ORIGINAL RESEARCH

CYP2D6 polymorphisms influence the efficacy of adjuvant tamoxifen in Thai breast cancer patients

Ekaphop Sirachainan[1]
Sureerat Jaruhathai[1]
Narumol Trachu[2]
Ravat Panvichian[1]
Thitiya Sirisinha[1]
Touch Ativitavas[1]
Vorachai Ratanatharathorn[1]
Montri Chamnanphon[3]
Chonlaphat Sukasem[3]

[1]Division of Medical Oncology, Department of Medicine, [2]Research Center, Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [3]Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Correspondence: Ekaphop Sirachainan
Division of Medical Oncology, Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Rama 6 Road, Phayathai, BKK, 10400, Thailand
Tel +66 2201 1671
Fax +66 2201 1671
Email ekaphop.sir@mahidol.ac.th

Chonlaphat Sukasem;
Division of Pharmacogenetics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Rama 6 Road, Phayathai, BKK, 10400, Thailand
Tel +66 2200 4331
Fax +66 2200 4332
Email chonlaphat.suk@mahidol.ac.th

Aim: We evaluated single nucleotide polymorphisms (SNPs) of *CYP2D6* to identify those that influence the efficiency of tamoxifen in adjuvant treatment of breast cancer through a matched case–control study.

Methods: Peripheral blood DNA was collected from 20 patients with disease recurrence during adjuvant tamoxifen treatment and from 19 patients who had completed 5 years of tamoxifen therapy without recurrence of breast cancer. *CYP2D6*4* (1846G > A; rs3892097), *CYP2D6*10* (100C > T, rs1065852), and *CYP2D6*5* (deletion) were genotyped. The correlation between disease-free survival (DFS) and genotype and clinical outcome were assessed using Kaplan–Meier analysis and a log-rank test.

Results: We found the allelic frequency of *CYP2D6*10* during this study. Patients with the *CYP2D6*10* homozygous variant T/T genotype had a significantly shorter median of DFS than those with C/T ($P = 0.036$), but DFS was not significantly different from that of patients with the C/C genotype ($P = 0.316$). One patient who was a carrier both of *CYP2D6* G/A (1846G > A) and T/T (100C > T) had DFS of 22.7 months.

Conclusions: This study demonstrated that *CYP2D6*10/*10* was significantly associated with shorter DFS in Thai breast cancer patients receiving tamoxifen. This was a pilot study investigating the correlation of *CYP2D6* polymorphisms and their influence on clinical outcomes in Thai estrogen receptor-positive breast cancer patients.

Keywords: breast cancer, *CYP2D6* polymorphism, pharmacogenetics, single-nucleotide polymorphism (SNP), tamoxifen

Introduction

Tamoxifen is a prodrug that requires conversion to active metabolites by the cytochrome P450 enzyme CYP2D6, which is one of the key enzymes for the formation of N-desmethyltamoxifen and 4-hydroxytamoxifen.[1,2] N-desmethyltamoxifen is further metabolized to endoxifen, which has more potent antiestrogenic effects than tamoxifen.[3] Breast cancer is currently the most common cancer in Thai women. Tamoxifen is the most commonly prescribed drug for the treatment and prevention of recurrence for patients with estrogen receptor (ER)-positive disease.[4,5] However, the clinical response to tamoxifen varies widely among patients; not all ER-positive breast cancer patients benefit from tamoxifen therapy.[6] Recent studies suggest that inherited variations in enzymatic activity responsible for the metabolism of tamoxifen affects the risk of breast cancer recurrence in patients receiving adjuvant tamoxifen.[7–9] Patients receiving tamoxifen who either carry genetic variants associated with low or absent *CYP2D6* activity have a significantly lower plasma level of endoxifen.[3,10–12]

The frequency of the *CYP2D6* poor metabolizer phenotype varies across different populations.[13] Caucasians have the highest frequency of the poor metabolizer phenotype at a frequency of 7%–10%, with *CYP2D6*4* (1846G > A) being a major variant.[14–18] Only 1%–3% of poor metabolizers from the Asian population have *CYP2D6*5* (gene deletion) as a major variant. However, the allele *CYP2D6*10* (100C > T) is a major variant that has been observed with a frequency of 40%–50% in Asian populations.[19–22]

Previous studies have shown that an association exists between *CYP2D6*4* genotypes and clinical outcomes in Caucasians.[23] Chinese women with the *CYP2D6*10* (100C > T) T/T genotype have a significantly worse disease-free survival (DFS) than those with the C/C or C/T genotype.[20] However, the association between the *CYP2D6* genotype and tamoxifen efficacy in Thai patients has never been reported. The present study examined which *CYP2D6* polymorphisms influence tamoxifen efficacy in Thai patients receiving adjuvant treatment for breast cancer. We conducted a matched case–control study in breast cancer patients who received adjuvant tamoxifen to determine the association of *CYP2D6* polymorphisms and DFS.

Methods
Patients and study design
We enrolled 39 breast cancer patients in Ramathibodi Hospital who met our inclusion criteria, including (1) histological diagnosis of estrogen- and/or progesterone receptor-positive breast cancer, (2) received tamoxifen as an adjuvant treatment for breast cancer, and (3) age at diagnosis > 18 years old. Exclusion criterion for patients in our study included if patients had started tamoxifen therapy along with other cytotoxic chemotherapy or any other endocrine therapy besides tamoxifen. Women taking other medicines known to inhibit CYP2D6 activity were also excluded. All subjects provided written consent before study entry. This study was approved by Ramathibodi's ethics committee.

Patients were classified into case and control groups. Case patients consisted of patients who had recurrence of breast cancer while receiving tamoxifen; patients who had already completed 5 years of adjuvant tamoxifen treatment were included as controls. Patient data was collected from medical records. We matched one control subject per case patient by age at diagnosis of breast cancer, menstruation status, type of surgery, date of surgery, ER/progesterone receptor (PgR) status, Her-2 status, histologic grading tumor, surgery margin status, lymphovascular involvement status, T stage of tumor, nodal involvement, number of nodes dissection, start and

stop date of chemotherapy either neoadjuvant or adjuvant setting, start and stop date of adjuvant radiotherapy, start and stop date of tamoxifen. Date and site of the first disease relapse were collected.

Genomic analysis
Genomic DNA was extracted and amplified by PCR. The *CYP2D6*4* (1846G > A; rs3892097) allele was detected by using restriction fragment length polymorphism (RFLP) techniques with the enzyme BstNI. The *CYP2D6*10* (100C > T; rs1065852) allele was detected using DNA sequencing techniques with Big Dye Terminator Cycle Sequencing Kit and the ABI 3100 Genetic Analyzer (all from Applied Biosciences, Foster City, CA). We analyzed sequences using Chroma software to compare the DNA sequence alignment with a wild type reference. Resequencing was conducted using a reverse primer. TaqMan® Copy Number Assays (Applied Biosystems) were used to genotype the *CYP2D6*5* (Hs.04083572_cn). Applied Biosystems CopyCaller™ Software was used for copy number analysis.

End points and statistical analyses of associations
An association between *CYP2D6* polymorphisms and any influence of tamoxifen efficacy in adjuvant treatment of breast cancer and DFS were analyzed in this study. DFS was defined as the time from surgery to the occurrence of breast event (ie, local, regional or distant occurrence or contralateral breast cancer) or death from any cause. Patients who were alive without a breast recurrence were censored at the date of their last disease evaluation. The overall distribution of DFS was estimated using Kaplan–Meier methods. A log-rank test was used to assess the association between the factor and the event of interest.

Results
Patient characteristics
Patient characteristics are shown in Table 1. Among the 39 subjects, median age was 46.12 years (range 30–66 years). Overall, patient baseline characteristics were similar, including age, menopausal status, tumor size, tumor grading, and ER status, although a higher percentage had lymphovascular involvement, progesterone receptor and Her-2 status positive in group 1. The numbers of pre- and postmenopausal patients were 31 and 8, respectively. Median DFS was 31.05 months and 96.4 months in case and control groups, respectively ($P < 0.005$). Median follow-up time was 52.3 and 97.4 months in case and control groups, respectively ($P = 0.02$).

Table 1 Patient characteristics

Characteristic	Patients arm I (case group) n = 20	Patient arm 2 (control group) n = 19	P-value
Age, years			
<35	14	2	
35–55	3	14	
>55	3	3	
Median age (years)	45.8	46.47	0.832
Median DFS (months)	31.05	96.4	<0.005
Median time F/U (months)	52.3	97.4	0.02
Menopausal status			
Premenopausal	16 (80%)	15 (78.9%)	0.935
Postmenopausal	4 (20%)	4 (21.1%)	
Tumor size (cm)			
<2	4 (20%)	3 (15.8%)	0.943
2–5	15 (75%)	15 (78.9%)	
>5	I (5%)	I (5.3%)	
Grading tumor			
I	3 (15%)	3 (15.8%)	0.831
2	11 (55%)	9 (47.4%)	
3	3 (15%)	2 (10.5%)	
Unknown	3 (15%)	5 (26.3%)	
No of positive nodes			
0	10 (50%)	9 (47.4%)	0.987
1–3	5 (25%)	5 (26.3%)	
≥4	5 (25%)	5 (26.3%)	
Lymphovascular invasion			
Positive	18 (90%)	10 (52.6%)	0.01
Negative	2 (10%)	9 (47.4%)	
ER status			
Positive	20 (100%)	19 (100%)	
Negative	0	0	
PgR status			
Positive	13 (65%)	4 (21%)	<0.005
Negative	3 (15%)	I (5.3)	
Unknown	4 (20%)	14 (73.7%)	
Her-2 status			
Positive 3+	2 (10%)	0 (0%)	<0.005
Negative	10 (50%)	2 (10.5%)	
Unknown	8 (40%)	17 (89.5%)	

Abbreviations: DFS, disease-free survival; ER, estrogen receptor; F/U, follow-up; PgR, progesterone receptor.

Genotype and allele frequency

We determined *CYP2D6*10* genotypes of the 39 patients. A total of 19 patients (19/39; 48.7%) were homozygous (T/T); 11 (11/20; 55%) and 8 (8/19; 42.1%) patients were in the case and control groups, respectively. A total of 14 patients (14/39; 35.9%) were heterozygous (C/T); 4 (4/20; 20%) patients were in the case and 10 (10/19; 52.6%) patients were in the control groups. Six patients (6/39; 15.3%)

were wild-type (C/C); 5 (25/20; 25%) patients were in the case group (1/19; 5.2%) and 1 patient was in the control group. No patients were homozygous for *CYP2D*4* (1846G > A). We found the heterozygous *CYP2D6*4* genotype in 1 patient (2.56%). Allele frequency of *CYP2D6*4* (1846G > A) was 1.28%. A total of 38 patients (97.43%) carried the wild-type allele. The *CYP2D6*5* (deletion) was not found in this population. Details of genotype and allele frequencies in each group are shown in Table 2.

Clinical outcome by genotype

Patients with *CYP2D6*10* homozygous variant (T/T; *CYP2D6*10/*10*) had a significantly shorter DFS than those with the heterozygous variant (C/T; *CYP2D6 Wt/*10*) (*P* = 0.036, Figure 1A). Furthermore, there was a significant difference in DFS between homozygous wild-type (C/C; CYP2D6 Wt/Wt and heterozygous variant (C/T; *CYP2D6 Wt/*10*) (*P* = 0.008, Figure 1C). In contrast to many previous studies, there was no significant difference in DFS between heterozygous variants, homozygous variants (T/T; *CYP2D6*10/*10*), and homozygous wild-type patients (C/C; *CYP2D6 Wt/Wt*) (*P* = 0.316, Figure 1B). Because we found the heterozygous *CYP2D6*4* G/A (1846G > A) only in one patient, we did not analyze this association. Notably, this patient also had *CYP2D6*10/*10* (T/T) and was classified as an intermediate metabolizer. Her DFS was only 22.7 months.

Discussion

Many recent studies have demonstrated a correlation between *CYP2D6* polymorphisms and the efficacy of tamoxifen treatment in ER-positive breast cancer.[13,24,25] Most studies were in Caucasians, which found that *CYP2D6*4* causes a poor metabolizer phenotype in

Table 2 Genotype and allele frequencies

Polymorphisms	Allele frequencies		P-value
	Case (n = 20)	Control (n = 19)	
*CYP2D6*10* (100C > T)			0.058
T/T	11 (55%)	8 (42.1%)	
C/T	4 (20%)	10 (52.6%)	
C/C	5 (25%)	I (5.3%)	
*CYP2D6*4* (1846G > A)			0.323
A/A	0 (0%)	0 (0%)	
G/A	I (5%)	0 (0%)	
G/G	19 (95%)	19 (100%)	
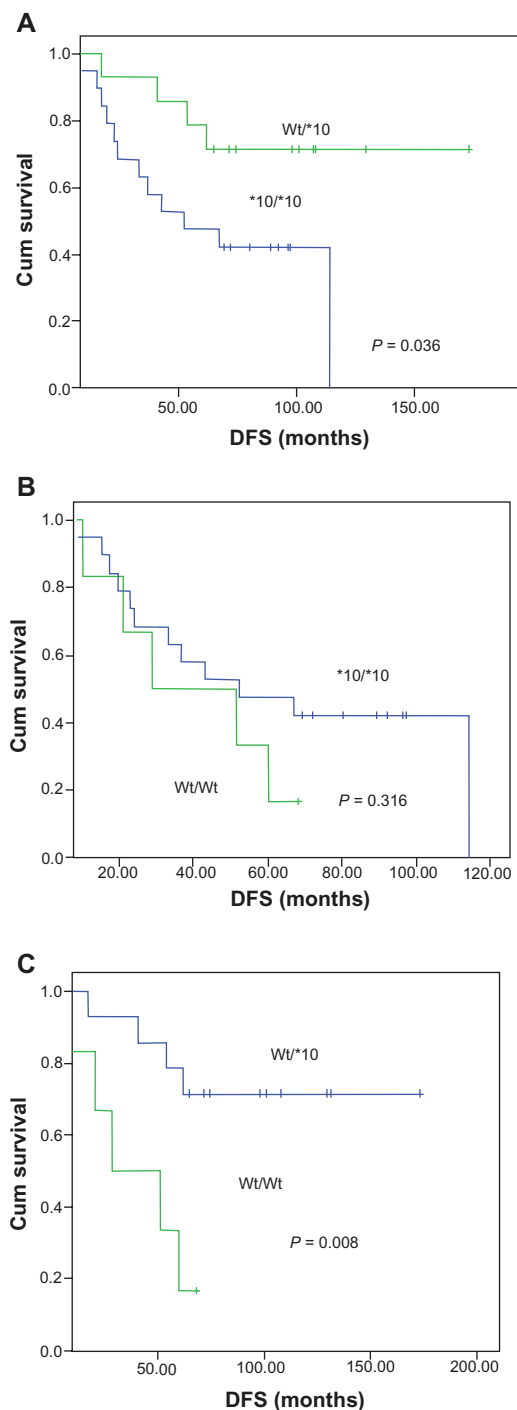
Figure I Kaplan–Meier estimates of disease-free survival (DFS) with the *CYP2D6*10* genotype in 39 Thai breast cancer patients receiving tamoxifen treatment. Patients with the *CYP2D6*10* homozygous variant T/T genotype were compared with patients with heterozygous C/T (**A**) or homozygous wild-type C/C genotype (**B**) and heterozygous C/T compared with homozygous wild-type C/C genotype (**C**).

12%–20% of the Caucasian population. *CYP2D6*4* was found in only 1%–2% of the Asian population, while *CYP2D6*10* (100C > T) causes the intermediate metabolizer phenotype and is found in 40%–50% of the Asian population.[11,26] Several studies have been performed primarily

in Caucasian women.[14–17] The relatively few studies in Asian women[19,20] have suggested that *CYP2D6* status is associated with outcomes in prevention, adjuvant, and metastatic settings. Recently, studies in Asian populations report a correlation between *CYP2D6*10* and the impact on the clinical outcome of ER-positive breast cancer.[19,20,22] However, whether *CYP2D6*10* genotypes influence the clinical effect in Thai breast cancer patients is still unclear.[21,22] This study was the first to explore this correlation in Thai patients. We designed this case–control study to investigate the relationship between *CYP2D6*10* and clinical outcomes.

Our study found a high frequency of the *CYP2D6*10* homozygous variant T/T genotype in 48.71% of patients, which agrees with the frequency of 40%–50% found in previous studies.[3] *CYP2D6*4/*4* (G/G; 1846G > A) was not detected in our study, which agrees with previous reports that *CYP2D6*4/*4* is rare in Asian populations (1%–3%).[19–22] The SNP of *CYP2D6*10* (100C > T) that we found in our study had previously been reported as a common SNP in Asian populations.[20–22] Our study found that patients with the T/T genotype had a significantly shorter DFS than those with the C/T genotype, but DFS was not significantly different compared with the C/C genotype. This is in contrast to previous reports.[21]

We then explored the baseline characteristics of all patients, including the addition of the chemotherapy regimen, which we thought might impact the outcome. We found that three of six *CYP2D6* (C/C; 100C > T) patients had involvement of more than four lymph nodes and that this proportion was higher than for the other two genotypes, but not statistically significant. The regimen of chemotherapy was not a confounding factor in this study. This may be due to the small number of patients in the study. However, we matched one control subject per case patient to avoid clinical confounding factors in this analysis. Other limitations of this case–control study include our inability to control other confounding factors, such as compliance with tamoxifen treatment and the family history of breast cancer, which may influence clinical outcome. However, we still found a trend of lower DFS in patients with the *CYP2D6*10* homozygous variant T/T genotype when analyzed and compared with the other two genotypes, although statistical significance was not reached. We consider that this is acceptable in a pilot study such as this, but future research should include a larger patient sample population and be prospective in order to accurately identify the correlation between *CYP2D6*10*, and *CYP2D6*5* polymorphisms and clinical outcome in Thai ER-positive breast cancer patients.

In summary, our findings demonstrated a high frequency of the *CYP2D6*10* homozygous variant T/T genotype, similarly to observations in other Asian populations. This genotype could lead to a reduced benefit of adjuvant tamoxifen and negatively affect the clinical outcome in Thai ER-positive breast cancer patients. In addition, it is reasonable to explore *CYP2D6*10* rather than *CYP2D6*4* due to the higher frequency of *CYP2D6*10*, even in a small patient sample, such as in this study.

Disclosure

The authors declare that they have no competing or conflicts of interest.

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