contribute to the overall metabolism of tamoxifen and its metabolites, albeit to different extents [4, 20–23].

On the basis of previous studies, we hypothesize that different functionally defective *CYP2D6* alleles which are operative in the Asian population [*CYP2D6**5 (*CYP2D6*del) and *10 (100C>T; rs1065852)] may impact on the pharmacokinetics of tamoxifen and its active metabolites (4-OHT and endoxifen) and hence affect therapeutic outcome. The objectives of the present study were to study the pharmacogenetics of *CYP3A5*, *CYP2C9*, *CYP2C19* and *CYP2D6* polymorphisms in healthy Asian subjects (Chinese, Malays, Indians) and to delineate the impact of known functional polymorphisms in these genes on the plasma concentrations as well as metabolic ratios of tamoxifen and its metabolites in Asian breast cancer patients.

Methods

Study subjects

The healthy subject population comprised of three ethnic groups predominant in the Asian population of Singapore, namely Chinese, Malays and Indians ($n = 76$ in each group). The ethnicities of all subjects were confirmed by individual screening and verified against their National Registry Identification Cards.

A total of 165 breast cancer patients were recruited prospectively. All patients were histologically diagnosed with ER and/or PR positive breast tumours and received 20 mg tamoxifen daily. Patients who had received inhibitors or inducers of *CYP2D6* within 4 weeks from the time of enrolment were excluded from the study. Patients with any of the following conditions were also excluded from the study: allergy to tamoxifen, pregnancy, expected survival of less than 6 months, prior malignancies other than basal cell carcinoma of the skin or carcinoma *in situ* of the uterine cervix, uncontrolled intercurrent illness such as ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that might limit compliance with the study requirements. The study was approved by the ethics review committee of the National Cancer Centre, Singapore. Written consent was obtained from both healthy subjects and patients for participation in this study.

Pharmacogenetic analyses

Purified genomic DNA was extracted from peripheral blood samples of healthy subjects and patients using the Gentra® Puregene Blood Kit (Qiagen, Inc.). A total of 228 healthy subjects and 165 breast cancer patients were screened for the following polymorphisms: *CYP2D6* [*2 (2850C>T; rs16947), *2A (–1584C>G), *3 (2549delA; rs35742686), *4 (1846G>A; rs3892097), *5 (*CYP2D6*del), *6 (1707delT; rs5030655), *7 (2935A>C; rs5030867), *8 (1758G>T), *9 (2615-2617delAAG; rs5030656), *10 (100C>T; rs1065852), *12 (124G>A; rs5030862), *14 (1758G>A), *17 (1023C>T; rs28371706), *29 (1659G>A; rs61736512), *41 (2988G>A; rs28371725) and *xN (*CYP2D6*dup)], *CYP3A5**3 (6986A>G; rs776746), *CYP2C9* [*2 (430C>T; rs1799853) and *3 (1075A>C; rs1057910)] and *CYP2C19* [*2 (681G>A; rs4244285), *3 (636G>A; rs4986893) and *17 (–3402C>T and –806C>T; rs12248560)]. The polymorphisms in *CYP2D6* were genotyped using the INFINITI™ CYP450 2D6I assay (AutoGenomics, Inc.) in accordance with the manufacturer's protocol. The polymorphic variants in *CYP3A5*, *CYP2C9* and *CYP2C19* were genotyped by amplification via polymerase chain reaction [24, 25], followed by purification by treatment with shrimp alkaline phosphatase and exonuclease I and direct sequencing on a ABI 3730 DNA Analyzer (Applied Biosystems, Inc.).

Pharmacokinetic analyses

Blood (3 ml) was collected from patients who had received a daily dose of 20 mg tamoxifen for 8 weeks or more. The samples were protected from light and immediately centrifuged at 2000 g for 10 min. Plasma was extracted and stored at –80°C until analysis.

The steady-state plasma concentrations of tamoxifen (C_{TAM}) and its metabolites, NDM (C_{NDM}), 4-OHT (C_{4-OHT}) and endoxifen (C_{END}) were quantified by reverse-phase high-performance liquid chromatography with fluorescence detection modified from the methods developed by Lee *et al.* [26] and Zhu *et al.* [27]. Tamoxifen ($\geq 99\%$), *trans*-4-hydroxytamoxifen ($\geq 98\%$) and propranolol (99%) were purchased from Sigma-Aldrich Co., Singapore. *N*-Desmethyltamoxifen and *trans*-endoxifen were kind gifts from Dr D.A. Flockhart at Indiana University School of Medicine (Indianapolis, IN, USA) and Dr Philip Lazarus at Pennsylvania State University College of Medicine (PA, USA), respectively. The analytes were extracted from 200 μ l of plasma with *n*-hexane : *n*-butanol (98:2, v : v), using propranolol as an internal standard. The chromatographic separation of the extract was performed on a C8 analytical column using a mobile phase consisting of acetonitrile and 100 mM ammonium acetate (pH 5.5) with a gradient profile of 30–80% (v : v) of acetonitrile. The limits of quantification for tamoxifen, NDM, 4-OHT and endoxifen were 10.0 ng ml^{–1}, 10.0 ng ml^{–1}, 0.5 ng ml^{–1} and 1.0 ng ml^{–1}, respectively. The calibration curves were linear over the concentration ranges of 10.0–800.0 ng ml^{–1} for tamoxifen ($r^2 = 0.9977$) and NDM ($r^2 = 0.9974$), 0.5–40 ng ml^{–1} for

4-OHT ($r^2 = 0.9993$), and 1.0–80.0 ng ml^{–1} for endoxifen ($r^2 = 0.9973$). The intra-day coefficient of variation was less than 7.0%, while inter-day coefficient of variation was less than 14.0%.

The plasma concentration ratios or metabolic ratios (MRs) were calculated as follows: $MR_{NDM-TAM} = C_{NDM}/C_{TAM}$, $MR_{4-OHT-TAM} = C_{4-OHT}/C_{TAM} (\times 10^2)$, $MR_{END-NDM} = C_{END}/C_{NDM} (\times 10^2)$, and $MR_{END-4-OHT} = C_{END}/C_{4-OHT}$. The total plasma concentration ratios or total metabolic ratios (TMRs) were calculated as follows: $TMR_{4-OHT} = C_{END}/C_{TAM} + C_{4-OHT} (\times 10^2)$ and $TMR_{NDM} = C_{END}/C_{TAM} + C_{NDM} (\times 10^2)$.

Statistical analyses

The chi-squared test was employed to assess for Hardy–Weinberg equilibrium between the genotype frequencies. Genotypic–phenotypic associations were performed using the non-parametric Kruskal–Wallis test and Mann–Whitney *U*-test. The level of significance was set at $P < 0.050$. Bonferroni corrections were applied for multiple comparisons between genotype groups. The levels of significance were set at $P < 0.017$ and $P < 0.005$ for multiple pairwise comparisons across three and five groups, respectively. The statistical analyses were carried out with SPSS version 14.0 (SPSS, Inc.).

Results

Patient demographics and plasma concentrations of tamoxifen and its metabolites

The median age of the breast cancer patients was 49.3 years (range 30–74 years) with 83.0% and 17.0% of patients being premenopausal and postmenopausal, respectively. The median height and weight were 156.0 cm (range 134.0–172.3 cm) and 58.9 kg (range 38.7–91.7 kg). The majority of the patients were Chinese (83.0%) followed by Indians (8.5%) and Malays (7.3%), consistent with the racial composition in Singapore.

The inter-individual variations in plasma concentrations of tamoxifen, NDM and 4-OHT were found to vary between 11- to 20-fold, while that for endoxifen varied by more than 24-fold. The median plasma concentrations of tamoxifen, NDM, 4-OHT and endoxifen in the breast cancer patients were 205.16 ng ml^{–1} (range 39.26–599.91 ng ml^{–1}), 304.05 ng ml^{–1} (40.82–802.98 ng ml^{–1}), 1.96 ng ml^{–1} (0.47–5.33 ng ml^{–1}) and 13.69 ng ml^{–1} (1.74–42.80 ng ml^{–1}), respectively. A high degree of interindividual variation was also observed for the MR (4- to 30-fold) and the TMR (17- to 20-fold). The median MRs and TMRs of the patient population were as follows: $MR_{NDM-TAM} = 1.56$ (range 0.62–2.76), $MR_{4-OHT-TAM} = 1.01$ (range 0.19–2.35), $MR_{END-NDM} = 4.97$ (range 0.92–27.81), $MR_{END-4-OHT} = 6.89$ (range 1.99–13.18), $TMR_{4-OHT} = 6.78$ (range 1.14–19.50) and $TMR_{NDM} = 2.76$ (range 0.51–10.69). None of the patients was prescribed *CYP2D6* inhibitors while they were receiving tamoxifen.

Pharmacogenetics of CYP2D6, CYP3A5, CYP2C and CYP2C19

The allelic frequency distributions of CYP2D6 polymorphisms in 228 healthy Asian subjects (Chinese, Malay and Indian; $n = 76$ each) and 165 Asian breast cancer patients are indicated in Table 1. The CYP2D6 genotypes of four patients could not be determined probably because of poor quality DNA samples.

Wide inter-ethnic variations were observed in the allelic frequencies of CYP2D6 polymorphisms in the healthy Asian populations (Table 1). Pairwise comparisons between the three Asian ethnic groups showed that the frequencies of the various CYP2D6 variants were similar between the Chinese and Malay ethnic groups but differed significantly from the Indians with regards to selected polymorphisms (Table 1). The allelic frequency of CYP2D6*10 (100C>T; rs1065852) was approximately five-fold higher in the healthy Chinese and Malay populations compared with the Indian population (Chinese vs. Malay vs. Indians: 0.56 vs. 0.57 vs. 0.11; $P < 0.001$). Similarly, the allelic frequency of CYP2D6*5 (CYP2D6del) was approximately twofold to threefold higher in the Chinese compared with the Malays and Indians (Chinese vs. Malay vs. Indians: 0.09 vs. 0.04 vs. 0.03; $P = 0.018$; Table 1). The Indian and Malay populations were also found to harbour significantly higher frequencies of CYP2D6*41 (2988G>A; rs28371725) compared with the Chinese population (Indians vs. Malay vs. Chinese: 0.15 vs. 0.09 vs. 0.01; Table 1).

The polymorphisms in CYP2C9 [*2 (430C>T; rs1799853) and *3 (1075A>C; rs1057910)] and CYP2C19 [*2 (681G>A; rs4244285), *3 (636G>A; rs4986893) and *17 (-3402C>T and -806C>T; rs12248560)] were noted to display significant interethnic variations and are depicted in Table 1. The pharmacogenetics of CYP3A5*3 (6986A>G; rs776746) were previously reported in healthy Chinese, Malay and Indian populations by Balram *et al.* [23] and are shown in Table 1 for the purpose of comparison. The allelic and genotypic distribution profiles of the polymorphisms in the candidate genes CYP2D6, CYP3A5, CYP2C9 and CYP2C19 observed in the patient population were found to be similar to those of the healthy Chinese population (Table 1).

Influence of CYP2D6 polymorphisms on the plasma concentrations of tamoxifen and its metabolites

The median (range) plasma concentrations of tamoxifen and the three metabolites observed in patients within each genotypic group are depicted in Table 2. Although the CYP2D6*10 (100C>T; rs1065852) polymorphism was not found to be significantly associated with plasma concentrations of tamoxifen and 4-OHT, a stepwise decrease in median 4-OHT concentration was observed when comparing patients with zero, one or two copies of the CYP2D6*10 (100C>T; rs1065852) allele (Figure 1A,C, Table 2). Significant gene-dose dependent effects were, however, observed

between the CYP2D6*10 (100C>T; rs1065852) allele and the plasma concentrations of NDM and endoxifen (Figure 1B,D, Table 2). The plasma concentration of NDM [median (range)] was approximately 1.6-fold and twofold higher in patients with the *1/*10 genotype [279.43 (115.41–502.13) ng ml⁻¹] and those with *10/*10 genotype [374.41 (84.77–802.98) ng ml⁻¹] compared with patients harbouring reference *1/*1 genotype [174.59 (40.82–448.65) ng ml⁻¹; $P < 0.001$; Figure 1B, Table 2]. Conversely, the median (range) plasma endoxifen concentrations were observed to be approximately 2.5-fold higher in patients carrying the *1/*1 [19.55 (4.18–39.47) ng ml⁻¹] or the *1/*10 [19.74 (7.26–33.24) ng ml⁻¹] genotypes compared with patients who were homozygous for *10 allele [8.03 (1.74–34.68) ng ml⁻¹; $P < 0.001$; Figure 1D, Table 2].

Although no significant associations were observed between CYP2D6*5 (CYP2D6del) polymorphism and the plasma concentrations of tamoxifen and the three metabolites (Table 2), patients harbouring the *1/*5 genotype displayed 30% to 50% higher median plasma concentrations of tamoxifen and NDM as well as 25% to 40% lower median plasma concentrations of 4-OHT and endoxifen compared with patients carrying the reference genotype (Table 2).

There were 12 compound heterozygotes (*5/*10) and their endoxifen concentrations [median (range)] were similar to patients harbouring the *10/*10 genotype [8.03 (1.74–34.68), $P = 0.232$] but significantly lower compared with patients homozygous [19.55 (4.18–39.47), $P < 0.001$] or heterozygous [*1/*5: 14.51 (10.73–26.04), $P = 0.001$; *1/*10: 19.74 (7.26–33.24), $P < 0.001$] for the reference allele (Figure 1D).

With respect to the CYP2D6*41 (2988G>A; rs28371725) allele, no significant trend in the variations of plasma concentrations of analytes was observed. However, patients who carried one or two copies of the *41 (2988G>A; rs28371725) alleles were found to have a 64.0% higher plasma NDM concentration ($P = 0.136$) and a 13.4% lower plasma endoxifen concentration ($P = 0.792$) compared with patients who were homozygous wild type (Table 2).

However, a total of seven patients carrying the CYP2D6*41 (2988G>A; rs28371725) allele were not included in this analysis as they were compound heterozygotes who also harboured other functional alleles such as *10 (100C>T; rs1065852), *14 (1758G>A) and *xN (CYP2D6dup). The presence of these variant alleles may confound the observation of the phenotypic effect of the *41 (2988G>A; rs28371725) allele. Likewise, the effect of CYP2D6 gene amplifications could not be assessed in the present study population as there was only one patient with the *1/*xN genotype. Although five other patients also carried CYP2D6 gene amplifications, the effect of this variant could not be evaluated because of the concomitant presence of *10 (100C>T; rs1065852), *14 (1758G>A) and *41 (2988G>A; rs28371725) variant alleles.

Table 1

Genotypic and allelic frequencies of polymorphisms in *CYP2D6*, *CYP3A5*, *CYP2C9* and *CYP2C19* in healthy Asians (*n* = 76 each) and Asian breast cancer patient (*n* = 165) populations

Polymorphisms	Genotypes	Genotypic frequencies				Allelic frequencies				Breast cancer patients	Indians	Malays	Chinese	Breast cancer patients	Overall P value
		Chinese	Malays	Indians	Breast cancer patients	Alleles	Chinese	Malays	Indians						
CYP2D6															
	CYP2D6*2 (2850C>T; rs16947)	*1/*1	37 (62.7)	47 (68.1)	23 (32.9)	92 (66.2)	*1	0.83	0.83	0.56	0.81	<0.001			
		*1/*2	21 (35.6)	20 (29.0)	33 (47.1)	37 (26.6)	*2	0.17	0.17§	0.44#	0.19				
		*2/*2	1 (1.7)	2 (2.9)	14 (20.0)	10 (7.2)									
	CYP2D6*2A (-1584C>G)	*1/*1	41 (67.2)	57 (81.4)	38 (52.8)	105 (75.6)	*1	0.85	0.90	0.72	0.87	0.002			
*1/*2A		19 (31.1)	12 (17.1)	27 (37.5)	28 (20.1)	*2A	0.15	0.10§	0.28#	0.13					
*2A/*2A		1 (1.6)	1 (1.4)	7 (9.7)	6 (4.3)										
CYP2D6*4 (1846G>A; rs3892097)	*1/*1	50 (98.0)	50 (92.6)	57 (89.1)	135 (99.3)	*1	0.98	0.96	0.91	1.00	0.199				
	*1/*4	1 (2.0)	4 (7.4)	3 (4.7)	1 (0.7)	*4	0.02	0.04	0.09	<0.01					
	*4/*4	0 (0.0)	0 (0.0)	4 (6.3)	0 (0.0)										
		61 (81.3)	70 (92.1)	72 (94.7)	139 (84.2)	*1	0.91	0.96	0.97	0.92	0.018				
CYP2D6*5 (CYP2D6 del)	*1/*5	14 (18.7)	6 (7.9)	4 (5.3)	26 (15.8)	*5	0.09	0.04	0.03#	0.08					
	*5/*5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)										
	*1/*1	50 (92.6)	61 (100.0)	69 (100.0)	98 (96.1)	*1	0.97	1.00	1.00	1.00	0.113				
	*1/*8	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	*8	0.01	–	–	–					
CYP2D6*8*14 (1758G>T/A)	*1/*14	2 (3.7)	0 (0.0)	0 (0.0)	4 (3.9)	*14	0.02	–	–	0.01					
	*8/*8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)										
	*14/*14	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)										
	*8/*14	0 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)										
CYP2D6*9 (2615_2617delAAG; rs5030656)	*1/*1	59 (100.0)	65 (100)	68 (98.6)	139 (100.0)	*1	1.00	1.00	0.99	1.00	0.405				
	*1/*9	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	*9	–	–	0.01	–					
	*9/*9	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)										
		14 (23.0)	12 (17.4)	56 (77.8)	42 (30.2)	*1	0.44	0.43	0.89	0.49	<0.001				
CYP2D6*10 (100C>T; rs1065852)	*1/*10	29 (47.5)	36 (52.2)	15 (20.8)	54 (38.9)	*10	0.56	0.57§	0.11#	0.51					
	*10/*10	18 (29.5)	21 (30.4)	1 (1.4)	43 (30.9)										
	*1/*1	61 (100.0)	68 (98.6)	72 (100.0)	139 (100.0)	*1	1.00	0.99	1.00	1.00	0.380				
	*1/*12	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	*12	–	0.01	–	–					
CYP2D6*12 (124G>A; rs5030862)	*12/*12	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)										
		56 (96.6)	56 (82.4)	51 (73.9)	125 (89.9)	*1	0.99	0.91	0.85	0.95	0.017				
	*1/*41	2 (3.4)	11 (16.2)	17 (24.6)	13 (9.4)	*41	0.01†	0.09	0.15‡	0.05					
	*41/*41	0 (0.0)	1 (1.5)	1 (1.4)	1 (0.7)										
CYP2D6*xN (multiple CYP2D6)	*1	61 (100.0)	69 (98.6)	71 (98.6)	133 (95.7)	*1	1.00	0.99	0.99	0.98	0.648				
	*1/*xN	0 (0.0)	1 (1.4)	1 (1.4)	6 (4.3)	*xN	–	0.01	0.01	0.02					

Table 1
Continued

Polymorphisms	Genotypic frequencies				Allelic frequencies				Breast cancer patients	Overall P value
	Genotypes	Chinese	Malays	Indians	Alleles	Chinese	Malays	Indians		
CYP3A5 CYP3A5*3 [¶] (6986A>G; rs776746)	*1/*1	(8.3)	(10.2)	(12.2)	14	(8.5)	0.30	0.41	0.30	–
	*1/*3	(32.4)	(57.1)	(56.7)	72	(43.6)	0.70	0.59	0.70	
	*3/*3	(59.3)	(32.7)	(31.1)	79	(47.9)				
CYP2C9 CYP2C9*2 (430C>T; rs1799853)	*1/*1	76	(100.0)	76	(100.0)	72	(94.7)	165	(100.0)	0.005
	*1/*2	0	(0.0)	0	(0.0)	4	(5.26)	0	(0.0)	
	*2/*2	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	
CYP2C9*3 (1075A>C; rs1057910)	*1/*1	74	(97.4)	69	(90.8)	63	(82.9)	151	(92.1)	<0.001
	*1/*3	2	(2.6)	7	(9.2)	12	(15.8)	12	(7.3)	
	*3/*3	0	(0.0)	0	(0.0)	1	(1.3)	1	(0.6)	
CYP2C19 CYP2C19*2 (681G>A; rs4244285)	*1/*1	38	(50.0)	44	(57.9)	28	(36.8)	73	(45.1)	<0.001
	*1/*2	28	(36.8)	29	(38.2)	38	(50.0)	66	(40.7)	
	*2/*2	10	(13.2)	3	(3.9)	10	(13.2)	23	(14.2)	
CYP2C19*3 (636G>A; rs4986893)	*1/*1	65	(85.5)	65	(85.5)	75	(98.7)	149	(90.3)	<0.001
	*1/*3	11	(14.5)	11	(14.5)	1	(1.3)	15	(9.1)	
	*3/*3	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.6)	
CYP2C19*17 (–3402C>T and –806C>T; rs12248560)	*1/*1	75	(98.7)	69	(90.8)	50	(65.8)	153	(93.3)	<0.001
	*1/*17	1	(1.4)	7	(9.2)	23	(30.3)	11	(6.7)	
	*17/*17	0	(0.0)	0	(0.0)	3	(3.9)	0	(0.0)	

†Chinese vs. Malay, P < 0.050. ‡Chinese vs. Indian, P < 0.050. §Malay vs. Indian, P < 0.050. ¶Genotypic and allelic frequencies were from previous study by Balam et al. [23].

Table 2
Effects of CYP2D6 polymorphisms on median (range) plasma concentrations of analytes and plasma metabolic ratios ($n = 111$)

Parameters		Genotypes *1/*1 ($n = 13$)		*1/*5 ($n = 9$)		*1/*10 ($n = 31$)		*10/*10 ($n = 40$)		*5/*10 ($n = 12$)		*1/*41 + *41/*41 ($n = 6$)	
Analytes		161.22		216.07		194.88		217.19		169.91		166.42	
Tamoxifen		(50.06–369.89)		(93.56–325.56)		(51.80–421.16)		(84.27–599.91)		(39.26–452.29)		(147.72–578.17)	
NDM		174.59		261.07		279.43		374.41		330.67		286.30	
4-OHT		(40.82–448.65)		(206.94–464.25)		(115.41–502.13)		(84.77–802.98)		(78.46–757.13)		(159.67–511.20)	
Endoxifen		2.49		(0.97–3.36)		1.92		(0.86–4.51)		1.87		2.55	
Plasma metabolic ratios (MRs)		19.55		(4.18–39.47)		19.74		(7.26–33.24)		7.46		16.93	
MR _{END-NDM}		(5.63–14.86)		(4.48–8.00)		6.62		(1.65–11.65)		2.15		(1.94–11.85)	
MR _{4-OHT-TAM}		1.34		(0.74–1.93)		1.08		(0.50–2.26)		0.94		(0.51–2.35)	
Total metabolic ratios (TMRs)		5.03		(2.94–7.45)		3.91		(0.98–7.24)		1.41		(1.35–7.37)	
TMR _{NDM}		11.05		(6.09–14.73)		9.18		(2.39–18.70)		4.29		(3.65–19.04)	
TMR _{4-OHT}													
Pairwise P values		*1/*1 vs. *1/*5		*1/*1 vs. *1/*10		*1/*10 vs. *10/*10		*1/*1 vs. *5/*10		*1/*10 vs. *5/*10		*1/*1 vs. *1/*41 + *41/*41	
Analytes		0.404		0.177		0.578		0.957		0.316		0.430	
Tamoxifen		(0.077)		(0.006)		(0.011)		(0.057)		(0.685)		(0.136)	
NDM		0.616		0.709		0.041		0.174		0.136		0.759	
4-OHT		0.243		0.827		<0.001		<0.001		<0.001		0.792	
Endoxifen													
Plasma metabolic ratios (MRs)		0.001		<0.001		<0.001		<0.001		<0.001		0.054	
MR _{END-NDM}		0.141		0.146		0.006		0.022		0.267		0.219	
MR _{4-OHT-TAM}													
Total metabolic ratios (TMRs)		0.001		<0.001		<0.001		<0.001		<0.001		0.035	
TMR _{NDM}		0.030		0.108		<0.001		<0.001		<0.001		0.054	
TMR _{4-OHT}

Figure 1

Association between CYP2D6*5- and *10-based genotypes and steady-state plasma concentrations of (A) tamoxifen, (B) NDM, (C) 4-OHT and (D) endoxifen

Influence of CYP2D6 polymorphisms on the metabolic ratios of tamoxifen and its metabolites

The median (range) $MR_{4-OHT-TAM}$ of patients carrying the *10/*10 genotype [0.83 (0.19–1.78)] was 1.25 to 1.5 times higher in patients harbouring the *1/*1 [1.34 (0.74–1.93)] or *1/*10 [1.08 (0.50–2.26)] genotypes (Table 2). The $MR_{4-OHT-TAM}$ was found to be comparable among patients harbouring the *1/*1 and *1/*10 genotypes (Figure 2B). Similarly, the median $MR_{END-NDM}$ was found to decrease as the number of *10 alleles increased. Patients with the *1/*1 genotype [10.11 (5.63–14.86)] were observed to have approximately 50% higher $MR_{END-NDM}$ than patients carrying *1/*10 [6.62 (1.62–11.65)], which were in turn twice of those in patients harbouring the *10/*10 genotype [2.31 (0.92–27.81); $P < 0.001$; Figure 2A]. Similar genotypic-phenotypic trends were observed with regards to TMR_{4-OHT} and TMR_{NDM} . The TMR_{4-OHT} median (range) was not found to differ significantly among patients who harboured *1/*1 [11.05 (6.09–14.73)] and *1/*10 [9.18 (2.39–18.70)] geno-

types but was found to be 2.7-fold and 2.2-fold higher in patients who harboured the *10/*10 genotype [4.16 (1.14–17.05); $P < 0.001$; Figure 2D]. A gradual decrease in TMR_{NDM} was observed across the different genotypes. Approximately 1.3-fold difference in TMR_{NDM} was observed between *1/*1 and *1/*10 carriers while a 2.8-fold difference in TMR_{NDM} was found between *1/*10 and *10/*10 carriers.

A non-significant 20% decrease in $MR_{4-OHT-TAM}$ [median (range)] was observed between patients carrying the *1/*1 [1.34 (0.74–1.93)] and *1/*5 [10.11 (5.63–14.86)] genotypes (Figure 2B). However, the $MR_{END-NDM}$ [median (range)] observed in patients who were heterozygous for the *5 allele [5.29 (4.48–8.00)] was found to be half of that observed in the homozygous wild type [10.11 (5.63–14.86)] patients (Figure 2A). Likewise, a 1.3 to 1.5 times lower TMR_{4-OHT} and TMR_{NDM} were observed in *1/*5 carriers compared with *1/*1 carriers (Figure 2C,D).

The median MRs and TMRs were found to be similar between patients carrying *10/*10 and *5/*10 genotypes

Figure 2

Association between *CYP2D6**5- and *10-based genotypes and plasma metabolic ratios: (A) MR_{END-NDM}, (B) MR_{4-OHT-TAM}, (C) TMR_{NDM} and (D) TMR_{4-OHT}

but significantly lower compared with patients who harboured *1/*1 genotypes (Figure 2A–D). The MR_{END-NDM} [median (range)] in *5/*10 [2.15 (1.06–3.30)] carriers was found to be 4.7-fold, 2.5-fold and 3.1-fold lower than that observed in *1/*1 [10.11 (5.63–14.86)], *1/*5 [5.29 (4.48–8.00)] and *1/*10 [6.62 (1.65–11.65)] carriers, respectively (Table 2). The relationship between TMR_{NDM} and genotypes was parallel to that observed with respect to MR_{END-NDM} (Figure 2A,C).

A non-significant relationship between *CYP2D6**41 (2988G>A; rs28371725) and median MRs was observed. The MR_{END-NDM} and MR_{4-OHT-TAM} were found to be 1.6 and 1.3 times lower in patients who were heterozygous or homozygous for *41 (2988G>A; rs28371725) compared with patients who were homozygous for the reference allele, respectively (Table 2). Similarly, the plasma TMRs were observed to be lower among *41 (2988G>A;

rs28371725) carriers. The TMR_{NDM} and TMR_{4-OHT} [median (range)] were found to be 1.53-fold and 1.68-fold lower in patients who harboured *1/*41 or *41/*41 genotypes [TMR_{NDM}: 3.27 (1.35–7.37); TMR_{4-OHT}: 6.59 (3.65–19.04)] compared with patients who were *1/*1 [TMR_{NDM}: 5.03 (2.94–7.45), $P = 0.035$; TMR_{4-OHT}: 11.05 (6.09–14.73), $P = 0.054$].

Influence of CYP3A5, CYP2C9 and CYP2C19 polymorphisms on the plasma concentrations of tamoxifen and its metabolites

The relationships between the polymorphic variants in *CYP3A5*, *CYP2C9* and *CYP2C19* and plasma concentrations of the analytes were not found to be statistically significant (Table 3). As there were no patients carrying the *CYP2C9**2 (430C>T; rs1799853) allele, the effect of *CYP2C9**2 (430C>T; rs1799853) could not be evaluated.

Table 3

Effects of CYP2C19 polymorphisms on median (range) plasma concentrations (ng mL⁻¹) and metabolic ratios of tamoxifen and its analytes (n = 165)

Parameters CYP2C19*2 (681G>A; rs4244285)	Genotypes		*1/*2 (n = 44)		*2/*2 (n = 22)		Pairwise P value		
	*1/*1 (n = 40)						*1/*1 vs. *1/*2	*1/*1 vs. *2/*2	*1/*2 vs. *2/*2
Analytes									
Tamoxifen	190.05	(50.06–578.17)	204.38	(39.26–599.91)	186.28	(84.27–421.16)	0.736	0.783	0.955
NDM	346.62	(40.82–734.94)	284.90	(78.46–802.98)	305.23	(84.77–757.13)	0.392	0.421	0.996
4-OHT	1.78	(0.70–5.02)	2.10	(0.47–5.33)	2.23	(0.87–4.51)	0.305	0.480	0.749
Endoxifen	10.47	(3.48–42.80)	14.51	(1.79–42.56)	16.34	(1.74–28.64)	0.358	0.246	0.553
Plasma metabolic ratios (MRs)									
MR _{NDM-TAM}	1.63	(0.82–2.76)	1.51	(0.62–2.65)	1.44	(1.01–2.10)	0.208	0.088	0.470
MR _{END-4-OHT}	6.63	(3.70–13.18)	7.05	(2.44–11.96)	6.97	(1.99–12.34)	0.793	0.428	0.613
Total metabolic ratios (TMRs)									
TMR _{NDM}	2.16	(0.65–7.46)	3.05	(0.51–10.69)	3.42	(1.03–5.86)	0.387	0.421	0.650
TMR _{4-OHT}	5.96	(1.71–19.50)	7.28	(1.14–18.00)	7.34	(2.04–15.33)	0.408	0.438	0.836
CYP2C19*3 (636G>A; rs4986893)	*1/*1 (n = 40)		*1/*3 + *3/*3 (n = 8)		*1/*1 vs. *1/*3 + *3/*3				
Analytes									
Tamoxifen	190.05	(50.06–578.17)	211.17	(164.79–364.69)			0.138		
NDM	346.62	(40.82–734.94)	323.23	(215.01–590.65)			0.415		
4-OHT	1.78	(0.70–5.02)	1.92	(1.10–3.82)			0.800		
Endoxifen	10.47	(3.48–42.80)	13.69	(4.74–23.04)			0.935		
Plasma metabolic ratios (MRs)									
MR _{NDM-TAM}	1.63	(0.82–2.76)	1.53	(1.06–1.95)			0.328		
MR _{END-4-OHT}	6.63	(3.70–13.18)	5.61	(3.74–11.69)			0.786		
Total metabolic ratios (TMRs)									
TMR _{NDM}	2.16	(0.65–7.46)	1.99	(0.75–5.52)			0.395		
TMR _{4-OHT}	5.96	(1.71–19.50)	4.55	(1.83–11.74)			0.405		
CYP2C19*17 (-3402C>T and -806C>T; rs12248560)	*1/*1 (n = 40)		*1/*17 (n = 6)		*1/*1 vs. *1/*17				
Analytes									
Tamoxifen	190.05	(50.06–578.17)	211.65	(100.24–369.89)			0.887		
NDM	346.62	(40.82–734.94)	318.49	(159.67–403.52)			0.404		
4-OHT	1.78	(0.70–5.02)	2.68	(0.96–4.84)			0.190		
Endoxifen	10.47	(3.48–42.80)	18.35	(6.37–31.05)			0.416		
Plasma metabolic ratios (MRs)									
MR _{NDM-TAM}	1.63	(0.82–2.76)	1.64	(0.92–1.77)			0.422		
MR _{END-4-OHT}	6.63	(3.70–13.18)	6.66	(4.50–10.06)			0.815		
Total metabolic ratios (TMRs)									
TMR _{NDM}	2.16	(0.65–7.46)	2.94	(1.67–7.37)			0.140		
TMR _{4-OHT}	5.96	(1.71–19.50)	6.78	(3.65–19.04)			0.281		

Influence of CYP3A5, CYP2C9 and CYP2C19 polymorphisms on the metabolic ratios of tamoxifen and its metabolites

The MRs were not found to differ between the different genotypes of CYP3A5*3 (6986A>G; rs776746). However, higher inter-individual variations in MRs and TMRs were observed as the number of CYP3A5*3 (6986A>G; rs776746) variant alleles increased. Similarly, the plasma metabolic ratios were not found to vary considerably between patients carrying different CYP2C9 and CYP2C19 (Table 3) variants.

Discussion

The metabolism of tamoxifen is complex and is mediated by multiple cytochrome P450 enzymes and polymorphisms in genes encoding these enzymes may influence the plasma concentrations of tamoxifen and its metabolites. In this exploratory study, we investigated the effects of previously reported polymorphisms in genes encoding the major enzymes involved in the metabolism of tamoxifen and its metabolites in Asian populations.

In the present study, the *CYP2D6**10 (100C>T; rs1065852) allele was correlated with higher NDM and a lower endoxifen concentrations in the plasma while associations with tamoxifen and 4-OHT were not found to be significant. A similar trend was observed when the phenotypic effect of *CYP2D6**5 (*CYP2D6*del) was examined in association with the *10 (100C>T; rs1065852) allele (Figure 1A–D). This is suggestive of an accumulation of NDM in the plasma when the metabolic conversion of NDM to endoxifen is impaired. This leads to the postulation that *CYP2D6*-mediated 4-hydroxylation of NDM is the rate-limiting step in the formation of endoxifen instead of the *CYP3A4/5*-catalysed N-demethylation of tamoxifen. The plasma concentrations of NDM and endoxifen were comparable between patients with *1/*5 and *1/*10 as well as *5/*10 and *10/*10 genotypes, implying that the functional impact of the *5 (*CYP2D6*del) allele is comparable with that of the *10 (100C>T; rs1065852) allele. These observations differed slightly from those reported by Lim *et al.* [19] in Koreans and Xu *et al.* [28] in Chinese. Lim *et al.* [19] demonstrated significant reductions in the steady-state plasma concentrations of both active metabolites (4-OHT and endoxifen) in patients with either *10 (100C>T; rs1065852), *5 (*CYP2D6*del) or both alleles compared with patients who were homozygous wild type. Likewise, Xu *et al.* [28] reported an association between the presence of two copies of the *10 (100C>T; rs1065852) allele and lower plasma concentrations of 4-OHT in a Chinese population. The discrepancy in the results obtained could be due to the difference in the *CYP2D6* alleles screened in each study. The *5 (*CYP2D6*del), *10 (100C>T; rs1065852) and *2 × *N* (*CYP2D6**2dup) alleles were screened in the Koreans while only the *10 (100C>T; rs1065852) allele was screened in the Chinese. In contrast, a comprehensive screening of 16 functionally important polymorphisms was performed using the INFINITI™ *CYP450* 2D6 assay in the current study. Recently, Schroth *et al.* [29] reported a discrepancy in the assigned phenotype status because of the difference in number of *CYP2D6* polymorphisms screened. The investigators concluded that comprehensive screening of *CYP2D6* polymorphisms provides more accurate prediction of *CYP2D6* phenotype status.

The major active metabolite, endoxifen, can be formed from tamoxifen via two pathways involving either NDM or 4-OHT. *CYP2D6* has been shown to play a major role in the 4-hydroxylation of NDM and tamoxifen to endoxifen and 4-OHT, respectively. To elucidate the influence of the *CYP2D6**5 (*CYP2D6*del) and *10 (100C>T; rs1065852) polymorphisms on the formation of the active metabolites, the associations between the two genetic variants and the MRs were examined. The metabolic ratios, MR_{4-OHT-TAM} and MR_{END-NDM} were found to decrease in a stepwise manner (Figure 2A,B) as the copies of variant alleles [*CYP2D6**5 (*CYP2D6*del) or *10 (100C>T; rs1065852)] increased. The effects were more apparent than those observed on the plasma concentrations of the analytes. These results

suggest a correlation between the presence of *5 (*CYP2D6*del), *10 (100C>T; rs1065852) or both alleles and a significantly lower rate of catalytic conversion of tamoxifen and NDM to 4-OHT and endoxifen, respectively.

To compare the relative impact of *CYP2D6* polymorphisms on the formation of endoxifen via the NDM or 4-OHT pathways, the associations between *CYP2D6**5 (*CYP2D6*del) and *10 (100C>T; rs1065852) polymorphisms and the TMRs were studied. Consistent with the observations in MRs, both TMRs were observed to be lower in patients who were homozygous variant or compound heterozygotes with a more dominant effect being observed in TMR_{NDM}. These observations further support that *CYP2D6* plays a critical role in the biosynthesis of endoxifen although other enzymes are involved. Furthermore, the data imply that *CYP2D6* polymorphisms are more influential in the 4-hydroxylation of NDM to form endoxifen.

Borges *et al.* [13] and Gjerde *et al.* [14] have previously examined the effects of *CYP2D6* polymorphisms on metabolic ratios in two studies involving Caucasian populations. In both studies, patients were classified as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) or ultrarapid metabolizers (UM) based on their *CYP2D6* genotype status. Correlations between *CYP2D6* genotypes and metabolic ratios were investigated indirectly by studying the relationships between the metabolizing status and the metabolic ratios. The patients who were EM or UM exhibited higher plasma concentrations of endoxifen and MR_{END-NDM} compared with patients who were IM or PM [13, 14]. In addition, Gjerde *et al.* [14] observed an association between the metabolizing status and MR_{4-OHT-TAM} in their study population, although the effect was not as prominent compared with the association between the metabolizing status and MR_{END-NDM}. The results of our study were consistent with those obtained by Borges *et al.* and Gjerde *et al.*

In addition, the present study evaluated the effect of *CYP2D6**41 (2988G>A; rs28371725) on the disposition profile of tamoxifen which was not investigated previously. Our study showed that *CYP2D6**41 (2988G>A; rs28371725) was associated with a non-significant increase in the plasma concentration of NDM and a corresponding decrease in the plasma concentration of endoxifen. The metabolic ratios except TMR_{NDM} were also found to be lower in *CYP2D6**41 (2988G>A; rs28371725) carriers suggesting lower rates of endoxifen formation in patients carrying the *CYP2D6**41 (2988G>A; rs28371725) allele. However, the number of study subjects carrying the *41 (2988G>A; rs28371725) allele was only six. Although additional *CYP2D6**41 (2988G>A; rs28371725) carriers were present, these patients were compound heterozygotes who harboured other functional alleles that may confound the phenotypic functional impact of the *CYP2D6**41 (2988G>A; rs28371725) allele. Similarly, the effect of *CYP2D6* gene amplifications in the present study population was not discernible because of its being present as compound

heterozygotes in several patients. Hence the impact of *41 (2988G>A; rs28371725) as well as *xN (CYP2D6dup) should be further evaluated in a larger homogenous patient population in which these two variants are more prevalent.

Besides CYP2D6, CYP3A5 is another major enzyme involved in the metabolism of tamoxifen. It catalyses the N-demethylation of tamoxifen to NDM or 4-OHT to endoxifen and the impact of the CYP3A5*3 (6986A>G; rs776746) polymorphism on the pharmacokinetics of tamoxifen was investigated. Although Jin *et al.* [9] previously observed a non-significant increase in endoxifen concentrations in patients harbouring *1/*1 and *1/*3 genotypes compared with patients carrying *3/*3 genotype, the plasma concentrations of endoxifen or other analytes were not found to vary considerably across different genotypes in our study population. Correspondingly, Tucker *et al.* [30] also did not find any significant association between CYP3A5*3 (6986A>G; rs776746) and the plasma concentrations of tamoxifen and its metabolites. It is likely that the functional impact of CYP3A5*3 (6986A>G; rs776746) on the metabolism of tamoxifen is nullified by the presence of other enzymes (CYP3A4, CYP1A2 and CYP2C9) which catalyse the N-demethylation of tamoxifen and 4-OHT (CYP3A4). Hence, further investigations on the influence of these polygenic determinants on the pharmacokinetics of tamoxifen are warranted.

CYP2C9 and CYP2C19 polymorphisms were investigated for associations with tamoxifen pharmacokinetics. No associations between the polymorphisms in CYP2C9 and CYP2C19 and the pharmacokinetics of tamoxifen were observed, consistent with reports from previous studies. Interestingly, Schroth *et al.* [5] reported a positive association between CYP2C19*17 (−3402C>T and −806C>T; rs12248560) and longer relapse-free time as well as disease-free survival in patients with one copy of the CYP2D6 null allele [CYP2D6*5 (CYP2D6del) or *4 (1846G>A; rs3892097)]. The polymorphism CYP2C19*17 (−3402C>T and −806C>T; rs12248560) has been reported to cause an increase in the rate of transcription of CYP2C19 leading to an increase in enzymatic activity. No statistically significant association was found in our study between CYP2C19*17 (−3402C>T and −806C>T; rs12248560) and plasma concentrations or MRs of the analytes. This may possibly be because of the lower allelic frequency in our small patient population compared with that observed in the Caucasian population (Asian Study population vs. Caucasian population: 0.06 vs. 0.24). Sub-analysis of the impact of CYP2C19*17 (−3402C>T and −806C>T; rs12248560) in patients carrying the CYP2D6*5 (CYP2D6del) allele was not possible as only one *1/*5 carrier harboured the CYP2C19*17 (−3402C>T and −806C>T; rs12248560) allele. The frequency of the CYP2C19*17 (−3402C>T and −806C>T; rs12248560) allele observed in the Indians was found to be comparable with the Caucasians [31]. Thus, the effect of the allele should be re-examined in a larger cohort of Indian breast cancer patients.

In conclusion, the present study indicates that CYP2D6*5 (CYP2D6del) and *10 (100C>T; rs1065852) are important factors that influence the plasma concentrations of tamoxifen and its metabolites, as well as metabolic ratios of tamoxifen, in a breast cancer population which consisted predominantly of Chinese subjects. The impact of other CYP2D6 polymorphic variants needs to be evaluated further in our minor ethnic groups (Malays and Indians) owing to the high inter-ethnic variation observed. In addition to the phase I metabolic enzymes investigated in the current study, the pharmacokinetics of tamoxifen may also be influenced by uridine 5'-diphosphoglucuronyltransferases (UGT) and sulfotransferases. Therefore, future studies should elucidate the impact of polymorphisms present in other candidate genes encoding the enzymes implicated in the metabolic pathway of tamoxifen.

Competing Interests

There are no competing interests to declare.

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