

# Association of *CYP2D6* and *CYP2C19* polymorphisms and disease-free survival of Thai post-menopausal breast cancer patients who received adjuvant tamoxifen

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**Purpose:** To investigate the impact of *CYP2D6* and *CYP2C19* polymorphisms in predicting tamoxifen efficacy and clinical outcomes in Thai breast cancer patients.

**Methods:** Polymorphisms of *CYP2D6* and *CYP2C19* were genotyped by the AmpliChip™ CYP450 Test (Roche Molecular Diagnostics, Branchburg, NJ, USA) for 57 patients, who were matched as recurrent versus non-recurrent breast cancers (n = 33 versus n = 24, respectively, with a 5-year follow-up).

**Results:** Based on the genotype data, five *CYP2D6* predicted phenotype groups were identified in this study including homozygous extensive metabolizer (13 of 57, 22.80%), extensive/intermediate metabolizer (23 of 57, 40.40%), extensive/poor metabolizer (3 of 57, 5.30%), homozygous intermediate metabolizer (14 of 57, 24.50%), and intermediate/poor metabolizer (4 of 57, 7.00%), and three *CYP2C19* genotype groups including homozygous extensive metabolizer (27 of 57, 47.40%), extensive/intermediate metabolizer (27 of 57, 47.40%), and homozygous poor metabolizer (3 of 57, 5.30%). The *CYP2D6* variant alleles were \*10 (52 of 114, 45.60%), \*5 (5 of 114, 4.40%), \*41 (2 of 114, 1.80%), \*4 (1 of 114, 0.90%), and \*36 (1 of 114, 0.90%); the *CYP2C19* variant alleles were \*2 (27 of 114, 23.70%) and \*3 (6 of 114, 5.30%). Kaplan–Meier estimates showed significantly shorter disease-free survival in patients with homozygous *TT* when compared to those with heterozygous *CT* or homozygous *CC* at nucleotides 100C>T and 1039C>T (*CYP2D6*\*10) post-menopausal (log-rank test; *P* = 0.046). They also had increased risk of recurrence, but no statistically significant association was observed (hazard ratio 3.48; 95% confidence interval 0.86–14.07; *P* = 0.080).

**Conclusion:** The *CYP2D6* and *CYP2C19* polymorphisms were not involved in tamoxifen efficacy. However, in the subgroup of post-menopausal women, the polymorphisms in *CYP2D6* and *CYP2C19* might be useful in predicting tamoxifen efficacy and clinical outcomes in breast cancer patients receiving adjuvant tamoxifen treatment. As the number of breast cancer patients was relatively small in this study, results should be confirmed in a larger group of prospective patients.

**Keywords:** *CYP2D6*, *CYP2C19*, disease-free survival, tamoxifen, pharmacogenetics, breast cancer

## Introduction

Tamoxifen is the most commonly prescribed and widely used treatment and adjuvant therapy drug for the prevention of estrogen receptor/progesterone receptor-sensitive breast cancers in pre- and post-menopausal women.<sup>1,2</sup> However, approximately 30%–50% of estrogen-positive breast cancer patients have recurrence of the disease and do not respond to tamoxifen treatment.<sup>3</sup>

Polymorphisms in *CYP2D6* and *CYP2C19* are clinically important in the metabolism of drugs, as certain allele variants demonstrate either altered activity or nonfunctional enzyme activity with the consequence of 4-hydroxy tamoxifen and endoxifen plasma concentrations.<sup>4</sup> Several studies have discovered the association between *CYP2D6* and *CYP2C19* polymorphisms and plasma concentrations of active metabolites as well as the clinical outcome of breast cancer patients receiving tamoxifen.<sup>5,6</sup>

It has been reported that European breast cancer patients who receive tamoxifen and are homozygous for *CYP2D6*\*4, thus a poor *CYP2D6* metabolizer, have a significantly lower level of endoxifen plasma concentration when compared with homozygous wild type *CYP2D6*\*1.<sup>7–9</sup> *CYP2D6*\*10 (100C>T) is the most common intermediate metabolizer allele in the Asian population, which has an allele frequency of approximately 40%–70%. In contrast, Caucasians and African Americans were reported as having approximately a 2%–5% and 3%–8% allele frequency, respectively.<sup>10–12</sup>

The *CYP2D6*\*10 homozygous variant genotype could affect the efficacy of tamoxifen, and it is associated with significantly lower plasma concentrations of 4-hydroxy tamoxifen when compared with the homozygous wild type genotype. Also, it was found that breast cancer patients with the *CYP2D6*\*10 homozygous variant genotype had a significantly worse disease-free survival (DFS) than those with heterozygous (CT) or homozygous wild type genotype.<sup>13–15</sup> Lim et al performed modeling analysis to investigate the influence of *CYP2D6*, genotype *CYP3A5*, *CYP2C9*, and *CYP2C19* polymorphisms on tamoxifen pharmacokinetics and found that *CYP2D6*\*5/\*10 and \*10/\*10 were significantly associated with lower concentrations of endoxifen and N-desmethyl tamoxifen.<sup>16</sup> The *CYP2C19* gene has two major poor metabolizer (PM) alleles that result in deficiency of the enzyme. However, information is limited on the possibility of the *CYP2C19* genotype affecting the efficacy of tamoxifen, but the result from van Schaik et al demonstrated that *CYP2C19* is associated with increased survival in breast cancer patients using tamoxifen.<sup>17</sup>

Therefore, this study aimed to identify the polymorphisms in *CYP2D6* and *CYP2C19* in patients with breast cancer and to investigate the impact of genetic polymorphisms on disease recurrence in patients who received adjuvant tamoxifen.

## Material and methods

### Clinical subjects

Fifty-seven participants in this retrospective study were recruited from a primary recurrent and non-recurrent breast cancer population enrolled between February 1997 and

January 2008 at the Department of Medicine, Ramathibodi Hospital in Bangkok, Thailand. All 57 patients were assigned randomly to receive 20 mg/day adjuvant tamoxifen for 5 years. This study was designed for 33 breast cancer recurrence and 24 breast cancer non-recurrence. The two groups were matched by the characteristics of the patients (Table 1). Patients receiving selective serotonin reuptake inhibitors were excluded in the post hoc analyses. Written informed consent forms were obtained from all patients. The study was approved by the Ramathibodi Hospital Ethics Committee.

### Patient characteristics

The use of adjuvant tamoxifen was similar in the two groups (cases and controls) (Table 1). The mean age of the subjects was  $48.9 \pm 10.6$  years. The median follow-up time of the case and control group was 93.5 months (range 59.0–172.0) and 22.0 months (range 2.0–62.0), respectively. The median follow-up time was 48.0 months (range 2.0–172.0). The number of pre- and post-menopausal patients was 38 and 19, respectively. All patients were estrogen receptor-positive except for one patient, who was estrogen receptor-negative but progesterone receptor-positive. Among the 33 patients with breast cancer recurrence, 6.06% (2/33) were human epidermal growth factor receptor-2 (Her-2)-positive and 60.60% (20/33) were of unknown status. Twenty-five (43.80%; 25/57) patients had positive axillary lymph nodes. Most patients were treated with a modified radical mastectomy. The adjuvant chemotherapy comprised cyclophosphamide, intravenous methotrexate, and 5-fluorouracil, and Adriamycin®-based and Adriamycin-taxane-based regimens. Three patients in this study did not receive adjuvant chemotherapy, despite their eligibility for treatment, because they had positive lymph node (N1) axillaries (two patients in the control arm and one patient in the case arm, respectively). There was no significant difference in patient characteristics between non-recurrent and recurrent breast cancers (Table 1).

### Analysis of polymorphisms in *CYP2D6* and *CYP2C19*

Genomic DNA was extracted from ethylenediaminetetraacetic acid blood and isolated by the salting out procedure.<sup>17</sup> The microarray technique (AmpliChip™ CYP450 Test; Roche Molecular Diagnostics, Branchburg, NJ, USA) was used for detection of polymorphisms in *CYP2D6* and *CYP2C19* according to the manufacturer's instructions. The main process of the test comprised polymerase chain reaction amplification, fragmentation and labeling, hybridization, staining, and scanning. The test explored

**Table 1** Characteristics of non-recurrent and recurrent breast cancer patients

| Clinical characteristics | n  | Non-recurrence | Recurrence  | P                   |
|--------------------------|----|----------------|-------------|---------------------|
| Number of patients       | 57 | 24             | 33          |                     |
| Age                      |    |                |             | 0.100 <sup>c</sup>  |
| ≤50 years                | 31 | 10 (41.67%)    | 21 (63.64%) |                     |
| >50 years                | 26 | 14 (58.33%)    | 12 (36.36%) |                     |
| Menstrual status         |    |                |             | 0.088 <sup>c</sup>  |
| Pre-menopause            | 38 | 13 (54.17%)    | 25 (75.76%) |                     |
| Post-menopause           | 19 | 11 (45.83%)    | 8 (24.24%)  |                     |
| Tumor size               |    |                |             | 0.718 <sup>b</sup>  |
| ≤2 cm                    | 9  | 5 (20.83%)     | 4 (12.12%)  |                     |
| 2.1–5 cm                 | 39 | 16 (66.67%)    | 23 (69.70%) |                     |
| >5 cm                    | 9  | 3 (12.50%)     | 6 (18.18%)  |                     |
| Estrogen receptor        |    |                |             | 1.000 <sup>b</sup>  |
| Positive                 | 56 | 24 (100.00%)   | 32 (96.97%) |                     |
| Negative                 | 1  | 0 (0.00%)      | 1 (3.03%)   |                     |
| Progesterone receptor    |    |                |             | 1.000 <sup>ab</sup> |
| Positive                 | 23 | 5 (20.83%)     | 18 (54.55%) |                     |
| Negative                 | 15 | 3 (12.50%)     | 12 (36.36%) |                     |
| Unknown                  | 19 | 16 (66.67%)    | 3 (9.09%)   |                     |
| Her-2                    |    |                |             | 1.000 <sup>ab</sup> |
| Positive                 | 2  | 0 (0.00%)      | 2 (4.17%)   |                     |
| Negative                 | 31 | 8 (33.33%)     | 23 (70.83%) |                     |
| Unknown                  | 24 | 16 (66.67%)    | 8 (25.00%)  |                     |
| Grading                  |    |                |             | 1.000 <sup>ab</sup> |
| 1                        | 5  | 2 (8.33%)      | 3 (9.09%)   |                     |
| 2                        | 24 | 9 (37.50%)     | 15 (45.45%) |                     |
| 3                        | 10 | 4 (16.67%)     | 6 (18.18%)  |                     |
| Unknown                  | 18 | 9 (37.50%)     | 9 (27.27%)  |                     |
| Lymph node status        |    |                |             | 0.658 <sup>c</sup>  |
| 0                        | 25 | 12 (50.00%)    | 13 (39.40%) |                     |
| 1–3                      | 15 | 5 (20.83%)     | 10 (30.30%) |                     |
| ≥4                       | 17 | 7 (29.17%)     | 10 (30.30%) |                     |
| LVI                      |    |                |             | 0.658 <sup>ac</sup> |
| Positive                 | 16 | 8 (33.33%)     | 8 (33.33%)  |                     |
| Negative                 | 24 | 11 (45.83%)    | 13 (54.17%) |                     |
| Unknown                  | 8  | 5 (20.84%)     | 3 (12.50%)  |                     |
| Margin                   |    |                |             | 0.720 <sup>b</sup>  |
| Positive                 | 9  | 3 (12.50%)     | 6 (18.18%)  |                     |
| Negative                 | 48 | 21 (87.50%)    | 27 (81.82%) |                     |
| Chemotherapy             |    |                |             | 0.131 <sup>b</sup>  |
| No chemotherapy          | 3  | 1 (4.17%)      | 2 (6.06%)   |                     |
| CMF                      | 28 | 15 (42.50%)    | 13 (39.39%) |                     |
| Adrinamycin base         | 21 | 8 (33.33%)     | 13 (39.39%) |                     |
| Adrinamycin–taxane base  | 5  | 0 (0.00%)      | 5 (15.15%)  |                     |
| Radiation                |    |                |             | 0.112 <sup>c</sup>  |
| Yes                      | 26 | 8 (33.33%)     | 18 (54.55%) |                     |
| No                       | 31 | 16 (66.67%)    | 15 (45.45%) |                     |

**Notes:** <sup>a</sup>The data were not included in *P*-value analysis; <sup>b</sup>Fisher's exact test; <sup>c</sup>Pearson's Chi-squared test.

**Abbreviations:** CMF, cyclophosphamide plus intravenous methotrexate plus 5-fluorouracil; Her-2, human epidermal growth factor receptor-2; LVI, lympho vascular invasion.

29 known polymorphisms in the *CYP2D6* gene, including gene deletion and duplication, and 33 different alleles were acceptable for identification. The *CYP2D6* genotypes were classified based on previous studies.<sup>18–20</sup> There were four phenotypic categories according to allele-related enzyme

activity: no enzyme activity alleles (PM) \*3, \*4, \*5, \*6, \*7, \*8, \*11, \*14A, \*15, \*19, \*20, \*36, \*40, and \*4XN; decreased enzyme activity alleles (intermediate metabolizer) \*9, \*10, \*17, \*29, \*41, \*10XN, \*17XN, and \*41XN; normal enzyme activity alleles (extensive metabolizer) \*1, \*2, and \*35; and increased enzyme activity alleles (ultra-rapid metabolizer) \*1XN, \*2XN, and \*35XN. The polymorphisms in *CYP2C19* were genotyped for \*1, \*2, and \*3.

## Statistical analysis

Descriptive statistics were used to describe the clinical characteristics of the subjects. Hardy–Weinberg equilibrium was conducted with Haploview 4.2 (Broad Institute of Harvard and MIT, Cambridge, MA, USA). Fisher's exact test or Pearson's Chi-squared test was used to compare the different alleles and patient characteristics between recurrent and non-recurrent breast cancers. DFS was defined as the time from surgery to the recurrence of breast cancer event (local, regional, or distant occurrence or contralateral breast cancer) or death from any cause. Patients who were alive without a breast cancer relapse were censored at the last follow-up date. Survival curves were estimated with the Kaplan–Meier method. Statistical significance of a relationship between breast cancer outcomes and each of the genetic polymorphisms was compared by the log-rank test. The univariate Cox proportion hazard model was used to estimate the hazard ratio (HR) for comparing the genotype of each group. All tests were two-sided and *P*-values of less than 0.05 were considered statistically significant. Statistical analyses were conducted using Stata<sup>®</sup> version 12 (StataCorp LP, College Station, TX, USA).

## Results

### Allele frequencies of the *CYP2D6* and *CYP2C19*

The polymorphisms observed in *CYP2D6* and *CYP2C19* were in Hardy–Weinberg equilibrium and they matched those in a previous report on Asian populations. Table 2 shows the frequencies of *CYP2D6* alleles among different ethnic groups. The *CYP2D6*\*10 and *CYP2D6*\*5 (gene deletion) alleles were the most variant and nonfunctional, respectively, in this study, with variance and allele frequency of 45.6% and 4.40%, respectively. Rare variant alleles found that *CYP2D6*\*36 and \*41 had a frequency of 0.90% and 1.80%, respectively. The results showed that the *CYP2D6*\*4 allele with a frequency of 0.90% was characterized by a 1846G>A mutation. The frequencies of *CYP2C19* alleles are shown in Table 2. The *CYP2C19*\*2 allele was the most common variant found in this study at 23.70%. There were no significant differences in allelic frequencies of *CYP2D6*

**Table 2** Frequencies of the *CYP2D6*<sup>10,11,24,25</sup> and *CYP2C19*<sup>26</sup> allele in different ethnic groups

| Alleles               | Major genetic variant     | Enzyme activity | SNP ID             | Current study n (%) | Asian           | Caucasian        | AA        |
|-----------------------|---------------------------|-----------------|--------------------|---------------------|-----------------|------------------|-----------|
| <b><i>CYP2D6</i></b>  |                           |                 |                    | <b>n = 114</b>      |                 |                  |           |
| *1                    | None                      | Normal          |                    | 40 (35.00%)         | 20–40           | 30–40            | 28–50     |
| *2                    | 2850C>T, 4180G>C          | Normal          | rs16947, rs1135840 | 11 (9.60%)          | 9–20            | 20–35            | 10–80     |
| *4                    | 1846G>A                   | None            | rs3892097          | 1 (0.90%)           | 0.5–3           | 12–23            | 2–7       |
| *5                    | Gene deletion             | None            |                    | 5 (4.40%)           | 4–6             | 1.5–7            | 0.5–6     |
| *10                   | 100C>T                    | Decreased       | rs1065852          | 52 (45.60%)         | 40–70           | 2–8              | 3–8       |
| *14B                  | 1758G>A                   | Decreased       | rs5030865          | 1 (0.90%)           |                 |                  |           |
| *35                   | 31G>A, 2850C>T, 4180G>C   | Normal          |                    | 1 (0.90%)           | 1               | 4–6              | –         |
| *36                   | Gene conversion           | Decreased       |                    | 1 (0.90%)           | –               | –                | 1         |
| *41                   | 1661G>C, 2850C>T, 4180G>C | Decreased       | rs1058164          | 2 (1.80%)           | 1.4–2.6         | 8                | 15        |
|                       |                           |                 |                    |                     | <b>SE Asian</b> | <b>Caucasian</b> | <b>AA</b> |
| <b><i>CYP2C19</i></b> |                           |                 |                    | <b>n = 114</b>      |                 |                  |           |
| *1                    | None                      | Normal          |                    | 81 (71.00%)         | 63.12           | 86.4             | 81        |
| *2                    | 681G>A                    | None            | rs4986893          | 27 (23.70%)         | 31.2            | 12.7             | 18.2      |
| *3                    | 636G>A                    | None            | rs4244285          | 6 (5.30%)           | 5.7             | 0.9              | 0.8       |

**Note:** The rs numbers are the accession numbers in the National Center for Biotechnology Information SNP database, (dbSNP).

**Abbreviations:** AA, African American; ID, identification; SE, Southeast; SNP, single nucleotide polymorphism.

and *CYP2C19* between recurrent and non-recurrent breast cancers (Table S1).

## Frequencies of the genotype and predicted phenotype of *CYP2D6* and *CYP2C19*

Most of the *CYP2D6* genotypes presented with heterozygous and homozygous intermediate metabolizer alleles. For example, *CYP2D6*\*1/\*10 and \*10/\*10 had allele frequencies of 28.10% (16/57) and 22.80% (13/57), respectively. Allele frequencies of the *CYP2D6* genotypes were 15.70% for *CYP2D6*\*1/\*1 (9/57), 3.50% for \*1/\*2 (2/57), 3.50% for \*1/\*5 (2/57), 1.80% for \*1/\*36 (1/57), 1.80% for \*1/\*41 (1/57), 3.50% for \*2/\*2 (2/57), 1.80% for \*2/\*4 (1/57), 7.00% for \*2/\*10 (4/57), 5.20% for \*10/\*5 (3/57), 1.80% for \*10/\*14B (1/57), 1.80% for \*10/\*35 (1/57), and 1.80% for \*10/\*41 (1/57) (Table S2). Additionally, no homozygous PM or multiple copy (ultra-rapid metabolizer) of *CYP2D6* alleles were observed in this study (Table 3).

Frequency of the homozygous *CYP2C19*\*1 and homozygous PM allele of the *CYP2C19* genotype was 47.40% and 5.30% for \*1/\*1 (27/57) and \*2/\*2 (3/57), respectively. Frequency of the remaining *CYP2C19* genotypes was 36.80% and 10.50% for \*1/\*2 (21/57) and \*1/\*3 (6/57), respectively (Table S2). In addition, Tables 3, S2, and S3 shows no significant difference in the distribution of *CYP2D6* and *CYP2C19* genotypes and predicted phenotypes between recurrent and non-recurrent breast cancers.

## *CYP2D6* and *CYP2C19* polymorphisms and breast cancer recurrence

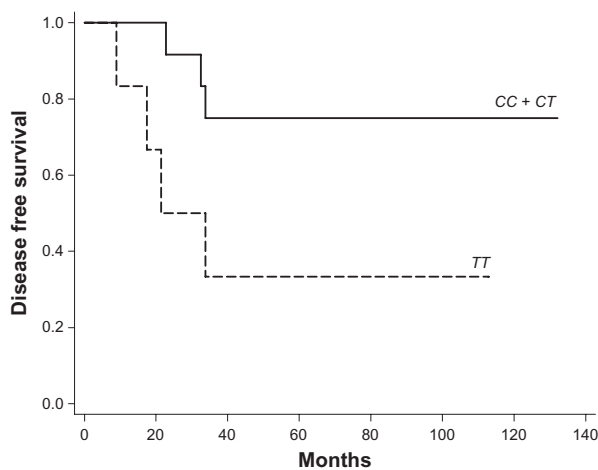
The time it took for the patients to develop breast cancer recurrence was evaluated using Kaplan–Meier analysis. Kaplan–Meier estimates showed significantly shorter DFS (Figure 1) in patients with homozygous *TT* when compared to those with heterozygous *CT* or homozygous *CC* at nucleotides 100C>T and 1039C>T (*CYP2D6*\*10) in post-menopausal women (log-rank test;  $P = 0.046$  and

**Table 3** *CYP2D6* and *CYP2C19* predicted phenotype according to non-recurrence and recurrence groups

| Predicted phenotype   | Genotype                                | Non-recurrence (n = 24) | Recurrence (n = 33) | P                  |
|-----------------------|-----------------------------------------|-------------------------|---------------------|--------------------|
| <b><i>CYP2D6</i></b>  |                                         |                         |                     |                    |
| EM/EM                 | *1/*1, *1/*2, *2/*2                     | 7 (29.20%)              | 6 (18.20%)          | 0.329 <sup>b</sup> |
| EM/IM                 | *1/*10, *2/*10, *10/*35, *1/*36, *1/*41 | 10 (41.70%)             | 13 (39.40%)         | 0.863 <sup>b</sup> |
| EM/PM                 | *1/*5, *2/*4                            | 0 (0.00)                | 3 (9.10%)           | 0.256 <sup>a</sup> |
| IM/IM                 | *10/*10, *10/*41                        | 5 (20.80%)              | 9 (27.30%)          | 0.577 <sup>b</sup> |
| IM/PM                 | *5/*10, *10/*14B                        | 2 (8.30%)               | 2 (6.00%)           | 1.000 <sup>a</sup> |
| <b><i>CYP2C19</i></b> |                                         |                         |                     |                    |
| EM/EM                 | *1/*1                                   | 10 (41.70%)             | 17 (51.50%)         | 0.462 <sup>b</sup> |
| EM/IM                 | *1/*2, *1/*3                            | 11 (45.80%)             | 16 (48.50%)         | 0.843 <sup>b</sup> |
| PM/PM                 | *2/*2                                   | 3 (12.50%)              | 0 (0.00)            | 0.069 <sup>a</sup> |

**Notes:** <sup>a</sup>Fisher's exact test; <sup>b</sup>Pearson's Chi-squared test.

**Abbreviations:** EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.



**Figure 1** Kaplan-Meier probabilities of disease-free survival in patients treated with adjuvant tamoxifen in relation with *CYP2D6* genotype, according to *CYP2D6* (100C>T and 1039C>T) homozygous CC and heterozygous CT versus homozygous TT in post-menopause.

**Note:**  $P = 0.046$ .

$P = 0.046$ ), in which two single nucleotide polymorphisms were in linkage disequilibrium. In addition, patients with *CYP2D6*\*10/\*10 followed a different trend for DFS when compared to heterozygous *CYP2D6*\*10 and homozygous wild type (*CYP2D6* Wt/Wt) in post-menopausal women, but there was no statistical significance ( $P = 0.087$ ).

Finally, no statistically significant difference in DFS was detectable in other nucleotides or genotypes of *CYP2D6* and *CYP2C19* (Tables S4 and S5).

### Risk estimation between genotypes of *CYP2D6* and *CYP2C19*

Patients with heterozygous GA at nucleotide 1846G>A (*CYP2D6*\*4) showed an increased risk of recurrence, but no overall statistically significant difference was observed in pre-menopausal patients (HR 5.82; 95% confidence interval [CI] 0.74–46.02;  $P = 0.094$  and HR 5.84; 95% CI 0.70–48.55;  $P = 0.102$ ). Overall, post-menopausal patients with homozygous TT at nucleotide 100C>T and 1039C>T (*CYP2D6*\*10) tended to have increased risk of recurrence, but no statistically significant association was observed. In contrast, pre-menopausal patients with homozygous TT at nucleotides 100C>T and 1039C>T tended to have decreased risk of recurrence, but no significant association was observed (Table S6). On the other hand, the results showed that pre-menopausal patients with heterozygous GC at nucleotide 4180G>C had decreased risk of developing recurrence when compared to patients with homozygous GG (HR 0.48; 95% CI 0.20–1.15;  $P = 0.099$ ). Table 4 shows that the genotype

**Table 4** Risk estimation between *CYP2D6* and *CYP2C19* genotypes and recurrences in breast cancer patients among overall, pre-menopausal, and post-menopausal groups

| Genotypes          | Overall |                  |       | Pre-menopause |                  |       | Post-menopause |                  |       |
|--------------------|---------|------------------|-------|---------------|------------------|-------|----------------|------------------|-------|
|                    | n       | HR (95% CI)      | P     | n             | HR (95% CI)      | P     | n              | HR (95% CI)      | P     |
| <b>CYP2D6</b>      |         |                  |       |               |                  |       |                |                  |       |
| Number of patients | 47      |                  |       | 31            |                  |       | 16             |                  |       |
| Wt/Wt              | 13      | 1.0 (ref)        |       | 6             | 1.0 (ref)        |       | 7              | 1.0 (ref)        |       |
| Wt/*10             | 21      | 1.17 (0.44–3.11) | 0.758 | 16            | 0.73 (0.23–2.31) | 0.594 | 5              | 0.86 (0.26–2.87) | 0.811 |
| *10/*10            | 13      | 1.93 (0.69–5.44) | 0.213 | 9             | 0.83 (0.23–2.94) | 0.770 | 4              | 2.16 (0.87–5.35) | 0.096 |
| Number of patients | 50      |                  |       | 33            |                  |       | 17             |                  |       |
| EM/EM              | 13      | 1.0 (ref)        |       | 6             | 1.0 (ref)        |       | 7              | 1.0 (ref)        |       |
| EM/IM              | 23      | 1.15 (0.44–3.05) | 0.768 | 17            | 0.67 (0.21–2.11) | 0.498 | 6              | 1.14 (0.42–3.00) | 0.792 |
| IM/IM              | 14      | 1.68 (0.60–4.73) | 0.325 | 10            | 0.69 (0.20–2.47) | 0.573 | 4              | 2.15 (0.87–5.31) | 0.097 |
| Number of patients | 57      |                  |       | 38            |                  |       | 19             |                  |       |
| Wt/Wt              | 13      | 1.0 (ref)        |       | 6             | 1.0 (ref)        |       | 7              | 1.0 (ref)        |       |
| Wt/V               | 26      | 1.33 (0.52–3.40) | 0.552 | 20            | 0.78 (0.23–2.38) | 0.667 | 6              | 1.13 (0.42–2.98) | 0.803 |
| V/V                | 18      | 1.59 (0.59–4.32) | 0.356 | 12            | 0.68 (0.20–2.34) | 0.546 | 6              | 1.97 (0.84–4.62) | 0.121 |
| <b>CYP2C19</b>     |         |                  |       |               |                  |       |                |                  |       |
| Number of patients | 57      |                  |       | 38            |                  |       | 19             |                  |       |
| Homo *1            | 27      | 1.0 (ref)        |       | 19            | 1.0 (ref)        |       | 8              | 1.0 (ref)        |       |
| Het *1             | 27      | 0.93 (0.47–1.84) | 0.829 | 17            | 1.03 (0.47–2.27) | 0.934 | 10             | 0.91 (0.45–1.81) | 0.779 |
| Homo *2            | 3       | 1.95e-16         | 1.000 | 2             | 2.01e-16         | 1.000 | 1              | 2.25e-08         | 1.000 |

**Note:** All  $P$ -values calculated by Pearson's Chi-squared test.

**Abbreviations:** CI, confidence interval; EM, extensive metabolizer; Het, heterozygous; Homo, homozygous; HR, hazard ratio; IM, intermediate metabolizer; V, variant; Wt, wild type.

of *CYP2D6* and *CYP2C19* had increased risk of developing recurrence, but no statistically significant association was observed.

## Discussion

This study aimed to investigate the association between *CYP2D6* and *CYP2C19* polymorphisms and breast cancer outcomes in Thai female breast cancer patients treated with tamoxifen. The characteristics of breast cancer patients may affect the clinical outcome.

Overall, the presence of variant *CYP2D6* and *CYP2C19* alleles had no significant difference in DFS between recurrent and non-recurrent breast cancers. However, Kaplan–Meier estimates showed a significant difference in DFS in patients with homozygous variant (*TT*) when compared with heterozygous variant (*CT*) or homozygous wild type (*CC*) at nucleotides 100C>T and 1039C>T (*CYP2D6\*10*) in post-menopausal patients (log-rank test  $P = 0.046$  and  $P = 0.046$ ), in which two single nucleotide polymorphisms were associated with linkage disequilibrium.

Previous studies investigated the association between polymorphisms in *CYP2D6* and tamoxifen efficacy and clinical outcomes in patients receiving adjuvant tamoxifen.<sup>14,15</sup> Goetz et al initially reported that breast cancer patients with decreased *CYP2D6* metabolism had a significantly shorter recurrence time (HR 1.91; 95% CI 1.05–3.45;  $P = 0.034$ ) and worse relapse-free survival (HR 1.74; 95% CI 1.10–2.74;  $P = 0.017$ ) when compared to patients with extensive *CYP2D6* metabolism. Patients with the PM phenotype (*CYP2D6\*4/\*4*) had a significantly higher risk of breast cancer relapse approximately three times that of patients with extensive metabolizers (*CYP2D6\*1/\*1* and *\*1/\*4*) (HR 3.12;  $P = 0.007$ ).<sup>22</sup> Xu et al showed that patients with the *CYP2D6\*10* homozygous *TT* genotype had significantly worse DFS than those with the heterozygous *CT* and homozygous *CC* genotype (HR 4.7; 95% CI 1.1–20.0;  $P = 0.004$ ).<sup>13</sup> Lim et al reported that patients with the *CYP2D6\*10/\*10* genotype had a significantly higher risk of breast cancer relapse within 10 years after surgery when compared to those with other genotypes (time to progression 5.03 versus 21.8 months,  $P = 0.0032$ ).<sup>23</sup> Kiyotani et al reported that patients with *CYP2D6\*10/\*10* and *CYP2D6\*1/\*10* showed significantly shorter recurrence-free survival when compared to those with *CYP2D6\*1/\*1* (HR 9.52; 95% CI 2.79–32.45;  $P = 0.000036$ ).<sup>24</sup>

In contrast, previous studies from both European and Asian populations showed no significant association between polymorphisms in *CYP2D6* and outcome of tamoxifen treatment. In the first, Okishiro et al reported no significantly

different relapse-free survival rates between breast cancer patients with *CYP2D6\*10/\*10* genotypes and those with *CYP2D6\*1/\*1* or *CYP2D6\*1/\*10* genotypes, nor was there a difference between patients with *CYP2C19* PM genotypes (*CYP2C19\*2/\*2*, *\*2/\*3*, or *\*3/\*3*) and those with *CYP2C19* extensive metabolizer genotypes (*CYP2C19 \*1/\*1*, *\*1/\*2*, or *\*1/\*3*).<sup>25</sup> Toyama et al demonstrated no significant correlation between patients with the *CYP2D6\*10/\*10* genotype and survival time (DFS, distant DFS, breast cancer-specific survival, and overall survival) when compared to those with *CYP2D6 \*1/\*1* and *\*1/\*10* genotypes.<sup>26</sup> In contrast, a report from Sweden showed that patients with the *CYP2D6\*4/\*4* genotype had significantly better DFS than those with heterozygous or homozygous *CYP2D6\*1* ( $P = 0.004$  and  $P = 0.005$ , respectively).<sup>27</sup>

The data in this study support the conclusion that *CYP2D6* and *CYP2C19* variants are not significantly associated with the clinical outcome in breast cancer patients taking adjuvant tamoxifen. Conversely, in a group of post-menopausal women, the polymorphisms in *CYP2D6\*10/\*10* might be useful in predicting tamoxifen efficacy and clinical outcomes when compared to heterozygous *CYP2D6\*10* and homozygous wild type (*CYP2D6 \*1/\*1*).

However, this study had some limitations. Primarily, the retrospective nature of the study design is weak, which it shares with all other available studies. This retrospective method also lacks data correlation between polymorphisms in *CYP2D6* and the plasma concentration of tamoxifen metabolites. While the small sample size and low number of PM phenotypes in this study may have given a low statistical power, all samples collected from the recruited were matched in a case–control manner.

It is possible that one or more of these variants are associated with a specific subgroup of breast cancer patients. The data in this study showed that the high frequency of *CYP2D6\*10* is similar to Asian populations reported previously,<sup>9,21</sup> and only nine variations include gene deletion, gene conversion, 1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T, and 4180G>C. No homozygous *CYP2D6* PM (*CYP2D6\*3*, *\*4*, and *\*5*) or homozygous ultra-rapid metabolizers (*CYP2D6\*1XN*, *\*2XN*, and *\*35XN*) in this study is due possibly to the small sample size.

## Conclusion

The variant alleles of *CYP2D6* and *CYP2C19* genes in this study were not involved in tamoxifen efficacy. However, in the subgroup of post-menopausal women, the polymorphisms

in *CYP2D6* and *CYP2C19* might be useful in predicting tamoxifen efficacy and clinical outcomes in breast cancer patients receiving adjuvant tamoxifen treatment. As the number of breast cancer patients was small in this study, results should be confirmed in a larger group of patients.

## Acknowledgments

This study was supported by a grant from the Thailand Center of Excellent Life Science (TCELS). The authors would like to thank Dr Suwit J Somponpun for assistance in the preparation and editing of this manuscript. The authors are also grateful to all participants who contributed to the study.

## Disclosure

The authors report no conflicts of interest in this work.

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## Supplementary data

**Table S1** *CYP2D6* and *CYP2C19* alleles frequency compared between groups

| Alleles               | Recurrence<br>(n = 33) | Non-recurrence<br>(n = 24) | P     |
|-----------------------|------------------------|----------------------------|-------|
| <b><i>CYP2D6</i></b>  |                        |                            |       |
| *1                    | 23                     | 17                         | 1.000 |
| *2                    | 4                      | 7                          | 0.198 |
| *4                    | 1                      | 0                          | 1.000 |
| *5                    | 3                      | 2                          | 1.000 |
| *10                   | 32                     | 20                         | 0.568 |
| *14B                  | 1                      | 0                          | 1.000 |
| *35                   | 1                      | 0                          | 1.000 |
| *36                   | 0                      | 1                          | 0.421 |
| *41                   | 1                      | 1                          | 1.000 |
| <b><i>CYP2C19</i></b> |                        |                            |       |
| *1                    | 50                     | 31                         | 0.215 |
| *2                    | 13                     | 14                         | 0.270 |
| *3                    | 3                      | 3                          | 0.695 |

**Table S2** *CYP2D6* and *CYP2C19* genotypes frequency compared between groups

| Genotypes             | Recurrence<br>n = 33 | Non-recurrence<br>n = 24 | Frequency<br>% (n) | P     |
|-----------------------|----------------------|--------------------------|--------------------|-------|
| <b><i>CYP2D6</i></b>  |                      |                          |                    |       |
| *1/*1                 | 5                    | 4                        | 15.70 (9)          | 1.000 |
| *1/*2                 | 1                    | 1                        | 3.50 (2)           | 1.000 |
| *1/*5                 | 2                    | 0                        | 3.50 (2)           | 0.504 |
| *1/*10                | 9                    | 7                        | 28.00 (16)         | 1.000 |
| *1/*36                | 0                    | 1                        | 1.80 (1)           | 0.421 |
| *1/*41                | 1                    | 0                        | 1.80 (1)           | 1.000 |
| *2/*2                 | 0                    | 2                        | 3.50 (2)           | 0.173 |
| *2/*4                 | 1                    | 0                        | 1.80 (1)           | 1.000 |
| *2/*10                | 2                    | 2                        | 7.00 (4)           | 1.000 |
| *5/*10                | 1                    | 2                        | 5.20 (3)           | 0.567 |
| *10/*10               | 9                    | 4                        | 22.80 (13)         | 0.524 |
| *10/*14B              | 1                    | 0                        | 1.80 (1)           | 1.000 |
| *10/*35               | 1                    | 0                        | 1.80 (1)           | 1.000 |
| *10/*41               | 0                    | 1                        | 1.80 (1)           | 0.421 |
| <b><i>CYP2C19</i></b> |                      |                          |                    |       |
| *1/*1                 | 17                   | 10                       | 47.40 (27)         | 0.593 |
| *1/*2                 | 13                   | 8                        | 36.80 (21)         | 0.782 |
| *1/*3                 | 3                    | 3                        | 10.50 (6)          | 0.689 |
| *2/*2                 | 0                    | 3                        | 5.30 (3)           | 0.069 |



**Table S3** Genotype frequencies of *CYP2D6* and *CYP2C19* of 33 breast cancer recurrence and 24 breast cancer non-recurrence cases

| Alleles             | n               | Non-recurrence<br>n (%) | Recurrence<br>n (%) | P     |
|---------------------|-----------------|-------------------------|---------------------|-------|
| <b>CYP2D6</b>       | <b>(n = 57)</b> | <b>(n = 24)</b>         | <b>(n = 33)</b>     |       |
| -1584C>G, rs1080985 |                 |                         |                     |       |
| CC                  | 47              | 19 (79.17)              | 28 (84.85)          | 0.578 |
| CG                  | 8               | 3 (12.50)               | 5 (15.15)           | 0.776 |
| GG                  | 2               | 2 (8.33)                | 0 (0.00)            | 0.091 |
| 100C>T, rs1065852   |                 |                         |                     |       |
| CC                  | 16              | 7 (29.17)               | 9 (27.27)           | 0.875 |
| CT                  | 27              | 12 (50.00)              | 15 (95.46)          | 0.734 |
| TT                  | 14              | 5 (20.83)               | 9 (27.27)           | 0.577 |
| 1039C>T, rs1081003  |                 |                         |                     |       |
| CC                  | 17              | 7 (29.17)               | 10 (30.30)          | 0.926 |
| CT                  | 26              | 12 (54.55)              | 14 (42.42)          | 0.571 |
| TT                  | 14              | 5 (20.83)               | 9 (27.27)           | 0.577 |
| 1661G>C, rs1058164  |                 |                         |                     |       |
| GG                  | 13              | 5 (20.83)               | 8 (24.24)           | 0.762 |
| GC                  | 22              | 10 (41.67)              | 12 (36.36)          | 0.685 |
| CC                  | 22              | 9 (37.50)               | 13 (39.40)          | 0.885 |
| 1846G>A, rs3892097  |                 |                         |                     |       |
| GG                  | 56              | 24 (100)                | 32 (96.97)          | 0.390 |
| GA                  | 1               | 0 (0.00)                | 1 (3.03)            | 0.390 |
| AA                  | 0               | 0 (0.00)                | 0 (0.00)            |       |
| 2850C>T, rs16947    |                 |                         |                     |       |
| CC                  | 45              | 18 (75.00)              | 27 (81.82)          | 0.533 |
| CT                  | 11              | 5 (20.83)               | 6 (18.18)           | 0.802 |
| TT                  | 1               | 1 (4.17)                | 0 (0.00)            | 0.237 |
| 4180G>C, rs1135840  |                 |                         |                     |       |
| GG                  | 12              | 4 (16.67)               | 8 (24.24)           | 0.489 |
| GC                  | 23              | 11 (45.83)              | 12 (36.36)          | 0.472 |
| CC                  | 22              | 9 (37.50)               | 13 (39.4)           | 0.885 |
| <b>CYP2C19</b>      |                 |                         |                     |       |
| 681G>A, rs4244285   |                 |                         |                     |       |
| GG                  | 32              | 12 (50.00)              | 20 (60.61)          | 0.426 |
| GA                  | 22              | 9 (37.50)               | 13 (39.39)          | 0.885 |
| AA                  | 3               | 3 (12.50)               | 0 (0.00)            | 0.069 |
| 636G>A, rs4986893   |                 |                         |                     |       |
| GG                  | 51              | 21 (87.50)              | 30 (90.91)          | 0.679 |
| GA                  | 6               | 3 (12.50)               | 3 (9.09)            | 0.679 |
| AA                  | 0               | 0 (0.00)                | 0 (0.00)            |       |

**Notes:** Fisher's exact test or Pearson's Chi-squared test was used to compare the different alleles and patient characteristics between recurrent and non-recurrent breast cancers; the rs numbers are the accession numbers in the National Center for Biotechnology Information single nucleotide polymorphism database, dbSNP.

**Table S4** Log-rank test of *CYP2D6* genotypes

| <i>CYP2D6</i><br>genotypes            | <i>P</i> (log-rank test) |               |                |
|---------------------------------------|--------------------------|---------------|----------------|
|                                       | Overall                  | Pre-menopause | Post-menopause |
| <b>(Wt/Wt versus Wt/V versus V/V)</b> |                          |               |                |
| -1584C>G                              | 0.380                    | 0.371         | 0.705          |
| 100C>T                                | 0.665                    | 0.503         | 0.168          |
| 1039C>T                               | 0.587                    | 0.310         | 0.168          |
| 1661G>C                               | 0.747                    | 0.566         | 0.427          |
| 1846G>A                               | 0.162                    | 0.187         | –              |
| 2850C>T                               | 0.632                    | 0.465         | 0.433          |
| 4180G>C                               | 0.532                    | 0.169         | 0.427          |
| <b>Wt/Wt versus (Wt/V + V/V)</b>      |                          |               |                |
| -1584C>G                              | 0.688                    | 0.805         | 0.492          |
| 100C>T                                | 0.972                    | 0.242         | 0.556          |
| 1039C>T                               | 0.805                    | 0.128         | 0.556          |
| 1661G>C                               | 0.694                    | 0.286         | 0.753          |
| 1846G>A                               | 0.162                    | 0.187         | –              |
| 2850C>T                               | 0.646                    | 0.904         | 0.433          |
| 4180G>C                               | 0.424                    | 0.060         | 0.753          |
| <b>(Wt/Wt + Wt/V) versus V/V</b>      |                          |               |                |
| -1584C>G                              | 0.176                    | 0.291         | 0.452          |
| 100C>T                                | 0.386                    | 0.838         | 0.046          |
| 1039C>T                               | 0.386                    | 0.838         | 0.046          |
| 1661G>C                               | 0.653                    | 0.668         | 0.201          |
| 1846G>A                               | –                        | –             | –              |
| 2850C>T                               | 0.346                    | 0.291         | –              |
| 4180G>C                               | 0.653                    | 0.668         | 0.201          |
| *1/*1 versus                          | 0.451                    | 0.689         | 0.097          |
| *1/*10 versus                         |                          |               |                |
| *10/*10                               |                          |               |                |
| Wt/Wt versus                          | 0.368                    | 0.863         | 0.087          |
| Wt/*10 versus                         |                          |               |                |
| *10/*10                               |                          |               |                |
| EM/EM versus                          | 0.553                    | 0.782         | 0.141          |
| EM/IM versus IM/IM                    |                          |               |                |
| Wt/Wt versus                          | 0.646                    | 0.831         | 0.180          |
| Wt/V versus V/V                       |                          |               |                |

**Abbreviations:** EM, extensive metabolizer; IM, intermediate metabolizer; V, variant; Wt, wild type.

**Table S5** Log-rank test of *CYP2C19* genotypes

| <i>CYP2C19</i> genotype             | <i>P</i> (log-rank test) |               |                |
|-------------------------------------|--------------------------|---------------|----------------|
|                                     | Overall                  | Pre-menopause | Post-menopause |
| <b>Wt/Wt versus Wt/V versus V/V</b> |                          |               |                |
| 681G>A                              | 0.247                    | 0.260         | 0.648          |
| 636G>A                              | 0.667                    | 0.669         | 0.269          |
| <b>Wt/Wt versus (Wt/V + V/V)</b>    |                          |               |                |
| 681G>A                              | 0.493                    | 0.292         | 0.764          |
| 636G>A                              | 0.667                    | 0.669         | 0.269          |
| <b>(Wt/Wt + Wt/V) versus V/V</b>    |                          |               |                |
| 681G>A                              | 0.096                    | 0.125         | 0.452          |
| 636G>A                              | –                        | –             | –              |
| homo*1 versus                       | 0.244                    | 0.308         | 0.722          |
| het*1 versus homo*2                 |                          |               |                |
| homo EM versus                      | 0.244                    | 0.308         | 0.722          |
| het EM versus                       |                          |               |                |
| homo PM                             |                          |               |                |

**Abbreviations:** EM, extensive metabolizer; homo, homozygous; het, heterozygous; PM, poor metabolizer; V, variant; Wt, wild type.

**Table S6** Risk estimation between genotypes and recurrences in breast cancer patients

| Genotype           | Overall |                   |       | Pre-menopause |                   |       | Post-menopause |                  |       |
|--------------------|---------|-------------------|-------|---------------|-------------------|-------|----------------|------------------|-------|
|                    | n       | HR (95% CI)       | P     | n             | HR (95% CI)       | P     | n              | HR (95% CI)      | P     |
| <b>CYP2D6</b>      |         |                   |       |               |                   |       |                |                  |       |
| Number of patients | 57      |                   |       | 38            |                   |       | 19             |                  |       |
| <b>-1584C&gt;G</b> |         |                   |       |               |                   |       |                |                  |       |
| CC                 | 47      | 1.0 (ref)         |       | 32            | 1.0 (ref)         |       | 15             | 1.0 (ref)        |       |
| CG                 | 8       | 1.17 (0.45–3.02)  | 0.753 | 5             | 1.64 (0.56–4.82)  | 0.369 | 3              | 0.83 (0.29–2.37) | 0.726 |
| GG                 | 2       | 1.59e-15          | 1.000 | 1             | 4.49e-15          | 1.000 | 1              | 3.79e-8          | 1.000 |
| CG + GG            | 10      | 0.82 (0.38–2.13)  | 0.689 | 6             | 1.14 (0.39–3.34)  | 0.807 | 4              | 0.70 (0.24–1.99) | 0.501 |
| <b>100C&gt;T</b>   |         |                   |       |               |                   |       |                |                  |       |
| CC                 | 16      | 1.0 (ref)         |       | 8             | 1.0 (ref)         |       | 8              | 1.0 (ref)        |       |
| CT                 | 27      | 0.89 (0.39–2.05)  | 0.791 | 22            | 0.58 (0.22–1.51)  | 0.262 | 5              | 0.74 (0.24–2.29) | 0.600 |
| TT                 | 14      | 1.30 (0.52–3.29)  | 0.575 | 8             | 0.60 (0.18–1.96)  | 0.396 | 6              | 1.69 (0.79–3.58) | 0.174 |
| CT + TT            | 41      | 1.01 (0.47–2.18)  | 0.972 | 30            | 0.58 (0.23–1.46)  | 0.250 | 11             | 1.24 (0.60–2.54) | 0.559 |
| <b>1039C&gt;T</b>  |         |                   |       |               |                   |       |                |                  |       |
| CC                 | 17      | 1.0 (ref)         |       | 9             | 1.0 (ref)         |       | 8              | 1.0 (ref)        |       |
| CT                 | 26      | 0.79 (0.35–1.77)  | 0.563 | 21            | 0.50 (0.20–1.27)  | 0.144 | 5              | 0.74 (0.24–2.29) | 0.600 |
| TT                 | 14      | 1.21 (0.49–2.98)  | 0.681 | 8             | 0.55 (0.17–1.74)  | 0.306 | 6              | 1.69 (0.79–3.58) | 0.174 |
| CT + TT            | 40      | 0.91 (0.43–1.92)  | 0.806 | 29            | 0.51 (0.21–1.24)  | 0.138 | 11             | 1.24 (0.60–2.54) | 0.559 |
| <b>1661G&gt;C</b>  |         |                   |       |               |                   |       |                |                  |       |
| GG                 | 13      | 1.0 (ref)         |       | 8             | 1.0 (ref)         |       | 5              | 1.0 (ref)        |       |
| GC                 | 22      | 0.75 (0.31–1.84)  | 0.528 | 15            | 0.62 (0.22–1.71)  | 0.352 | 7              | 0.86 (0.32–2.30) | 0.768 |
| CC                 | 22      | 0.98 (0.40–2.36)  | 0.958 | 15            | 0.60 (0.21–1.70)  | 0.339 | 7              | 1.43 (0.61–3.36) | 0.406 |
| GC + CC            | 44      | 0.85 (0.38–1.89)  | 0.695 | 30            | 0.61 (0.24–1.54)  | 0.294 | 14             | 1.14 (0.51–2.53) | 0.754 |
| <b>1846G&gt;A</b>  |         |                   |       |               |                   |       |                |                  |       |
| GG                 | 56      | 1.0 (ref)         |       | 37            | 1.0 (ref)         |       | 19             |                  |       |
| GA                 | 1       | 5.82 (0.74–46.02) | 0.094 | 1             | 5.84 (0.70–48.55) | 0.102 | 0              | –                | –     |
| AA                 | 0       | –                 | –     | 0             | –                 | –     | 0              | –                | –     |
| GA + AA            | 1       | 5.82 (0.74–46.02) | 0.094 | 1             | 5.84 (0.70–48.55) | 0.102 | 0              | –                | –     |
| <b>2850C&gt;T</b>  |         |                   |       |               |                   |       |                |                  |       |
| CC                 | 45      | 1.0 (ref)         |       | 30            | 1.0 (ref)         |       | 15             | 1.0 (ref)        |       |
| CT                 | 11      | 0.93 (0.38–2.25)  | 0.865 | 7             | 1.37 (0.51–3.66)  | 0.532 | 4              | 0.66 (0.23–1.90) | 0.445 |
| TT                 | 1       | 5.94e-16          | 1.000 | 1             | 6.11e-16          | 1.000 | 0              | –                | –     |
| CT + TT            | 12      | 0.81 (0.34–1.97)  | 0.648 | 8             | 1.06 (0.40–2.84)  | 0.905 | 4              | 0.66 (0.23–1.90) | 0.445 |
| <b>4180G&gt;C</b>  |         |                   |       |               |                   |       |                |                  |       |
| GG                 | 12      | 1.0 (ref)         |       | 7             | 1.0 (ref)         |       | 5              | 1.0 (ref)        |       |
| GC                 | 23      | 0.62 (0.25–1.52)  | 0.296 | 16            | 0.48 (0.20–1.15)  | 0.099 | 7              | 0.86 (0.32–2.30) | 0.768 |
| CC                 | 22      | 0.86 (0.35–2.07)  | 0.731 | 15            | 0.44 (0.15–1.25)  | 0.121 | 7              | 1.43 (0.61–3.36) | 0.406 |
| GC + CC            | 45      | 0.72 (0.33–1.61)  | 0.429 | 31            | 0.42 (0.16–1.07)  | 0.070 | 14             | 1.14 (0.51–2.53) | 0.754 |
| <b>CYP2C19</b>     |         |                   |       |               |                   |       |                |                  |       |
| Number of patients | 57      |                   |       | 38            |                   |       | 18             |                  |       |
| <b>681G&gt;A</b>   |         |                   |       |               |                   |       |                |                  |       |
| GG                 | 32      | 1.0 (ref)         |       | 22            | 1.0 (ref)         |       | 10             | 1.0 (ref)        |       |
| GA                 | 22      | 0.94 (0.47–1.90)  | 0.871 | 14            | 0.79 (0.35–1.80)  | 0.576 | 8              | 1.21 (0.60–2.42) | 0.597 |
| AA                 | 3       | 1.46e-15          | 1.000 | 2             | 4.88e-16          | 1.000 | 1              | 2.56e-8          | 1.000 |
| GA + AA            | 25      | 0.78 (0.39–1.58)  | 0.496 | 16            | 0.65 (0.29–1.47)  | 0.299 | 9              | 1.11 (0.55–2.23) | 0.764 |
| <b>636G&gt;A</b>   |         |                   |       |               |                   |       |                |                  |       |
| GG                 | 51      | 1.0 (ref)         |       | 34            | 1.0 (ref)         |       | 17             | 1.0 (ref)        |       |
| GA                 | 6       | 0.77 (0.24–2.53)  | 0.669 | 4             | 1.30 (0.39–4.37)  | 0.672 | 2              | 1.36e-8          | 1.000 |
| AA                 | 0       | –                 | –     | 0             | –                 | –     | 0              | –                | –     |
| GA + AA            | 6       | 0.77 (0.24–2.53)  | 0.669 | 4             | 1.30 (0.39–4.37)  | 0.672 | 2              | 1.36e-8          | 1.000 |

**Note:** All P-values calculated by Pearson's Chi-squared test.

**Abbreviations:** CI, confidence interval; HR, hazard ratio.

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