Joint Effects of the *CYP1A1 MspI*, *ERα PvuII*, and *ERα XbaI* Polymorphisms on the Risk of Breast Cancer: Results from a Population-Based Case-Control Study in Shanghai, China

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Abstract

Estrogen-metabolizing gene and estrogen receptor (ER) genes are the possible risk factors implicated in the initiation and development of breast through estrogen tumorigenesis pathway. We examined whether *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* genetic polymorphisms could increase the risk of breast cancer among Chinese women and gene-gene joint effect on the breast cancer risk in a subset from a population-based case-control study conducted in urban Shanghai from January 1, 1998 and November 31, 2001. PCR-RFLP method based on buccal cells was used to examine the three candidate polymorphisms in 282 breast cancer cases and 298 controls. Compared with *CYP1A1 MspI m1/m1*, the risk of breast cancer was doubled for genotypes *CYP1A1 MspI m1/m2* [odds ratio (OR), 1.83; 95% confidence interval (95% CI), 1.24-2.69] and *CYP1A1 MspI m2/m2* (OR, 2.22; 95% CI, 1.26-3.85). The association seemed to be stronger among cases diagnosed older than 45 years and women without a family history of breast cancer. *ERα PvuII pp* and *ERα XbaI xx* polymorphisms, which are in possible linkage disequilibrium, were both associated with a nonsignificantly elevated risk in all subjects; the associations seemed to be stronger among women with a family history of breast cancer. There seems to be a joint effect on the breast cancer risk between *CYP1A1 MspI* and *ERα XbaI* genotypes (m2/m2 and xx; OR, 5.87; 95% CI, 1.38-24.98), between *CYP1A1 MspI* and *ERα PvuII* genotypes (m2/m2 and pp; OR, 2.39; 95% CI, 0.81-7.07), and among all three genotypes (m2/m2, pp, and xx; OR, 8.07; 95% CI, 1.45-44.77). Results of this study indicate that estrogen-metabolizing genes and estrogen receptor may jointly play a role in the etiology of breast cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(2):342–7)

Introduction

Breast cancer is the most common malignant tumor among women in the United States and Europe. In recent decades, the incidence of breast cancer has been increasing in China, especially, in big cities, such as Shanghai, Beijing, and Tianjin (1, 2). The age-adjusted incidence rate of breast cancer has increased >50% from 18.3 per 10^5 during 1972 to 1974 to 27.5 during 1993 to 1994, making breast cancer the most common cancer among women, Shanghai (2). Although some risk factors, such as early onset of menstruation, nulliparity, delayed age at first childbirth, short duration of breast-feeding, late menopause, family history of breast cancer, and consumption of alcohol, have been identified (3-6), the etiology of breast cancer is still largely elusive. There is increasing evidence that genetic susceptibility may play an important role in the etiology of breast cancer. One of the areas related to the genetic susceptibility is the polymorphisms of estrogen-metabolizing enzyme genes and estrogen receptor genes.

CYP1A1, a phase-one enzyme, metabolizes estrogen and a variety of environmental carcinogens, including polyaromatic hydrocarbons, and produces many reactive intermediates (i.e., catechol estrogen). Estradiol is metabolized through three pathways to form inactive 2-hytroxyestrone, active 4-hytroxyestrone, and active 16α-hytroxyestrone (7). Some studies have shown that estrogen metabolites can bind to DNA and trigger damage, suggesting that estrogen might be a complete carcinogen (8-10). Polymorphisms in the *CYP1A1* gene have been associated with increased metabolic capacity that could affect the level of carcinogens exposure in the breast cells. Therefore, polymorphisms of *CYP1A1* may change individual's susceptibility of breast cancer.

The estrogen receptor α (ERα) is an important mediator of the hormonal response in estrogen-sensitive tissues, such as breast, endometrium, and bone. Although the results were not consistent, a few studies have showed that the polymorphisms in the *ERα* gene, such as *ERα PvuII* and *ERα XbaI*, are associated with the risk of breast cancer (11-13).

Polymorphisms of *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* are the possible risk factors implicated in the initiation and development of the breast cancer through estrogen tumorigenesis pathway (3-6, 11-25). Hence, we examined whether polymorphisms of these two genes that are related to estrogen metabolism and its effect increases the risk of breast cancer among Chinese women.

Materials and Methods

Subjects. Study subjects were a subset of those involved in a large population-based case-control study of risk factors for breast cancer conducted between January 1, 1998 and November 31, 2001 in urban Shanghai. In this large study, 1,595 histologically confirmed breast cancer cases who were 25 to 55 years of age at the time of diagnosis were obtained from Shanghai Cancer Registry Center. A total of 1,794 controls of female permanent residents with no prior history of any cancer were selected randomly from urban Shanghai population records, and they were frequency matched to cases by age (±3 years). All study participants were interviewed in-person by

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trained interviewers and asked to provide buccal cell specimen (cotton swab method) for DNA analysis after signing an informed consent form. A structured questionnaire was administrated, which included detailed information on hormone use; diet intakes during various periods of their life; known risk factors of breast cancer; a completed history of reproduction; contraception and menstruation; a history of breast diseases; a family history of cancers, including breast, ovarian, and other reproductive cancers; and demographic and socioeconomic factors. Ultimately, 1,517 cases (95.11%) and 1,573 controls (87.68%) successfully completed the interview. We also collected oral buccal cell specimen from 1,370 cases (85.89%) and 1,354 controls (75.47%).

Given the constraint of the budget for DNA assay, we randomly selected 300 cases and controls, respectively, from the parent study. After excluding the failed specimens, ultimately, 282 cases and 298 controls were included into the study. Our study sample was comparable with the study population in the parent study in the distribution of many social-demographic characteristics and risk factors for breast cancer.

DNA Extraction and Genotyping. Genetic DNA was extracted from the buccal cell using phenol/chloroform method. Each buccal cell sample was collected by three cotton swabs and stored in the absolute alcohol at −70°C. Polymorphisms of the *CYP1A1 MspI* (Fig. 1) and *ERα PvuII* and *ERα XbaI* (Figs. 2 and 3) genes were revealed by PCR-RFLP assays, as described by Kawajiri et al. (14) and Nishio et al. (26), respectively. The assay results were detected by two independent experiment staff who were blind to the case-control status. Apart from the insufficient DNA from some buccal cell samples, unsuccessful PCR amplification and failed RFLP experiments, 518 samples for *CYP1A1 MspI* (89.3%), 521 samples for *ERα PvuII* (88.3%), and 523 samples for *ERα XbaI* (88.6%) gave rise to the final experiment results. Totally, 587 subjects (259 cases and 278 controls) contributed to the final analysis.

Statistical Analysis. The Hardy-Weinberg equilibrium (HWE) assumption was assessed for defined groups using Pearson's χ^2 test by comparing the observed numbers of different genotypes with those expected under HWE in that group and also for cases by assuming that the control genotypes were under HWE. Estimate of pairwise linkage disequilibrium, such as Lewontin's D', and the correlation coefficient r between *ERα PvuII* and *ERα XbaI* alleles in control women were calculated, as described by Weir (27). Odds ratios (OR) and 95% confidence interval (95% CI) were used to measure the association between the *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* genotypes and breast cancer after adjustment for possible confounders, such as age, education status, family history of breast cancer, age at first birth, and age at menarche using unconditional logistic regression (28). Joint effect on breast cancer of *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* genotypes was also assessed. All analyses were done using SAS software package (version 8.1; SAS Institute, Cary, NC).

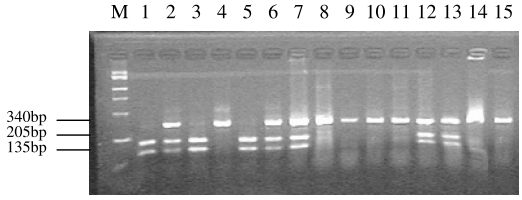

Figure 1. *CYP1A1 MspI* PCR-RFLP genetic polymorphism. *M,* DGL2000Marker; *lanes 1, 3*, and *5, m2/m2,* homozygote; *lanes 2, 6, 7, 12*, and *13, m1/m2,* heterozygote; *lanes 4, 8-11, 14*, and *15, m1/m1,* wild type.

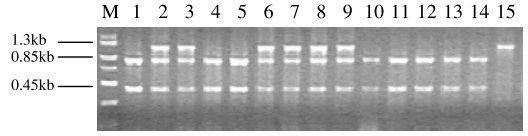

Figure 2. *ERα PvuII* PCR-RFLP genetic polymorphism. *M,* DGL2000Marker; *lanes 1, 4, 5*, and *10-14, pp,* homozygote; *lanes 2-3, 6-7*, and *8-9, Pp,* heterozygote; *lane 15, PP,* wild type.

Results

Characteristics between Cases and Controls. The distribution of selected demographic characteristics and major risk factors for breast cancer are shown in Table 1. Breast cancer cases and controls were comparable in age, educational level, and economic status. Although not all statistically significant, major risk factors of breast cancer, which have been reported from studies elsewhere, occurred more frequently among the cases than among the controls.

HWE Test and Linkage Disequilibrium Estimate. Table 2 shows that the genotype distribution of *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* polymorphisms in case and control. Except *CYP1A1 MspI* in cases and *ERα XbaI* in controls, other alleles did not deviate from HWE. There was an indication of strong linkage disequilibrium between pair of *ERα PvuII* and *ERα XbaI* single nucleotide polymorphisms ($D' = 0.8481$, $r = 0.5960$).

CYP1A1 *MspI* Genetic Polymorphism and Breast Cancer. In this study population, the frequencies of *CYP1A1 MspI m1/m1, CYP1A1 MspI m1/m2, CYP1A1 MspI m2/m2* in the controls were 47.8%, 40.7%, and 11.5%, respectively. Compared with *CYP1A1 MspI m1/m1*, the risk of breast cancer associated with genotype *CYP1A1 MspI m1/m2* (OR, 1.83; 95% CI, 1.24-2.69) and *CYP1A1 MspI m2/m2* (OR, 2.21; 95% CI, 1.26-3.85) was almost doubled, after adjusted for the risk factors for breast cancer and potential confounders. The association seemed to be stronger for breast cancer cases diagnosed at age older than 45 years, and among women without a family history of breast cancer, those differences reached statistical significance (Table 3).

***ERα PvuII* Genetic Polymorphism and Breast Cancer.** For the control population, the frequencies of *ERα PvuII PP, ERα PvuII Pp*, and *ERα PvuII pp* were 15.7%, 45.3%, and 39.0%, respectively. Compared with *ERα PvuII PP* wild type, although not statistically significant, an increased risk of breast cancer was found for the genotypes *ERα PvuII Pp* (OR, 1.47; 95% CI, 0.85-2.54) and *ERα PvuII pp* (OR, 1.38; 95% CI, 0.79-2.42), after adjusted for potential confounders. The association between breast cancer risk and the *ERα PvuII Pp* or *ERα PvuII pp* genotypes seemed to be stronger among women with a family history of breast cancer than among women with no family history of breast cancer, although this difference was not statistical significant (Table 4).

***ERα XbaI* Genetic Polymorphism and Breast Cancer.** The frequencies of *ERα XbaI XX, Xx,* and *xx* in the control subjects were 7.6%, 31.5%, and 60.9%, respectively.

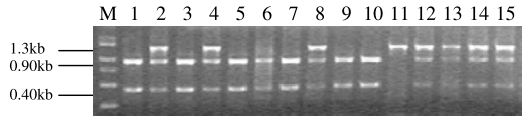

Figure 3. *ERα XbaI* PCR-RFLP genetic polymorphism. *M,* DGL2000Marker; *lanes 1, 3, 5-7*, and *9-10, xx,* homozygote; *lanes 2, 4, 8*, and *12-15, Xx,* heterozygote; *lane 11, XX,* wild type.

Table 1. Characteristics of cases and controls of Shanghai Breast Cancer Study, 2000-2001

	Cases, *n* (%)	Controls, *n* (%)	χ^2	*P*
Age (y)				
<40	36 (13.9)	54 (19.4)	3.419	0.3315
40-44	78 (30.1)	72 (25.9)		
45-49	92 (35.5)	94 (33.8)		
50-55	53 (20.5)	58 (20.9)		
Education level				
Junior middle school	120 (46.3)	139 (50.0)	1.072	0.5852
High school	108 (41.7)	112 (40.3)		
College or higher	31 (12.0)	27 (9.7)		
Income per person (10^3 RMB, last year)				
<6	107 (41.3)	129 (46.4)	1.410	0.2350
≥6	152 (58.7)	149 (53.6)		
Age at menarche (y)				
<15	127 (49.0)	124 (44.6)	1.057	0.3038
≥15	132 (51.0)	154 (55.4)		
Self-reported benign breast illness				
No	113 (43.6)	193 (69.4)	40.292	<0.001
Fibrocystic disease	122 (47.1)	79 (28.4)		
Other	24 (9.3)	6 (2.2)		
Family history of breast cancer*				
No	187 (72.2)	246 (88.5)	22.780	<0.001
Yes	72 (27.8)	32 (11.5)		
Age at first birth (y)				
≤25	21 (8.1)	44 (15.8)	10.300	<0.05
26-29	143 (55.2)	140 (50.4)		
>29	85 (32.8)	76 (27.3)		
Nulliparity	10 (3.9)	18 (6.5)		
Fresh vegetable intake in the past 5 y (kg/y)				
≤73	135 (52.1)	150 (54.0)	0.181	0.6706
>73	124 (47.9)	128 (46.0)		
Fresh fruit intake in the past 5 y (kg/y)				
≤41.6	125 (48.3)	143 (51.4)	0.541	0.4620
>41.6	134 (51.7)	135 (48.6)		

*A history of breast cancer among first-degree and second-degree relatives.

Compared with *ERα XbaI* XX wild type, an increased risk of breast cancer, although not statistically significant, was also found for the genotypes *ERα XbaI Xx* (OR, 1.51; 95% CI, 0.70-3.25) and *ERα XbaI xx* (OR, 1.36; 95% CI, 0.65-2.85), after adjusted for potential confounders. The increased risk of breast cancer associated with genotypes of Xx or xx seemed to be greater among women with a family history of breast cancer than among those without such a family history (Table 5).

Gene-Gene Joint Effects among *CYP1A1 MspI, ERα PvuII,* and *ERα XbaI*. To evaluate whether susceptible genotypes for *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* have joint effects, we compared the population with dose of the susceptible genotypes to the population carrying no susceptible genotypes. Table 6 presents the risk of breast cancer associated with various combinations of susceptible genotypes. The joint risk effects could be observed between *CYP1A1 MspI* and *ERα PvuII* genotypes, between *CYP1A1 MspI* and *ERα XbaI* genotypes, and among all three genotypes. For the combination of *CYP1A1 MspI* and *ERα PvuII* genotype, compared with women carrying no susceptible genotypes, women carrying one to three susceptible alleles (other genotype combination) or four susceptible alleles (m2/m2 and pp) had more than twice increased risk of breast cancer. With increasing number of variant alleles of both *CYP1A1 MspI* and *ERα XbaI*, a corresponding increase in breast cancer risk was also observed (trend test, $P < 0.05$). Compared with the women carrying both m1/m1 and xx wild types, the adjusted OR for the women carrying both m2/m2 and xx homozygote was 5.87 (95% CI, 1.38-24.98). A combination of variant alleles at all three loci (*m2/m2, pp*, and *xx*) increased breast cancer even further (OR, 8.07; 95% CI, 1.45-44.77; trend test, $P < 0.05$). No significant joint risk effect was found between *ERα PvuII* and *ERα XbaI* genotype.

Table 2. Distribution of *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* genotypes in the control and case groups

	Genotype (*n*)			Allele frequency in control group(%)		HWE χ^2	*P*
	wt/wt (%)	wt/vt (%)	vt/vt (%)	w	v		
CYP1A1 MspI							
Control	128 (47.8)	109 (40.7)	31 (11.5)	68.1	31.9	1.097	0.2952
Case	83 (33.2)	125 (50.0)	42 (16.8)			22.593	0.0000
*ERα PvuII**							
Control	43 (15.7)	124 (45.3)	107 (39.0)	38.3	61.7	0.499	0.4801
Case	29 (11.7)	120 (48.6)	98 (39.7)			1.721	0.1896
*ERα XbaI**							
Control	21 (7.6)	87 (31.5)	168 (61.9)	23.4	76.6	3.968	0.0464
Case	14 (5.7)	84 (34.0)	149 (60.3)			0.353	0.5526

Abbreviations: wt, wild type; vt, variant type.
*Lewontin $D' = 0.8481$, $r = 0.5960$.

Table 3. *CYP1A1 MspI* Genotypes in relation to breast cancer risk

Genotype *CYP1A1 MspI*	Case (%), n = 250	Control (%), n = 268	OR* (95% CI)
m1/m1	83 (33.2)	128 (47.8)	1.00
m1/m2 + m2/m2	167 (76.8)	140 (52.8)	1.91 (1.32-2.76)
m1/m2	125 (50.0)	109 (40.7)	1.83 (1.24-2.69)
m2/m2	42 (16.8)	31 (11.5)	2.21 (1.26-3.85)
Older than 45 y †			
m1/m1	46 (33.2)	76 (47.8)	1.00
m1/m2	69 (50.0)	54 (40.7)	2.32 (1.37-3.92)
m2/m2	24 (16.8)	16 (11.5)	2.77 (1.31-5.86)
≤45 y †			
m1/m1	37 (33.3)	52 (42.6)	1.00
m1/m2	56 (50.5)	55 (45.1)	1.28 (0.71-2.32)
m2/m2	18 (16.2)	15 (12.3)	1.50 (0.64-3.51)
Family history of breast cancer ‡			
m1/m1	26 (36.6)	16 (50.0)	1.00
m1/m2	36 (50.7)	11 (34.4)	1.45 (0.53-3.98)
m2/m2	9 (12.7)	5 (15.6)	0.98 (0.25-3.89)
No family history of breast cancer ‡			
m1/m1	57 (31.8)	112 (47.5)	1.00
m1/m2	89 (49.7)	98 (41.5)	1.76 (1.15-2.71)
m2/m2	33 (18.5)	26 (11.0)	2.46 (1.34-4.53)

*Adjusted for age, education level, family history of breast cancer, self-reported benign breast illness, age at first birth, and age at menarche.
†Not adjusted for age.
‡Not adjusted for family history of breast cancer.

Discussion

Most of the studies on *CYP1A1* gene polymorphisms and breast cancer were conducted in the developed countries (21, 22, 29-31). There were a few studies on the relationship between *CYP1A1 MspI* genotype and breast cancer among Asian women. Results thus far have not been consistent (21-23, 29, 30). In our study, the genetic frequency distribution of *CYP1A1 MspI m1/m1, m1/m2,* and *m2/m2* genotypes among women in Shanghai is similar to that in Huang et al. (23), a study of women in Taiwan. We found that women who are homozygous for *CYP1A1 MspI* (*m2/m2*) had increased risk of breast cancer. The increased susceptibility to the risk of breast cancer associated with the genotype seems to show a slight dose-response relationship. The association seems to be stronger among breast cancer cases diagnosed older than 45 years and among women without a family history. This finding of variation of the association by women's age was also consistent with Huang's another report that the *CYP1A1 MspI* polymorphism was more significantly associated with the increased risk of breast cancer in postmenopausal women (24). CYP1A1 is one of the major enzymes involving in estrogen hydroxylation. Recent studies have shown that the estrogen metabolites, such as catechol estrogen, 4-hytroxyestrone, and 16α-hytroxyestrone, are able to bind to DNA, creating adducts and causing gene mutations (7). Two studies (32, 33) have reported that the CYP1A1 activity is more readily inducible in lymphocytes with *m2/m2* genotype than in *m1/m1* lymphocytes, which means that the women who carry the *m2/m2* genotype are likely to produce more estrogen metabolites leading to DNA damage than other women.

Because ERα is an important mediator of the hormonal response in estrogen sensitive tissues, the genetic polymorphisms on the ER were therefore postulated as the potential risk factors of breast cancer. Studies in western and Asian women have revealed, however, inconsistent associations of *ERα PvuII* and *ERα XbaI* polymorphisms with breast cancer. Parl et al. (34) found that the pp genotype of *Pvu*II was related to younger breast cancer patients, but no such correlation was found in a later study by Yaich et al. (35). Andersen et al. (11)

Table 4. *ERα PvuII* Genotypes in relation to breast cancer risk

Genotype *ERα PvuII*	Case (%) n = 247	Control (%) n = 274	OR* (95%CI)
PP	29 (11.7)	43 (15.7)	1.00
Pp/pp	218 (88.3)	231 (84.3)	1.43 (0.85-2.40)
Pp	120 (45.6)	124 (45.3)	1.47 (0.85-2.54)
pp	98 (39.7)	107 (39.0)	1.38 (0.79-2.42)
Older than 45 y			
PP	20 (14.5)	28 (18.6)	1.00
Pp	63 (45.7)	61 (40.7)	1.51 (0.76-3.00)
pp	55 (39.8)	61 (40.7)	1.32 (0.66-2.65)
≤45 y			
PP	9 (8.3)	15 (12.1)	1.00
Pp	57 (52.3)	63 (51.8)	1.21 (0.47-3.09)
pp	43 (39.4)	46 (37.1)	1.36 (0.52-3.56)
Family history of breast cancer			
PP	6 (9.0)	8 (25.8)	1.00
Pp	34 (50.8)	13 (41.9)	2.46 (0.61-9.88)
pp	27 (40.2)	10 (32.3)	3.04 (0.73-12.67)
No family history of breast cancer			
PP	23 (12.8)	35 (14.4)	1.00
Pp	86 (47.8)	111 (45.6)	1.21 (0.66-2.21)
pp	71 (39.4)	97 (40.0)	1.12 (0.61-2.07)

*Adjusted for age, education level, family history of breast cancer, Self-reported benign breast illness, age at first birth, and age at menarche.

Table 5. *ERα XbaI* genotypes in relation to breast cancer risk

Genotype *ERα XbaI*	Case (%), n = 247	Control (%), n = 276	OR* (95% CI)
XX	14 (5.7)	21 (7.6)	1.00
Xx/xx	233 (94.3)	255 (92.4)	1.41 (0.69-2.42)
Xx	84 (34.0)	87 (31.5)	1.51 (0.70-3.25)
xx	149 (60.3)	168 (60.9)	1.36 (0.65-2.85)
Older than 45 y			
XX	8 (5.8)	11 (7.3)	1.00
Xx	43 (30.9)	47 (31.3)	1.41 (0.50-3.92)
xx	88 (63.3)	92 (61.4)	1.46 (0.54-3.89)
≤45 y			
XX	6 (5.6)	10 (7.9)	1.00
Xx	41 (38.0)	40 (31.8)	1.52 (0.48-4.79)
xx	61 (56.4)	76 (60.3)	1.20 (0.39-3.66)
Family history of breast cancer			
XX	3 (4.5)	5 (15.6)	1.00
Xx	23 (34.3)	10 (31.3)	3.42 (0.49-23.93)
xx	41 (61.2)	17 (53.1)	4.20 (0.65-27.28)
No family history of breast cancer			
XX	11 (6.1)	16 (6.6)	1.00
Xx	61 (33.9)	77 (31.6)	1.14 (0.49-2.66)
xx	108 (60.0)	151 (61.8)	0.99 (0.44-2.24)

*Adjusted for age, education level, family history of breast cancer, Self-reported benign breast illness, age at first birth, and age at menarche.

reported that the frequency of the *x* allele of the *Xba*I polymorphisms among breast cancer patients was 1.4 times higher than that among the controls ($P < 0.05$). Recently, two case-control studies were conducted in Asian women. Shin et al. (12) reported that compared with those carrying *XX* genotype, South Korean women carrying the *xx* genotype of *Xba*I has 2.5 times the risk of breast cancer; however, no difference in the *Pvu*II polymorphism distribution was detected between the breast cancer patients and the controls. However, a large-scale population-based case-control study conducted in urban Shanghai by Cai et al. (13) showed that the *pp* genotype of *Pvu*II was associated with a relative risk of 1.4 (95% CI, 1.1-1.8) when compared with women with *PP* genotype. The *XbaI* polymorphism was associated with a nonsignificantly elevated risk; however, for *Xx* and *xx* genotype, a higher elevated risks were mainly confined to older or postmenopausal women. Our results indicated that both *ERα PvuII Pp/pp* and *ERα XbaI Xx/xx* genotypes may increase the risk of breast cancer, and the women with a family history of breast cancer may be more susceptible to the variants. However, this finding needs to be further confirmed. For the *ERα PvuII Pp/pp* and *ERα XbaI Xx/xx* genotypes, we did not observe a variation of the association by age.

Although three genetic polymorphisms have been reported to be associated with breast cancer individually, their joint effects on breast cancer have not been well examined. Our study now provides some evidence that certain combination of the *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* polymorphisms may jointly increase the susceptibility to breast cancer probably through the pathway of estrogen-metabolizing and ER estrogen-binding response.

Examination of gene-gene interaction requires a large sample size. Thus far, only a few studies have examined effect of genetic polymorphism of multiple genes on the risk of breast cancer. Huang et al. (23) in 1999 reported that three estrogen-metabolizing genes had the joint effect of increasing the risk of breast cancer. Recently, Mitrunen et al. (36) in Finland reported that the combination of *COMT-L* allele with the *GSTM1* null genotype posed a significantly increased risk of breast cancer (OR, 9.10; 95% CI, 1.84-45.0). Zheng et al. (37) reported that there was a joint effect of ERβ sequence polymorphism [C(33390)G] and endogenous estrogen exposure

Table 6. Combined effect of genotypes of *CYP1A1 MspI, ERα PvuII*, and *ERα XbaI* in relation to breast cancer

	Case (%)	Control (%)	OR* (95%CI)
ERα PvuII PP/CYP1A1 MspI m1/m1	10 (4.2)	22 (8.3)	1.00
One to three susceptive alleles †	213 (89.5)	228 (86.4)	2.24 (1.01-4.98)
ERα PvuII pp/CYP1A1 MspI m2/m2	15 (6.3)	14 (5.3)	2.39 (0.81-7.07)
Trend test, P = 0.1068			
ERα XbaI XX/CYP1A1 MspI m1/m1	3 (13)	13 (4.8)	1.00
One to three susceptive alleles ‡	213 (89.5)	235 (88.4)	4.67 (1.26-17.30)
ERα XbaI xx/CYP1A1 MspI m2/m2	22 (9.2)	18 (6.8)	5.87 (1.38-24.98)
Trend test, P = 0.0480			
ERα PvuII PP/ERα XbaI XX	10 (4.1)	16 (5.9)	1.00
Other genotype combinations §	144 (58.5)	157 (57.5)	1.57 (0.67-3.69)
ERα PvuII pp/ERα XbaI xx	92 (37.4)	100 (36.6)	1.55 (0.65-3.69)
Trend test, P = 0.6343			
CYP1A1 MspI m1/m1/ERα PvuII PP/ERα XbaI XX	2 (0.9)	13 (4.9)	1.00
One to five susceptive alleles ‖	220 (92.8)	237 (90.2)	6.89 (1.48-32.11)
CYP1A1 MspI m2/m2/ERα PvuII pp/ERα XbaI xx	15 (6.3)	13 (4.9)	8.07 (1.45-44.77)
Trend test, P = 0.0458			

*Adjusted for age, education level, family history of breast cancer, Self-reported benign breast illness, age at first birth, and age at menarche.
†*PPm1/m2, PPm2/m2, Ppm1/m1, Ppm1/m2, Ppm2/m2, ppm1/m1*, and *ppm1/m2* genotype combinations.
‡*XXm1/m2, XXm2/m2, Xxm1/m1, Xxm1/m2, Xxm2/m2, xxm1/m1*, and *xxm1/m2* genotype combinations.
§*PPXx, PPxx, Ppxx, PpXx, Ppxx, ppXX*, and *ppXx* genotype combinations.
‖The other three genotype combinations excepting *m1/m1, PP*, and *XX* combination and *m2/m2, pp*, and *xx* combination.

on the risk of breast cancer among Chinese women. Those and our studies indicated that multiple genes may be involved in increasing the risk of breast cancer.

A few limitations need to be kept in mind when interpreting the results. First, the sample size of this study was relatively small which prevented some observed effects of genetic polymorphisms from reaching statistical significance. Second, due to the strong linkage disequilibrium between the *ERα PvuII* and *ERα XbaI* single nucleotide polymorphisms, it may be possible that the observed effect associated with *ERa PvuII* and *ERa XbaI* polymorphism reflected the effect of the same disease allele in linkage disequilibrium with both *ERa PvuII* and *ERa XbaI* alleles. This may explain the absence of joint risk effect for *ERα PvuII* and *ERα XbaI* genotypes. Third, the original study was designed to study risk factors of breast cancer among relatively young breast cancer cases, where all our cases were 55 years of age or younger. Therefore, we were not able to examine whether the association between polymorphisms of those two genes and the risk of breast cancer varied between premenopausal and postmenopausal women.

In summary, in this Chinese population, we observed a >2-fold increased risk of breast cancer associated with susceptible genotypes of *CYP1A1 MspI* compared with wild genotypes of *CYP1A1 MspI*. The *ERα PvuII* and *ERα XbaI* polymorphisms were associated with a nonsignificantly elevated risk of breast cancer. There was some indication that women's age at diagnosis of breast cancer and a family history may modify the association between these polymorphisms and breast cancer risk. The risk of breast cancer was further increased when combination of those susceptible genotypes was present simultaneously. Our findings suggest that polymorphism of genes involving estrogen-metabolizing pathway and estrogen receptor pathway may play an important role in the etiology of breast cancer.

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