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Original Article

S4646 polymorphism in *CYP19A1* gene is associated with the efficacy of hormone therapy in early breast cancer

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Abstract: Purpose: The aim was to verify the potential association between *CYP19A1* genetic polymorphisms and clinical outcome of hormone therapy in hormone receptor (HR)-positive early breast cancer. Methods: Genotyping for *CYP19A1* rs4646 (C/A) polymorphism was performed on 287 women with HR-positive early breast cancer. Associations were evaluated between *CYP19A1* rs4646 genotypes and disease-free survival (DFS). Results: Totally, women with the minor allele (AA or AC) had an improved DFS when compared with those carrying the homozygous common allele (CC) (AA or AC vs. CC: 62.7 months versus 55.6 months; Hazard ratio (HR), 0.745; 95% CI, 0.562-0.988; *P* = 0.04). The difference was further demonstrated by multivariate analyses (HR, 0.681; 95% CI, 0.506-0.917; *P* = 0.011). In premenopausal women, AA genotype was associated with a prolonged DFS (AA versus CC or AC: 98.2 months versus 56.2 months; HR, 0.425; 95% CI, 0.198-0.914; *P* = 0.024). In addition, women with the A allele had an improved DFS when compared with those carrying the homozygous C allele (AA or AC vs. CC: 62.7 months versus 55.6 months; HR, 0.709; 95% CI, 0.516-0.975; *P* = 0.033). These findings were further confirmed by the Cox regression model (HR, 0.336, 0.670; 95% CI, 0.160-0.836, 0.479-0.938; *P* = 0.017, 0.019). In postmenopausal women, rs4646 genotypes were significantly associated with DFS (AA versus AC versus CC: 32.7 months versus not reached versus 56.3 months; *P* = 0.011). Women carrying AA variant had a poorer DFS than those with CC or AC genotypes (32.7 months versus 70.6 months; HR, 3.613; 95% CI, 1.380-9.457; *P* = 0.005). Furthermore, being adjusted by the patients features in multivariate analyses, AA genotype remained an independent prognostic factor for DFS (HR, 3.614; 95% CI, 1.308-9.991; *P* = 0.013). Conclusions: The homozygous minor allele (AA) of *CYP19A1* rs4646 is significantly associated with improved clinical outcome of hormone therapy in premenopausal HR-positive early breast cancer patients, but with a worse impact on postmenopausal women. The findings are novel, if confirmed, genotyping for *CYP19A1* rs4646 polymorphism may provide predictive information for better selection of endocrine treatment.

Keywords: Breast cancer, aromatase, genetic polymorphisms, predictive role

Introduction

Breast cancer is one of the most prevalent malignancies in women worldwide [1], and has become the most common cause of cancer-specific mortality and morbidity in women [2]. Two thirds of primary breast cancer overexpress estrogen receptors (ER) and/or progesterone receptors (PgR) [3]. Accordingly, hormone-based treatment, such as tamoxifen or aromatase inhibitors, has turned to be one of the mainstream treatment in hormone receptor (HR)-positive breast cancer, and brought about a great improvement in disease-free survival (DFS) and overall survival (OS) [4, 5]. However, adjuvant hormone therapy does not work as intended for a considerable amount of breast cancer patients [4, 6, 7], and thus, identifica-

Table 1. Clinical and pathological characteristics of the patients

Parameters	n (%)
Menopausal status	
Premenopausal	217 (75.6)
Postmenopausal	70 (24.4)
Tumor size (cm)	
≤ 2	102 (35.5)
> 2	168 (58.5)
Unknown	17 (5.9)
Lymph nodes	
Negative	82 (28.6)
Positive	199 (69.3)
Unknown	6 (2.1)
Estrogen Receptor	
Negative	36 (12.5)
Positive	245 (85.4)
Unknown	6 (2.1)
Progesterone Receptor	
Negative	65 (22.6)
Positive	216 (75.3)
Unknown	6 (2.1)
HER-2 status	
Negative	174 (60.6)
Positive	70 (24.4)
Unknown	43 (15.0)
BMI	
≤ 24	159 (55.6)
> 24	128 (44.4)
Adjuvant hormone therapy	
Tamoxifen	250 (87.1)
Aromatase inhibitors	37 (12.9)
Adjuvant chemotherapy	
Yes	274 (95.5)
No	13 (4.5)

tion of markers for better selection of endocrine treatment is demanded.

It has been suggested that the response to tamoxifen therapy may depend on the *CYP2D6* gene polymorphisms, however, the results are widely heterogeneous [8-11]. Consequently, *CYP2D6* genotyping before tamoxifen administration is currently not recommended [5, 9].

Approximately two thirds of human breast cancer express aromatase protein or display aromatase enzyme activity [12-15]. Polymorphisms in the aromatase *CYP19A1* gene have been shown to alter aromatase activity as well as circulating steroid hormone levels in postmenopausal women [16-21]. Hence, it is biologically plausible that the *CYP19A1* polymorphisms may be correlated with the response to hormone therapy. However, no definite evidence between *CYP19A1* polymorphisms and therapeutic efficacy of hormone therapy in breast cancer has yet been established. Colomer et al. [22] revealed that time to progression (TTP) was significantly prolonged in patients with the rare T allele of *CYP19A1* rs4646 when compared with those carrying the homozygous common allele (GG) in the postmenopausal metastatic breast cancer (MBC) women treated with letrozole. On the contrary, the same variants (GT and TT) were evident to be correlated with a poorer benefit from letrozole therapy (a shorter progression-free survival, PFS) when evaluated in the neoadjuvant setting [23]. More recently, a study conducted by Kuo et al. [24] indicated that the combined high risk A allele of *CYP19A1* rs4646 polymorphism was significantly in relation to a shorter distant disease-free survival (DDFS) ($P < 0.05$) and marginally associated with a poorer overall survival (OS) ($P = 0.06$) and DFS ($P = 0.07$) in lymph node (LN)-negative, HR-positive women with hormone therapy.

In the present study, we performed a genetic analysis of *CYP19A1* polymorphisms in a cohort of HR-positive early breast cancer to elucidate whether *CYP19A1* gene rs4646 variants were associated with clinical outcome of hormone therapy.

Patients and methods

Study cohort and sources of information

287 HR-positive early breast cancer were enrolled in the study between April 1, 2004 and July 31, 2010. The pathologic review, archiving of tumor tissues and blood samples, and genetic studies were approved by the institutional review board of Zhejiang Cancer Hospital. A 2 mL blood sample was extracted and stored in polypropylene cryotubes at -80°C until extraction of genomic DNA. All patients were provided written informed consent according to guidelines of the ethics committee of Zhejiang Cancer Hospital.

DNA preparation and genotyping

Genomic DNA was isolated from peripheral blood with the AxyPrep Blood Genomic DNA

Table 2. Association of CYP19A1 rs4646 genotypes with disease-free survival

CYP19A1 polymorphisms	n	HR (95% CI)	P	HR (95% CI)*	P*
All patients					
CC	152	1.0 (reference)	0.106	1.0 (reference)	0.044
AC	115	0.766 (0.570-1.028)		0.716 (0.528-0.970)	
AA	20	0.641 (0.351-1.170)		0.585 (0.311-1.099)	
CC/AC	267	1.0 (reference)	0.266	1.0 (reference)	0.187
AA	20	0.716 (0.396-1.293)		0.657 (0.352-1.227)	
CC	152	1.0 (reference)	0.040	1.0 (reference)	0.011
AC/AA	135	0.745 (0.562-0.988)		0.681(0.506-0.917)	
Premenopausal patients					
CC	115	1.0 (reference)	0.026	1.0 (reference)	0.013
AC	87	0.786 (0.566-1.091)		0.746 (0.533-1.044)	
AA	15	0.384 (0.177-0.834)		0.323 (0.140-0.741)	
CC/AC	202	1.0 (reference)	0.024	1.0 (reference)	0.017
AA	15	0.425 (0.198-0.914)		0.336 (0.160-0.836)	
CC	115	1.0 (reference)	0.033	1.0 (reference)	0.019
AC/AA	102	0.709 (0.516-0.975)		0.670 (0.479-0.938)	
Postmenopausal patients					
CC	37	1.0 (reference)	0.011	1.0 (reference)	0.015
AC	28	0.681 (0.348-1.334)		0.517 (0.245-1.091)	
AA	5	3.115 (1.159-8.375)		2.575 (0.900-7.371)	
CC/AC	65	1.0 (reference)	0.005	1.0 (reference)	0.013
AA	5	3.613 (1.380-9.457)		3.614 (1.308-9.991)	
CC	37	1.0 (reference)	0.648	1.0 (reference)	0.287
AC/AA	33	0.868 (0.473-1.594)		0.687(0.348-1.354)	

Note: HR, hazard ratio; CI, confidence interval. *Adjusted by positive lymph nodes, tumor size >2 cm, negative hormone receptor status, HER-2-postive status, chemotherapy, BMI > 24 in multivariate analyses.

Miniprep kit (Axygen Biosciences, Union City, CA). Genotyping was performed through the SEQUENOM MassARRAY matrix-assisted laser desorption/ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA) [25]. Primers (5'-TCTCTTGTAGCCTGGTTCTC-3' and 5'-GTGACAACCCATAGGAGGTA-3') for PCR and single base extension were designed by the Assay Designer's software version 3.0 (Sequenom) and synthesized with Sangon Biotech (Shanghai, China).

Purified primer extension reaction products were spotted onto a 384-well spectroCHIP with the MassARRAY Nanodispenser and determined by the matrix-assisted laser desorption-ization time-of-flight mass spectrometer. Genotype analysis was conducted in real time with MassARRAY RT software version 3.0.0.4 and analyzed through the MassARRAY Typer software version 3.4.

Statistical analysis

Follow-up data available as of July 31, 2014, were analyzed. DFS was calculated from the date of the original surgery for breast cancer to the date of locoregional or distant recurrence or death for any causes [26]. Kaplan-Meier method was utilized to measured survival. Differences in survival were compared using the log-rank test.

Cox regression analyses were performed to estimate hazard ratio (HR) and the corresponding 95% confidence interval (CI) for each variable. The multivariate-adjusted HR of relapse associated with the individual genotypes was examined for the groups after adjusting for tumor size, lymph nodes involvement, ER and PR status, HER-2 status, Body Mass Index (BMI) and chemotherapy. All analyses were performed with SPSS 17.0 for Windows (SPSS Inc,

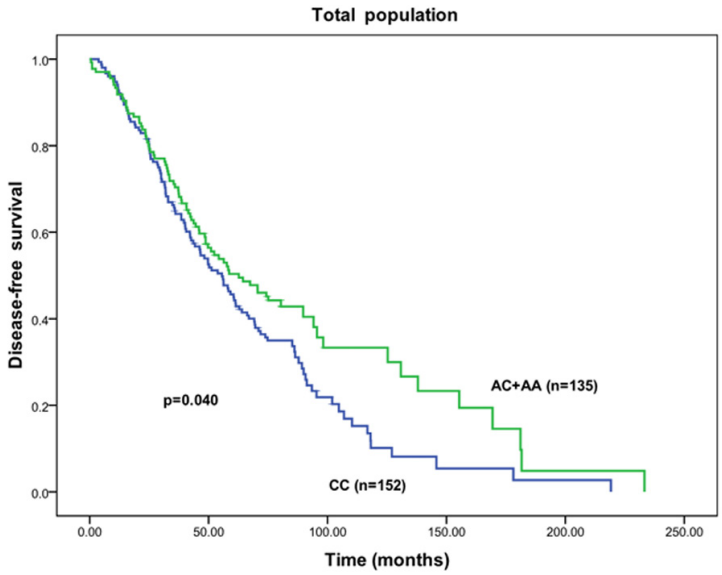

Figure 1. DFS of the whole cohort segregated on the absence or presence of *CYP19A1* rs4646 SNP variant (AA + AC vs. CC). Log-rank *P* = 0.04.

Chicago, IL). Two-sided values less than 0.05 were considered statistically significant.

Deviation from Hardy-Weinberg equilibrium (HWE) was calculated by Pearson's chi-squared test with means of the Finetti program [27].

Results

Clinical features

Two hundred and eighty-seven HR-positive patients were included in the study, and the median age was 46 years (range 20-73 years). As shown in **Table 1**, 217 women were premenopausal and 70 postmenopausal. The clinicopathologic characteristics and treatments were also listed in **Table 1**. Briefly, 250 patients received tamoxifen therapy, 37 patients with the third generation aromatase inhibitors administration. Two hundred and seventy-four (95.5%) received adjuvant chemotherapy.

CYP19A1 Rs4646 polymorphism and DFS in the whole cohort

Based on the analysis of all patients, no significant differences were observed between rs4646 polymorphism and DFS (AA versus AC versus CC: 52.37 months versus 62.7 months versus 55.6 months; *P* = 0.106) (**Table 2**). When the population was subgrouped into two cohorts, one with AA variant, the other carrying AC or CC genotypes, there was no relationship between the genotypes and DFS (AA versus AC or CC: 52.37 months versus 57.67 months; HR, 0.716; 95% CI, 0.396-1.293; *P* = 0.266) (**Table 2**). However, women with the minor allele (AA or AC) had an improved DFS when compared with those carrying the homozygous common allele (CC) (AA or AC versus CC: 62.7 months versus 55.6 months; HR, 0.745; 95% CI, 0.562-0.988; *P* = 0.04) (**Table 2**; **Figure 1**). Furthermore, being adjusted by positive lymph nodes, tumor size > 2 cm, negative hormone receptor status, HER-2-postive status, chemotherapy and BMI > 24 in multivariate analyses, AA or AC genotype remained an independent prognostic factor for DFS (HR, 0.681; 95% CI, 0.506-0.917; *P* = 0.011) (**Table 2**).

CYP19A1 Rs4646 polymorphism and DFS in premenopausal women

In premenopausal women, rs4646 genotypes were significantly associated with DFS (AA versus AC versus CC: 98.2 months versus 58.6 months versus 55.6 months; *P* = 0.026) (**Table 2**; **Figure 2A**). While the study patients were clustered into two groups, one with AA variant, the other carrying CC or AC genotypes, AA genotype was associated with prolonged DFS (AA versus CC or AC: 98.2 months versus 56.2 months; HR, 0.425; 95% CI, 0.198-0.914; *P* = 0.024) (**Table 2**; **Figure 2B**). Furthermore, being adjusted by the patients features, AA genotype remained an independent prognostic factor for DFS (HR, 0.336; 95% CI, 0.160-0.836; *P* = 0.017) (**Table 2**). In addition, women with the minor allele had an improved DFS when compared with those carrying the homozygous common allele (AA or AC versus CC: 62.7 months versus 55.6 months; HR, 0.709; 95% CI, 0.516-0.975; *P* = 0.033) (**Table 2**; **Figure 2C**). Being adjusted by clinicopathologic characteristics and treatments in multivariate analyses, AA or AC genotypes remained an independent prognostic factor for DFS (HR, 0.670; 95% CI, 0.479-0.938; *P* = 0.019) (**Table 2**).

CYP19A1 Rs4646 polymorphism and DFS in postmenopausal women

In postmenopausal women, rs4646 genotypes were significantly associated with DFS (AA versus AC versus CC: 32.7 months versus not

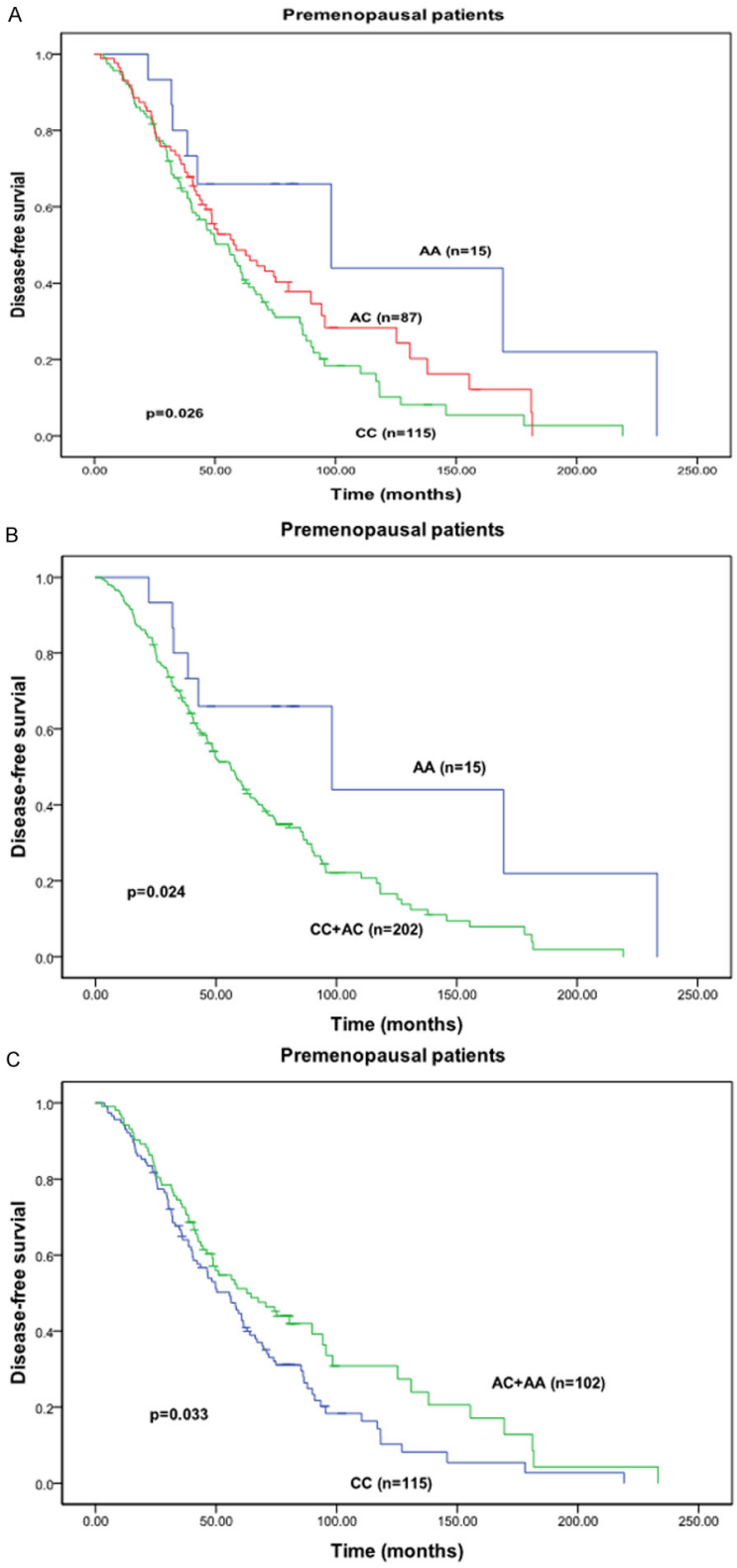

Figure 2. Survival curves for premenopausal patients. A. DFS of premenopausal women stratified by *CYP19A1* rs4646 genotypes (CC vs. AC vs. AA). Log-rank *P* = 0.026. B. DFS of premenopausal women grouped according to *CYP19A1* rs4646 polymorphisms (AA vs. AC + CC). Log-rank *P* = 0.024. C. DFS of premenopausal women grouped according to *CYP19A1* rs4646 genotypes (AA + AC vs. CC). Log-rank *P* = 0.033.

reached versus 56.3 months; *P* = 0.011) (**Table 2**; **Figure 3A**). When the population was subgrouped into two cohorts, women carrying AA variant had a poorer DFS (AA versus CC or AC: 32.7 months versus 70.6 months; HR, 3.613; 95% CI, 1.380-9.457; *P* = 0.005) (**Table 2**; **Figure 3B**). Furthermore, being adjusted by clinicopathologic patients features in multivariate analyses, AA genotype remained an independent prognostic factor for DFS (HR, 3.614; 95% CI, 1.308-9.991; *P* = 0.013) (**Table 2**). However, there were no significant differences in DFS between women harbor the minor allele and those with the homozygous common allele (AA or AC versus CC: 58.23 months versus 56.3 months; HR, 0.868; 95% CI, 0.473-1.594; *P* = 0.648) (**Table 2**).

Discussion

We described a relationship between polymorphic variants of the aromatase gene and the efficacy of adjuvant hormone therapy in women with HR-positive early breast cancer. In the present study, we demonstrated that, women with the minor allele (AA or AC) of *CYP19A1* rs4646 polymorphism had an improved DFS when compared with those carrying the homozygous common allele (CC). Notably, this prognostic effect of *CYP19A1* rs4646 was also evident in premenopausal patients. However, postmenopausal women carrying AA variant had a poorer DFS than those with AC or CC genotype. These differences were further confirmed by multivariate analyses.

Population-based studies of *CYP19A1* polymorphisms have revealed inconsistent results

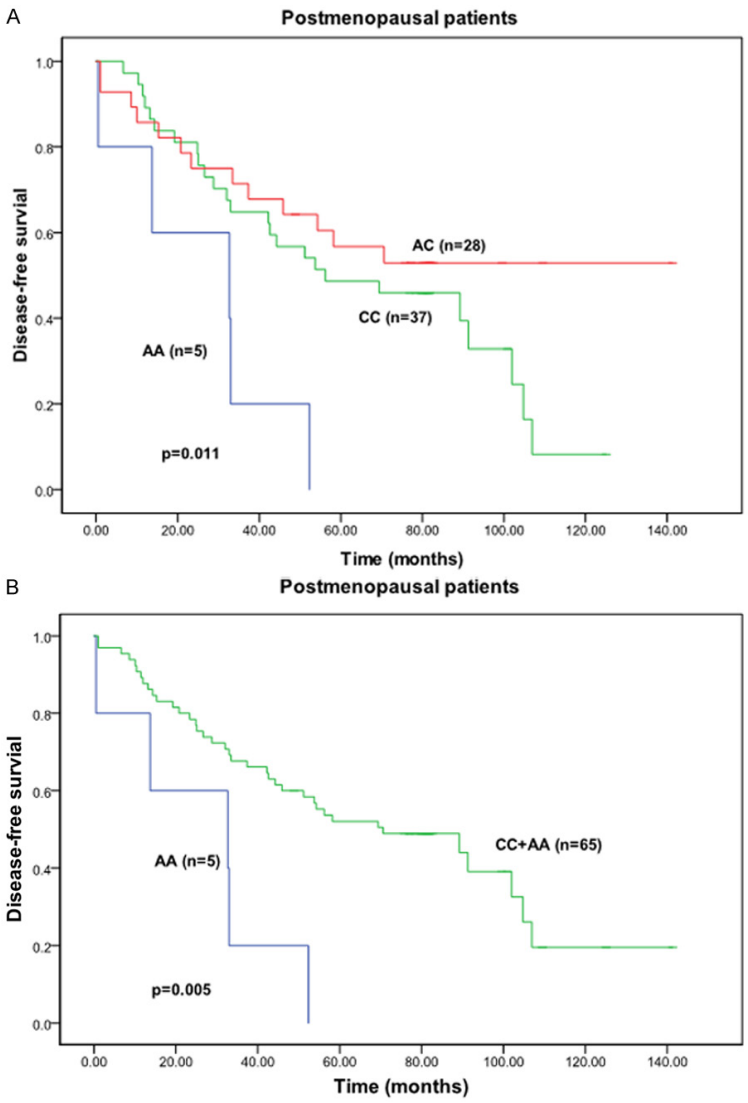

Figure 3. Survival curves for postmenopausal patients. A. DFS of postmenopausal women grouped by CYP19A1 rs4646 genotypes (CC vs. AC vs. AA). Log-rank P = 0.011. B. DFS of postmenopausal women stratified by CYP19A1 rs4646 genotypes (AA vs. AC+CC). Log-rank P = 0.005.

with respect to their potential association with the efficacy of AIs or survival. The data in a cohort of 67 HR-positive MBC women with letrozole administration has demonstrated that patients carrying the rare T allele of rs4646 had a longer TTP which was thrice that of those with homozygotes for the wild-type allele (GG) [22]. Notably, almost half (46%) of the study patients had the variant form of the gene. In addition, the frequency of the variant allele was significantly higher in the responder group (61% versus 40%), and thus, the authors suggested that this finding can be of considerable clinical relevance [22]. Identically, Liu et al. [28] evaluated *CYP19A1* gene polymorphisms in a cohort of 272 patients with MBC who received anastrozole treatment, and revealed that TTP was significantly improved in patients with the variant alleles of rs4646 when compared with those carrying the wild-type allele. Moreover, the rs4646 variant alleles were significantly associated with longer OS. On the contrary, the same variants (GT and TT) were significantly associated with a shorter PFS in the neoadjuvant setting [23]. Besides, the genotypic variants of rs4646 were more frequently represented in the nonresponder subgroup (48% versus 26%) [23]. The study including 296 patients with LN-negative, HR-positive breast cancer has established that the variant alleles of *CYP19A1* rs4646 were significantly related to a shorter DDFS ($P < 0.05$) and marginally associated with a poorer OS ($P = 0.06$) and inferior DFS ($P = 0.07$) [24]. A population-based study with 482 stage I-II and operable stage III Taiwanese breast cancer patients enrolled has demonstrated that a long repeat of the TTTA polymorphism was correlated with a longer survival in premenopausal breast cancer patients but not in postmenopausal women [29]. Similarly, a British population-based study showed that a long repeat of the TTTA polymorphism was in relation with a superior survival [30]. Consequently, the authors speculated that premenopausal patients carrying a longer allele might have a higher level of circulating estrogen and that the changes in estrogen levels among women with different alleles could be more remarkable in premenopausal women [29]. In note, it has been demonstrated that *CYP19A1* polymorphisms were significantly associated with hormone levels [16, 17, 31]. And what's more, some data have suggested that CYP19 gene rs4646 variants were in correlation with higher circulating steroid hormone levels [22, 28].

As mentioned above, we postulate that premenopausal patients with AA genotype may

harbor higher estrogen levels, and, most importantly, the decrease in levels of circulating estrogen caused by therapy may be more evident among patients with AA variant than those carrying AC or CC genotype. Therefore, hormone therapy could be more effective in premenopausal patients with AA variant. However, the changes in estrogen levels is not so great between postmenopausal patients with AA genotype and those carrying AC or CC variant, and thus, the AA genotype is evident to be associated with inferior DFS in postmenopausal patients.

Because majority of the patients (87.1%) in this study received tamoxifen for hormonal therapy, germline single nucleotide polymorphisms (SNPs) of tamoxifen-metabolizing genes may affect their survival. There are two important metabolites of tamoxifen, 4-hydroxy tamoxifen and 4-hydroxy-N-desmethyl tamoxifen, or endoxifen [32]. Tamoxifen is metabolized to endoxifen through cytochrome P450 2D6 (*CYP2D6*). For women who were wild type for *CYP2D6*, the 5-year DFS rates were similar to or perhaps even superior with tamoxifen than with aromatase inhibitors [11]. Most recently, a meta-analysis of ten previous clinical reports (n = 5183) has established that genetic polymorphisms of *CYP2D6* might be important predictors of the clinical outcomes of adjuvant tamoxifen treatment for breast cancer patients [33]. Sulfotransferase 1A1 (*SULT1A1*) catalyzes the sulfation of 4-hydroxy tamoxifen [34]. The risk for breast cancer death among women with homozygous low-activity alleles of *SULT1A1* was three times that of patients carrying homozygous or heterozygous for the common allele [35]. Further validation in a larger prospective cohort should incorporate these genes together in order to evaluate the combined effect of these polymorphisms in patients with tamoxifen hormone therapy.

To conclude, we have demonstrated that the minor allele of *CYP19A1* rs4646 was significantly associated with improved clinical outcome of hormone therapy in premenopausal patients, but a worse effect for postmenopausal women. Testing for the *CYP19A1* rs4646 SNP as a predictive tool for early breast cancer patients with hormone therapy based on a larger independent prospective cohort is warranted.

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Disclosure of conflict of interest

None.

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