RESEARCH ARTICLE

Menopausal Status Modifies Breast Cancer Risk Associated with ESR1 PvuII and XbaI Polymorphisms in Asian Women: a HuGE Review and Meta-analysis

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Abstract

Background: Published data on the association between single nucleotide polymorphisms (SNPs) in the ESR1 gene and breast cancer susceptibility are inconclusive or controversial. The aim of this Human Genome Epidemiology (HuGE) review and meta-analysis was to derive a more precise estimation of this relationship. Methods: A literature search of Pubmed, Embase, Web of science and CBM databases was conducted from inception through September 1th, 2012. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association. Results: A total of five studies including 1,678 breast cancer cases and 1,678 general population controls in Asian populations were involved in this meta-analysis. When all the eligible studies were pooled into the meta-analysis, the higher transcriptional activity variant allele T of ESR1 PvuII (C>T) (rs2234693) in pre-menopausal breast cancer women showed a significant relation to increased risk (OR = 1.13, 95%CI: 1.01-1.28, P = 0.040) in contrast to their post-menopausal counterparts which showed non-significant increased risk (OR = 1.01, 95%CI: 0.87-1.18, P = 0.858). Nevertheless, no significant association between ESR1 XbaI (A>G) (rs9340799) polymorphism and the risk of breast cancer was observed in pre-menopausal and post-menopausal individuals. Conclusion: Based on a homogeneous Asian population, results from the current meta-analysis indicates that the ESR1 PvuII (C>T) polymorphism places pre-menopausal breast cancer women at risk for breast cancer, while ESR1 XbaI (A>G) polymorphism is not likely to predict the risk of breast cancer.

Keywords: Breast cancer - estrogen receptor 1 - single nucleotide polymorphism - meta-analysis

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Introduction

Breast cancer, as the most frequent cancer among females worldwide, has been estimated that over one million women are diagnosed annually and more than 410,000 will die from the disease (Jemal et al., 2011), which is the leading cause of cancer-related mortality and accounts for approximate 14% of all cancer deaths (Ferlay et al., 2010). While breast cancer incidence has increased over the past 30-40 years, the mortality has remained stable or even decreased in the last 10-15 years probably owing to the earlier detection and improved treatment (Stuckey, 2011). The survival rate for female breast cancer is higher than for most other types of cancer, with the majority of patients remaining alive for at least 5 years following diagnosis (Baade et al., 2011). To date, various etiologies of elevated breast cancer risks have been proposed to consist of extrinsic factors, such as environmental carcinogens, tobacco consumption, body mass index, alcohol drinking and exogenous hormone use, which contribute to a partial increase in breast cancer risk, and intrinsic factors such as hereditary family histories, genetic variants, reproductive patterns and menopausal status (McCormack and Boffetta, 2011). Menopausal characteristics, also known as hormonal factors, are the key risk factors for breast cancer and may synergistically interact with genetic factors in triggering the development and progression of breast cancer through estrogen synthesis, metabolism and signal transduction (Butt et al., 2012). For example, Pabalan et al found that the MPO gene mutation might place post-menopausal women at higher breast cancer susceptibility than pre-menopausal women in Caucasian population (Pabalan et al., 2012). Up till now, studies regarding associations of genetic mutations with breast cancer risk in relation to hormone receptor status, or menopausal status have attracted more and more attention and a wide range of genes, such as BRCA1/2, BRIP1, PALB2, TP53, ATM, CHEK2 and ESR1 have been identified to be implicated in the increased susceptibility to breast cancer (Campeau et al., 2008).

Estrogen receptor 1 (ESR1) gene, located on chromosome 6p25.1, spanning nearly 300 kb in length and consisting of 8 exons and 7 introns (Parker et al., 1997), is a newly recognized breast cancer susceptibility gene, which is responsible for the stimulation of mammary epithelial tissue proliferation and the alteration of

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corresponding downstream genes expression (Laudanski et al., 2004). In the progress of breast carcinogenesis, ESR1 serves as a ligand-activated transcription factor and is capable of binding both endogenous and exogenous hormones in order to drive cell proliferation, control cell growth, program cell death and thus increase the opportunity of accumulation of genetic mutations that occur during breast cancer cell division (Zhang and Yu, 2007). Therefore, the ESR1 gene variants associated with higher ESR1 expression may be correlated with elevated susceptibility to breast cancer (Tsezou et al., 2008).

So far, a large number of studies indicating the potential relationship between ESR1 gene polymorphisms and breast cancer risk in respect of menopausal status have been reported. For instance, Sobti et al indicated that there appeared to be a positive impact of ESR1 codon 594 genotypes on breast cancer risk. Moreover, a significantly higher risk was observed in pre-menopausal patients with ESR1 polymorphism that had undergone menopause above the age of 50 years (Sobti et al., 2012). Ding et al also provided support for the diverse roles of ESR1 polymorphism in determining susceptibility in different stages of breast cancer (Ding et al., 2010). However, the findings of several other studies examining the ESR1 SNPs with risks of breast cancer are inconsistent. Neither of Einarsdottir et al and Tsezou et al observed significant difference in the frequency distribution of corresponding ESR1 gene mutations between patients and controls and supported a strong association between variants in the ESR1 genes and breast cancer susceptibility, tumor characteristics or survival (Einarsdottir et al., 2008; Tsezou et al., 2008). It is notable that Jeon et al similarly came to the conclusion that no significant correlation existed between breast cancer risk and the genetic polymorphisms of ESR1, but when ESR1 P325P was analyzed together with CDK7, women carrying both the CDK7 TT and ESR1 P325P CC genotypes showed increased breast cancer risk (Jeon et al., 2010). The controversial results of the prognostic value of ESR1 are probably due to the small sample size and the differences between studies, such as ethnic backgrounds, geographical locations, and the baseline characteristics of the included patients (age, histological type, menopausal status, differentiation or tumor stage) (Li et al., 2010). Thus, we attempt to perform a meta-analysis of all eligible case-control studies with breast cancer risk and try to reveal the exact associations between ESR1 gene polymorphisms and breast cancer susceptibility, which might be further utilized as a potential biomarker in predicting breast cancer or as a powerful diagnostic tool for accurate determination of therapeutic strategies in breast cancer treatment.

Materials and Methods

Publication search

Relevant papers published before September 1th, 2012 were identified through a search of Pubmed, Embase, Web of science and CBM databases using the following terms: ("genetic polymorphism" or "polymorphism" or "SNP" or "gene mutation" or "genetic variants") and ("breast neoplasms" or " breast neoplasm" or " breast cancer" or " cancer of breast ") and ("estrogen receptor alpha" or " estrogen receptor alpha, human " or "ER alpha" or "Estrogen Receptor 1" or "ESR1"). The references of the eligible articles or textbooks were also reviewed to check through manual searches to find other potentially studies. Any disagreement was resolved by discussion between the authors.

Inclusion and Exclusion Criteria

Studies included in our meta-analysis have to meet the following criteria: (i) case-control study or cohort study focused on associations between ESR1 gene polymorphisms and breast cancer susceptibility; (ii) data were stratified by menopausal status among case and control ; (iii) all patients with the diagnosis of breast cancer confirmed by pathological or histological examination; (iv) sufficient published data about the size of the sample, odds ratio (OR), and their 95% confidence interval (CI); (v) published in English or Chinese language. Studies were excluded when they were: (i) not case-control study or cohort study; (ii) data were not stratified by menopausal status among case and control ; (iii) duplicate of previous publication; (iv) based on incomplete data; (v) meta-analyses, letters, reviews or editorial articles.

Data Extraction

Using a standardized form, data from published studies were extracted independently by two authors to populate the necessary information. For each study, the following characteristics were collected: the first author, year of publication, country, language, ethnicity, study design, numbers of subjects, source of cases and controls, detecting sample, genotype method, allele and genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, an agreement was reached following a discussion between the authors.

Quality assessment of included studies

Two authors independently assessed the quality of papers according to modified STROBE quality score systems (da Costa et al., 2011; Zhang et al., 2011). Forty assessment items related with the quality appraisal were used in this meta-analysis, scores ranging from 0 to 40. Scores of 0-20, 20-30 and 30-40 were defined as low, moderate and high quality, respectively. Disagreement was resolved by discussion between the authors.

Statistical Analysis

The strength of the association between ESR1 gene polymorphisms and breast cancer susceptibility was measured by ORs with 95%CIs under five genetic models, including allele model, dominant model, recessive model, homozygous model, and heterozygous model. The statistical significance of the pooled OR was examined by Z test. Between-study variations and heterogeneities were estimated using Cochran's Q-statistic, and $P < 0.05$ was considered to be manifestation of statistically significant heterogeneity (Higgins and Thompson, 2002). We also quantified the effect of heterogeneity by using I^2 test, which ranges from 0 to 100% and represents the

Table 1. Characteristics of Included Studies in This Meta-analysis

First author	Year	Country	Language	Ethnicity	Number		Source		Sample	Genotype method	SNP	Alias name	Quality scores
					Case	Control	Case	Control					
Cai et al	2003	USA	English	Asian	1069	1166	PB	PB	Blood	PCR-RFLP	rs2234693 (C/T)	PvuII	28
							PB	PB	Blood	PCR-RFLP	rs9340799 (A/G)	XbaI	
Shin et al	2003	Korea	English	Asian	201	195	HB	HB	Blood	PCR-RFLP	rs9340799 (A/G)	XbaI	26
							HB	HB	Blood	PCR-RFLP	rs2234693 (C/T)	PvuII	
Lu et al	2005	China	Chinese	Asian	138	140	HB	HB	Blood	PCR-RFLP	rs9340799 (A/G)	XbaI	25
							HB	HB	Blood	PCR-RFLP	rs2234693 (C/T)	PvuII	
Shen et al	2006	China	English	Asian	247	274	PB	PB	Tissue	PCR-RFLP	rs2234693 (C/T)	PvuII	28
							PB	PB	Tissue	PCR-RFLP	rs9340799 (A/G)	XbaI	
Song et al	2006	China	Chinese	Asian	113	113	HB	PB	Blood	PCR-SSCP	rs2234693 (C/T)	PvuII	24
							HB	PB	Blood	PCR-SSCP	rs9340799 (A/G)	XbaI	

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single strand conformation polymorphism; HB, hospital-based; PB, population-based

Table 2. The Genotype Distribution of ESR1 PvuII and XbaI Polymorphisms

First author	Year	SNPs	Subgroup	Case group								Control group								HWE test	
				Total	1	2	1/1	1/2	2/2	TA	MAF	Total	1	2	1/1	1/2	2/2	TA	MAF	P value	Test
Cai et al	2003	rs2234693 (C/T)	Premenopausal	715	526	904	93	340	282	1430	0.63	746	593	899	119	355	272	1492	0.60	0.86	HWE
			Postmenopausal	349	265	433	45	175	129	698	0.62	417	331	503	71	189	157	834	0.60	0.28	HWE
		rs9340799 (A/G)	Premenopausal	715	394	1036	26	342	347	1430	0.72	746	397	1095	31	335	380	1492	0.73	0.00	non-HWE
			Postmenopausal	349	173	525	10	153	186	698	0.75	417	207	627	17	173	227	834	0.75	0.02	non-HWE
Shin et al	2003	rs9340799 (A/G)	Premenopausal	122	51	193	6	39	77	244	0.79	109	60	158	3	54	52	218	0.72	0.01	non-HWE
			Postmenopausal	79	31	127	5	21	53	158	0.80	81	52	110	3	46	32	162	0.68	0.01	non-HWE
		rs2234693 (C/T)	Premenopausal	122	98	146	21	56	45	244	0.60	109	94	124	18	58	33	218	0.57	0.38	HWE
			Postmenopausal	79	63	95	14	35	30	158	0.60	81	61	101	8	45	28	162	0.62	0.10	HWE
Lu et al	2005	rs9340799 (A/G)	Premenopausal	86	40	132	4	32	50	172	0.77	89	48	130	3	42	44	178	0.73	0.06	HWE
			Postmenopausal	52	20	84	2	16	34	104	0.81	51	33	69	3	27	21	102	0.68	0.13	HWE
		rs2234693 (C/T)	Premenopausal	86	63	109	12	39	35	172	0.63	89	72	106	15	42	32	178	0.60	0.85	HWE
			Postmenopausal	52	40	64	7	26	19	104	0.62	51	39	63	6	27	18	102	0.62	0.39	HWE
Shen et al	2006	rs2234693 (C/T)	Premenopausal	109	75	143	9	57	43	218	0.66	124	93	155	15	63	46	248	0.63	0.35	HWE
			Postmenopausal	138	103	173	20	63	55	276	0.63	150	117	183	28	61	61	300	0.61	0.08	HWE
		rs9340799 (A/G)	Premenopausal	108	53	163	6	41	61	216	0.75	126	60	192	10	40	76	252	0.76	0.16	HWE
			Postmenopausal	139	59	219	8	43	88	278	0.79	150	69	231	11	47	92	300	0.77	0.16	HWE
Song et al	2006	rs2234693 (C/T)	Premenopausal	79	56	102	8	40	31	158	0.65	83	62	104	16	30	37	166	0.63	0.04	HWE
			Postmenopausal	34	34	34	8	18	8	68	0.50	30	21	39	3	15	12	60	0.65	0.59	HWE
		rs9340799 (A/G)	Premenopausal	79	133	25	55	23	1	158	0.16	83	125	41	48	29	6	166	0.25	0.58	HWE
			Postmenopausal	34	53	15	21	11	2	68	0.22	30	51	9	22	7	1	60	0.15	0.64	HWE

SNP, single nucleotide polymorphism; TA, total alleles; MAF, minor allele frequency; 1 = wild allele; 2 = mutant allele; 1/1 = wild homozygote; 1/2 = heterozygote; 2/2 = mutant homozygote; HWE, Hardy-Weinberg equilibrium

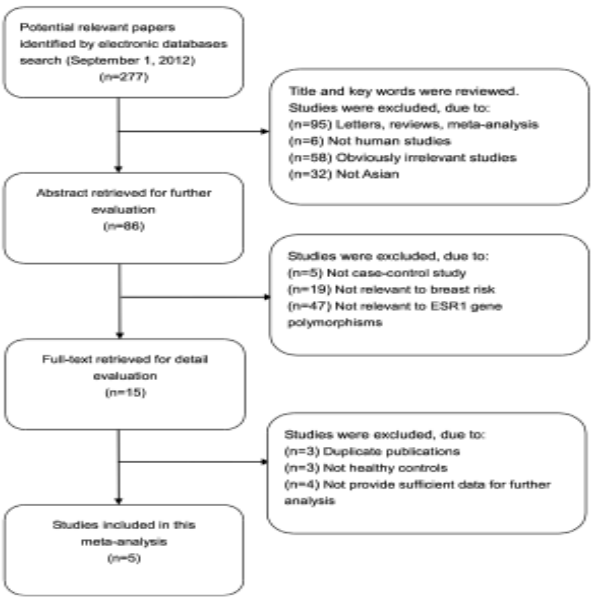

Figure 1. Flow Chart Shows Study Selection Process

proportion of inter-study variability that can be contributed to heterogeneity rather than by chance (Zintzaras and Ioannidis, 2005). When a significant Q-test ($P < 0.05$) or $I^2 > 50\%$ indicated that heterogeneity among studies existed, the random effects model (DerSimonian Laird method) was conducted for meta-analysis. Otherwise, the fixed effects model (Mantel-Haenszel method) was used. To establish the effect of heterogeneity on the conclusions of meta-analyses, we also performed subgroup analysis by menopausal status, source of controls, HWE test. We tested whether genotype frequencies of controls were in HWE using the χ^2 test. Sensitivity was performed by omitting each study in turn to assess the stability of results. Begger's funnel plots were used to detect publication bias. In addition, Egger's linear regression test which measures funnel plot asymmetry using a natural logarithm scale of OR was used to evaluate the publication bias (Peters et al., 2006). All the P values were two-sided. All analyses were calculated using STATA Version 12.0 software (Stata Corp, College Station, TX).

Results

The characteristics of included studies

We identified 277 potentially relevant articles from our search of the published literature, of which 272 articles were excluded, only 5 eligible studies (Cai et al., 2003; Shin et al., 2003; Lu et al., 2005; Shen et al., 2006; Song et al., 2006) involving breast cancer cases 1,768 and 1,788 healthy controls met our inclusion criteria for this meta-analysis. Figure 1 shows the strategy used for PubMed search as of September 2012. During the extraction of data, four articles (Zhang et al., 2004; Surekha et al., 2007; Deng, 2011; Sakoda et al., 2011) were excluded because their data were not stratified by menopausal status among case and control. The studied focusing on ESR1 PvuII and XbaI polymorphisms and breast cancer risk among Asians

Table 3. Meta-analysis of the Association Between ESR1 PvuII and XbaI Polymorphisms and Breast Cancer Risk Among Asians

SNPs/Subgroups	2 allele vs. 1 allele (allele model)				2/2 + 1/2 vs. 1/1 (dominant model)				2/2 vs. 1/1 + 1/2 (recessive model)				2/2 vs. 1/1 (homozygous model)				2/2 vs. 1/2 (heterozygous model)			
	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph
PvuII																				
Overall	1.09	0.99-1.20	0.084	0.835	1.22	1.02-1.47	0.034	0.727	1.06	0.93-1.22	0.369	0.838	1.24	1.01-1.51	0.039	0.653	1.01	0.88-1.17	0.852	0.669
Menopausal status																				
Pre-menopausal	1.13	1.01-1.28	0.04	0.999	1.29	1.02-1.63	0.036	0.148	1.13	0.95-1.34	0.752	0.808	1.35	1.04-1.75	0.022	0.98	1.07	0.89-1.28	0.464	0.493
Post-menopausal	1.01	0.87-1.18	0.858	0.451	1.12	0.84-1.51	0.442	0.415	0.97	0.78-1.20	0.166	0.707	1.08	0.78-1.49	0.657	0.252	0.93	0.74-1.17	0.523	0.665
Source of healthy controls																				
Population-based	1.09	0.98-1.21	0.098	0.533	1.32	1.07-1.62	0.008	0.49	1.03	0.89-1.20	0.674	0.573	1.29	1.03-1.62	0.025	0.479	0.96	0.82-1.13	0.628	0.551
Hospital-based	1.06	0.85-1.32	0.587	0.843	0.88	0.58-1.35	0.599	0.556	1.22	0.89-1.67	0.222	0.965	1.02	0.64-1.63	0.932	0.672	1.29	0.92-1.80	0.141	0.951
HWE test																				
HWE	1.09	0.99-1.20	0.091	0.758	1.19	0.99-1.44	0.069	0.453	1.08	0.94-1.24	0.284	0.844	1.22	0.99-1.50	0.059	0.595	1.04	0.90-1.20	0.627	0.794
non-HWE	1.09	0.69-1.71	0.722	-	2.12	0.85-5.28	0.106	-	0.8	0.43-1.50	0.492	-	1.68	0.63-4.44	0.299	-	0.63	0.32-1.23	0.176	-
XbaI																				
Overall	1.18	0.99-1.41	0.063†	0.038	1.19	0.86-1.64	0.297	0.772	1.29	0.99-1.69	0.064†	0.001	1.23	0.89-1.71	0.211	0.865	1.29	0.97-1.74	0.085†	<0.001
Menopausal status																				
Pre-menopausal	1.15	0.91-1.44	0.238†	0.128	1.19	0.79-1.79	0.417	0.408	1.21	0.87-1.69	0.255†	0.038	1.19	0.78-1.81	0.413	0.508	1.2	0.84-1.73	0.324†	0.024
Post-menopausal	1.24	0.89-1.73	0.201†	0.041	1.19	0.71-1.99	0.513	0.793	1.4	0.83-2.37	0.210†	0.002	1.3	0.77-2.20	0.328	0.876	1.43	0.80-2.56	0.225†	0.001
Source of healthy controls																				
Population-based	1.01	0.88-1.15	0.923	0.354	1.33	0.93-1.91	0.116	0.731	0.95	0.82-1.10	0.511	0.499	1.29	0.89-1.85	0.176	0.645	0.92	0.79-1.07	0.285	0.713
Hospital-based	1.55	1.21-2.00	0.001	0.49	0.71	0.33-1.52	0.378	0.818	2.08	1.48-2.94	<0.001	0.304	1.02	0.47-2.21	0.955	0.784	2.26	1.55-3.28	<0.001	0.265
HWE test																				
HWE	1.17	0.90-1.51	0.235†	0.029	1.08	0.73-1.61	0.699	0.535	1.37	0.88-2.13	0.163†	<0.001	1.12	0.74-1.68	0.596	0.887	1.44	0.87-2.37	0.153†	<0.001
non-HWE	1.22	0.94-1.58	0.145†	0.109	1.4	0.82-2.42	0.221	0.676	1.24	0.88-1.76	0.217	0.118	1.47	0.85-2.56	0.169	0.608	1.2	0.84-1.72	0.312	0.126

OR, odds ratios; 95%CI, 95% confidence interval; Ph, P value of heterogeneity test; †, estimates for random effects model

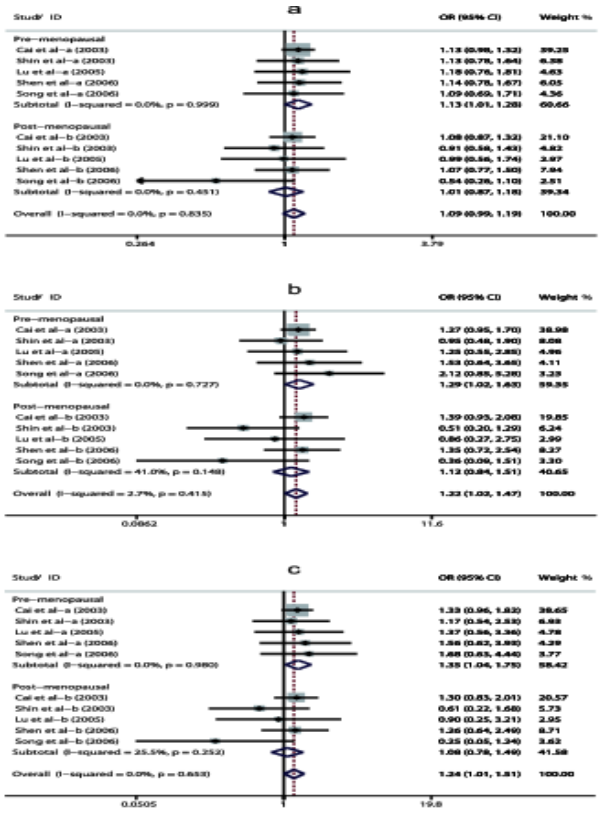

Figure 2. Forest plot showed the association between ESR1 PvuII (C>T) polymorphism and breast cancer risk modified by menopausal status among Asians. a: allele model; b: dominant model; c: homozygous model

were chosen. All patients fulfilled the diagnosis criteria of breast cancer confirmed by pathological examination of the surgical specimen. In addition, controls were mainly matched on age and/or gender, of which three were population-based and two were hospital-based. HWE test was conducted on genotype distribution of the controls in all included studies. There were 2 studies (Cai et al., 2003; Shin et al., 2003) where, upon recalculation, there was evidence of Hardy-Weinberg disequilibrium. All quality scores of included studies were higher than 20 (moderate high quality). The basic information including first author, published year, original country, ethnicity of the study populations, the number of cases and controls, source of controls, genotyping method, and HWE test of controls of each study are listed in Table 1. The genotype distribution of ESR1 PvuII and XbaI polymorphisms were presented in Table 2.

Association between ESR1 PvuII (C>T) and XbaI (A>G) polymorphisms and breast cancer stratified by menopausal status among Asians

Here we investigated the breast cancer associated with ESR1 PvuII (C>T) and XbaI (A>G) polymorphisms status in ethnically homogenous Asian women. The evaluations of the associations between these two common polymorphisms and breast cancer susceptibility were present in Table 3.

For PvuII (C>T) polymorphism, eligible studies included 1,763 breast cancer patients and 1,880 control subjects. Initial meta-analysis has shown that individuals with the TT/CT and TT genotypes had a 1.22 and 1.24 fold increased breast cancer compared with the CC genotype respectively (dominant model: OR = 1.22, 95%CI: 1.02-1.47, P = 0.034; homozygous model: OR = 1.24, 95%CI: 1.01-1.51, P = 0.039; respectively). In a stratified analysis by menopausal status, under the allele, dominant and homozygous models, the pre-menopausal women carrying the variant p allele, were found to be at significantly increased breast cancer risk (allele model: OR = 1.13, 95%CI: 1.01-1.28, P = 0.040; dominant model: OR = 1.29, 95%CI: 1.02-1.63, P = 0.036; homozygous model: OR = 1.35, 95%CI: 1.04-1.75, P = 0.022; respectively) (Table 3). However, these similar associations were not found among post-menopausal breast cancer women (Figure 2). According to source of controls, significant effects were also observed in population-based studies (dominant model: OR = 1.32, 95%CI: 1.07-1.62, P= 0.008) (Table 3).

For XbaI (A>G) polymorphism, the G allele was not associated with breast cancer susceptibility compared to the A allele (allele model: OR = 1.18, 95%CI: 0.99-1.20, P = 0.091). Similar results were observed in other model,

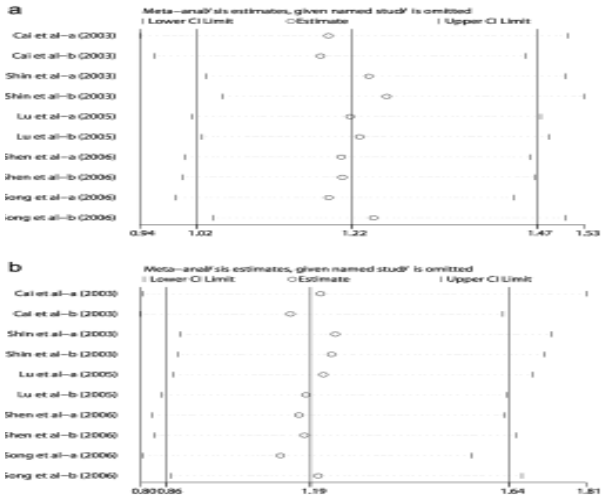

Figure 3. Sensitivity Analysis of the Summary Odds Ratio Coefficients on the Association Between ESR1 Gene Polymorphisms and Breast Cancer Risk among Asians under Dominant Model. a: PvuII (C>T); b: XbaI (A>G)

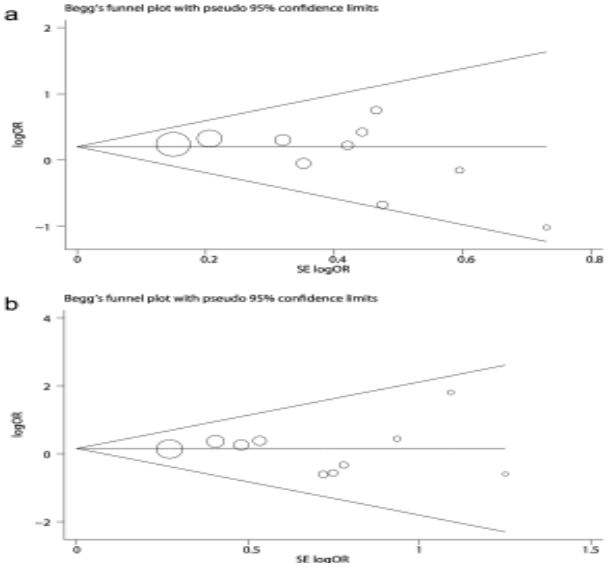

Figure 4. Begger's Funnel Plot of the Meta-analysis of Between ESR1 Gene Polymorphisms and Breast Cancer Risk among Asians. a: PvuII (C>T); b: XbaI (A>G)

which did not seem to be associated with breast cancer susceptibility in overall genetic models (dominant model: OR = 1.19, 95%CI: 0.86-1.64, P = 0.297; recessive model: OR = 1.29, 95%CI: 0.99-1.69, P = 0.064; homozygous model: OR = 1.23, 95%CI: 0.89-1.71, P = 0.211; heterozygous model: OR = 1.29, 95%CI: 0.97-1.74, P = 0.085; respectively) (Table 3). Furthermore, it was also performed subgroup analysis in studies by menopausal status. The results displayed that the XbaI polymorphism had non-significant with breast cancer in pre-menopausal and post-menopausal individuals. The source of controls findings , however, significant effects were observed in hospital-based studies (allele model: OR = 1.55, 95%CI: 1.21-2.00, P = 0.001) (Table 3).

In overall population, there was non-significant heterogeneity in ESR1 PvuII (C>T) polymorphism for any genetic model. Nevertheless, the Q-test of heterogeneity was significant in ESR1 XbaI (A>G) polymorphism for allele, recessive and heterozygous models, and conducted analysis using random effect models. After subgroup analysis by menopausal status, the heterogeneity was not effectively removed under the same models. The detailed data were presented in Table 3.

Sensitivity analysis

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential removal of individual studies. The analysis results suggested that no individual study significantly altered the pooled ORs in both ESR1 PvuII (C>T) and XbaI (A>G) polymorphisms under dominant model (Figure 3), confirming the stability of the results. Hence, results of the sensitivity analysis suggest that the data in this meta-analysis are relatively stable and credible.

Publication bias

Begger's funnel plot and Egger's linear regression test were performed to assess the publication bias of included studies. The shapes of the funnel plots in dominant models did not reveal any evidence of obvious asymmetry in both ESR1 PvuII (C>T) and XbaI (A>G) polymorphisms under dominant model (Figure 4). Thus, Egger's test also showed that there was no significantly statistical evidence of publication bias (PvuII: t = -1.39, P = 0.203; XbaI: t = -0.27, *P* = 0.795).

Discussion

Estrogen receptor alpha (ER-α), encoded by estrogen receptor 1 (ESR1) gene, is a ligand-activated transcription factor essential for sexual development, reproductive function and hormonal response in estrogen-sensitive tissues, such as breast, endometrium, and bone (Cai et al., 2003). ER-α plays an important role in the proliferation of mammary epithelial tissue by interacting with estrogens and altering expressions of downstream genes (Li et al., 2010). In agreement with this, potentially critical dysregulation of ER-α expression has been suggested to be involved in pathological processes of several human diseases including breast cancer (Shin et al., 2003). Recently, it has been reported that the abnormal ER-α expression is mainly caused by genetic polymorphisms of ESR1 gene and the transcript variants, different in their 5' UTRs, can stimulate growth and mediate differentiation of normal mammary tissue through high affinity binding to ERs (Jeon et al., 2010). Therefore, the expression of ER-α and its downstream signaling are likely altered by such mechanism, and it comes out that higher expression of circulating ER-α, as well as prolonged estrogen exposure, has been associated with increased breast cancer risk (Key et al., 2002).

ESR1 gene, located on chromosome 6p25.1, spanning approximately 300 kb in length and consisting of at least 8 exons and 7 introns (Parker et al., 1997), is responsible for inducing cell proliferation, managing cell growth, programming cell death and accumulating genetic mutations during cell division in the progress of breast carcinogenesis by binding both endogenous and exogenous hormones (Zhang and Yu, 2007). In the last decade, the correlations of genetic variants in ESR1

gene, such as rs2234693 (PvuII), rs9340799 (XbaI) and rs1801132 (Li et al., 2010), with the susceptibility to breast cancer in respect of menopausal status have become the subject of increasing interest, because menopausal characteristics, as one of the hormonal factors, can synergistically cooperate with genetic polymorphisms and affect the breast cancer risk (Butt et al., 2012). The differences in menopausal status are indicated to have an impact on estrogen synthesis, metabolism and signal transduction, accordingly, the dysfunction of which might trigger the development and progression of breast cancer. Considering the menopausal status in the risk of breast cancer, pre-menopausal women are suggested not to have the same hormonal risk factors as do the post-menopausal women (Pabalan et al., 2012).

Since the ESR1 is an important mediator of the hormonal response in estrogen-sensitive tissue such as the breast, endometrium and bone (Weiderpass et al., 2000), we hypothesized that polymorphisms in the ESR1 gene highly interesting in the search for susceptibility for breast cancer. In the recent years, studies in western and Asian women suggested that the association between ESR1 gene and breast cancer (Cai et al., 2003; Zhang et al., 2004; Surekha et al., 2007; Deng, 2011; Sakoda et al., 2011). However, these studies have appeared in the literature either supporting or negating the significant association, inconsistent associations of ESR1 PvuII (C>T) and XbaI (A>G) polymorphisms with breast cancer. Cai et al reported that the PvuII polymorphism was associated with breast cancer patients, but no such correlation was found in a later study by Sakoda et al. Recently, a hospital-based case-control studies were conducted in South Korea women, the most interesting finding in this study was that increased risk was observed prominently in post-menopausal women, suggesting that XbaI polymorphism may be associated with the breast cancer of late-onset or of onset after menopause, while the XbaI polymorphism was associated with a non-significantly elevated risk by Sakoda et al.

In order to provide the comprehensive and reliable conclusion, we perform the present meta-analysis of 5 independent case-control studies, including 1,768 breast cancer cases and 1,788 healthy controls. The main finding of this systematic review confirmed a positive association between ESR1 PvuII (C>T) polymorphism and susceptibility to breast cancer, and could be used as one of the genetic markers for screening in a Eastern population of subject who are at high-risk for development of breast cancer, specifically, among pre-menopausal women. Nevertheless, ESR1 XbaI (A>G) polymorphism is not likely to predict the risk of breast cancer.

A recent meta-analysis (Li et al., 2010), which investigated risk associated with ESR1 PvuII (C>T) polymorphism only modified by ethnicity and regardless of the menopausal status reported no association with breast cancer. There is some evidence to suggest that the altered breast cancer risk observed by menopausal status may be partly explained by the differences in age and levels of estrogen production between pre-menopausal and post-menopausal women (Li et al., 2009). Thus, the strengths of our study include (i) the statistically significant pooled findings which were homogeneous and (ii) the data were stratified by menopausal status among case and control.

Although our primary result of the current meta-analysis is suggestive, some limitations need to be addressed. Firstly, the sample size is still relatively small and might not provide sufficient power to estimate the association between ESR1 gene polymorphisms and breast cancer risk. In addition, the selection bias may exist because of the differences in the source of controls or detection samples. Besides, our meta-analysis was based on unadjusted ORs estimates because not all published presented adjusted ORs or when they did, the ORs were obtained with a failure to take account of important confounding factors, such as ethnicity, age, gender, other environmental risk factors, etc. Nevertheless, it is well acknowledged that in complex diseases, the genotype may be only one of the diseases. Finally, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that may have influenced our results.

Based on the limitations of the present study listed above, our meta-analysis still had some strength. To the best of our knowledge, this is the first meta-analysis of the relationship of ESR1 gene polymorphisms and breast cancer risk modified by menopausal status among Asians. It is worthwhile to mention that we established perfectly searching and selecting strategy based on computer-assisted and manual search, which increased the statistical power of this meta-analysis. By this means, the quality of studies included in current meta-analysis was satisfactory according to our selection criteria. Besides, explicit methods for study selection, data extraction, and data analysis were well designed before initiating. Last but not the least, Begger's test also no evidence of potential publication bias in this meta-analysis and the sensitivity analysis, indicating that the preferential publication of positive results does not occur and results are statistically robust.

In summary, the current meta-analysis of the 5 studied strongly indicated that ESR1 PvuII (C>T) polymorphism might be risk factor for breast cancer among Asians, specifically, among pre-menopausal women, while ESR1 XbaI (A>G) polymorphism is not likely to predict the risk of breast cancer. Such relationship would promisingly provide a more comprehensive mechanism of how ESR1 gene mutations function in the development of breast cancer and help to design therapy targets for corresponding treatment. However, it is critical that larger and well-designed studies are warranted to re-evaluate the potential association between ESR1 gene polymorphism with other genes polymorphisms and breast cancer risk.

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