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ORIGINAL ARTICLE

Association between estrogen receptor 1 (*ESR1*) genetic variations and cancer risk: a meta-analysis

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Summary

Purpose: *Emerging published reports on the association between estrogen receptor 1 (ESR1) genetic variation and cancer susceptibility are inconsistent. This review and meta-analysis was performed to achieve a more precise evaluation of this relationship.*

Methods: *A literature search of PubMed database was conducted from the inception of this study through April 1st 2014. Crude odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to assess the association.*

Results: *87 studies were enrolled in this meta-analysis. The results indicated that PvuII (T>C) polymorphism was associated with an increased risk of hepatocellular carcinoma (HCC) and prostate cancer, in contrast with the decreased risk of gallbladder cancer. No significant association was found in Asian and Caucasian populations. Furthermore, XbaI (A>G) genetic variation was only associated with an increased risk of prostate cancer, but was not related with race. In addition, T594T (G>A) polymorphisms were significantly associated with an increased risk of cancer, especially in Asian populations.*

Conclusions: *This meta-analysis indicated that PvuII (T>C) genetic variation may be risk factor for HCC, prostate cancer and gallbladder cancer. Meanwhile, XbaI (A>G) polymorphism may be potential prognostic factor for prostate cancer. Furthermore, T594T (G>A) was closely related with cancer susceptibility, especially in Asian populations.*

Key words: *cancer risk, estrogen receptor 1, genetic variation, meta-analysis*

Introduction

Cancer is one of the most serious diseases threatening human public health and represents the second cause of death after cardiovascular diseases. The incidence of cancer is also increasing worldwide owing to the increase of the population and aging [1]. Generally, it is known that cancer is a multifactorial disease induced by complex interactions between genetic and environmental factors [2]. Evidence from epidemiological and genetic studies have provided more information on the inherited susceptibility to cancer. Among these factors, hormonal factors have been proved to play a critical role in carcinogenesis through estrogen synthesis, metabolism and signal transduction pathways [3]. In recent years, accumulating evidence indicates that genetic variations in hormonal genes are key players in carcinogenesis [4,5].

ESR1, located on chromosome 6q25.1, is one of the hormonal genes encoding estrogen receptor α with approximately 300 kb in length, consisting of 8 exons and 7 introns [6]. *ESR1* functions as a ligand-activated transcription factor consisted of several domains important for DNA binding, hormone regulation, and activation of transcription. *ESR1* is also closely correlated with estrogens which can stimulate the proliferation of mammary epithelial tissues and alter the ex-

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pression of downstream genes [7]. Therefore, aberrant expression of *ESR1* often occurs in a number of human epithelial cancers. Recently, several *ESR1* gene polymorphisms have been identified as candidates for cancer susceptibility; ESR1 PvuII (rs2234693 T>C), XbaI (rs9340799 A>G) and T594T (rs2228480 G>A) polymorphisms have been reported to be significantly associated with the development of cancer. Meanwhile, these three genetic variations can affect *ESR1* transcription activity and possibly contribute to carcinogenesis [3,8,9]. However, several recent studies observed no association between these genetic polymorphisms of *ESR1* and cancer risk [7,10]. The inconsistent conclusions between *ESR1* gene mutations and cancer risk may be due to the limitations in the sample size of the corresponding studies or the inadequate statistical power in genetic studies with complex characteristics, like age, race, gender, tumor stage, tumor grade and research methodology. Therefore, we performed a search of the relevant literature and carried out a meta-analysis to achieve a more accurate evaluation of the association between *ESR1* genetic polymorphisms and cancer risk.

Methods

Publication selection

Studies were identified by an electronic search of PubMed using the following terms: "Estrogen Receptor 1", "Estrogen Receptor α", "*ESR1*", "*ESRa*", "polymorphism", "cancer", "tumor" or "carcinomas". Meanwhile, we also searched manually the references of these publications in order to retrieve additional studies. Only those published as full-text articles were included as candidates. The search finished on April 2014.

Inclusion and exclusion criteria

Studies evaluating the association between *ESR1* genetic polymorphisms and cancer risk had to meet all of the following criteria: 1) they were original epidemiological studies on the association between *ESR1* genetic polymorphisms and cancer susceptibility; 2) case-control studies; 3) effective information provided to estimate ORs with 95% CI; 4) published in English only. Case-only studies, case reports, duplicated studies, unpublished data, letters, comments and reviews were excluded.

Data extraction

For every eligible study, two investigators (HLS and QWD) using a standardized and uniform method of data extraction collected carefully information regarding the first author's last name, country of origin, year of publication, race of the study population, cancer type, the source of control, genotyping method, polymorphism site and the numbers of cases and controls. All disagreements about eligibility were resolved by discussion after data collection and got consensus following with the opinion of another reviewer.

Statistics

Every eligible study was evaluated by Hardy-Weinberg equilibrium (HWE) using the goodness-of-fit x^2 test. ORs with the corresponding 95% CIs were

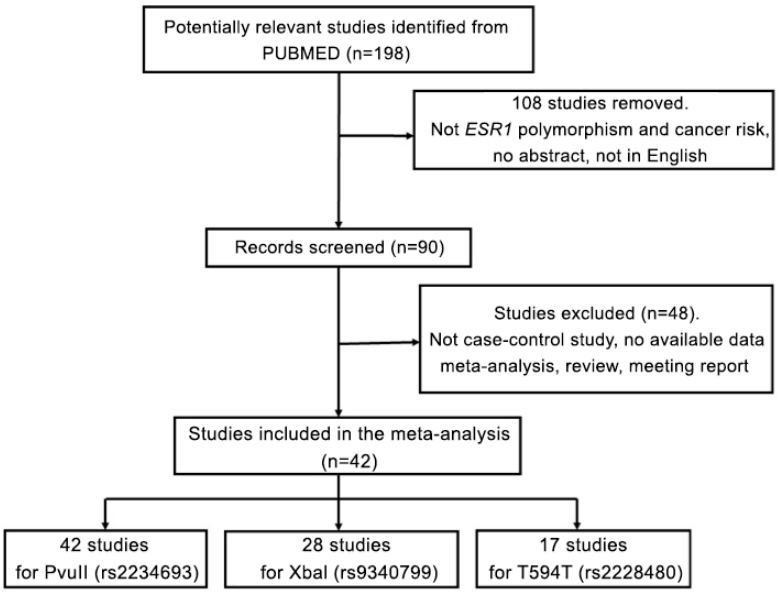

Figure 1. Flow chart of studies identified according to the inclusion and exclusion criteria.

used to estimate the strength of association between ESR1 PvuII (T>C), XbaI (A>G) and T594T (G>A) polymorphisms and cancer risk. The pooled ORs were also assessed for PvuII (T>C) by homozygous, heterozygous, recessive and dominant models as well as allele comparison and so were XbaI (A>G) and T594T (G>A). Subsequently, stratified analyses were also performed by cancer type and ethnicity (if one cancer type contained less than two individual studies, it would have been stratified into the "others" group).

Chi square-based Q test was used to assess the heterogeneity across studies [11]. The random-effects model (DerSimonian and Laird method) was chosen [12]. Otherwise, a fixed-effect model (Mantel-Haenszel method) was chosen if $p_{heterogeneity}$ (p_h) > 0.05 for the Q test [13]. In addition, in the sensitivity analysis the stability of results was estimated by excluding each study individually and recalculating the ORs with the corresponding 95% CIs for the remaining ones. The publication bias was assessed by Funnel plots and Egger's linear regression test [14]. All p values were two-sided and p<0.05 was considered as significant. All statistical tests were performed with STATA, version 10.0 (College Station Corporation, TX, USA).

Results

Characteristics of studies

Based on the inclusion criteria, 51 eligible articles were enrolled into this meta-analysis (Figure 1). For PvuII (T>C), only 42 studies with available data were enrolled into the pooled analysis.

Table 1. Characteristics of studies included in the meta-analysis

Cancer	*First author with ref. no.*	*Year*	*Country*	*Ethnicity*	*Source*	*Genotyping method*	*Polymorphism site*	*Case/Control*	*HWE*
Breast	Anghel[34]	2009	Romania	Caucasian	HB	RFLP, Hybridization probes	PvuII, XbaI, T594T	101/90, 102/90, 103/84	0.33, 0.20, 0.60
	Dunning[35]	2009	UK	Caucasian	PB	Taqman	PvuII, XbaI	4362/4548, 4170/4447	0.25, 0.35
	Gallicchio[36]	2006	USA	Caucasian	PB	Taqman	T594T	80/1240	0.70
	Han[37]	2011	China	Asian	PB	Taqman	PvuII	859/877	0.17
	Jeon[38]	2010	Korean	Asian	HB	MALDI-TOF	T594T	774/675	0.58
	Kallel[39]	2009	Tunisia	Caucasian	HB	RFLP	T594T	142/240	0.10
	Kjaergaard[40]	2007	Denmark	Caucasian	HB	RFLP	PvuII	1256/2489	0.62
	Mancha[41]	2008	Spain	Caucasian	PB	Sequence	PvuII	444/704	0.44
	Modugno[21]	2005	USA	Caucasian	PB	RFLP	PvuII, XbaI	248/3901, 247/3935	0.01, 0.44
	Sakoda[26]	2010	China	Asian	HB	SnaPshot	PvuII, XbaI	612/874, 614/876	0.29, 0.60
	Sangrajrang[22]	2009	Thai	Asian	HB	Taqman	PvuII, XbaI, T594T	566/485, 563/484, 562/481	0.01, 0.56, 0.07
	Slattery[19]	2008	USA	Caucasian	PB	Taqman	XbaI	1163/1328, 574/725	0.82, 0.45
	Sobti[42]	2012	India	Asian	HB	RFLP	T594T	150/150	0.20
	Sonestedt[43]	2009	Sweden	Caucasian	PB	MassArray	PvuII	539/1073	0.67
	Tang[44]	2013	China	Asian	HB	MALDI-TOF	PvuII	794/845	0.08
	Wang[45]	2007	USA	Caucasian	PB	Sequence	PvuII, XbaI	392/783, 392/788	0.86, 0.89
	Wedren[46]	2004	Sweden	Caucasian	PB	Sequence	PvuII, XbaI	1292/1384, 1291/1348	0.25, 0.99
Colorectal	Rudolp[47]	2011	Germany	Caucasian	PB	KASPar	PvuII, XbaI	670/672, 676/676	0.27, 0.50
	Lin[27]	2010	USA	Caucasian	HB	Sequence	PvuII, XbaI	151/540, 148/526	0.82, 0.71
Endometrial	Ashton[48]	2009	Australia	Caucasian	HB	RFLP	PvuII, XbaI	191/290	0.09, 0.75
	Iwamoto[49]	2003	Japan Sweden USA	Asian	HB	RFLP	PvuII, XbaI	92/65	0.41, 0.14
	Lundin[18]	2012	Italy	Caucasian	HB	Taqman	PvuII	391/709	0.22

	Sasaki[24]	2002	USA	Asian	PB	Sequence	PvuII	113/200	0.06
	Wedren[50]	2008	Sweden	Caucasian	PB	Sequence	PvuII, XbaI	662/1368	0.50, 0.57
	Weiderpass[51]	2000	Sweden	Caucasian	PB	RFLP	PvuII, XbaI	261/380	0.86, 0.62
	Yoneda[52]	2013	Japan	Asian	PB	RFLP	PvuII, XbaI	125/200	0.65, 0.80
Gallbladder	Srivastava[53]	2012	India	Asian	HB	RFLP	PvuII	410/220	0.07
	Park[17]	2010	China	Asian	PB	Taqman	PvuII	235/778	0.66
HCC	Anghel[16]	2009	Romania	Caucasian	HB	Hybridization probes	PvuII, XbaI	12/114	0.31, 0.16
	Zhai[54]	2006	China	Asian	PB	RFLP	PvuII, XbaI	244/237, 244/235	0.46, 0.94
Prostate	Cancel-Tassin[55]	2003	France	Caucasian	PB	DHPLC	T594T	96/96	0.30
	Chae[7]	2009	USA	Caucasian	HB	Taqman	T594T	219/379	0.90
	Fukatsu[56]	2004	Japan	Asian	HB	SSCP	PvuII, XbaI	116/238, 117/242	0.38, 0.26
	Gupta[57]	2009	India	Asian	PB	RFLP	PvuII, XbaI	157/170	0.05, 0.44
	Hernandez[15]	2006	USA	other	HB	Taqman	PvuII, XbaI	47/213	0.37, 0.23
	Hernandez[15]	2006	USA	Caucasian	HB	Taqman	PvuII, XbaI	551/885	0.54, 0.91
	Low[8]	2006	UK	Caucasian	HB	Taqman	PvuII	75/158	0.27
	Modugno[58]	2001	USA	Caucasian	PB	RFLP	PvuII, XbaI	81/237	0.44, 0.17
	Onsory[59]	2008	India	Asian	HB	RFLP	PvuII	100/100	0.49
	Sissung[31]	2011	USA	Caucasian	HB	RFLP	PvuII, XbaI	128/126, 129/127	0.95, 0.12
	Sobti[60]	2008	India	Asian	HB	RFLP	PvuII	157/170	0.05
	Sonoda[61]	2010	Japan	Asian	HB	Taqman	PvuII	180/177	_
	Tanaka[23]	2003	Japan	Asian	HB	Sequence	PvuII, T594T	115/200	0.06, 0.01
Others	Anghel[16]	2009	Romania	Caucasian	HB	Hybridization probes	PvuII, XbaI, T594T	15/114	0.31, 0.16, 0.48
	Anghel[16]	2009	Romania	Caucasian	HB	Hybridization probes	PvuII, XbaI, T594T	18/114	0.31, 0.16, 0.48
	Anghel[16]	2009	Romania	Caucasian	HB	Hybridization probes	T594T	12	0.48
	Denschlag[62]	2006	Germany	Caucasian	HB	Sequence	PvuII	130	0.07
	Ferlin[10]	2010	Italy	Caucasian	HB	RFLP	PvuII, XbaI	234/218	0.13, 0.07
	Maha[20]	2009	Tunisia	Caucasian	HB	RFLP	T594T	106	0.17
	Park[17]	2010	China	Asian	PB	Taqman	PvuII	123/778	0.66
	Park[17]	2010	China	Asian	PB	Taqman	PvuII	47/778	0.66
	Park[17]	2010	China	Asian	PB	Taqman	T594T	235/781	0.65
	Park[17]	2010	China	Asian	PB	Taqman	T594T	125/781	0.65
	Park[17, 24]	2010	China	Asian	PB	Taqman	T594T	47/781	0.65
	Sasaki[24]	2002	USA	Asian	PB	Sequence	T594T	113/200	0.01
	Srivastava[53]	2012	India	Asian	HB	RFLP	XbaI	410/220	0.14

PB: population based, HB: hospital based, RFLP: restriction fragment length polymorphism, AS-PCR: allele-specific PCR, MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight, DASH: dynamic allele-specific hybridization, HWE: Hardy-Weinberg equilibrium; -:HWE not shown due to no valid data for control

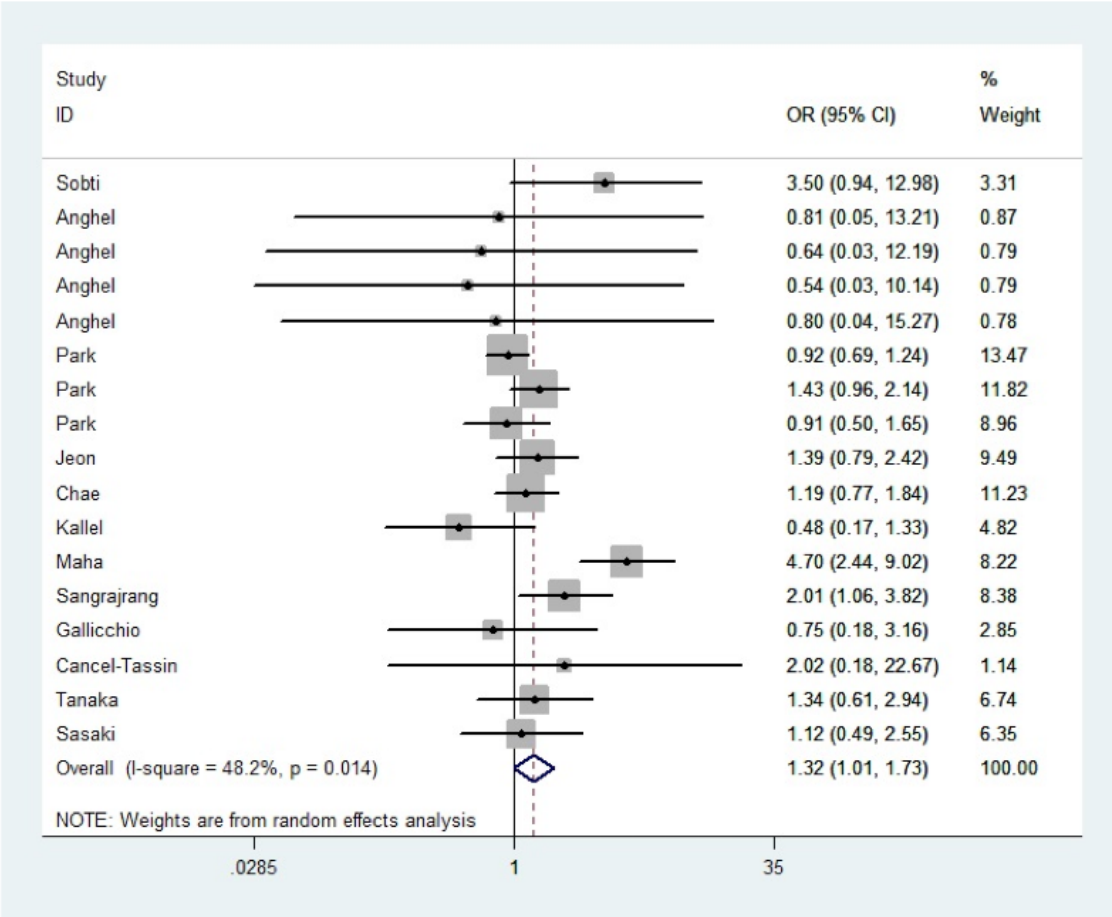

Figure 2. Forest plots of effect estimates for ESR1 T594T polymorphism (A/A vs G/A+G/G). For each of the studies, the estimation of OR and its 95% CI is plotted with a box and a horizontal line. White diamond shows the pooled OR and its 95% CI.

The population of one paper contained two ethnic groups, being in fact two separate studies [15] and two publications were dealing with three cancer types providing three independent studies [16,17]. Breast cancer (12 studies), colorectal cancer (2 studies), endometrial cancer (7 studies), gallbladder cancer (2 studies), HCC (2 studies), prostate cancer (11 studies) and the "others" were included in the pooled analysis. In addition, in the 42 studies, populations were divided into two racial groups (Asian, Caucasian) and the group of the "other descendents" [15,18] with only one racial group (Table 1).

For XbaI (A>G) polymorphism, 24 publications with 28 studies were chosen for eligibility, and were classified into breast cancer (9 studies), colorectal cancer (2 studies), endometrial cancer (5 studies), HCC (2 studies), prostate cancer (6 studies) and "the others" in the Asian and Caucasian populations. One papers with three cancer types provided three studies [16], and two papers [15,19] with two races offered two independent studies (Table 1).

For T594T (G>A) polymorphism, 17 studies were case-control studies with available data, which consisted of Asian (8 studies) and Caucasian (9 studies) populations related to breast cancer, prostate cancer and other cancers. Meanwhile, two papers [16,17] with different cancer types provided three independent studies (Table 1).

Main results

PvuII (T>C).

The overall results for the PvuII (T>C) polymorphism and cancer risk are shown in Tables 2 and 3. Results of the pooled analysis did not indicate significantly increased or decreased risk between PvuII (T>C) polymorphism and overall cancer risk. However, in a stratified analysis by cancer type, a statistically significant association was observed for gallbladder cancer (homozygous: OR=0.65, 95% CI=0.46-0.91, P_h =0.161; het-

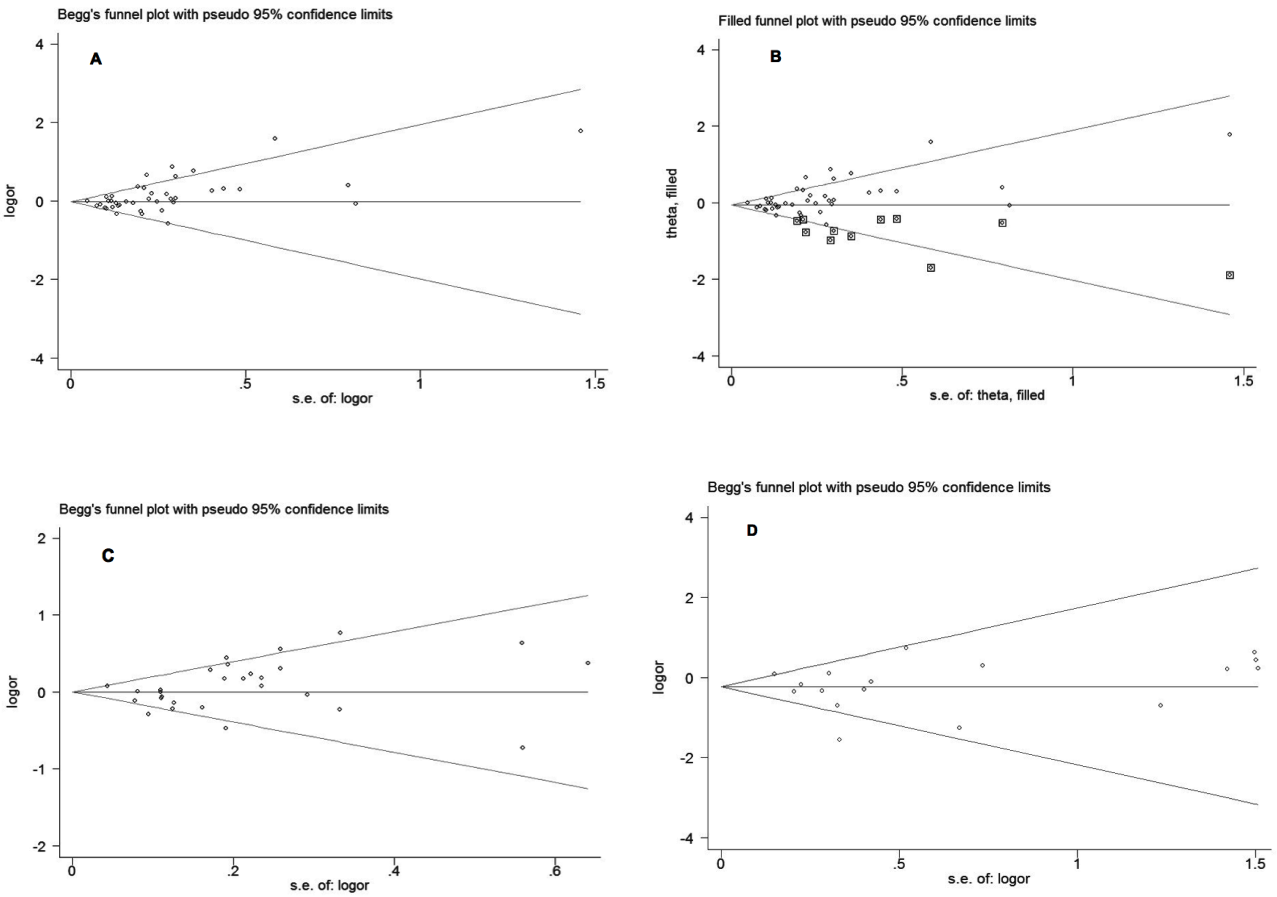

Figure 3. Begg's funnel plot of Egger's test for publication bias for three polymorphisms. Each circle represents an independent study for the indicated association. Log [OR], natural logarithm of OR. Horizontal lines mean effect size. **A:** Begg's funnel plot of publication bias test for ESR1 PvuII (T>C) polymorphism. **B:** Begg's funnel plot of publication bias test after trim-and-fill method. **C:** Begg's funnel plot of publication bias test for XbaI (A>G) polymorphism. **D:** Begg's funnel plot of publication bias test for T594T (G>A) polymorphism.

erozygous: OR=0.70, 95% CI=0.59-0.98; dominant: OR=0.68, 95% CI=0.50-0.93, Ph =0.373 and allele: OR=0.83, 95% CI=0.71-0.97, Ph=0.159), and we found a significantly increased risk between PvuII (T>C) polymorphism and HCC risk (homozygous: OR=1.99, 95% CI=1.18-3.34, P_h =0.263; recessive: OR=1.67, 95% CI=1.06-2.61 and allele: OR=1.36, 95% CI=1.06-1.74, P_h =0.222) as well as prostate cancer risk (homozygous: OR=1.50, 95% CI=1.24-1.82, P_h =0.119; heterozygous: OR=1.18, 95% CI=1.02-1.36, P_h =0.493; recessive: OR=1.32, 95% CI=1.13-1.55, P_h =0.204 and allele: OR=1.21, 95% CI=1.10-1.33, P_h =0.190). Ethnicity subgroup analysis revealed that PvuII (T>C) polymorphism was not related with cancer risk in Caucasian and Asian populations [Tables 2,3].

XbaI (A>G).

The overall results for the XbaI (A>G) polymorphism and cancer risk are shown in Tables 2 and 3. Results of the pooled analysis indicated no significant association between XbaI (A>G) polymorphism and overall cancer risk. In the subgroup analysis, a statistically significant association was found for prostate cancer (dominant: OR=1.19, 95% CI=1.02-1.39, P_h =0.145 and allele: OR=1.15, 95% CI=1.02-1.29, P_h =0.235).

T594T (G>A).

The overall results for the T594T (G>A) polymorphism and cancer risk are shown in Tables 2 and 3. Increased risk association was observed in the overall pooled analysis with the comparison of the recessive model (OR=1.32, 95% CI=1.01-1.73, P_h =0.014) shown in Figure 2. In the subgroup analysis by race, T594T (G>A) was significantly associated with increased risk of Asian populations (homozygous: OR=1.44, 95% CI=1.10-1.89, P_h =0.162 and recessive: OR=1.20, 95% CI=1.00-1.44, P_h =0.097, p=0.048).

Table 2. Meta-analysis of the association between ESR1 PvuII, XbaI, T594T polymorphisms and cancer risk

Variables	*No. of studies*	*Homozygous*			*Heterozygous*			*Allele*		
		OR (95% CI)	P^{het}	I^2(%)	*OR (95% CI)*	P^{het}	I^2(%)	*OR (95% CI)*	P^{het}	I^2(%)
		C/C vs T/T			*T/C vs T/T*			*C vs T*		
For PvuII (T>C)										
All	42	1.04 (0.94-1.16)	0.000	58.1	1.00 (0.93-1.07)	0.023	32.8	1.01 (0.96-1.06)	0.000	55.9
Cancer type										
Breast	12	0.93 (0.83-1.04)	0.027	49.3	0.96 (0.91-1.02)	0.080	39.1	0.97 (0.94-1.00)	0.104	35.7
CRC	2	0.91 (0.69-1.19)	0.096	63.8	0.99 (0.80-1.22)	0.072	69.1	0.96 (0.84-1.09)	0.079	67.7
Endometrial	7	1.05 (0.76-1.44)	0.007	66.2	0.98 (0.85-1.12)	0.110	42.1	1.02 (0.87-1.19)	0.007	66.4
Gallbladder	2	**0.65 (0.46-0.91)**	0.161	49.2	**0.70 (0.59-0.98)**	0.681	0.0	**0.83 (0.71-0.97)**	0.159	49.5
HCC	2	**1.99 (1.18-3.34)**	0.263	20.2	1.23 (0.84-1.82)	0.912	0.0	**1.36 (1.06-1.74)**	0.222	33.0
Prostate	11	**1.50 (1.24-1.82)**	0.119	36.2	**1.18 (1.02-1.36)**	0.493	0.0	**1.21 (1.10-1.33)**	0.190	27.6
Others	6	0.94 (0.69-1.27)	0.715	0.0	0.93 (0.71-1.22)	0.156	37.6	0.95 (0.82-1.10)	0.416	0.0
Race										
Asian	18	1.08 (0.88-1.33)	0.001	59.4	0.99 (0.91-1.09)	0.652	0.0	1.03 (0.94-1.12)	0.005	53.5
Caucasian	22	1.02 (0.89-1.16)	0.000	60.9	1.02 (0.92-1.13)	0.001	53.8	1.01 (0.96-1.06)	0.000	61.1
Other	2	1.12 (0.81-1.55)	0.281	14.0	0.86 (0.65-1.13)	0.568	0.0	1.05 (0.89-1.23)	0.236	28.9
For XbaI (A>G)		G/G *vs* A/A			A/G *vs* A/A			G *vs* A		
All	28	1.05 (0.92-1.19)	0.009	43.0	1.00 (0.93-1.08)	0.018	39.4	1.02 (0.96-1.09)	0.000	55.8
Cancer type										
Breast	9	1.01 (0.92-1.11)	0.243	22.5	1.00 (0.94-1.06)	0.492	0.0	1.00 (0.96-1.05)	0.142	34.4
CRC	2	0.94 (0.69-1.28)	0.164	48.4	1.05 (0.87-1.28)	0.074	68.7	1.00 (0.87-1.14)	0.072	69.2
Endometrial	5	0.93 (0.56-1.53)	0.007	71.6	0.89 (0.77-1.02)	0.105	47.7	0.96 (0.75-1.23)	0.002	76.3
HCC	2	2.13 (0.99-4.57)	0.799	0.0	1.10 (0.75-1.60)	0.827	0.0	1.28 (0.95-1.71)	0.748	0.0
Prostate	6	1.27 (0.98-1.66)	0.459	0.0	1.17 (1.00-1.38)	0.169	35.8	**1.15 (1.02-1.29)**	0.235	26.6
Others	4	1.41 (0.95-2.08)	0.235	29.6	0.88 (0.50-1.56)	0.014	71.6	1.02 (0.69-1.49)	0.022	68.7
Race										
Asian	8	1.22 (0.95-1.57)	0.286	18.3	1.04 (0.92-1.18)	0.769	0.0	1.07 (0.97-1.17)	0.220	26.2
Caucasian	19	1.00 (0.87-1.16)	0.007	50.0	0.98 (0.89-1.07)	0.011	47.8	1.00 (0.93-1.07)	0.000	61.0
Other	1	1.81 (0.60-5.50)			**2.24 (1.13-4.41)**			**1.60 (1.00-2.57)**		
For T594T (G>A)		A/A *vs* G/G			G/A *vs* G/G			A *vs* G		

All	17	1.46 (0.93-2.31)	0.000	70.4	1.29 (0.95-1.74)	0.000	81.3	1.22 (0.98-1.52)	0.000	83.6
Cancer type										
Breast	6	1.36 (0.71-2.60)	0.036	58.1	1.19 (0.80-1.77)	0.000	84.5	1.15 (0.84-1.57)	0.000	83.1
Prostate	3	1.26 (0.84-1.90)	0.812	0.0	1.14 (0.87-1.49)	0.437	0.0	1.16 (0.96-1.40)	0.506	0.0
Others	8	1.47 (0.55-3.96)	0.000	82.5	1.35 (0.61-2.95)	0.000	84.2	1.22 (0.75-1.99)	0.000	88.6
Race										
Asian	8	**1.44 (1.10-1.89)**	0.162	33.4	1.17 (0.84-1.64)	0.000	75.4	1.18 (0.98-1.42)	0.003	67.3
Caucasian	9	1.37 (0.46-4.08)	0.000	81.4	1.43 (0.80-2.54)	0.000	85.6	1.20 (0.73-1.97)	0.000	89.1

CRC: colorectal cancer, HCC: hepatocellular carcinoma. Statistically significant results are in bold

Test of heterogeneity

Significant heterogeneity was revealed among overall studies for the T594T (G>A) polymorphism and cancer risk (recessive: P_h =0.014). Hence, the random-effect model was applied to generate CIs for the genetic model comparison (P_h <0.05). Otherwise, the fixed-effect model was used when P_h>0.05.

Sensitivity analysis

Sensitivity analysis was conducted to assess the stability of the results and the source of heterogeneity by sequential removal of each eligible study. For PvuII (T>C) and XbaI (A>G) polymorphisms, the results were stable by sensitivity analysis. For T594T (G>A) polymorphism, the results indicated that the study of Rebai et al. [20] was the main source of heterogeneity. By removing this study, the heterogeneity was decreased (AA vs GG+GA: P_h =0.521).

Despite the genotype distributions in four studies disobeying HWE [21-24], the corresponding pooled ORs were not significantly altered by omitting the studies above.

Publication bias

Funnel plot and Egger's test were performed to assess the publication bias. The shape of the funnel plot showed an obviously asymmetry in PvuII dominant model comparison and Egger's test was used to provide statistical evidence of funnel plot asymmetry (t=2.63, p=0.012) (Figure 3A), which suggested the existence of publication bias in this meta-analysis. To adjust the bias, the trim-and-fill method by Duval and Tweedie [25] was utilized (Figure 3B). The conclusion with or without the trim-and-fill method didn't change, which indicated that the results were robust. Meanwhile, the models of XbaI and T594T did not show publication bias (p>0.05) (Figures 3C and 3D).

Discussion

The association between *ESR1* polymorphisms and cancer risk has been investigated by many researchers. However, the results remained inconsistent for different types of cancer. Moreover, the results were contradictory for the same cancer in many studies. In the current case-control study, associations of three *ESR1* polymorphisms (PvuII rs2234693 T>C, XbaI rs9340799 A>G and T594T rs2228480 G>A) and cancer risk were assessed. The polymorphisms of *ESR1* may play a critical role in tumorigenesis, development and prognosis of several kinds of cancer, such as colorectal, prostate, breast and endometrial cancer [23,24,26,27].

We concluded that PvuII polymorphism was not associated with cancer risk in the overall pooled results from 42 studies. Stratified analysis by cancer type indicated that rs2234693 increased the risk of HCC and prostate cancer, which was inconsistent with previous studies [28,29], and which could be attributed to the limited number of studies enrolled in the present meta-analysis. Different inclusion and exclusion criteria should also be considered to influence the final pooled results. Meanwhile, no significant association was observed in breast, colorectal and endometrial cancer, which revealed that rs2234693 polymorphisms might have different effects on distinct cancers. However, for gallbladder cancer, rs2234693 C allele was a protective factor, which resulted from

Table 3. Meta-analysis of the association between ESR1 PvuII, XbaI, T594T polymorphisms and cancer risk by recessive and dominant models

		Recessive			*Dominant*		
Variables	*No. of studies*	*OR (95% CI)*	P^{het}	I^2(%)	*OR (95% CI)*	P^{het}	I^2(%)
		C/C vs (T/C+T/T)			*(C/C+T/C) vs T/T*		
For PvuII (T>C)							
All	42	1.01 (0.93-1.09)	0.000	48.6	1.01 (0.94-1.08)	0.001	46.2
Cancer type							
Breast	12	0.96 (0.91-1.02)	0.123	33.4	0.96 (0.91-1.01)	0.052	43.8
CRC	2	0.91 (0.72-1.16)	0.389	0.0	1.05 (0.66-1.67)	0.049	74.1
Endometrial	7	0.99 (0.78-1.27)	0.015	61.8	1.03 (0.83-1.29)	0.038	55
Gallbladder	2	0.84 (0.67-1.05)	0.108	61.3	**0.68 (0.50-0.93)**	0.373	0.0
HCC	2	**1.67 (1.06-2.61)**	0.136	54.9	1.40 (0.97-2.03)	0.614	0.0
Prostate	11	**1.32 (1.13-1.55)**	0.204	26.0	1.25 (1.09-1.43)	0.318	13.2
Others	6	0.94 (0.75-1.18)	0.248	24.8	0.92 (0.71-1.18)	0.373	0.0
Race							
Asian	18	1.06 (0.90-1.24)	0.003	55.9	1.00 (0.92-1.09)	0.195	21.8
Caucasian	22	1.01 (0.93-1.09)	0.014	4.1	1.02 (0.92-1.13)	0.000	60.5
Other	2	1.24 (0.94-1.62)	0.338	0.0	0.93 (0.72-1.20)	0.138	0.0
For XbaI (A>G)		G/G *vs* (A/G+A/A)			(G/G+A/G) *vs* A/A		
All	28	1.03 (0.95-1.10)	0.188	18.9	1.02 (0.94-1.10)	0.001	52.2
Cancer type							
Breast	9	1.01 (0.92-1.10)	0.499	0.0	1.00 (0.94-1.06)	0.242	22.7
CRC	2	0.91 (0.68-1.22)	0.417	0.0	1.03 (0.85-1.24)	0.052	73.5
Endometrial	5	0.94 (0.63-1.39)	0.049	58.1	0.95 (0.72-1.27)	0.014	68.2
HCC	2	2.05 (0.97-4.30)	0.870	0.0	1.21 (0.85-1.73)	0.764	0.0
Prostate	6	1.20 (0.93-1.54)	0.728	0.0	**1.19 (1.02-1.39)**	0.145	39.1
Others	4	**1.50 (1.04-2.17)**	0.665	0.0	0.93 (0.52-1.65)	0.009	74.2
Race							
Asian	8	1.19 (0.94-1.52)	0.380	6.5	1.06 (0.94-1.19)	0.484	0.0
Caucasian	19	1.01 (0.93-1.09)	0.143	26.1	0.98 (0.89-1.09)	0.001	58.8
Other	1	1.21 (0.43-3.44)			**2.15 (1.12-4.13)**		
For T594T (G>A)		A/A *vs* (G/A+G/G)			(A/A+G/A) *vs* G/G		
All	17	**1.32 (1.01-1.73)**	0.014	48.2	1.31 (0.97-1.77)	0.000	83.3
Cancer type							
Breast	6	1.39 (0.99-1.95)	0.143	39.4	1.20 (0.80-1.79)	0.000	85.8
Prostate	3	1.24 (0.85-1.81)	0.891	0.0	1.18 (0.92-1.52)	0.459	0.0
Others	8	1.33 (0.83-2.13)	0.003	68.0	1.34 (0.60-3.02)	0.000	86.6
Race							
Asian	8	**1.20 (1.00-1.44)**	0.188	30.1	1.23 (0.89-1.69)	0.000	75.2
Caucasian	9	1.20 (0.59-2.43)	0.011	59.5	1.39 (0.76-2.57)	0.000	88.1

For abbreviation see footnote of Table 2. Statistically significant results are in bold

rs2234693 polymorphism affecting receptor function via altering splicing of *ESR1* mRNA or affecting the levels of *ESR1* expression among different cancers [30]. In addition, bad dietary habits could contribute to the induction of gallbladder cancer and HCC, including eating high-fat and high-protein food, processed food, smoking and drinking. Moreover, in people with family history of these cancers, cancer risk would be affected according the epidemiology reports, while for prostate cancer, sexual activity, fat intake, race and family history were the main source of risk factors. The varied mechanisms may have different effects on the rs2234693 polymorphism leading to different results. Subsequently, stratified analysis by race indicated no significant association in Caucasian and Asian populations, possibly attributed to the small sample size or the different frequencies of rs2234693 C allele variant in this study.

Previous studies have shown that XbaI (A>G) displayed obvious association with the increased cancer risk [15,31]. Our results showed that XbaI (A>G) polymorphism was not associated with cancer risk in the overall pooled ORs among all models. Cancer type by subgroup analysis indicated that increased cancer risk was only found in prostate cancer, which was consistent with previous studies by different genetic models [28,32]. The contradictory results could be generated by the different inclusion and exclusion criteria. However, no significant association was observed in Asian and Caucasian populations, which was inconsistent with the Zhou et al. study [33]. As described above, the genetic background and frequencies of rs9340799 G allele in different races contributed to these results. However, there were only 28 studies enrolled in present study. Well-designed, unbiased, large case-control studies should be performed to acquire a more precise association between XbaI (A>G) polymorphism and cancer risk for the two ethnic populations.

As for T594T (G>A) polymorphism, there has been no meta-analysis concerning the association between the T594T (G>A) polymorphism and cancer risk up to now. Our results indicated T594T (G>A) polymorphism had significant association with increased cancer risk by the recessive model in the overall pooled analysis. And subgroup analysis by ethnicity and cancer type revealed that T594T AA genotype was a risk factor in Asian populations. Meanwhile, a similar association with increased cancer risk was also observed after comparison of A/A vs (G/A+G/A) in Asians. The results suggested different races might lead to distinct effects of T594T polymorphism. In addition, only 17 studies were enrolled in the analysis of T594T polymorphism, which could affect the results, owing to the small number of studies. To acquire a more accurate conclusion, more well-designed studies are needed to further clarify the association of T594T (G>A) polymorphism and cancer risk.

Some limitations of this meta-analysis should be acknowledged. First, all eligible studies were limited to papers written in English only. So some studies were missed for not being written in English, although they met the inclusion criteria. Second, controls were not uniformly defined. Some of them might be patients, although the healthy populations were the main source of the controls. Third, publication bias was detected in T594T polymorphism, while in some papers in which such bias was not detected might also appear in other polymorphisms owing to the small number of studies. Fourth, the number of cases and controls in the subgroup analysis was relatively small in different cancers, not having sufficient statistical power to estimate the real association. Therefore, our results should be interpreted with caution based on unadjusted estimates, and further studies are needed to confirm our unadjusted estimates.

In conclusion, we performed this meta-analysis to evaluate the association between three *ESR1* polymorphisms and cancer risk. Despite the aforementioned limitations, our results showed that PvuII polymorphism was associated with cancer risk, especially with HCC and prostate cancer, in contrast with gallbladder cancer. Moreover, XbaI G allele was significantly associated with increased risk of prostate cancer. In addition, T594T polymorphism was risk factor in the overall pooled analysis by the recessive model, especially in Asian populations. It is essential to conduct more large trials using standardized unbiased design, homogeneous cancer patients and well-matched controls. More important, gene-environment and gene-gene interactions should also be taken into account in the analysis to achieve better and comprehensive estimates of the three *ESR1* polymorphisms and cancer risk.

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Authors' contributions

Conceived and designed the experiments: SKW, QWD and BSH

Performed the experiments: HLS, QWD, YQP, HQY, JC and XL

Analyzed the data: HLS and QWD

Contributed to reagents/materials/analysis tools: HLS and QWD

Wrote the manuscript: SKW, HLS

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