

RESEARCH ARTICLE

Estrogen Receptor Alpha Gene Polymorphisms and Breast Cancer Risk: a Case-control Study with Meta-analysis Combined

Hong Lu¹, Dong Chen², Li-Ping Hu¹, Lian-Lian Zhou¹, Hui-Ying Xu¹, Yong-Heng Bai³, Xiang-Yang Lin^{1*}

Abstract

Molecular epidemiological studies have shown that gene polymorphisms of estrogen receptor alpha gene (*ESR- α*) are associated with breast cancer risk. However, previous results from many molecular studies have been inconsistent. In this study, we examined two polymorphisms (*PvuII* and *XbaI* RFLPs) of the *ESR- α* gene in 542 breast cancer cases and 1,016 controls from China. Associations between the polymorphisms and breast cancer risk were calculated with an unconditional logistic regression model. Linkage disequilibrium and haplotypes were analyzed with the SHEsis software. In addition, we also performed a systematic meta-analysis of 24 published studies evaluating the association. No significant associations were found between the *PvuII* polymorphism and breast cancer risk. However, a significantly decreased risk of breast cancer was observed among carriers of the *XbaI* 'G' allele (age-adjusted OR = 0.80; 95% CI = 0.66- 0.97) compared with carriers of the 'A' allele. Haplotype analysis showed significantly decreased cancer risk for carriers of the 'CG' haplotype (OR = 0.79; 95% CI = 0.66- 0.96). In the systematic meta-analysis, the *XbaI* 'G' allele was associated with an overall significantly decreased risk of breast cancer (OR = 0.90, 95% CI = 0.82- 1.00). In addition, the *PvuII* 'C' allele showed a 0.96- fold decreased disease risk (95% CI = 0.92- 0.99). In subgroup analysis, an association between the *PvuII* 'C' and *XbaI* 'G' alleles and breast cancer risk was significant in Asians ('C' vs. 'T': OR = 0.93, 95% CI = 0.85- 1.00; 'G' vs. 'A': OR = 0.82, 95% CI = 0.68- 0.98), but not in Euro-Americans. Thus, our results provide evidence that *ESR- α* polymorphisms are associated with susceptibility to breast cancer. These associations may largely depend on population characteristics and geographic location.

Keywords: Estrogen receptor - polymorphism - breast cancer - risk - meta-analysis

Asian Pac J Cancer Prev, 14 (11), 6743-6749

Introduction

Cumulative, excessive estrogen exposure from both endogenous and exogenous sources can lead to pathological consequences in multiple human tumors, including breast cancer (Crooke et al., 2011). Experiments have shown that estrogen is an important regulator of growth and differentiation in the normal mammary gland and is important in the development and progression of breast carcinoma (Medina et al., 2001). Epidemiological evidence has provided support for the association between estrogen levels and breast cancer in postmenopausal women (Russo et al., 1998; Key et al., 2002). In vivo, the biological actions of estrogen are mediated by estrogen receptors (*ESRs*), which interact with other cell-signaling pathways to influence cell behavior. There are two major types of *ESRs*, including *ESR- α* and *ESR- β* . In breast cells, the *ESR- α* plays an important role in regulating cell proliferation and differentiation through a paracrine mechanism (Mallepell et al., 2006). Mammary glands from the *ESR- α* knockout mouse do not undergo ductal

morphogenesis and alveolar development. Disrupted *ESR- α* signaling may result in reduced estrogen-responsive gene products in the mammary gland (Bocchinfuso et al., 2000). A recent study by Liu et al. found that *ESR- α* plays an important role in regulating p53 activity. *ESR- α* binding to p53 leading to functional inactivity of wild-type p53 could be one reason for the inability of wild-type p53 to inhibit tumor growth and metastasis in *ESR*-positive breast cancer (Liu et al., 2006). Thus, genetic variations in genes controlling estrogen activity, including *ESR- α* , could reveal a potential risk for breast cancer.

The rs2234693 (*PvuII*, C/T) and rs9340799 (*XbaI*, G/A) polymorphisms of the *ESR- α* gene are most commonly reported as associated with breast cancer. In 1992, the first report to evaluate these associations was published by Yaich and his colleagues (Yaich et al., 1992). They identified a random subset of 257 cases of primary breast cancer and 140 controls without breast cancer in the United States. Based on their analysis, the *PvuII* polymorphism was not associated with estrogen receptor content or patient age at tumor diagnosis. Although this

¹Department of Laboratory Medicine, ³Wenzhou Key Laboratory of Surgery, First Affiliated Hospital of Wenzhou Medical University,

²Wenzhou Center of Disease Control and Prevention, Wenzhou, China *For correspondence: greatsailor@163.com

conclusion was also supported by Wedrén et al. in Sweden (Wedren et al., 2004), other researchers, such as Cai et al. (2003) and Onland-Moret et al. (2005), have failed to show this same result with different populations. Another polymorphic variant *XbaI* did not show any association with breast cancer risk in a Shanghai (China) population (Cai et al., 2003; Shen et al., 2006), but it was strongly correlated with susceptibility to breast cancer in Korean women (Shin et al., 2003). The inconsistent association outcomes are probably due to differences in the study populations. In the present study, we have carried out a case-control study to investigate the relationship between the *PvuII* and *XbaI* polymorphisms of *ESR-α* gene and breast cancer risk in the Chinese population, as one salient characteristic of China's population that it possesses a large base of diverse genetic backgrounds. The present study can provide a platform to help explain the pathological mechanism of breast cancer and a better understanding of the geographic and ethnic differences associated with disease incidence and mortality.

Since it can be difficult for individual studies to achieve sufficient statistical power to detect associations between the *ESR-α* gene polymorphisms and breast cancer risk, a meta-analysis that combines data from all published studies may detect genetic associations more accurately. In addition, a reduced probability of false-negatives might also be achieved (Egger et al., 2003). Therefore, a systematic meta-analysis of population-based studies was performed to investigate the association between the *ESR-α* polymorphisms and breast cancer risk.

Materials and Methods

Study population in our study

Between March 2007 and October 2010, a total of 542 female breast cancer patients with a mean age of diagnosis of 50.60 years (range 25-83 years) were enrolled in the study from the Zhejiang region in China. All cases diagnosed were confirmed by pathological examination. In addition, 1,016 unrelated healthy women with a mean age of 48.64 years (range 18-84 years) were recruited as controls. The controls were screened to ensure that there had never been a diagnosis of cancer. All subjects were informed about the contents of the study and gave their informed consent. This study was approved by the Ethics Committee of Wenzhou Medical University, Wenzhou, China.

Genotyping and quality control

Blood samples were collected with the anticoagulant EDTA K2 and stored at -20°C. Genomic DNA was isolated using a DNA Extraction Kit (TaKaRa Bio Group, Japan) and stored at -20°C. *ESR-α* genotypes were determined by a PCR-RFLP method reported earlier (Kobayashi et al., 1996). The specific primers for analysis were 5'-CTG CCA CCC TAT CTG TAT CTT TTC CTA TTC TCC-3' (forward) and 5'-TCT TTC TCT GCC ACC CTG GCG TCG ATT ATC TGA-3' (reverse). For the positive internal control, the primer 5'-TCC ACC ACC CTG TTG CTG TA-3' (forward) and 5'-ACC ACA GTC CAT GCC ATC AC-3' (reverse) coding for human GAPDH gene was

used. The negative control utilized the same reagents as those used with actual samples, but without the DNA templates. In addition, a total of 155 samples (about 10%) were randomly selected and genotyped and confirmed by DNA sequencing by a second investigator.

Meta-analysis

To examine the association between the *ESR-α* polymorphisms and breast cancer risk, a search of the MEDLINE database (from January 1990 to March 2010), EMBASE, Cochrane, and the US National Library of Medicine's PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>) was performed. In addition, various scientific research tools available on the web were used to search relevant references such as Google (<http://scholar.google.com/>) and Scirus (<http://www.scirus.com/>). We focused on the two well-characterized polymorphic variants: *PvuII* and *XbaI*. Keywords used in searches included 'estrogen receptor' in combination with the terms 'polymorphism', 'genotype', 'allele', 'breast cancer' or 'risk'.

Papers selected for this meta-analysis included a case-control study and complete data. All relevant references that met the inclusive criterion were required to be published as articles or abstracts and to contain original data. Case-only studies, studies without complete data, or studies with inadequate control groups were excluded.

To estimate associations with breast cancer risk, various genotypic models were selected. Both the Peto Mantel-Haenszel fixed-effects model and the DerSimonian Laird random-effects model were used to calculate summary ORs, and both within- and between-study variations were considered (DerSimonian et al., 2007). A P-value of less than 0.10 was considered statistically significant when comparing trials showing heterogeneity, and random-effects analysis was selected for such trial; in contrast, fixed-effects analysis was used for comparing trials showing homogeneity. Inverted funnel plots were used to examine asymmetry; the ORs were plotted on a logarithmic scale against the inverse of their corresponding standard errors (Oxman et al., 1993). In the presence of publication bias, the funnel plot was asymmetric, and the data showed remarkable skewness. There may be many reasons for this, most notably that some studies with negative findings are not published. In contrast, the plots were symmetric when bias was absent.

Statistical analysis

Tests for Hardy-Weinberg equilibrium were performed separately for each SNP among case and control subjects. An independent samples t-test was used to determine differences according to age, and the chi-square or Fisher's exact test was performed to calculate the clinical parametric distributions. Unconditional logistic regression analysis models were used to evaluate the relationships between different genotypes and disease risk [odds ratios (OR), 95% confidence intervals (95% CI)] adjusted by age. Linkage disequilibrium and haplotypes were analyzed with the SHEsis software (Shi et al., 2005; Li et al., 2009).

All of the statistical analyses were performed in the Statistical Package for Social Sciences (SPSS, version

Table 1. Clinic and Demographic Characteristics of Study Participants at Time of Joining the Cohort

Characteristics	Cases		Controls		OR(95%CI)	P value
	N	%	N	%		
Ages (year)						
mean ^a	50.60±9.75		48.64±10.12			0.113
<40	63	11.6	262	25.8		
40~49	191	35.1	354	34.8		
50~59	192	35.6	252	24.8		
≥60	96	17.7	148	14.6		
Smoking status						
Nonsmoking	511	0.94	928	0.91	1.49(0.71-3.10)	0.286 ^b
Smoking	10	0.02	27	0.03		
NR	21	0.04	61	0.06		
Alcohol intake						
No alcohol	495	0.91	931	0.92	0.99(0.58-1.68)	0.973 ^b
Drinking	22	0.04	41	0.04		
NR	25	0.05	44	0.04		
Expression of ESR						
(+)	262	48.4				
(-)	160	29.5				
NR	120	22.1				
Expression of PR						
(+)	220	40.6				
(-)	198	36.5				
NR	124	22.9				
Metastasis						
(+)	174	32.1				
(-)	290	53.5				
NR	78	14.4				
Tumor type						
Ductal	412	0.76				
Parathyroid	91	0.17				
Others	39	0.07				

^aData are expressed as mean±standard deviation (SD), *P* values are calculated using unpaired t-test; ^bBased on chi-square test; NR, not reported

13.0) and Review Manager (version 4.2, The Cochrane Collaboration). A *P*-value of less than 0.05 was considered statistically significant, and all of the *P* values were two-sided.

Results

ESR-α polymorphisms and breast cancer risk in a Chinese population

Table 1 shows the demographic and clinical characteristics of the subjects (542 breast cancer patients and 1,016 non-cancer controls). No significant differences in age, smoking status, or alcohol intake were observed between cases and controls. In the present study, two polymorphisms, *PvuII* and *XbaI*, located at the 5' end of *ESR-α* gene were evaluated; each SNP was in Hardy-Weinberg equilibrium, as shown in Table 2. Statistical analysis revealed a significant difference in the frequency of the *XbaI* genotype (*P* = 0.044), while the *PvuII* polymorphism did not show any significant differences. Compared to data for the *XbaI* 'AA', subjects with the 'GG' genotypes were associated with a decreased risk, assessed by chi-square statistics (OR = 0.59, 95% CI = 0.35- 0.99, *P* = 0.042, table not shown). After adjustment for age, the OR value was 0.80 (95% CI = 0.64- 1.02, *P* = 0.069). The *XbaI* 'G' allelic frequency occurred at 18.5% in cancer patients, which was significantly lower than that observed for controls (22.3%), indicating a decreased

Table 2. Distribution of ESR-α Genotypes, Alleles and Haplotypes Between Breast Cancer Cases and Controls

Models	Cases (freq)	Controls (freq)	<i>P</i> value*	OR (95%CI)
<i>PvuII</i> genotype				
<i>TT</i> (wt)	227 (0.42)	425 (0.42)	1.00 (ref)	
<i>CT</i> (ht)	258 (0.48)	454 (0.44)	0.55	1.07 (0.85-1.34)
<i>CC</i> (mut)	57 (0.10)	137 (0.14)	0.251	0.81 (0.57-1.16)
Trend test				
<i>T</i>	712 (0.66)	1,304 (0.64)	1.00 (ref)	
<i>C</i>	372 (0.34)	728 (0.36)	0.542	0.95 (0.81-1.12)
<i>XbaI</i> genotype				
<i>AA</i> (wt)	363 (0.67)	623 (0.613)	1.00 (ref)	
<i>AG</i> (ht)	158 (0.29)	332 (0.327)	0.069	0.80 (0.64-1.02)
<i>GG</i> (mut)	21 (0.04)	61 (0.060)	0.131	0.67 (0.40-1.13)
Trend test				
<i>A</i>	884 (0.82)	1,578 (0.78)	1.00 (ref)	
<i>G</i>	200 (0.18)	454 (0.22)	0.022	0.80 (0.66-0.97)
Haplotypes				
<i>CA</i>	180.4 (0.17)	297 (0.15)	0.145	1.16 (0.95~1.42)
<i>CG</i>	191.6 (0.18)	431 (0.21)	0.016	0.79 (0.66~0.96)
<i>TA</i>	703.6 (0.64)	1,281 (0.63)	0.362	1.08 (0.92~1.26)
<i>TG</i>	8.4 (0.01)	23 (0.01)	-	-

*Adjusted for age; wt, homozygote wild type; ht, heterozygote mutated; mut, homozygote mutated

disease risk associated with this allele (OR = 0.80, 95% CI: 0.66- 0.97, *P* = 0.022). In addition, the associations of the *XbaI* or *PvuII* polymorphisms with breast cancer risk according to different clinical stages (lymph node status), expression of estrogen receptor, or stratified by the average age of 50 years were also evaluated, but no significant differences were observed (Table not shown). In this report, statistical significance of linkage disequilibrium was detected among these two polymorphisms, and the *P* value was 6.7×10^{-16} (*D'* = 0.926, *r*² = 0.417). Haplotypes with the two-loci of *ESR-α* gene polymorphisms were analyzed with the SHEsis software. The frequency of haplotype 'CG' was 17.7% in cases, significantly lower than that observed for controls (21.2%, *P* = 0.016), suggesting that 'CG' indicates a decreased disease risk.

Characteristics and qualitative assessment of included studies in the meta-analysis

According to the criteria defined above, 24 published studies relevant to the *ESR-α* gene and breast cancer risk were reviewed. Ten of these papers were excluded due to insufficient clarity in data presentation, repeated literature, or significant differences present in the study design compared to the other papers identified. The remaining 14 eligible case-control studies (listed in Table 3) were included in a meta-analysis to investigate the associations of *PvuII* and *XbaI* polymorphisms with breast cancer risk. In 13 of these studies, 10,419 cases and 16,178 controls were analyzed for the *PvuII* polymorphism, while 11 studies included 8,542 cases and 12,941 controls analyzed for the *XbaI* polymorphism. Among controls, the frequency of the 'T' allele at the *PvuII* site ranged from 48.8% in a Utrecht population of the Netherlands to 64.2% in a Zhejiang population of China (Hu et al., 2007). In contrast, the frequency of the 'A' allele at the *XbaI* site among controls ranged from 53.9% in a Dutch population to 77.7% in a Chinese population (Onland-Moret et al., 2005; Hu et al., 2007).

Table 3. ESR- α PvuII and XbaI Genotypes and Alleles in Breast Cancer Cases and Controls Included in the Meta-analysis

SNPs	First author	Year	Region and country	Case/control	Genotype distribution										Adjusted P_{HWP}	
					Cases					Controls						
					GG	AG	AA	G	A	GG	AG	AA	G	A		
PvuII	Yaich L	1992	Tennessee, USA	257/145	61	134	62	256	258	34	75	36	143	147	Yes	0.676
	Cai Q	2003	Shanghai, China	1,069/1,166	138	516	415	792	1346	190	546	430	926	1,406	Yes	0.452
	Shin A	2003	South Korea	201/195	35	91	75	161	241	26	105	64	157	233	Yes	0.095
	Wedren S	2004	Sweden	1,292/1,348	268	634	390	1,170	1,414	313	651	384	1,277	1,419	Yes	0.248
	Lu X	2005	Beijing, China	138/140	19	65	54	103	173	21	69	50	111	169	NR	0.723
	Onland-Moret NC	2005	Netherlands	308/337	69	150	89	288	328	96	153	88	345	329	Yes	0.093
	Shen Y	2006	Shanghai, China	247/274	29	120	98	178	316	43	124	107	210	338	Yes	0.48
	Hu Z	2007	Shanghai, China	113/113	16	58	39	90	136	19	45	49	83	143	NR	0.128
	Kjaergaard AD	2007	Denmark	1,256/2,489	245	613	398	1,103	1,409	537	1,225	727	2,299	2,679	Yes	0.621
	Gonzalez-Mancha R	2008	Spain	444/704	82	209	153	373	515	150	361	193	661	747	Yes	0.435
	Gonzalez-Zuloeta Ladd	2008	Netherlands	190/3,703	24	94	72	142	238	453	1,648	1,602	2,554	4,852	Yes	0.452
Dunning AM	2009	Caucasians	4,362/4,548	938	2,164	1,260	4,040	4,684	934	2,296	1,318	4,164	4,932	NR	0.254	
This study	2011	Zhejiang, China	542/1,016	57	228	227	372	712	137	454	425	728	1,304	Yes	0.368	
XbaI	Andersen TI	1994	Norway	274/204	22	95	157	139	409	28	74	102	130	278	NR	0.019
	Cai Q	2003	Shanghai, China	1,069/1,167	36	497	536	569	1,569	49	508	610	606	1,728	Yes	0
	Shin A	2003	South Korea	201/195	11	60	130	82	320	7	102	86	116	274	Yes	0
	Wedren S	2004	Sweden	1,291/1,348	143	560	588	846	1,736	161	610	577	932	1,764	Yes	0.991
	Lu X	2005	Beijing, China	138/140	6	48	84	60	216	6	69	65	81	199	NR	0.019
	Onland-Moret NC	2005	Netherlands	307/335	55	130	122	240	374	61	151	123	273	397	Yes	0.223
	Shen Y	2006	Shanghai, China	247/276	14	84	149	112	382	21	87	168	129	423	Yes	0.046
	Hu Z	2007	Shanghai, China	113/110	3	34	76	40	186	7	35	68	49	171	No	0.395
	Gonzalez-Zuloeta Ladd	2008	Netherlands	190/3,703	46	96	48	188	192	800	1,815	1,088	3,415	3,991	Yes	0.403
	Dunning AM	2009	Caucasians	4,170/4,447	521	1,967	1,682	3,009	5,331	526	2,048	1,873	3,100	5,794	NR	0.347
	This study	2011	Zhejiang, China	542/1,016	21	158	363	200	884	61	332	623	454	1,578	Yes	0.063

PHWP, P value in controls for Hardy-Weinberg proportion

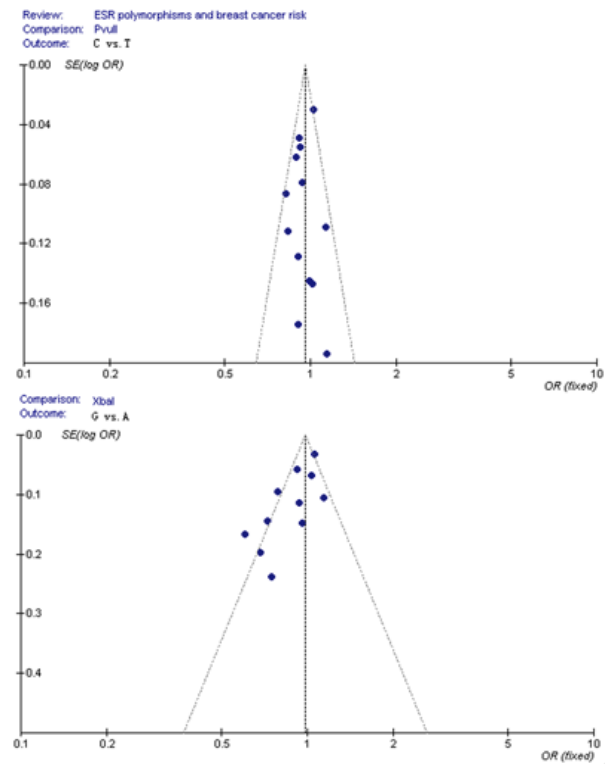


Figure 1. A funnel Plot was Used to Estimate the Publication Bias of the Studies Included in the Meta-analysis Performed

Assessment of Hardy-Weinberg proportion is regarded as an important criterion for evaluating genetic association studies; caution should be exercised when interpreting the studies included in the meta-analysis (Little et al., 2002). Most of the studies included in this meta-analysis reported genotype frequencies in their control groups that were consistent with Hardy-Weinberg proportions ($P > 0.05$). Deviations from Hardy-Weinberg proportions in controls

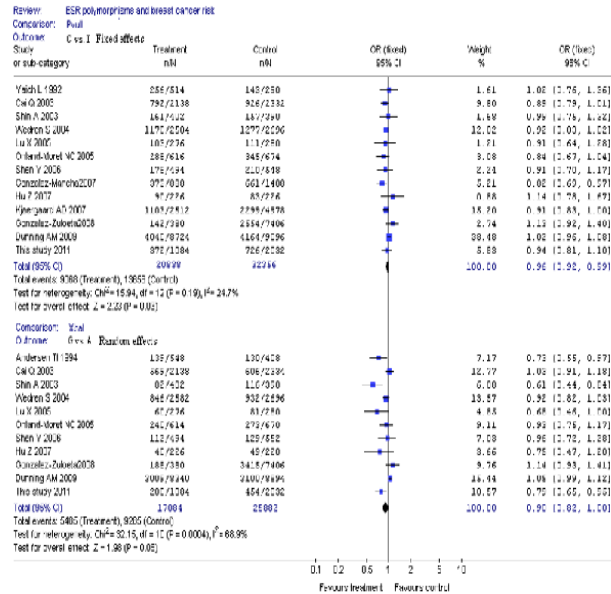


Figure 2. Forest Plot of the Meta-analysis Performed to Investigate the Association Between the PvuII and XbaI Polymorphisms of ESR- α Gene and Breast Cancer Risk

were observed only in four studies for XbaI (Little et al., 2002; Cai et al., 2003; Lu et al., 2005; Shen et al., 2006).

Funnel plotting was performed to evaluate whether publication bias was present in the meta-analysis performed. As shown in Figure 1, the shape of the funnel plots obtained appears to be symmetrical in models ('C' vs. 'T') but unsymmetrical in model ('G' vs. 'A'). We hypothesized that the publication bias may be the reason for this heterogeneity.

Meta-analysis of the ESR- α polymorphisms and breast cancer risk

A heterogeneity test of potential associations between

Table 4. Summary ORs and 95% CI in the ESR-α Gene Stratified by Race

SNPs	Race	Model	Total No. cases	Total No. controls	Fixed effects		Random effects		P value ^b
					OR (95%CI)	P value ^a	OR (95%CI)	P value ^a	
<i>PvuII</i>	Mixed	CC vs. TT	1,981/3,332	2,952/5,474	0.91 (0.84-0.98)	0.01	0.88 (0.79-0.97)	0.01	0.19
		CC vs. (CT+TT)	1,981/6,514	2,952/10,410	0.92 (0.85-0.98)	0.01	0.86 (0.75-0.97)	0.02	0.008
		(CC+CT) vs. TT	7,087/3,332	10,704/5,474	0.95 (0.89-1.01)	0.12	0.95 (0.90-1.01)	0.11	0.31
		C vs. T	9,068/11,770	13,656/18,700	0.96 (0.92-0.99)	0.03	0.95 (0.90-0.99)	0.03	0.19
	European	CC vs. TT	1,687/2,424	2,517/4,348	0.94 (0.86-1.02)	0.12	0.89 (0.77-1.03)	0.11	0.07
		CC vs. (CT+TT)	1,687/6,422	2,517/10,757	0.96 (0.89-1.03)	0.29	0.93 (0.83-1.03)	0.17	0.16
		(CC+CT) vs. TT	5,685/2,424	8,926/4,348	0.95 (0.89-1.02)	0.16	0.94 (0.85-1.04)	0.22	0.11
		C vs. T	7,372/8,846	11,443/15,105	0.97 (0.93-1.01)	0.13	0.95 (0.88-1.02)	0.15	0.04
	Asian	CC vs. TT	294/908	435/1,126	0.80 (0.68-0.96)	0.01	0.80 (0.68-0.96)	0.01	0.82
		CC vs. (CT+TT)	294/2,016	435/2,469	0.81 (0.69-0.95)	0.009	0.81 (0.69-0.95)	0.009	0.52
(CC+CT) vs. TT		1,402/908	1,778/1,126	0.96 (0.85-1.07)	0.44	0.96 (0.85-1.07)	0.44	0.64	
C vs. T		1,696/2,924	2,213/3,595	0.93 (0.85-1.00)	0.06	0.93 (0.85-1.00)	0.06	0.88	
<i>XbaI</i>	Mixed	GG vs. AA	878/3,935	1,727/5,383	0.98 (0.88-1.08)	0.66	0.89 (0.74-1.06)	0.18	0.08
		GG vs. (AG+AA)	878/7,664	1,727/11,214	0.98 (0.89-1.08)	0.67	0.93 (0.81-1.07)	0.32	0.2
		(GG+AG) vs. AA	4,607/3,935	7,558/5,383	0.98 (0.92-1.04)	0.42	0.87 (0.76-1.01)	0.42	<0.001
		G vs. A	5,485/11,599	9,285/16,597	0.98 (0.94-1.03)	0.43	0.90 (0.82-1.00)	0.05	<0.001
	European	GG vs. AA	787/2,597	1,576/3,763	1.01 (0.91-1.12)	0.87	1.01 (0.91-1.13)	0.86	0.6
		GG vs. (AG+AA)	787/5,445	1,576/8,461	1.02 (0.92-1.13)	0.75	0.99 (0.85-1.15)	0.9	0.2
		(GG+AG) vs. AA	3,635/2,597	6,232/3,763	1.02 (0.95-1.09)	0.61	0.97 (0.84-1.12)	0.7	0.06
		G vs. A	4,422/8,042	7,850/12,224	1.01 (0.96-1.07)	0.6	0.97 (0.87-1.09)	0.64	0.02
	Asian	GG vs. AA	91/1,338	151/1,620	0.73 (0.55-0.95)	0.02	0.73 (0.55-0.96)	0.03	0.8
		GG vs. (AG+AA)	91/2,219	151/2,753	0.76 (0.58-1.00)	0.05	0.76 (0.58-1.00)	0.05	0.59
(GG+AG) vs. AA		972/1,338	1,284/1,620	0.88 (0.78-0.98)	0.02	0.76 (0.58-1.01)	0.06	<0.001	
G vs. A		1,063/3,557	1,435/4,373	0.89 (0.81-0.97)	0.01	0.82 (0.68-0.98)	0.03	0.01	

^aTest for overall effect; ^bTest for heterogeneity

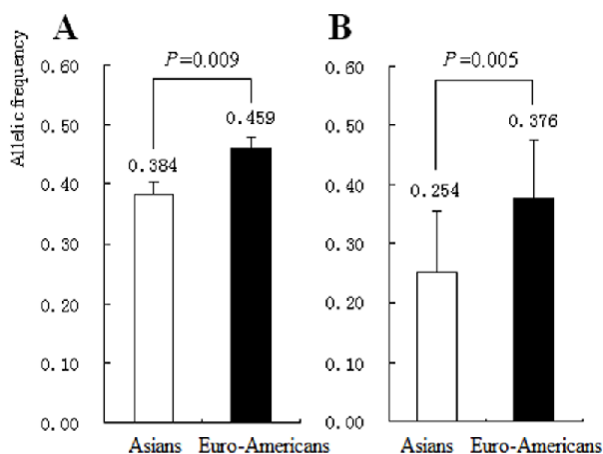


Figure 3. Allelic Frequencies of the *PvuII* 'C' (A) and *XbaI* 'G' (B) in Different Populations

the *PvuII* and *XbaI* polymorphisms and breast cancer risk are presented in Table 4 and Figure 2.

Examining the *PvuII* polymorphism first, only 13 studies to date have investigated the relationship between the *PvuII* polymorphism and breast cancer risk, and all of these studies were in Hardy-Weinberg equilibrium (Yaich et al., 1992; Cai et al., 2003; Shin et al., 2003; Wedren et al., 2004; Lu et al., 2005; Onland-Moret et al., 2005; Shen et al., 2006; Hu et al., 2007; Kjaergaard et al., 2007; Gonzalez-Mancha et al., 2008; Gonzalez-Zuloeta et al., 2008; Dunning et al., 2009). Except for the model ('CC' vs. 'CT+TT', $P = 0.008$), there was little evidence of statistical heterogeneity. Individuals carrying the 'C' allele (OR = 0.96; 95% CI = 0.92- 0.99, $P = 0.03$; $P = 0.19$ for heterogeneity, $I^2 = 24.7\%$) were associated with a significant decrease in breast cancer diagnosis compared

to patients carrying the 'T' allele (Figure 2). In addition, the model ('CC' vs. 'TT') showed a significant association with breast cancer risk, with the associated ORs being 0.91 (95% CI = 0.84- 0.98, $P = 0.01$; $P = 0.19$ for heterogeneity, $I^2 = 25.5\%$). Compared to the 'TT' genotype, subgroup analysis revealed that genotype 'CC' was associated with a 0.80- fold risk of breast cancer risk in Asians ($P = 0.01$, fixed effects) but not in Euro-Americans ($P = 0.11$, random effects, Table 4).

A total of 11 studies were included in the meta-analysis performed to examine the associations between the *XbaI* polymorphism and breast cancer risk; four studies of these were not consistent with Hardy-Weinberg proportions (Cai et al., 2003; Shin et al., 2003; Wedren et al., 2004; Lu et al., 2005; Onland-Moret et al., 2005; Shen et al., 2006; Hu et al., 2007; Gonzalez-Zuloeta et al., 2008; Dunning et al., 2009). Since there is heterogeneity in model ('G' vs. 'A'), random-effects analysis was selected. We observed that individuals carrying the 'G' genotype were associated with a 0.82- fold decreased risk in Asians (95% CI = 0.68- 0.98, $P = 0.03$; $P = 0.01$ for heterogeneity, $I^2 = 65.7\%$) compared to the data for the 'A' genotype. However, no significant differences were observed between this polymorphism and breast cancer risk in Euro-Americans.

Discussion

Worldwide, the incidence of clinical breast cancer shows a strong dependence on sex, age, race, and geography. For example, the incidence in African-Americans is significantly higher than that in Asians, especially the Chinese population (Smigal et al., 2006; American Cancer Society, 2011). Recent epidemiological

studies in different populations have indicated that differences in *ESR-α* genotype frequency may play an important role in the risk of breast cancer. Thus, this study was undertaken to assess whether the *PvuII* and *XbaI* polymorphisms in the *ESR-α* gene are associated with breast cancer risk in a Chinese population.

In the present study, we observed that the *XbaI* 'G' allele was associated with an almost 0.80- fold decreased risk for developing breast cancer compared to patients carrying the 'A' allele in a Chinese population. Results from the meta-analysis with 11 studies included 8,542 cases and 12,941 controls, it showed that the *XbaI* 'G' allele was associated with a 0.82- fold decreased risk in Asians. However, no significant association was observed between the *XbaI* 'G' allele and breast cancer risk in Euro-Americans. Although no significant association with breast cancer risk was observed in our study population, the *PvuII* polymorphism was closely related to breast cancer risk in Asians. The meta-analysis showed that the *PvuII* 'CC' genotype was linked to a 0.80- fold decreased risk of breast cancer. However, we also did not observe any association between the *PvuII* polymorphism and breast cancer risk in Euro-Americans. The significant difference between the *PvuII* or *XbaI* polymorphisms and breast cancer risk in Asians but not in Euro-Americans, suggests that race, geographical location, and lifestyle may be involved in carcinogenesis.

Although a single genotype or allele may influence the occurrence and development of disease, haplotype might play a more important role considering the strong linkage disequilibrium between these two polymorphisms. We analyzed haplotypes with two-locus (*PvuII* and *XbaI*) of *ESR-α* gene polymorphisms using the SHEsis software and found that the haplotype 'CG' indicates a decreased risk of breast cancer in our study population. Our result supports the conclusion that the *PvuII* 'C' or *XbaI* 'G' allele is associated with a decreased risk of breast cancer. At present, few studies have investigated association of this haplotype and the risk of breast cancer. Thus, this finding needs to be further confirmed.

To make a comprehensive and deep analysis of the underlying reason, we evaluated the genotype and allelic frequencies of the *ESR-α* gene in different populations. We found that the allelic frequency of *PvuII* 'C' in Asian populations (38.4%, in control) was significantly lower than that observed for Euro-Americans (45.9%, Figure 3A). The allelic frequency of the *XbaI* 'G' allele in Asians was 25.4% (in control), significantly lower than that found in Euro-Americans (37.6%, Figure 3B). Interestingly, we found the lower frequencies of the *PvuII* 'C' or *XbaI* 'G' allele occurred mainly in Asians, which was consistent with the lower incidence of breast cancer in these regions (Smigal et al., 2006). The higher allelic frequencies occurred in Euro-Americans with higher cancer incidence. We hypothesized that the allelic distribution of the *PvuII* and *XbaI* may be an important factor resulting in the difference of breast cancer incidence in different regions of the world.

However, the reasons and the underlying molecular mechanisms that the *ESR-α* gene polymorphisms influence the occurrence of breast cancer remain unknown.

The *PvuII* and *XbaI* polymorphic variants are located on the untranslated intron 1 and do not seem to alter the amino acid sequence. However, evidence from several studies shows that the *PvuII* and *XbaI* polymorphisms may affect the receptor function through differential splicing of mRNA (Dotzlaw et al., 1992; Fuqua et al., 1992) or alteration of transcriptional elements within introns (Roodi et al., 1995). A study by Herrington and colleagues (Herrington et al., 2002) found suggests that the *PvuII* 'C' allele, a potential binding site for myb transcription factors, had a higher transcription of the *ESR-α* gene compared to the 'T' allele. Additionally, a significant interaction between levels of estradiol (E2) and the *PvuII* genotype were observed by Onland-Moret et al. (2005), who reported that women with Low E2 levels and the 'C' allele had a decreased risk of breast cancer compared to women with high E2 levels and the 'T' allele. Besides the E2 levels, the *PvuII* 'C' allele was also associated with decreased levels of androstenedione (Weiderpass et al., 2000). Based on these observations, we believe that the *ESR-α* gene polymorphisms may indirectly influence the binding activity to the hormone response element on the target gene by regulating gene expression or receptor function, and then influence the transcriptional regulation of its downstream genes including TP53 (Rasti et al., 2012), causing the origination of the breast cancer.

In summary, the present study indicates that the *ESR-α* gene polymorphisms may be associated with breast cancer risk. These associations may largely depend on population characteristics and geographic location. Thus, these results help provide laboratory basis for molecular epidemiological studies of breast cancer and a better understanding of the geographic and ethnic differences associated with disease incidence and mortality.

Acknowledgements

This study was sponsored by Zhejiang Provincial Top Key Discipline in Surgery. The author(s) declare that they have no competing interests.

References

- American Cancer Society. Cancer Facts and Figures 2011. [Available online at: <http://www.cancer.org/docroot/home/index.asp>]
- Andersen TI, Heimdal KR, Skrede M, et al (1994). Oestrogen receptor (*ESR*) polymorphisms and breast cancer susceptibility. *Hum Genet*, **94**, 665-70.
- Bocchinfuso WP, Lindzey JK, Hewitt SC, et al (2000). Induction of mammary gland development in estrogen receptor-alpha knockout mice. *Endocrinology*, **141**, 2982-94.
- Cai Q, Shu XO, Jin F, et al (2003). Genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev*, **12**, 853-9.
- Crooke PS, Justenhoven C, Brauch H, et al (2011). Estrogen Metabolism and Exposure in a Genotypic-Phenotypic Model for Breast Cancer Risk Prediction. *Cancer Epidemiol Biomarkers Prev*, **20**, 1502-15.
- DerSimonian R, Kacker R (2007). Random-effects model for meta-analysis of clinical trials: an update. *Contemp Clin Trials*, **28**, 105-14.

- Dotzlaw H, Alkhalaf M, Murphy LC (1992). Characterization of estrogen receptor variant mRNAs from human breast cancers. *Mol Endocrinol*, **6**, 773-85.
- Dunning AM, Healey CS, Baynes C, et al (2009). Association of *ESR1* gene tagging SNPs with breast cancer risk. *Hum Mol Genet*, **18**, 1131-9.
- Egger M, Juni P, Bartlett C, et al (2003). How important are comprehensive literature searches and the assessment of trial quality in systematic reviews? Empirical study. *Health Technol Assess*, **7**, 1-76.
- Fuqua SA, Fitzgerald SD, Allred DC, et al (1992). Inhibition of estrogen receptor action by a naturally occurring variant in human breast tumors. *Cancer Res*, **52**, 483-6.
- Gonzalez-Mancha R, Galan JJ, Crespo C, et al (2008). Analysis of the ERalpha germline *PvuII* marker in breast cancer risk. *Med Sci Monit*, **14**, CR136-43.
- Gonzalez-Zuloeta Ladd AM, Vasquez AA, Rivadeneira F, et al (2008). Estrogen receptor alpha polymorphisms and postmenopausal breast cancer risk. *Breast Cancer Res Treat*, **107**, 415-9.
- Herrington DM, Howard TD, Brosnihan KB, et al (2002). Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation*, **105**, 1879-82.
- Hu Z, Song CG, Lu JS, et al (2007). A multigenic study on breast cancer risk associated with genetic polymorphisms of ER Alpha, COMT and CYP19 gene in BRCA1/BRCA2 negative Shanghai women with early onset breast cancer or affected relatives. *J Cancer Res Clin Oncol*, **133**, 969-78.
- Key T, Appleby P, Barnes I, et al (2002). Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst*, **94**, 606-16.
- Kjaergaard AD, Ellervik C, Tybjaerg-Hansen A, et al (2007). Estrogen receptor alpha polymorphism and risk of cardiovascular disease, cancer, and hip fracture: cross-sectional, cohort, and case-control studies and a meta-analysis. *Circulation*, **115**, 861-71.
- Kobayashi S, Inoue S, Hosoi T, et al (1996). Association of bone mineral density with polymorphism of the estrogen receptor gene. *J Bone Miner Res*, **11**, 306-11.
- Li Z, Zhang Z, He Z, et al (2009). A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (<http://analysis.bio-x.cn>). *Cell Res*, **19**, 519-23.
- Little J, Bradley L, Bray MS, et al (2002). Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. *Am J Epidemiol*, **156**, 300-10.
- Liu W, Konduri SD, Bansal S, et al (2006). Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. *J Biol Chem*, **281**, 9837-40.
- Lu X, Li B, Wei JM, et al (2005). [The *XbaI* and *PvuII* gene polymorphisms of the estrogen receptor alpha gene in Chinese women with breast cancer]. *Zhonghua Wai Ke Za Zhi*, **43**, 290-3.
- Mallepell S, Krust A, Chambon P, et al (2006). Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proc Natl Acad Sci U S A*, **103**, 2196-201.
- Medina D, Sivaraman L, Hilsenbeck SG, et al (2001). Mechanisms of hormonal prevention of breast cancer. *Ann N Y Acad Sci*, **952**, 23-35.
- Onland-Moret NC, van Gils CH, Roest M, et al (2005). The estrogen receptor alpha gene and breast cancer risk (The Netherlands). *Cancer Causes Control*, **16**, 1195-202.
- Oxman AD, Guyatt GH (1993). The science of reviewing research. *Ann N Y Acad Sci*, **703**, 125-33; discussion 33-4.
- Rasti M, Arabsofghar R, Khatooni Z, et al (2012). p53 Binds to estrogen receptor 1 promoter in human breast cancer cells. *Pathol Oncol Res*, **18**, 169-75.
- Roodi N, Bailey LR, Kao WY, et al (1995). Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. *J Natl Cancer Inst*, **87**, 446-51.
- Russo IH, Russo J (1998). Role of hormones in mammary cancer initiation and progression. *J Mammary Gland Biol Neoplasia*, **3**, 49-61.
- Shen Y, Li DK, Wu J, et al (2006). Joint effects of the CYP1A1 MspI, ERalpha PvuII, and ERalpha XbaI polymorphisms on the risk of breast cancer: results from a population-based case-control study in Shanghai, China. *Cancer Epidemiol Biomarkers Prev*, **15**, 342-7.
- Shi YY, He L (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*, **15**, 97-8.
- Shin A, Kang D, Nishio H, et al (2003). Estrogen receptor alpha gene polymorphisms and breast cancer risk. *Breast Cancer Res Treat*, **80**, 127-31.
- Smigal C, Jemal A, Ward E, et al (2006). Trends in breast cancer by race and ethnicity: update 2006. *CA Cancer J Clin*, **56**, 168-83.
- Wedren S, Lovmar L, Humphreys K, et al (2004). Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. *Breast Cancer Res*, **6**, R437-49.
- Weiderpass E, Persson I, Melhus H, et al (2000). Estrogen receptor alpha gene polymorphisms and endometrial cancer risk. *Carcinogenesis*, **21**, 623-7.
- Yaich L, Dupont WD, Cavener DR, et al (1992). Analysis of the *PvuII* restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. *Cancer Res*, **52**, 77-83.