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# Association of CYP2C19*3 gene polymorphism with breast cancer in Chinese women

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**ABSTRACT.** Cytochrome P450 (CYP) 2C19 metabolizes arachidonic acid to biologically active epoxyeicosatrienoic acids, which significantly promote proliferation of cancer cells in *vitro* and in *vivo*. We looked for a possible association between human CYP2C19*3 gene polymorphism and breast cancer in the Chinese Han population. In a Chinese Han case-control study of breast cancer patients (N = 600) and age- and gender-matched healthy controls (N = 600), we investigated polymorphism in the CYP2C19 gene by PCR-RFLP analysis. The CYP2C19*3 AG + AA genotype was significantly more prevalent in breast cancer patients than in control subjects (6.67 *vs* 3.00%; P = 0.003). The odds ratio for carriers of AG + AA genotype for breast cancer was 2.31 (95% confidence interval = 1.27-4.43). Among patients, estrogen receptor, tumor size, histologic grade, presence of primary lymphonode metastases, progesterone receptor positivity, and age at diagnosis were not found to be significantly associated with CYP2C19*3 genotypes (all P > 0.05). We conclude that the CYP2C19*3 gene polymorphism is associated with breast cancer risk in Chinese Han women.

**Key words:** Polymorphism; CYP2C19; Breast cancer; Genetics

 

## INTRODUCTION

Cytochrome P450 (CYP) epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs) (Node et al., 2001; Fichtlscherer et al., 2004; Lundell and Wikvall, 2008; Fava et al., 2008). CYP2C19 is one of the human cytochrome P450 enzymes, and it is abundantly expressed in endothelial and smooth muscle cells (Imig et al., 2000; Ercan et al., 2008). CYP2C19 is the key enzyme of EET synthesis. The addition of exogenous EETs has been shown to markedly promote the proliferation of cancer cells in *vitro* and in *vivo* (Jiang et al., 2005). In neoplastic cell lines, EET increased the activation of MAPK and PI3K/Akt pathways and enhanced the phosphorylation of epidermal growth factor receptor (EGFR). EETs also inhibited carcinoma cell apoptosis through up-regulation of the antiapoptotic proteins Bcl-2 and Bcl-xL and down-regulation of the proapoptotic protein Bax (Jiang, et al., 2005). These previous results suggested that the CYP2C19 plays a previously unrecognized role in the promotion of the neoplastic phenotype and in the pathogenesis of a variety of human cancers. In addition, genetic variability may impact the expression and activity of CYP2C19 and can therefore influence EET levels.

Recently, a common genetic variant, CYP2C19*17, was reported to be associated with decreased breast cancer risk in a German population (Justenhoven et al., 2009). However, this variant is common in Europeans but rare in Asians. Another variant, CYP2C19*3 (CYP2C19_G636A), is common in Asians but rare in Europeans according to the record in the NCBI database (http://www.ncbi.nlm.nih.gov/SNP). This polymorphism, 636 G→A substitution in the exon 4 region, changes the tryptophan codon to the termination codon, which stops protein synthesis earlier and results in a protein with a defective heme-binding region. However, the role of this gene variant in breast cancer has not been sufficiently investigated.

Therefore, in this study, we investigated the potential association between CYP2C19*3 and breast cancer in a Chinese Han population.

## MATERIAL AND METHODS

### Subjects

This study included 600 consecutive female patients with histologically confirmed prevalent breast cancers without any other cancer diagnosis besides breast cancer, and a population-based and age-matched control group of 600 healthy women. All patients were recruited between January 2006 and July 2010 from patients seen at the General Surgery Department, First Affiliated Hospital of Chongqing Medical University. For each patient, one healthy female age-matched (±1 year) control subject was included. This study has been approved by the local ethical committee. Written informed consent was obtained from all participating subjects.

### Genotyping

DNA was extracted from peripheral venous blood leukocytes with a whole blood genome extraction kit (Beijing Boiteke Corporation, Beijing, China). Genotyping was confirmed by polymerase chain reaction (PCR)-restriction fragment length polymorphism

(RFLP) analysis. The primer of *CYP2C19* was designed by the Primer Premier 5.0 software. The forward primer was 5'-CATCCTGGGCTGTGCT-3'; and the reverse primer was 5'-AGGGCTTTGGAGTTTAGTG-3'; annealing temperature was 52°C. The PCR product (15 µL) was incubated with 5 U *Bam*HI (Fermentas Corporation) in a total volume of 25 µL overnight at 37°C, and the resulting fragments were separated on a 1.5% agarose gel. The presence of the G636A variant creates a *Bam*HI site producing two fragments of 263, and 133 bp. To confirm the results, we used sequenced genomic DNAs as positive controls in our assays.

### Statistical data analysis

Statistical analysis was done using SPSS 11.0 for Windows. Numerical values were analyzed by the *t*-test, and proportions of groups were compared by the $\chi^2$ test. Odds ratios and 95% confidence intervals were calculated by logistic regression to estimate the risk of breast cancer. Threshold for significance was $P < 0.05$.

## RESULTS

### Subject

The demographics of the cases and controls enrolled in this study are summarized in Table 1. There were no statistically significant differences between the cases and controls for age (P = 0.728), and this suggested that the matching based on these two variables was adequate.

**Table 1.** Characteristics of patients with breast cancer and healthy controls.

| Characteristics | | Patients (N = 600) | Controls (N = 600) | P |
|---|---|---|---|---|
| Tumor size (cm) | <2 | 318 (53.00) | - | - |
| | >2 | 282 (47.00) | - | - |
| Histologic grade | 1 and 2 | 272 (45.33) | - | - |
| | 3 and 4 | 328 (54.67) | - | - |
| Lymph node metastases | Negative | 229 (38.17) | - | - |
| | Positive | 371 (61.83) | - | - |
| Estrogen receptor | Negative | 226 (37.67) | - | - |
| | Positive | 374 (62.33) | - | - |
| Progesterone receptor | Negative | 354 (59.00) | - | - |
| | Positive | 246 (41.00) | - | - |
| Age, y (mean ± SD) | | 53.1 ± 12.3 | 53.2 ± 11.8 | 0.728 |

Data are reported as number with percent in parentheses.

### Distribution of the CYP2C19*3 genotype

We genotyped 600 patients with breast cancer and 600 control individuals. Genetic data are summarized in Table 2. The frequency of the *CYP2C19*3* A allele was significantly higher in breast cancer patients than in controls (3.75 *vs* 1.75%; P = 0.003). The CYP2C19*3 AG + AA genotype was significantly more prevalent in breast cancer patients than in control subjects (6.67 *vs* 3.00%; P = 0.003). The odds ratio for carriers of the AG + AA genotype for breast cancer was 2.31 (95%CI = 1.27-4.43). The odds ratio for carriers of A allele for breast cancer was 2.19 (95%CI = 1.87-4.08).

**Table 2.** Genotype distribution of CYP2C19*3 polymorphism.

| Group | N | Allele (%) | | OR (95% CI) | P | Genotype (N, %) | | | P | OR* (95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | G | | | AA | AG | GG | | |
| Cases | 600 | 3.75 | 96.25 | 2.19 (1.87-4.08) | 0.003 | 5 (0.83) | 35 (5.83) | 560 (93.33) | 0.003 | 2.31 (1.27-4.43) |
| Controls | 600 | 1.75 | 98.25 | | | 3 (0.50) | 15 (2.50) | 582 (97.00) | | |

*P indicated (AA+AG) *vs* GG; *OR indicated (AA + AG) *vs* GG.

## CYP2C19*3 genotype and characteristics of breast cancer

Among patients with breast cancer, the CYP2C19*3 genotypes showed no association with tumor size, histologic grade, estrogen receptor positivity, presence of primary lymph node metastases, progesterone receptor positivity, or age at diagnosis (Table 3).

**Table 3.** CYP2C19*3 polymorphism and characteristics of patients with breast cancer.

| Characteristic | | Genotypes | | P (AA + AG) *vs* GG |
|---|---|---|---|---|
| | | AA + AG (N = 40) | GG (N = 560) | |
| Tumor size (cm) | <2 | 21 (52.5) | 297 (53.0) | 0.948 |
| | >2 | 19 (47.5) | 263 (47.0) | |
| Histologic grade | 1 and 2 | 18 (45.0) | 254 (45.4) | 0.965 |
| | 3 and 4 | 22 (55.0) | 306 (54.6) | |
| Lymph node metastases | Negative | 15 (37.5) | 214 (38.2) | 0.536 |
| | Positive | 25 (62.5) | 346 (61.8) | |
| Estrogen receptor | Negative | 14 (35.0) | 212 (37.9) | 0.429 |
| | Positive | 26 (65.0) | 348 (62.1) | |
| Progesterone receptor | Negative | 24 (60.0) | 330 (58.9) | 0.517 |
| | Positive | 16 (40.0) | 230 (41.1) | |
| Age at diagnosis, years (means ± SD) | | 53.4 ± 12.2 | 52.6 ± 12.4 | 0.456 |

## DISCUSSION

In this case-control study we evaluated the association between the polymorphism of the CYP2C19*3 and breast cancer in a Han Chinese population. Our data showed significant differences in both allele and genotype frequencies between breast cancer patients and healthy controls.

The relationship between breast cancer and the CYP gene family was recently reported, including CYP1B1(Economopoulos and Sergentanis, 2010; Ozbek et al., 2010), CYP2J2 (Jiang et al., 2007, 2009), CYP2D6 (Stingl et al., 2010; González-Tejera et al., 2010; Thompson et al., 2011), CYP2C8 (Knüpfer et al., 2004; Dixit, et al., 2007; Jernström et al., 2009), CYP2C9 (Ekhart et al., 2008; Jernström et al., 2009) and CYP2C19 (Justenhoven et al., 2009; Ruiter et al., 2010; Goetz and Suman, 2010). Several studies have reported different conclusions for the relationship with CYP2C19. Ruiter et al. reported that CYP2C19*2 but not CYP2C19*3 polymorphism is associated with increased survival in breast cancer patients using tamoxifen (Ruiter et al., 2010). Justenhoven et al. suggested CYP2C19*17 but not CYP2C19*3 and CYP2C19*2 is associated with decreased breast cancer risk (Justenhoven et al., 2009). Both of the last two groups explored the association of CYP2C19 genetic polymorphisms with breast cancer in European subjects. However, both CYP2C19*2 (CYP2C19_681_G>A, rs4244285) and CYP2C19*17 (CYP2C19_-806_C>T, rs12248560) are rare in the Chinese population accord-

ing to the HapMap database (http://snp.cshl.org/). The CYP2C19*3 (CYP2C19_636_G>A, rs57081121) is common in the Chinese population but rare in European subjects. Therefore, the selection of CYP2C19*3 for the present study was reasonable.

To our knowledge, this is the first study to report the relationship between *CYP2C19*3* polymorphism and breast cancer in a Chinese Han population. The *CYP2C19*3 mutant A allele changes the tryptophan codon to the termination codon which results in protein synthesis stopping earlier and consequently a truncated protein with a defective heme-binding region (Ercan et al., 2008). Cytochrome P450 epoxygenases metabolize arachidonic acid to EETs. Some of the EETs are reported to have potential antimigratory, antioxidant, and antiapoptotic effects (Sun et al., 2002; Gauthier et al., 2004), and these effects help to explain the association of CYP2C19 with the mechanism of breast cancer.

In the present study, we found the carriers of A allele of CYP2C19*3 have 2.19-fold risk for breast cancer compared to the G allele carriers. However, the CYP2C19*3 polymorphism was not associated with tumor size, histologic grade, presence of primary lymph node metastases, estrogen receptor positivity, progesterone receptor positivity, or age at diagnosis.

The present study included incident as well as prevalent cases, and therefore, a survival bias cannot be excluded. Nevertheless, due to the rather modest effect and the low frequency of the A risk-allele, it is unlikely that the results of the present study were strongly distorted due to survival bias.

## CONCLUSION

We conclude that the CYP2C19*3 polymorphism is associated with the risk of breast cancer and may be a genetic marker for breast cancer in the Chinese Han population.

### Competing interests

The authors declare that they have no competing interests.

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