Genetic Polymorphisms in the Estrogen Receptor α Gene and Risk of Breast Cancer: Results from the Shanghai Breast Cancer Study[1]

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Abstract

Estrogen receptor (ER) is a ligand-activated transcription factor that mediates estrogen actions in target tissues. Several common polymorphisms of the *ER-α* gene have been reported to be associated with alterations in receptor expression and function. We evaluated the hypothesis that genetic polymorphisms in the *ER-α* gene may be associated with breast cancer risk in a population-based case-control study conducted in urban Shanghai during 1996–1998. Two RFLPs at the *ER-α* gene locus, denoted as *Pvu*II and *Xba*I, were examined in 1069 breast cancer cases and 1166 age frequency-matched controls. *Pvu*II polymorphism was associated with an increased risk of breast cancer with the age-adjusted odds ratios for genotypes *Pp* and *pp* being 1.3 [95% confidence interval (CI), 1.0–1.7) and 1.4 (95% CI, 1.1–1.8), respectively, comparing to genotype *PP*. The *Xba*I polymorphism was associated with a nonsignificantly elevated risk. The odds ratios for genotypes *Xx* and *xx* were 1.2 (95% CI, 0.7–1.9) and 1.3 (95% CI, 0.8–2.0), respectively, and the elevated risks were mainly confined to older or postmenopausal women. No apparent synergetic effect of these two polymorphisms was identified. Results of this study indicate that genetic polymorphisms in the *ER-α* gene may play a role in the etiology of breast cancer.

Introduction

Estrogen influences the growth, differentiation, and function of many target tissues, including the breast, uterus, vagina, ovary, testis, epididymis, and prostate (1). The biological effect of estrogens such as stimulating growth and differentiation of normal mammary tissue is mediated primarily through high-affinity binding to ERs[3] (2). ERs are nuclear receptor proteins that have an estrogen binding domain and a DNA binding domain (3, 4). There are two types of ERs, ER-α and ER-β. The *ER-α* gene is localized on chromosome 6q25.1 (5), and the *ER-β* gene is localized on chromosome 14q22-24 (6). Among the steroid receptors, ER-α and the ER-regulated PR are of special interest because their protein levels are elevated in premalignant and malignant breast cells (7). Both ER and PR have been demonstrated to be significant prognostic factors for breast cancer (8). Consequently, inhibition of the ER-α has become one of the major strategies for the prevention and treatment of breast cancer (9).

The association of genetic polymorphisms in the *ER-α* gene and the risk of diseases, including breast cancer, have been the subject of increasing interest. Several DNA sequence variations in the *ER-α* gene have been reported. Two case-case studies showed that *Pvu*II polymorphism was related to a younger age at breast cancer diagnosis (10, 11). Two case-control studies reported that *Xba*I, not *Pvu*II, polymorphism was related to breast cancer risk (12, 13). The sample sizes from these studies, however, were small in general, and potential selection bias in hospital-based case-control study was a concern.

In this article, we report results from the Shanghai Breast Cancer Study, a large-scale population-based case-control study that examined the association of *ER-α* gene *Pvu*II and *Xba*I polymorphisms with the risk of breast cancer. The associations of these polymorphisms with ER/PR status among breast cancer cases were also evaluated.

Materials and Methods

Study Subject. Case patients and control subjects in this study were participants of the Shanghai Breast Cancer Study, a population-based case-control study (14–16). This study included 1459 women who were between the ages of 25–64 years and diagnosed with breast cancer from August 1996 through March 1998, as well as 1556 age frequency-matched control women. The study protocol was approved by committees of relevant institutions for the use of human subjects in research. All case patients and control subjects were permanent residents of urban Shanghai who had no histories of breast cancer. Through a rapid case ascertainment system supplemented by the population-based Shanghai Tumor Registry, 1602 eligible case patients with breast cancer were identified during the study period, and in-person interviews were completed for 1459 (91%) of them. The major reasons for nonparticipation were refusal (109 case patients, 6.8%), death before the interview (17 case patients, 1.1%), and the inability to locate (17 case patients,

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[3] The abbreviations used are: ER, estrogen receptor; PR, progesterone receptor; QC, quality control; OR, odds ratio; CI, confidence interval; FAR, floating absolute risk; BMI, body mass index; WHR, waist-to-hip ratio; PI3K, phosphatidylinositol 3′-kinase.

Table 1 Comparison of cases and controls by selected demographic factors and major risk factors for breast cancer from the Shanghai Breast Cancer Study, 1996–1998

	Subjects included in whole study		Subjects with genotying data		*P*		
	Cases (n = 1459) (1)	Controls (n = 1556) (2)	Cases (n = 1069) (3)	Controls (n = 1166) (4)	Case (3 *versus* 1)	Control (4 *versus* 2)	Case *versus* control (3 *versus* 4)
Demographic factors							
Age (yr)[a]	47 (42, 53)	46 (40, 54)	47 (42, 53)	46 (40, 54)	0.088	0.666	0.230
Education ≥ high school, %	45.2	44.3	43.7	42.7	0.346	0.274	0.642
Major risk factor							
First-degree relative with breast cancer, %	3.7	2.4	3.3	2.3	0.510	0.853	0.168
Ever diagnosed with breast fibroadenoma, %	9.6	5.0	9.9	5.2	0.759	0.783	<0.001
Smoking, %	2.6	2.5	2.4	2.7	0.797	0.811	0.734
Drinking, %	4.0	4.1	3.4	4.0	0.298	0.921	0.464
Hormone replacement therapy, %	2.9	2.7	2.7	2.5	0.788	0.721	0.838
No regular leisure physical activity, %	81.3	74.8	80.9	74.4	0.782	0.758	<0.001
WHR[a]	0.81 (0.77, 0.84)	0.80 (0.76, 0.84)	0.81 (0.77, 0.84)	0.80 (0.76, 0.83)	0.209	0.495	<0.001
WHR >0.84, %	26.7	21.3	26.3	20.6	0.769	0.554	0.001
BMI[a]	23.2 (21.2, 25.5)	22.8 (20.7, 25.1)	23.1 (21.2, 25.6)	22.9 (20.8, 25.2)	0.892	0.296	0.035
≥25, %	30.4	25.8	31.0	26.9	0.694	0.412	0.036
Age at menarche (yr)[a]	14 (13, 16)	15 (13, 16)	14 (13, 16)	15 (13, 16)	0.650	0.596	0.001
Age at menarche ≤13 yr, %	31.6	26.5	31.6	25.9	0.981	0.637	0.003
Premenopausal, %	65.5	63.8	67.2	64.1	0.263	0.824	0.130
Age at menopause ≥50 yr[b], %	40.5	37.2	41.0	37.2	0.906	0.966	0.282
No live birth, %	5.1	3.9	4.9	4.1	0.811	0.787	0.393
Age at first live birth ≥30 yr[a], %	22.2	16.5	22.0	16.3	0.900	0.906	<0.001
Calorie intake (Kcal)[a]	1796 (1541, 2128)	1782 (1529, 2075)	1795 (1536, 2132)	1784 (1535, 2087)	0.994	0.678	0.278
Fat (g)[a]	32.9 (25.0, 43.5)	32.5 (24.6, 43.2)	33.1 (24.9, 44.0)	32.5 (24.5, 43.0)	0.787	0.958	0.088
Protein (g)[a]	73.5 (60.2, 91.0)	71.9 (59.5, 87.1)	73.3 (60.2, 91.0)	71.8 (59.1, 87.0)	0.737	0.969	0.013
Carbohydrate (g)[a]	313.0 (272.7, 366.1)	310.2 (269.8, 360.0)	313.9 (272.8, 366.5)	312.9 (270.3, 361.5)	0.999	0.534	0.852

[a] Median (25th, 75th percentile) are presented, *P* were derived from test.
[b] Among postmenopausal women.
[c] Among parous women.

1.1%). Cancer diagnoses for all patients were confirmed by two senior study pathologists through a review of tumor slides. Detailed information on cancer diagnosis and treatment, including ER and PR status, was abstracted from medical charts. The information on ER/PR status was obtained from 956 of the 1459 breast cancer cases. Of those, 52.7% were ER+/PR+, 11.2%, ER+/PR−, 10.6%, ER−/PR+, and 25.5%, ER−/PR−.

Control subjects were randomly selected from the female general population and were frequency matched to case patients by age (5-year intervals). The number of control subjects in each age-specific stratum was determined in advance according to the most recent data on the age distributions of the breast cancer patients available from the Shanghai Tumor Registry. The Shanghai Resident Registry, which keeps registry cards for all adult residents in urban Shanghai, was used to randomly select control subjects. For each age-predetermined control subject, a registry card identifying a potential control subject in the same 5-year age group was randomly selected. Only the women who lived at the address during the study period were considered to be eligible for the study. In-person interviews were completed for 1556 (90%) of the 1724 eligible control subjects identified. Excluded from the study were 168 potential control subjects because of refusal (n = 166; 9.6%) and death or a prior cancer diagnosis (n = 2; 0.1%).

A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive histories, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. All participants were also measured for their current weight, circumferences of the waist and hip, and heights while sitting and standing. Blood samples were obtained from 1193 (82%) case patients and 1310 (84%) control subjects who completed the in-person interviews. These samples were used for the genotyping assays in this study.

DNA Extraction. Genomic DNA was extracted from buffy coat fractions using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. DNA concentration was measured by PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR). Ten ng of genomic DNA were used for each PCR.

PCR-RFLP. *ER-α* genotypes were determined with a PCR-RFLP method reported earlier (10, 17) with some modification. The primers for analysis were: 5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′ (forward) and 5′-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3′ (reverse). These primers generated a 1.3-kb fragment. The PCR was performed in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc., Waltham, MA). Each 35 μl of PCR mixture contained 10 ng of DNA, 1× PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0)], 2.5 mM $MgCl_2$, 0.16 mM deoxynucleoside triphosphate, 0.5 μM of each primer, and 1.5 unit of TaqDNA polymerase.

The reaction mixture was initially denatured at 94°C for 3 min, followed by 36 cycles of 94°C for 45 s, 61°C for 45 s, and 72°C for 2 min. The PCR was completed by a final extension cycle at 72°C for 7 min. The product contains a part of *intron 1* and *exon 2* of the *ER-α* gene.

The PCR products were digested by the *Pvu*II and *Xba*I restriction endonucleases, respectively. The DNA fragments were then separated using 1.5% agarose gel and detected by ethidium bromide staining. *PP* and *XX*, signifying the absence of restriction sites, gave one 1.3-kb fragment. *pp*, signifying the presence of *Pvu*II restriction sites on both alleles, was digested into two fragments (0.85 and 0.45 kb). The *xx* genotype was revealed by *Xba*I digestion into two fragments (0.9 and 0.4 kb).

The laboratory staff was blind to the identity of the subject. QC samples were included in genotyping assays. Each 96-well plate contains one water, two Centre d'Etude du Polymorphisme Humain (CEPH) 1347-02 DNA, two blinded QC DNA, and two unblinded QC DNA samples. The blinded and unblended QC samples were taken from the second tube of study samples included in the study. The *ER-α* genotypes determined for the QC samples were in complete agreement with the genotypes determined for the study samples.

Genotyping data were obtained from 1069 (89.6%) cases and 1166 (89.0%) controls who have blood samples, which represent 67% (1069 of 1602) of eligible case patients and 67% (1166 of 1724) of eligible control subjects. The major reasons for incomplete genotyping were insufficient DNA and unsuccessful PCR amplification.

Statistic Analysis. χ^2 statistics were used to evaluate case-control difference in the distribution of allele types and genotypes. To accommodate the age frequency-matched study design, we used logistic regression models conditioned on age to estimate ORs and 95% CIs to measure the strength of the association between *ER-α* gene polymorphisms and breast cancer risk (18, 19). Although unconditional logistic regression could also be used by including age variable in the model, conditional logistic regression is more direct and intuitive in the control for the effect of matched variables and is more parsimonious because it does not include matched variables in the model. To better assess CIs for estimates based on the small reference group, the FAR CIs were analyzed (20). Analyses stratified by age and menopausal status were conducted to check the homogeneity of the association. Additional analyses stratifying by indicators of endogenous estrogen exposure and lifestyle factors were conducted to evaluate the potential modifying effects of these variables on the association between *ER-α* genotypes and breast cancer risk. All statistical tests were two-sided.

Results

The distributions of selected demographic characteristics and major risk factors for breast cancer are shown in Table 1. These data are presented separately for subjects with genotyping data and those included in the whole study to evaluate the consistency of the distribution of these factors between these two groups. There was no appreciable difference between cases included in the genotyping study and the whole study. The same was true for the two control groups. Breast cancer cases and controls were comparable in age and education level. Although not all statistically significant, elevated risks of breast cancer were observed for all major risk factors that have been reported from studies conducted elsewhere (21). The associations of these risk factors with breast cancer were similar in the subjects with genotyping data and subjects in the whole study (Table 1).

Table 2 Polymorphisms of *ER-αPvu*II and *Xba*I and breast cancer risk, Shanghai Breast Cancer Study, 1996–1998

	Case (%) (n = 1069)	Control (%) (n = 1166)	OR[a]	95% CI	FAR 95% CI
*Pvu*II					
PP	12.9	16.3	1.0	(ref)[b]	
Pp/pp	87.1	83.7	1.4	(1.1–1.7)	
Pp	48.3	46.8	1.3	(1.0–1.7)	(1.2–1.5)
pp	38.8	36.9	1.4	(1.1–1.8)	(1.2–1.6)
Trend test			P = 0.042		
*Xba*I					
XX	3.4	4.2	1.0	(ref)	
Xx/xx	96.6	95.8	1.2	(0.8–1.9)	
Xx	46.5	43.6	1.3	(0.8–2.0)	(1.1–1.4)
xx	50.1	52.3	1.2	(0.7–1.8)	(1.0–1.3)
Trend test			P = 0.660		
*Pvu*II and *Xba*I					
PPXX	2.7	3.2	1.0	(ref)	
PPXx	8.0	9.5	1.0	(0.6–1.7)	(0.7–1.3)
PPxx	2.3	3.7	0.7	(0.3–1.4)	(0.4–1.2)
PpXX	0.6	0.7	1.0	(0.3–3.4)	(0.4–3.0)
PpXx	27.9	27.2	1.2	(0.7–2.0)	(1.0–1.4)
Ppxx	19.7	18.8	1.3	(0.7–2.1)	(1.0–1.5)
ppXX	0	0	–		
ppXx	10.6	7.3	1.7	(1.0–3.1)	(1.3–2.3)
ppxx	28.2	29.6	1.1	(0.7–1.9)	(1.0–1.3)

[a] Adjusted for age.
[b] ref., reference.

Allele frequencies of *ER-α* gene *Pvu*II and *Xba*I polymorphisms in the control group were similar to those reported previously from other studies conducted in Asian populations (13, 17). The *P* allele was slightly more prevalent among controls (39.7%) than cases (37.1%) in the *Pvu*II polymorphism (P = 0.07). Approximately 16.3% of controls and 12.9% of cases were homozygous, and 46.8% of controls and 48.3% of cases were heterozygous for this allele (P = 0.08). Age-adjusted ORs for genotypes *Pp* and *pp* were 1.3 (95% CI, 1.0–1.7) and 1.4 (95% CI, 1.1–1.8), respectively, comparing to genotype *PP* (Table 2). There was no appreciable difference in allele frequency or genotype of the *Xba*I polymorphism between controls and cases (Table 2). When the two *ER-α* polymorphisms were analyzed jointly, no consistent synergistic effect was noted. The genotype combinations, including one or two *p* alleles, were, in general, associated with an elevated risk, and the highest OR was observed for the genotype with *pp* and *Xx* combination (OR = 1.7, 95% CI, 1.0–3.1). The ORs were essentially unchanged after additional adjustment of physical activity, BMI, WHR, menarche, live girth, age at first birth, and breast cancer family history. Also presented in Table 2 are FAR. The range of these risk estimates are narrower than the 95% CI estimated for point estimate of risk.

Table 3 showed the associations of *ER-α* genotypes with breast cancer risk by age and menopausal status. The *Pvu*II polymorphism was associated with elevated breast cancer risk in all strata defined by age and menopausal status. For the *Xba*I polymorphism, however, the elevated risks were mainly confined to older (>45 years) or postmenopausal women.

Additional analyses were conducted to evaluate the association of the *ER-α* gene polymorphisms and risk of breast cancer by indicators of endogenous estrogen exposure (Table 4). These indicators included years of menstruation, a prior history of breast fibroadenoma, BMI, and WHR. The risk

Table 3 Polymorphisms of *ER-α PvuII* and *XbaI* and breast cancer risk by age at diagnosis and menopausal status, Shanghai Breast Cancer Study, 1996–1998

	Age ≤45					Age >45				
	Case % (n = 471)	Control % (n = 558)	OR[a]	95% CI	FAR 95% CI	Case % (n = 598)	Control % (n = 608)	OR[a]	95% CI	FAR 95% CI
*Pvu*II										
PP	12.2	15.5	1.0	(ref)[b]		13.5	17.1	1.0	(ref)	
Pp/pp	87.8	84.5	1.4	(1.0–2.0)		86.5	82.9	1.3	(1.0–1.8)	
Pp	46.7	48.2	1.3	(0.9–1.9)	(1.1–1.5)	49.7	45.5	1.4	(1.0–1.9)	(1.2–1.6)
pp	41.2	36.4	1.5	(1.0–2.2)	(1.2–1.9)	36.9	37.4	1.3	(0.9–1.8)	(1.0–1.5)
Trend test			P = 0.036					P = 0.406		
*Xba*I										
XX	4.1	4.5	1.0	(ref)		2.9	3.8	1.0	(ref)	
Xx/xx	95.9	95.5	1.1	(0.6–2.0)		97.1	96.2	1.4	(0.7–2.6)	
Xx	48.6	43.1	1.2	(0.6–2.3)	(1.0–1.5)	44.8	44.0	1.4	(0.7–2.6)	(1.1–1.6)
xx	47.3	52.3	1.0	(0.5–1.8)	(0.8–1.2)	52.4	52.2	1.4	(0.7–2.6)	(1.2–1.6)
Trend test			P = 0.203					P = 0.548		

	Premenopausal					Postmenopausal				
	Case % (n = 715)	Control % (n = 746)	OR[a]	95% CI	FAR 95% CI	Case % (n = 349)	Control % (n = 417)	OR[a]	95% CI	FAR 95% CI
*Pvu*II										
PP	13.0	15.9	1.0	(ref)		12.9	17.0	1.0	(ref)	
Pp/pp	87.0	84.1	1.3	(1.0–1.8)		87.1	83.0	1.4	(0.9–2.1)	
Pp	47.6	47.6	1.3	(0.9–1.8)	(1.1–1.5)	50.0	45.3	1.4	(0.9–2.2)	(1.2–1.8)
pp	39.4	36.5	1.4	(1.0–1.9)	(1.2–1.7)	37.1	37.7	1.3	(0.8–2.0)	(1.0–1.7)
Trend test			P = 0.057					P = 0.448		
*Xba*I										
XX	3.6	4.1	1.0	(ref)		2.9	4.1	1.0	(ref)	
Xx/xx	96.4	95.9	1.0	(0.6–1.8)		97.1	95.9	1.5	(0.7–3.3)	
Xx	47.8	44.9	1.1	(0.6–1.9)	(1.0–1.3)	43.8	41.4	1.5	(0.7–3.5)	(1.2–1.9)
xx	48.5	51.0	1.0	(0.6–1.7)	(0.9–1.2)	53.3	54.5	1.4	(0.6–3.3)	(1.2–1.8)
Trend test	P = 0.456							P = 0.899		

[a] Adjusted for age.
[b] ref, reference.

associated with the p allele of the *Pvu*II polymorphism was elevated in all strata, and no apparent modifying effect of endogenous estrogen exposure indicators was observed. We also evaluated the association of the *ER-α* gene polymorphisms and risk of breast cancer by lifestyle factors. The proportions of smokers, drinkers, and hormone replacement therapy user were very small, making it difficult to evaluate their modifying effects. No apparent modifying effects were found for physical activity, calorie intake, fat intake, protein intake, and carbohydrate intake (Table 4). Similarly, no modifying effect of endogenous estrogen exposure indicators and lifestyle factors on the association between *Xba*I polymorphism and breast cancer risk was found.

We also evaluated the association of *ER-α* gene *Pvu*II and *Xba*I polymorphisms with ER/PR status among breast cancer cases. No apparent association was observed (Table 5).

Discussion

The association of *ER-α* genetic polymorphisms with breast cancer risk attracts much attention because ER functions as a hormone-dependent transcriptional regulator, which, in turn, plays a significant role in the development of breast cancer (1, 22). Several *ER-α* gene polymorphisms have been reported, among which *Pvu*II and *Xba*I polymorphisms are the most studied. Several diseases, including breast cancer (10–13), endometrial cancer (23), Alzheimer's disease (24), obesity (25), multiple sclerosis (26), endometriosis (27), adenomyosis (28), leiomyomas (27, 28), and bone mineral density (17, 19–34), have been evaluated for possible linkage with *Pvu*II and *Xba*I polymorphisms.

Both *Pvu*II and *Xba*I polymorphisms are located in *intron 1* of the *ER-α* gene and are 50 bp apart (10, 35). Parl *et al.* (11) found that the *pp* genotype of *Pvu*II was related to a younger age at breast cancer diagnosis. Yaich *et al.* (10) examined the *Pvu*II polymorphism in the tumor tissue of 257 primary breast cancer patients and 140 peripheral blood DNA samples from women without breast cancer. Breast cancer patients with a *pp* genotype were significantly younger than women with *PP* or *Pp* genotype at the time of cancer diagnosis. However, this finding was found in only one of the two study hospitals (10). In a study of 360 breast cancer patients from a hospital in Norway and 672 convenient controls, Andersen *et al.* (12) found that allele frequencies of the *Pvu*II polymorphism did not differ between cases and controls. The frequency of the x allele of the *Xba*I polymorphism among breast cancer patients, however, was 1.4 times of that for controls (95% CI, 1.0–1.9; Ref. 12). Among the breast cancer patients, there was an association of borderline significance between the *Xba*I restriction site and older age at onset (12). In a hospital-based case-control study (201 cases and 201 controls) conducted in South Korea, Shin *et al.* (13) reported that OR associated with the *xx* genotypes of *Xba*I was 2.38 (95% CI, 1.58–3.58) compared with women with *XX* genotype. In our large-scale population-based case-control study, we found that the polymorphism at the *Pvu*II restriction site (*p* allele) was associated with an elevated risk of breast cancer. The associations were, in general, weak with the

Table 4 Polymorphisms of *ER-α PvuII* and *XbaI* and breast cancer risk by selected estrogen exposure-related factors and lifestyle factors, Shanghai Breast Cancer Study, 1996–1998

	PvuII polymorphism				*XbaI* polymorphism			
	PP		Pp or pp		XX		Xx or xx	
	Case/control	OR[a] (95% CI)	Case/control	OR[a] (95% CI)	Case/control	OR[a] (95% CI)	Case/control	OR[a] (95% CI)
Age at menarche (yr)								
≤13	98/140	1.0 (ref)	619/707	1.3 (1.0–1.7)	29/37	1.0 (ref)[b]	698/813	1.1 (0.6–1.8)
>13	38/47	1.0 (ref)	298/250	1.5 (1.0–2.4)	7/11	1.0 (ref)	327/289	1.5 (0.6–4.1)
Age at menopause (yr)[c]								
<50	26/45	1.0 (ref)	176/209	1.4 (0.8–2.4)	7/13	1.0 (ref)	196/246	1.5 (0.6–3.9)
≥50	18/24	1.0 (ref)	122/128	1.3 (0.7–2.4)	3/4	1.0 (ref)	139/148	1.3 (0.3–5.7)
Years of menstruation[d]								
<Median (30 yr)	54/85	1.0 (ref)	396/470	1.4 (0.9–2.0)	19/26	1.0 (ref)	431/538	1.0 (0.6–1.9)
≥Median (30 yr)	82/102	1.0 (ref)	523/489	1.3 (1.0–1.8)	17/22	1.0 (ref)	596/566	1.4 (0.7–2.6)
Ever diagnosed with breast fibroadenoma								
No	119/179	1.0 (ref)	830/906	1.4 (1.1–1.8)	32/46	1.0 (ref)	924/1046	1.2 (0.8–2.0)
Yes	16/8	1.0 (ref)	89/53	0.9 (0.4–2.2)	4/2	1.0 (ref)	102/58	0.9 (0.2–5.0)
BMI								
<25	93/136	1.0 (ref)	635/706	1.4 (1.0–1.8)	29/34	1.0 (ref)	704/807	1.0 (0.6–1.6)
≥25	43/51	1.0 (ref)	284/253	1.3 (0.9–2.1)	7/14	1.0 (ref)	323/297	2.1 (0.9–5.4)
BMI[c]								
<25	22/43	1.0 (ref)	168/211	1.6 (0.9–2.7)	6/10	1.0 (ref)	185/245	1.4 (0.5–3.8)
≥25	22/26	1.0 (ref)	130/126	1.3 (0.7–2.4)	4/7	1.0 (ref)	150/149	1.8 (0.5–6.4)
WHR								
≤0.84	102/154	1.0 (ref)	674/757	1.4 (1.0–1.8)	27/38	1.0 (ref)	756/878	1.2 (0.7–2.0)
>0.84	34/33	1.0 (ref)	245/202	1.2 (0.7–2.0)	9/10	1.0 (ref)	271/226	1.3 (0.5–3.2)
Regular exercise during past 10 years								
Yes	26/56	1.0 (ref)	176/240	1.5 (0.9–2.6)	4/8	1.0 (ref)	199/286	1.3 (0.4–4.3)
No	110/131	1.0 (ref)	742/719	1.3 (1.0–1.7)	32/40	1.0 (ref)	827/818	1.2 (0.8–2.0)
Caloric intake								
<Medium	64/94	1.0 (ref)	452/484	1.4 (1.0–1.9)	17/24	1.0 (ref)	505/556	1.3 (0.7–2.5)
≥Medium	72/93	1.0 (ref)	467/475	1.3 (1.0–1.9)	19/24	1.0 (ref)	522/548	1.1 (0.6–2.0)
Fat intake								
<Medium	62/83	1.0 (ref)	436/490	1.2 (0.8–1.7)	16/26	1.0 (ref)	489/550	1.5 (0.8–2.8)
≥Medium	74/104	1.0 (ref)	483/469	1.5 (1.1–2.1)	20/22	1.0 (ref)	538/554	1.0 (0.6–1.9)
Protein intake								
<Medium	60/95	1.0 (ref)	440/482	1.4 (1.0–2.0)	17/26	1.0 (ref)	489/553	1.4 (0.7–2.5)
≥Medium	76/92	1.0 (ref)	479/477	1.3 (0.9–1.8)	19/22	1.0 (ref)	538/551	1.1 (0.6–2.0)
Carbohydrate intake								
<Medium	62/98	1.0 (ref)	458/480	1.5 (1.0–2.1)	20/19	1.0 (ref)	505/556	0.9 (0.5–1.6)
≥Medium	74/89	1.0 (ref)	461/479	1.2 (0.9–1.7)	16/29	1.0 (ref)	522/548	1.6 (0.9–3.0)

[a] Adjusted for age.
[b] ref, reference.
[c] Among postmenopausal women only.
[d] Years of menstruation = menopausal age or age at interview for premenopausal women − menarche age.

highest OR of 1.5. The *Xba*I polymorphism was associated with a nonsignificant increased risk for breast cancer only among older or postmenopausal women. The differences in study design (hospital based *versus* population based) and study population (Caucasian *versus* Chinese) may have contributed to the inconsistency between findings from previous and current studies.

How breast cancer risk is affected by the intronic *Pvu*II polymorphism of the *ER-α* gene remains unclear. Possible explanations include: (*a*) the intronic polymorphism may be in linkage disequilibrium with *exon* alteration, which affects ER protein function (17); (*b*) the *Pvu*II polymorphism in the *ER-α* gene may be linked with the alteration of another unidentified gene adjacent to the *ER-α* gene, which increases breast cancer risk (17); (*c*) intronic changes in gene sequence may have an impact on the expression of other genes by influencing the transcription and/or stability of mRNA of those genes (36, 37); (*d*) and some *introns* contain regulatory sequences such as enhancers, which affect the levels of expression through transcriptional regulation (38, 39). For example, it has been shown that a polymorphism within the first *intron* of Drosophila β-3-tubulin gene has a significant effect on the level of the protein synthesis (39). The *Pvu*II polymorphism has been previously reported to be associated with ER expression in a study of 188 breast cancer patients (40). Along with two later studies (10, 12), we did not find this association in our study. It has also been indicated that the *Pvu*II restriction site (*p* allele) is associated with PR negativity (12). Additional functional analyses of this polymorphism are needed to better understand how the *Pvu*II polymorphism is involved in breast cancer development.

Other genetic polymorphisms of *ER-α* gene may also be associated with breast cancer risk. In a hospital-based study, a statistically significant association was found between the polymorphism in *ER-α* codon 325 [CCC→CCG (Pro)] and a self-reported family history of breast cancer (2). However, no association was found between *ER-α* gene polymorphism in codon 325 and the risk of breast cancer in a population-based case-control study of breast cancer among younger women (<40 years) conducted in Australia (41). Recently, Kang *et al.* (42) reported that this silent mutation in *exon 4* was associated

Table 5 Association of *ER-α PvuII* and *XbaI* polymorphisms with ER/PR status among breast cancer patients, Shanghai Breast Cancer Study, 1996–1998

	*Pvu*II (%)			*Xba*I (%)		
	PP	Pp	pp	XX	Xx	xx
ER status						
Positive	13.5	48.6	37.9	3.0	46.0	51.0
Negative	12.8	45.0	42.2	3.1	48.1	48.8
	$P = 0.516$			$P = 0.857$		
PR status						
Positive	14.4	46.6	39.0	2.7	46.5	50.8
Negative	11.6	48.2	40.2	3.6	47.0	49.4
	$P = 0.599$			$P = 0.788$		
ER/PR status						
Both positive	14.6	48.3	37.1	3.0	46.3	50.7
One positive	10.9	43.8	45.3	2.1	46.1	51.8
Both negative	12.6	47.5	39.9	3.8	48.1	48.1
Unknown	23.8	50.4	37.5	4.0	46.0	50.0
	$P = 0.639$			$P = 0.940$		

with tumor PR and ER expression. Additional studies are needed to investigate the association of these polymorphisms, as well as their interaction with *Pvu*II and *Xba*I polymorphisms. In addition, growth factors and their signaling molecules are important for breast cancer growth and progression. There is considerable cross-talk between ER and growth factors pathways (43). For example, the PI3K pathway mediates cell survival and proliferation signals coming from growth factors such as insulin, the insulin-like growth factors, and epidermal growth factor family (44). ER directly interacts with PI3K in the cell membrane to activate it (45). The PI3K/AKT pathway can also directly modulate the ER by phosphorylating ER at serine 167 (46). The mitogen-activated protein kinase and stress response pathways also interact with ER (43). It would be useful to study the interaction of the *ER-α* gene with genes involved in these pathways.

The current study has many strengths. First, the high participation rate and population based study design substantially reduced selection bias. Second, Chinese women living in Shanghai are relatively homogeneous in ethnic backgrounds; over 98% of them are classified into a single ethnic group (Han Chinese). Therefore, the potential confounding effect by ethnicity is not a major concern in our study. Third, the extensive information on lifestyle factors allowed a comprehensive evaluation of their interaction with the genetic polymorphisms.

In summary, in this population-based case-control study, we found that *Pvu*II polymorphism in the *ER-α* gene was associated with breast cancer risk. Additional studies are needed to understand the nature of the association.

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