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*Original Article*

# Association between estrogen receptor-alpha gene PvuII and XbaI polymorphisms and osteoarthritis risk: a meta-analysis

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**Abstract:** Estrogen receptor-alpha (ER-α) gene PvuII (T/C) and XbaI (A/G) polymorphisms have been hypothesized to be associated with osteoarthritis (OA) risk by several epidemiological studies, however, the available results were inconclusive and conflicting. We conducted a meta-analysis of 10 case-control studies that included 3328 osteoarthritis cases and 6390 case-free controls. We assessed the strength of the association, using odds ratios (ORs) with 95% confidence intervals (CIs). This meta-analysis showed that the ER-α PvuII and XbaI polymorphisms were not associated with OA risk in overall population. For the PvuII (T/C) polymorphism, however, in the subgroup analysis by country, a significantly reduced risk was observed among Chinese (TC vs. CC: OR = 0.73, 95% CI 0.54-0.99, $I^2$ = 0%, $P_{heterogeneity}$ = 0.498; dominant model, OR = 0.73, 95% CI = 0.55-0.98, $I^2$ = 0%, $P_{heterogeneity}$ = 0.555). For the XbaI (A/G) polymorphism, when stratifying by sample size, a significantly elevated risk was found in sample size ≤ 500 (AA vs. GG: OR = 2.60, 95% CI 1.10-6.18, $I^2$ = 42.9%, $P_{heterogeneity}$ = 0.135; dominant model: OR = 2.04, 95% CI 1.12-3.71, $I^2$ = 11.4%, $P_{heterogeneity}$ = 0.341; and recessive model: OR = 1.69, 95% CI 1.12-2.55, $I^2$ = 40.2%, $P_{heterogeneity}$ = 0.154). No publication bias was found in the present study. This meta-analysis suggests that ER-α PvuII (T/C) polymorphism may be associated with a reduced OA risk among Chinese and the XbaI (A/G) polymorphism may not be associated with OA risk, while the observed increase in OA risk for XbaI polymorphism may be due to small-study bias.

**Keywords:** Meta-analysis, estrogen receptor-alpha, gene polymorphisms, osteoarthritis

## Introduction

Primary osteoarthritis (OA) is the most prevalent type of arthritis and the sixth main cause of years lived with disability at the global level, accounting for 3% of the total global years lived with disability [1]. It is a complex, multifactorial disorder, in which the interplay between environmental, hormonal, and genetic factors might be relevant [2, 3]. The incidence and prevalence of OA was significantly higher in postmenopausal women than men. This found has led to the hypothesis that sex hormones may play a critical role in the etiology of OA [4]. Although several studies demonstrated no statistically significant relationship between OA and estrogens [5, 6], other studies showed a protective role for estrogens against the development of OA in women [7, 8]. It has been reported that estrogen primarily exerts its beneficial effect on articular cartilage [9, 10], but the exact mechanisms are not fully understood.

Estrogen receptor-α (ER-α) is a critical mediator of signal transduction pathway in the estrogen endocrine system. The expression of ER-α protein is found in a variety of cell types, including human bone cells [11] and articular chondrocytes [12]. The human ER-α gene/ESR1 is localized at chromosome 6q25, and several variations in the ER-α DNA sequence have been reported. These polymorphisms may lead to changes in activity or structure of ER-α protein and would therefore also result in differences in the influence of estrogen on the development of OA. To date, however, no common DNA variations have been found in the coding region of the ER-α gene. The most widely polymorphisms studied involve PvuII (rs2234693) and XbaI (rs9340799) in the ER-α gene, which are localized at the first intron and separated by only 46 bp. The PvuII (T397C) polymorphism is caused by a T > C transition in intron 1, whereas the XbaI (G351A) polymorphism is caused by a G > A transition located 50 bp downstream of the PvuII polymorphic site [13]. The PvuII and XbaI

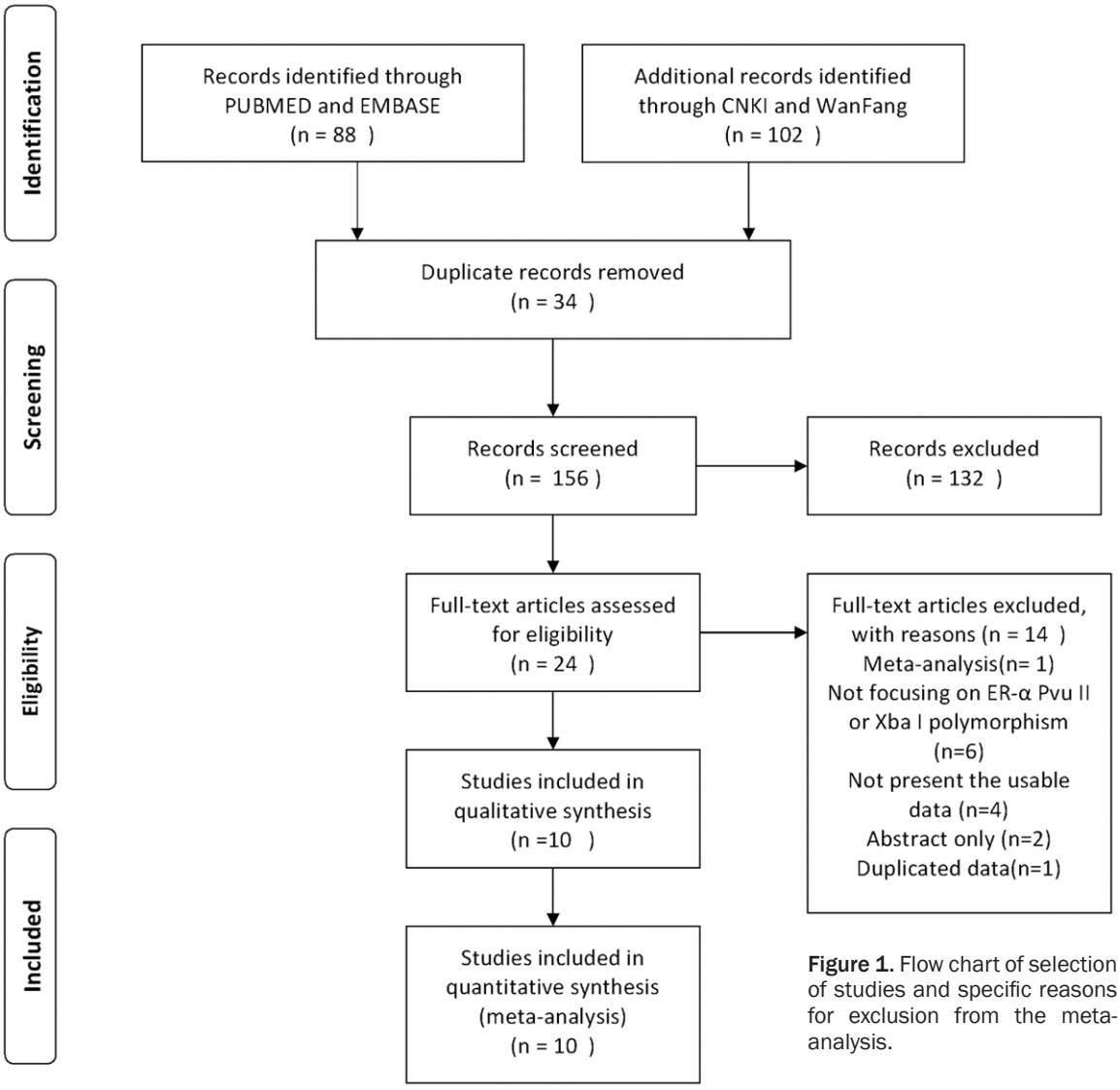


**Figure 1.** Flow chart of selection of studies and specific reasons for exclusion from the meta-analysis.

polymorphisms of the ER-α gene have been reported for possible relationship with several clinical outcomes, including osteoarthritis [14].

In recent years, several reports have examined the association between the PvuII and XbaI polymorphisms of ER-α gene and OA [14-23], however, the findings are controversial. Dai and co-workers [23] found associations between a genotype of PvuII and XbaI polymorphisms in intron 1 of the ER-α gene and knee OA in the Chinese Han population. Bergink and co-workers [14] reported that an ER-α haplotype of PvuII and XbaI polymorphisms was associated with radiographic OA of the knee. However, Wise and co-workers [20] found no association between ER-α gene polymorphisms and hand OA. Thus, in this study we conducted a meta-analysis to combine all studies available and validate whether the ER-α gene polymorphisms contributes to OA susceptibility.

## Materials and methods

### *Publication search*

We searched for relevant studies up to June 2014 through the Embase, PubMed, China National Knowledge Infrastructure Platform (CNKI; http://www.cnki.net), Wanfang (http://www.wanfangdata.com.cn) database with the following terms and their combinations: "osteoarthritis", "polymorphism/variant", and "estrogen receptor-α". We tried to identify potential relevant studies from the whole reference lists by orderly reviewing title, abstract and full text. All the studies must meet the following criteria: (1) case-control study; (2) the outcome had to

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

| First author | Year | Country | Ethnicity | Genotyping method | Sample size (case/control) | Case (genotype) | | | Control (genotype) | | | Quality score | $P_{HWE}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | TT | TC | CC | TT | TC | CC | | |
| Bergink | 2003 | Netherlands | Caucasian | PCR-RFLP | 1483/687 | 434 | 737 | 312 | 225 | 333 | 129 | 8 | 0.768 |
| Jin | 2004 | Korea | Asian | PCR-RFLP | 151/397 | 61 | 68 | 22 | 152 | 183 | 62 | 8 | 0.575 |
| Xue | 2005 | China | Asian | PCR-RFLP | 55/176 | 17 | 23 | 15 | 57 | 87 | 32 | 8 | 0.905 |
| Kang | 2007 | Korea | Asian | PCR-RFLP | 100/74 | 40 | 46 | 14 | 29 | 32 | 13 | 8 | 0.425 |
| Lian | 2007 | USA | Caucasian | Hybridization | 567/4133 | 188 | 277 | 102 | 1162 | 2067 | 884 | 11 | 0.533 |
| Tian | 2009 | China | Asian | PCR-RFLP | 38/40 | 16 | 15 | 7 | 15 | 16 | 9 | 10 | 0.251 |
| Wise | 2009 | USA | Caucasian | PCR-RFLP | 304/211 | 101 | 145 | 58 | 65 | 100 | 46 | 9 | 0.519 |
| Yang | 2009 | China | Asian | PCR-RFLP | 41/40 | 14 | 17 | 10 | 12 | 23 | 5 | 8 | 0.239 |
| Borgonio-Cuadra | 2012 | Mexico | Caucasian | PCR-RFLP | 115/117 | 52 | 49 | 14 | 51 | 50 | 16 | 10 | 0.507 |
| Dai | 2014 | China | Asian | Taqman | 469/514 | 167 | 217 | 85 | 198 | 242 | 74 | 11 | 0.997 |

HWE: Hardy-Weinberg Equilibrium.

**Table 2.** Characteristics of the XbaI studies included in this meta-analysis

| First author | Year | Country | Ethnicity | Genotyping method | Sample size (case/control) | Case (genotype) | | | Control (genotype) | | | Quality score | $P_{HWE}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | AA | AG | GG | AA | AG | GG | | |
| Bergink | 2003 | Netherlands | Caucasian | PCR-RFLP | 1483/687 | 643 | 682 | 158 | 372 | 263 | 52 | 8 | 0.561 |
| Jin | 2004 | Korea | Asian | PCR-RFLP | 151/397 | 98 | 49 | 4 | 256 | 126 | 15 | 8 | 0.918 |
| Xue | 2005 | China | Asian | PCR-RFLP | 55/176 | 21 | 24 | 10 | 40 | 82 | 54 | 8 | 0.409 |
| Kang | 2007 | Korea | Asian | PCR-RFLP | 100/74 | 65 | 31 | 4 | 46 | 28 | 0 | 8 | 0.045 |
| Lian | 2007 | USA | Caucasian | Hybridization | 569/4123 | 257 | 250 | 62 | 1700 | 1932 | 491 | 11 | 0.104 |
| Tian | 2009 | China | Asian | PCR-RFLP | 38/40 | 18 | 16 | 4 | 6 | 21 | 13 | 10 | 0.599 |
| Wise | 2009 | USA | Caucasian | PCR-RFLP | 307/214 | 148 | 116 | 43 | 85 | 99 | 30 | 9 | 0.891 |
| Yang | 2009 | China | Asian | PCR-RFLP | 41/40 | 28 | 11 | 2 | 24 | 13 | 3 | 8 | 0.516 |
| Borgonio-Cuadra | 2012 | Mexico | Caucasian | PCR-RFLP | 115/117 | 70 | 41 | 4 | 62 | 47 | 8 | 10 | 0.821 |
| Dai | 2014 | China | Asian | Taqman | 469/522 | 288 | 152 | 29 | 348 | 155 | 19 | 11 | 0.736 |

HWE: Hardy-Weinberg Equilibrium.

**Table 3.** Quantitative analyses of the ER-α PvuII T/C polymorphism on osteoarthritis risk

| Variables | N[a] | TC versus CC | | TT versus CC | | TT/TC versus CC (dominant) | | TT versus TC/CC (recessive) | |
|---|---|---|---|---|---|---|---|---|---|
| | | OR (95% CI) | P[b] | OR (95% CI) | P[b] | OR (95% CI) | P[b] | OR (95% CI) | P[b] |
| Total | 10 | 0.98 (0.86-1.12) | 0.410 | 1.00 (0.81-1.25) | 0.077 | 1.00 (0.88-1.13) | 0.164 | 1.03 (0.91-1.15) | 0.338 |
| Ethnicities | | | | | | | | | |
| Caucasian | 4 | 1.05 (0.90-1.23) | 0.555 | 1.11 (0.79-1.56) | 0.024 | 1.06 (0.92-1.23) | 0.156 | 1.06 (0.84-1.33) | 0.041 |
| Asian | 6 | 0.83 (0.64-1.07) | 0.433 | 0.85 (0.65-1.11) | 0.585 | 0.84 (0.66-1.06) | 0.416 | 0.97 (0.81-1.17) | 0.926 |
| Country | | | | | | | | | |
| China | 4 | 0.73 (0.54-0.99) | 0.498 | 0.74 (0.54-1.02) | 0.741 | 0.73 (0.55-0.98) | 0.555 | 0.92 (0.74-1.16) | 0.853 |
| Other | 6 | 1.06 (0.91-1.23) | 0.797 | 1.12 (0.87-1.44) | 0.087 | 1.07 (0.93-1.23) | 0.362 | 1.05 (0.94-1.18) | 0.139 |
| Sample size | | | | | | | | | |
| > 500 | 5 | 1.00 (0.87-1.15) | 0.382 | 1.02 (0.76-1.36) | 0.011 | 1.00 (0.82-1.23) | 0.081 | 1.02 (0.85-1.23) | 0.044 |
| ≤ 500 | 5 | 0.85 (0.56-1.28) | 0.332 | 0.96 (0.63-1.47) | 0.651 | 0.89 (0.59-1.33) | 0.367 | 1.06 (0.78-1.43) | 0.989 |

The numbers in bold indicated statistically significant values. [a]Number of comparisons. [b]P value of Q-test for heterogeneity test.

**Table 4.** Quantitative analyses of the ER-α XbaI A/G polymorphism on osteoarthritis risk

| Variables | N[a] | AG versus GG | | AA versus GG | | AA/AG versus GG (dominant) | | AA versus AG/GG (recessive) | |
|---|---|---|---|---|---|---|---|---|---|
| | | OR (95% CI) | P[b] | OR (95% CI) | P[b] | OR (95% CI) | P[b] | OR (95% CI) | P[b] |
| Total | 10 | 0.96 (0.80-1.15) | 0.404 | 1.25 (0.79-1.97) | <0.001 | 1.08 (0.77-1.51) | 0.014 | 1.18 (0.90-1.54) | <0.001 |
| Ethnicities | | | | | | | | | |
| Caucasian | 4 | 0.94 (0.77-1.15) | 0.615 | 1.03 (0.62-1.70) | 0.003 | 0.96 (0.69-1.32) | 0.094 | 1.07 (0.71-1.62) | <0.001 |
| Asian | 6 | 1.01 (0.68-1.50) | 0.191 | 1.56 (0.58-4.21) | 0.001 | 1.30 (0.61-2.78) | 0.018 | 1.34 (0.88-2.04) | 0.004 |
| Country | | | | | | | | | |
| China | 4 | 1.04 (0.67-1.61) | 0.165 | 2.09 (0.56-7.82) | <0.001 | 1.52 (0.59-3.94) | 0.009 | 1.71 (0.78-3.75) | 0.001 |
| Other | 6 | 0.94 (0.77-1.15) | 0.526 | 1.02 (0.64-1.60) | 0.007 | 0.96 (0.71-1.31) | 0.129 | 1.07 (0.78-1.45) | <0.001 |
| HWE in controls | | | | | | | | | |
| Yes | 9 | 0.97 (0.81-1.16) | 0.482 | 1.30 (0.82-2.06) | <0.001 | 1.10 (0.79-1.53) | 0.015 | 1.19 (0.89-1.59) | <0.001 |
| No | 1 | 0.12 (0.01-2.38) | — | 0.16 (0.01-2.99) | — | 0.14 (0.01-2.72) | — | 1.13 (0.61-2.11) | — |
| Sample size | | | | | | | | | |
| > 500 | 5 | 0.91 (0.75-1.10) | 0.599 | 0.87 (0.57-1.33) | 0.004 | 0.87 (0.65-1.16) | 0.101 | 0.96 (0.71-1.29) | <0.001 |
| ≤ 500 | 5 | 1.45 (0.84-2.49) | 0.484 | 2.60 (1.10-6.18) | 0.135 | 2.04 (1.12-3.71) | 0.341 | 1.69 (1.12-2.55) | 0.154 |

The numbers in bold indicated statistically significant values. [a]Number of comparisons. [b]P value of Q-test for heterogeneity test. HWE: Hardy-Weinberg Equilibrium.

be osteoarthritis; and (3) at least two comparison groups (osteoarthritis group vs. control group). The major exclusion criteria were: (1) duplicate data, (2) abstract, comment, review and editorial and (3) no sufficient data were reported.

*Data extraction*

Data were extracted from each study by two investigators (W.H. and F.S.) independently according to the inclusion criteria listed above. Characteristics abstracted from the studies included the first author's name, year of publication, country of origin, ethnicity, definition of study patients (cases), genotyping method, total number of cases and controls, and genotype distributions in cases and controls. Quality of studies was assessed according to the predefined criteria based on previous observational studies [24, 25] (Table S1).

*Statistical analysis*

Hardy-Weinberg equilibrium (HWE) was assessed by the chi-square test in controls and a $P < 0.05$ was considered as significant disequilibrium. The strength of the association between the ER-α polymorphism and osteoarthritis risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The significance of the pooled OR was determined by the Z-test, and $P < 0.05$ was considered as statistically significant. The meta-analysis examined the association between PvuII and XbaI polymorphisms and osteoarthritis risk in co-dominant model, dominant model and recessive model. Subgroup analyses were done by ethnicity, country, HWE and sample size.

Heterogeneity among studies was assessed by using the chi-square-based Q test and $I^2$ statistics [26]. When $P > 0.10$, the pooled OR of each

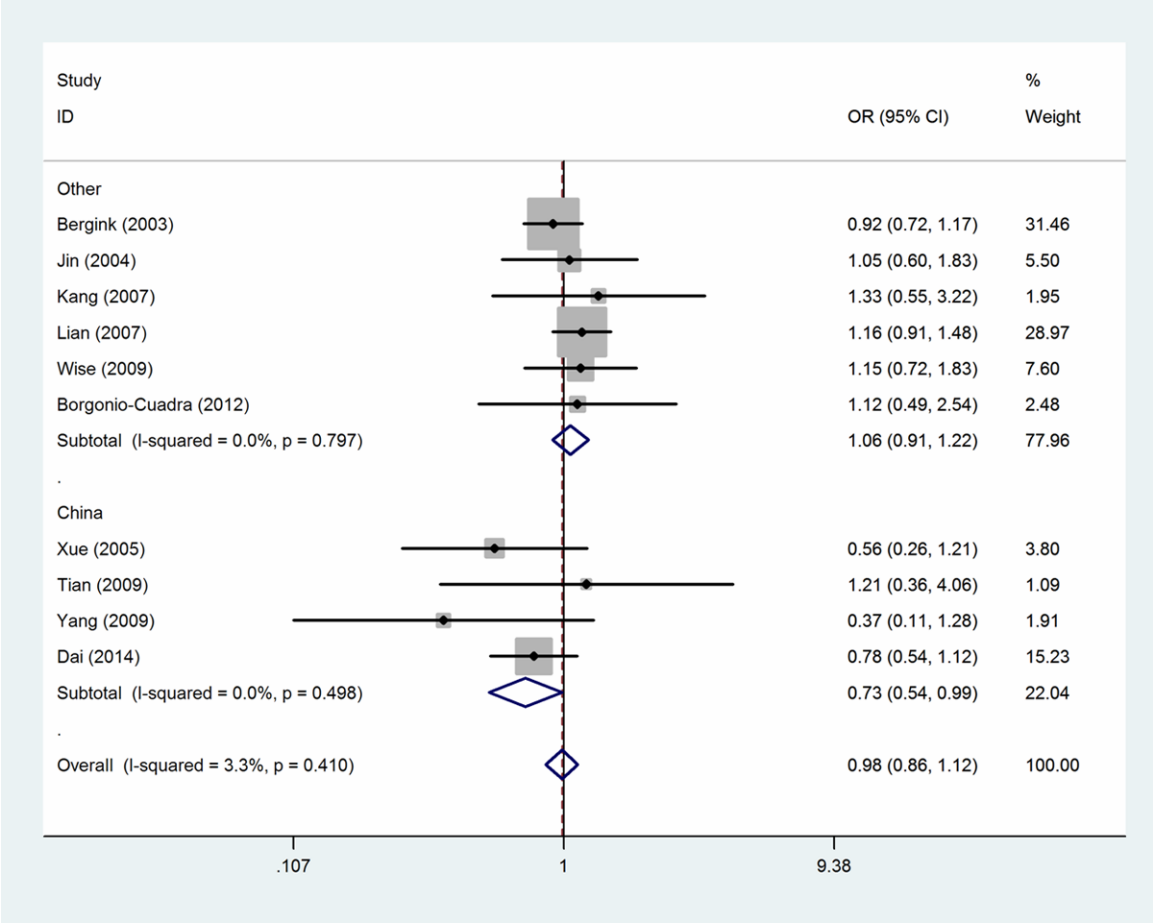


**Figure 2.** Meta-analysis for the association between OA risk and the ER-α PvuII polymorphism (TC vs CC) is illustrated in subgroup analysis by country. OR: odds ratio; CI: confidence interval; $I^2$, measure to quantify the degree of heterogeneity in meta-analyses.

study was calculated by using the fixed-effects model [27]; otherwise, the random-effects model [28] was used. Relative influence of each study on the pooled estimate was assessed by omitting one study at a time for sensitivity analysis.

The Begg's rank correction method and the Egger's weighted regression method were used to statistically assess publication bias [29] ($P < 0.05$ was regarded as representative of statistical significance). All analyses were performed using STATA 11.0 (STATA Corp., College Station, TX, USA), using two-sided *P*-values.

## Results

### *Characteristics of the studies*

A total of 10 eligible studies were identified based on our criteria [14-23]. The detailed flow chart of the inclusion/exclusion process is presented in **Figure 1**. PCR- RFLP, Probe hybridization or TaqMan was used for examining the mutations of ESR-α PvuII and XbaI polymorphisms. The genotype distributions among the controls of all studies were consistent with HWE except for one study [17] (**Table 2**). The characteristics of the included studies for ESR-α PvuII and XbaI polymorphisms are listed in **Tables 1**, **2**. Among those 10 studies, there were 4 Caucasian and 6 Asian studies, respectively.

### *Quantitative synthesis*

Two common SNPs occurred in estrogen receptor-α gene sequences were included in quantitative synthesis, and detail results were shown in **Tables 3** and **4**. For the PvuII (T/C) polymorphism, no obvious associations were was observed in the overall comparison (TT vs.

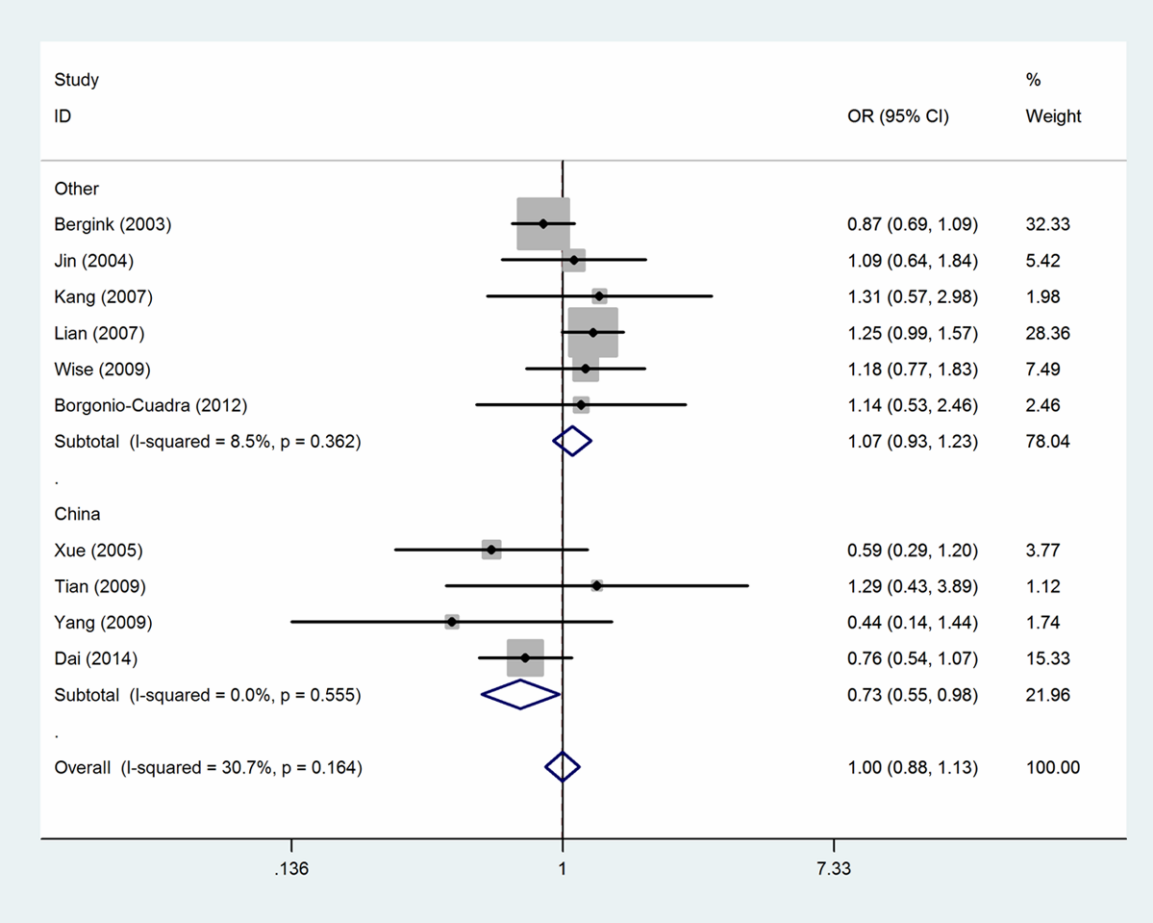


**Figure 3.** Meta-analysis for the association between OA risk and the ER-α PvuII polymorphism (TT + TC vs CC) is illustrated in subgroup analysis by country. OR: odds ratio; CI: confidence interval; $I^2$, measure to quantify the degree of heterogeneity in meta-analyses.

CC: OR = 1.00, 95% CI 0.81-1.25, $I^2$ = 42%, $P_{heterogeneity}$ = 0.077; TC vs. CC:OR = 0.98, 95% CI 0.86-1.12, $I^2$ = 3.3%, $P_{heterogeneity}$ = 0.410; dominant model: OR = 1.00, 95% CI 0.88-1.13, $I^2$ = 30.7%, $P_{heterogeneity}$ = 0.164; and recessive model: OR = 1.03, 95% CI 0.91-1.15, $I^2$ = 11.4%, $P_{heterogeneity}$ = 0.338) (**Table 3**). When stratifying by ethnicity and study sample size, still no obvious associations were found. However, in the subgroup analysis by country, a significantly reduced risk was observed among Chinese (TC vs. CC: OR = 0.73, 95% CI 0.54 -0.99, $I^2$ = 0%, $P_{heterogeneity}$ = 0.498; dominant model, OR = 0.73, 95% CI = 0.55-0.98, $I^2$ = 0%, $P_{heterogeneity}$ = 0.555) (**Figures 2** and **3**). For the XbaI (A/G) polymorphism, also no obvious associations were found when all studies were pooled into the meta-analysis (AG vs. GG: OR = 0.96, 95% CI 0.80-1.15, $I^2$ = 3.9%, $P_{heterogeneity}$ = 0.404; AA vs. GG:OR = 1.25, 95% CI 0.79-1.97, $I^2$ = 74.7%, $P_{heterogeneity}$ < 0.001; dominant model: OR = 1.08, 95% CI 0.77-1.51, $I^2$ = 56.5%, $P_{heterogeneity}$ = 0.014; and recessive model: OR = 1.18, 95% CI 0.90-1.54, $I^2$ = 81%, $P_{heterogeneity}$ < 0.001) (**Table 4**). In the subgroup analysis by ethnicity, country, HWE in controls and study sample size, still no obvious associations were found. However, when stratifying by sample size, a significantly elevated risk was found in sample size ≤ 500 (AA vs. GG: OR = 2.60, 95% CI 1.10-6.18, $I^2$ = 42.9%, $P_{heterogeneity}$ =0.135; dominant model: OR = 2.04, 95% CI 1.12-3.71, $I^2$ = 11.4%, $P_{heterogeneity}$ = 0.341; and recessive model: OR = 1.69, 95% CI 1.12-2.55, $I^2$ = 40.2%, $P_{heterogeneity}$ = 0.154) (**Table 4**).

*Sensitivity analysis*

Sensitivity analysis was performed by examining the influence of each study on the overall OR, and the result indicated that no individual study influenced the overall OR dominantly,

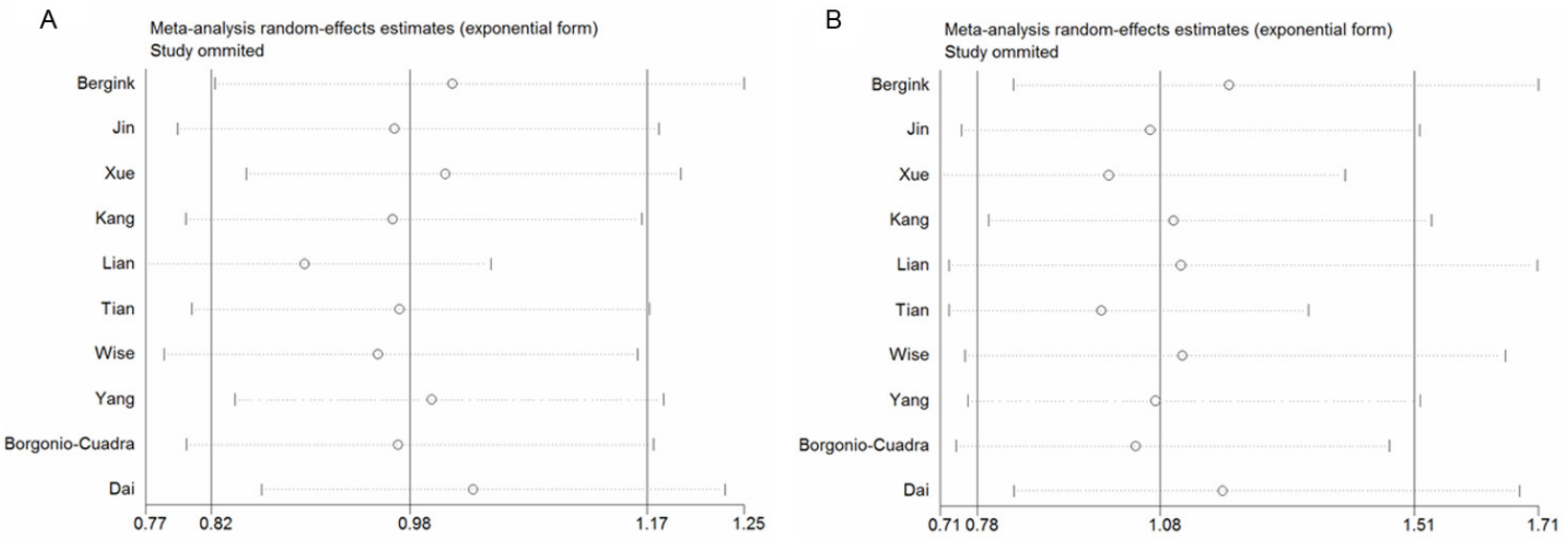


**Figure 4.** Sensitivity analysis of ER-α PvuII polymorphism for a dominant model (A: CC + TC vs. TT) and XbaI polymorphism for a dominant model (B: GG + AG vs. AA).

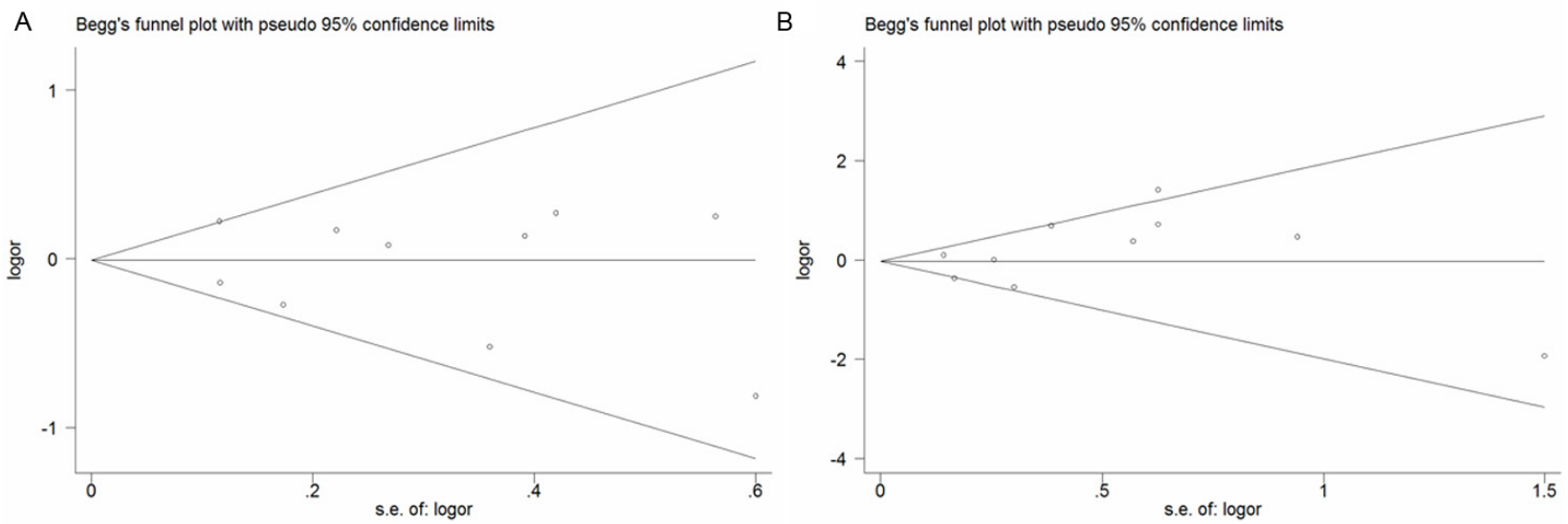


**Figure 5.** Funnel plots of ER-α PvuII polymorphism for a dominant model (A: CC + TC vs. TT) and XbaI polymorphism for a dominant model (B: GG + AG vs. AA).

since the omission of any single study made no substantial difference (**Figure 4**). This procedure proved our overall result was reliable and stable.

*Publication bias*

Begg's funnel plot and Egger's test were performed to assess publication bias among the literatures. The funnel plots have been shown that the ESR-α pvuII and XbaI are no evidence of publication bias. The results of Egger's and Begg's test also indicated that the pvuII (Egger's test P = 0.656, Begg's test P = 1.000) (**Figure 5A**) and the XbaI (Egger's test P = 0.386, Begg's test P = 0.592) (**Figure 5B**) are no evidence of publication bias.

## Discussion

Estrogens are the main female sex hormones secreted by ovaries and natural estrogens easily diffuse through the cell membrane, like all steroid hormones. Once the binding and activation of the estrogen receptor, the ER complex binds to a particular DNA sequences known as hormone response element to trigger the transcription of some ER-regulated genes [30]. Estrogen receptors are categorized as Type I receptors largely located in the cytosol. The ER's 12-helix domains play an important role in determination of interactions with co-activating and co-repressing factors and, therefore, influence the respective agonist or antagonist ligand [31, 32]. The ER-α expression is in stromal cells, chondrocytes, and osteoblasts [33], which may indicate that the bone and cartilage is regulated by ER-α gene. There have been some studies on the relationship between two common ER-α gene polymorphisms (PvuII and XbaI) and OA risk, and the results were controversial [34]. Therefore, in this study, we performed a meta-analysis to examine the genetic association between ESR1 gene polymorphisms and OA susceptibility.

In the present meta-analysis, we identified 10 eligible studies, including 3328 osteoarthritis cases and 6390 controls, and analyzed the relationship between ER-α PvuII and XbaI polymorphisms and susceptibility to osteoarthritis. To the best of our knowledge, this is the first systematic review of the literature by a meta-analysis so far exploring the association between two ER-α Polymorphisms and OA risk.

We found that ER-α PvuII and XbaI polymorphisms were not associated with OA risk in overall population. For PvuII (T/C) polymorphism, obvious associations were found among Chineses and for XbaI (A/G) polymorphisms, the significant association was mainly observed among studies with small sample size.

ER-α PvuII and XbaI polymorphisms are located in intron 1, however, it is unclear how the intron polymorphism may affect the metabolism of articular cartilage. Recent evidence indicates that, in the human genome, the intron and exon portion, are subject to selection pressure of the same degree, and intron portion has similar level of functional importance to exon [35]. Therefore, intron polymorphism could have functional consequences in OA. ER-α is a key mediator in the signal transduction pathway [36]; it is possible that particular ER genotypes may influence expression in chondrocytes through transcriptional regulation leading to increased cartilage formation preventing the development of OA.

In interpreting the current results, there were still some limitations which need to be addressed. Firstly, OA is a complex disease related to genetic and environmental risk factors. However, insufficient environmental information limited us to further investigate the gene-environment interaction. Secondly, only published studies were considered in this meta-analysis, some so called "grey literatures" might be still missed, which may have biased our results. Thirdly, the number of studies and samples in this meta-analysis are relatively small.

In conclusion, this meta-analysis suggests that ER-α PvuII (T/C) polymorphism may be associated with a reduced OA risk among Chinese and the XbaI (A/G) polymorphism may not be associated with OA risk, while the observed increase in OA risk for XbaI polymorphism may be due to small-study bias. More well designed studies with adequately sized populations are necessary to validate our findings.

## Disclosure of conflict of interest

None.

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**Table S1.** Scale for quality assessment of molecular association studies of osteoarthritis.

| Criteria | Score |
|---|---|
| Representativeness of cases | |
| Consecutive/randomly selected from case population with clearly defined sampling frame | 2 |
| Consecutive/randomly selected from case population without clearly defined sampling frame or with extensive inclusion/exclusion criteria | 1 |
| No method of selection described | 0 |
| Representativeness of controls | |
| Controls were consecutive/randomly drawn from the same sampling frame as cases | 2 |
| Controls were consecutive/randomly drawn from a different sampling frame as cases | 1 |
| Not described | 0 |
| Ascertainment of osteoarthritis | |
| Clearly described objective criteria for diagnosis of osteoarthritis | 2 |
| Diagnosis of osteoarthritis by patient self-report or by patient history | 1 |
| Not described | 0 |
| Quality control of genotyping methods | |
| Clearly described a different genotyping assay to confirm the data | 1 |
| Not described | 0 |
| Hardy-Weinberg equilibrium | |
| Hardy-Weinberg equilibrium in controls | 2 |
| Hardy-Weinberg disequilibrium in controls | 1 |
| No checking for Hardy-Weinberg disequilibrium | 0 |