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Estrogen receptor alpha gene *Pvu*II polymorphism and risk of fracture in postmenopausal women: a meta-analysis

W. Deng, J.C. Han, L. Chen and W.L. Qi

Department of Orthopaedics,
The Affiliated Hospital to Changchun University of Chinese Medicine,
Changchun, Jilin, China

Corresponding author: W.L. Qi
E-mail: qiwangli1234@126.com

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ABSTRACT. Numerous studies have evaluated the association between estrogen receptor alpha (*ESR1*) gene *Pvu*II polymorphism and fracture risk in postmenopausal women. However, the results have been inconsistent. We performed a meta-analysis to examine the association between the *ESR1* gene *Pvu*II polymorphism and fracture risk in postmenopausal women. Studies published from PubMed, Google Scholar, and China National Knowledge Infrastructure data were retrieved. Pooled odds ratios with 95% confidence intervals were calculated using fixed- or random-effects models. A total of 6 case-control studies containing 592 patients and 705 controls were included in this meta-analysis. We found no association between the *Pvu*II polymorphism in the *ESR1* gene and fracture in postmenopausal women. Taking into account the effect of ethnicity, further stratified analyses were performed. In the subgroup analysis, no significant association was found in Caucasians and in Asians. No publication bias was found in the present study (all $P > 0.05$). In conclusion, the *ESR1* gene *Pvu*II polymorphism may not

be associated with fracture risk in postmenopausal women. Additional larger studies are needed to confirm this conclusion.

Key words: Estrogen receptor alpha; Fracture; Gene polymorphism; Meta-analysis

INTRODUCTION

Osteoporosis is a common skeletal disorder characterized by compromised bone strength and increased bone fragility, with a consequent increase in fracture susceptibility; osteoporosis affects up to 40% of postmenopausal women and 15% of men (NIH Consensus Development Panel, 2001). With increases in aging populations, the number of osteoporosis cases is expected to increase to 2.6 million by 2025 and to 4.5 million by 2050 (Abrahamsen et al., 2009). This condition is associated with impaired functions, decreased quality of life, increased nursing home admission, and increased mortality, imposing a major economic burden on society (Pongchaiyakul et al., 2005). Epidemiological studies have shown that fracture is influenced by various environmental factors, such as older age (>75 years), low heel quantitative ultrasound stiffness index (<78%), history of fracture, recent fall, and a failed chair test. In addition, many reports have identified genes underlying the development and progression of fracture**,** and the pathogenesis of fracture may be influenced by multiple genetic factors (Michaelsson et al., 2005).

Estrogen plays an important role in regulating bone homeostasis and in the prevention of post-menopausal bone loss (Kjaergaard et al., 2007). Estrogen deficiency has important effects on the pathogenesis of postmenopausal osteoporosis, when serum estrogen levels fall into postmenopausal range <30 pg/mL (Ettinger et al., 1998). The action of estrogen is mediated through estrogen receptor alpha and beta present on bone cells, implying that these receptors have a functional role in bone metabolism (Tomkinson et al., 1997). The human estrogen receptor alpha (*ESR1*) gene is located on chromosome 6q25. It is comprised of 8 exons separated by 7 intronic regions and spans more than 140 kb. The *ESR1* gene encodes ligand-activated transcription factor, which belongs to the nuclear receptor superfamily (Bagger et al., 2000; Gennari et al., 2005).

The *Pvu*II polymorphism of the *ESR1* gene is located in an intron, but whether this polymorphism has functional consequences is unknown. A previous meta-analysis suggested no significant association between the *ESR1* gene *Pvu*II polymorphism and fracture (Ioannidis et al., 2002). However, this specific association in postmenopausal women remains controversial. Thus, to resolve this issue, we performed a meta-analysis of all available studies to determine the association between the *ESR1* gene *Pvu*II polymorphism and the susceptibility to fracture in post-menopausal women.

MATERIAL AND METHODS

Selection of studies

Two investigators (Deng and Han) independently screened each of the titles, abstracts, and full texts. The results were compared and disagreements were resolved by consensus. We searched the PubMed, Google Scholar, and China National Knowledge Infrastructure data-

bases to retrieve papers linking the *Pvu*II polymorphism of the *ESR1* gene and fracture risk available through March 2014 without language restrictions, using the following key words: "*Pvu*II", "fracture", "polymorphism", "single nucleotide polymorphism", and "genetic polymorphism". The reference lists of major textbooks, reviews, and articles included were identified through manual searches to identify other potentially eligible studies. Studies reported by the same authors, although published in different journals, were checked for possible overlapping participant groups. When pertinent data were not included, or data that presented were unclear, the authors were contacted directly.

Inclusion and exclusion criteria

The following inclusion criteria were required to be met: 1) case-control studies that addressed fracture cases and healthy controls; 2) studies that evaluated the association between the *ESR1* gene *Pvu*II polymorphism and fracture risk; 3) all patients with clinically diagnosed fracture; 4) studies that included sufficient genotype data for extraction; 5) the studies contained at least 2 comparison groups (cancer group *vs* control group); 6) the studies included detailed genotyping data. In addition, the following exclusion criteria were used: 1) not case-control studies that evaluated the association between the *ESR1* gene *Pvu*II polymorphism and fracture risk; 2) case reports, letters, reviews, meta-analysis, and editorial articles; 3) studies that were based on incomplete raw data or no usable data reported; 4) duplicated publications.

Data extraction

Using a standardized form, data from published studies were extracted independently by 2 reviewers to populate the necessary information (Chen and Qi). Disagreements were resolved through discussion. From each of the articles included, the following information was extracted: first author, year of publication, country, nationality, number of patients and controls, polymorphisms of gene, and evidence of Hardy-Weinberg equilibrium (HWE). In the case of conflicting evaluations, an agreement was reached through discussion.

Statistical analysis

We assessed HWE in the controls for each study using χ^2 test; $P < 0.05$ was considered to indicate significant disequilibrium. The strength of the association between the *ESR1* gene *Pvu*II polymorphism and fracture risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs) under 4 genetic models, including a homozygote comparison (PP *vs* pp), a heterozygote comparison (PP *vs* Pp), a dominant model (Pp + pp *vs* PP), and a recessive model (PP + Pp *vs* pp) between groups. In addition, we conducted stratification analysis in Asians and Europeans, which was used to determine the diversity among the results of different studies (Ding et al., 2011). The I^2 test was performed to evaluate whether variation was due to heterogeneity or by chance, and I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively (Wang and Pan, 2012). If heterogeneity was observed among the studies, the pooled OR was estimated using the fixed-effects model ($P > 0.10$ or $I^2 < 50\%$). Otherwise, the random-effects model was used to estimate the pooled OR. Sensitivity analysis was performed through the random effect model values compared to the fixed effect to ensure the reliability of the results. Begg's test was used to measure publication bias ($P < 0.05$ was

considered to indicate significant publication bias). All analyses were conducted using the STATA version 12.0 software (Stata Corp, College Station, TX, USA).

RESULTS

Study characteristics

Forty-six papers were retrieved through the literature search, of which 6 full-text papers were included in this meta-analysis and 40 studies were excluded (Aerssens et al., 2000; Langdahl et al., 2000; Dong et al., 2002; Mitra et al., 2006; Ge et al., 2009; Erdogan et al., 2011). The flow chart of study selection is summarized in Figure 1. These 6 case-control studies included a total of 592 cases and 705 healthy controls. All studies included were case-control studies that evaluated the association between *Pvu*II polymorphism in the *ESR1* gene and susceptibility to fracture. The published year of the studies included ranged from 2000 to 2014. The source of controls was based on healthy populations. The HWE test was performed to determine the genotype distribution of the controls, and all were in agreement with HWE. Study characteristics are presented in Table 1.

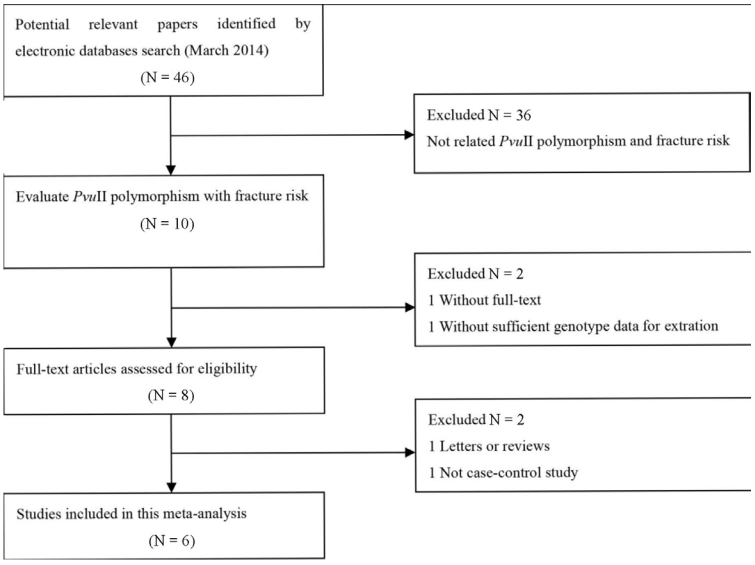

Figure 1. Flow chart of study study selection based on the inclusion and exclusion criteria.

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

Study included	Year	Area	Race	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test
					PP	Pp	pp	PP	Pp	pp	
Aerssens et al.	2000	Belgium	Europe	135/239	27	66	2	47	125	67	0.41
Langdahl et al.	2000	Denmark	Europe	80/80	11	37	32	21	34	25	0.19
Dong et al.	2002	China	Asian	140/60	25	77	38	7	26	27	0.85
Mitra et al.	2006	India	Asian	119/97	22	46	51	22	44	31	0.40
Ge et al.	2009	China	Asian	72/199	13	36	23	35	89	75	0.34
Erdogan et al.	2011	Turkey	Europe	46/30	13	20	13	10	18	2	0.11

Quantitative synthesis

The combined results of *Pvu*II polymorphism and fracture risk are summarized in Figure 2 and Table 2. The distribution of genotypes in the controls was in HWE (Table 1). Meta-analysis results revealed no significant association between the *ESR1* gene *Pvu*II polymorphism and susceptibility to fracture (PP *vs* pp: OR = 1.17, 95%CI = 0.44-3.15; PP *vs* Pp: OR = 0.94, 95%CI = 0.69-1.28; dominant model: OR = 0.99, 95%CI = 0.74-1.32; recessive model: OR = 1.22, 95%CI = 0.56-2.68). When stratified according to ethnicity, we detected no significant association in Asians (PP *vs* pp: OR = 1.17, 95%CI = 0.54-2.57; PP *vs* Pp: OR = 0.99, 95%CI = 0.63-1.56; dominant model: OR = 0.97, 95%CI = 0.64-1.48; recessive model: OR = 1.20, 95%CI = 0.59-2.43) and in Caucasians (PP *vs* pp: OR = 1.16, 95%CI = 0.08-17.06; PP *vs* Pp: OR = 0.90, 95%CI = 0.59-1.37; dominant model: OR = 1.15, 95%CI = 0.50-2.64; recessive model: OR = 1.30, 95%CI = 0.11-14.94). Sensitivity analyses were conducted by altering the statistical models. No material alteration was detected, indicating that our results were statistically robust.

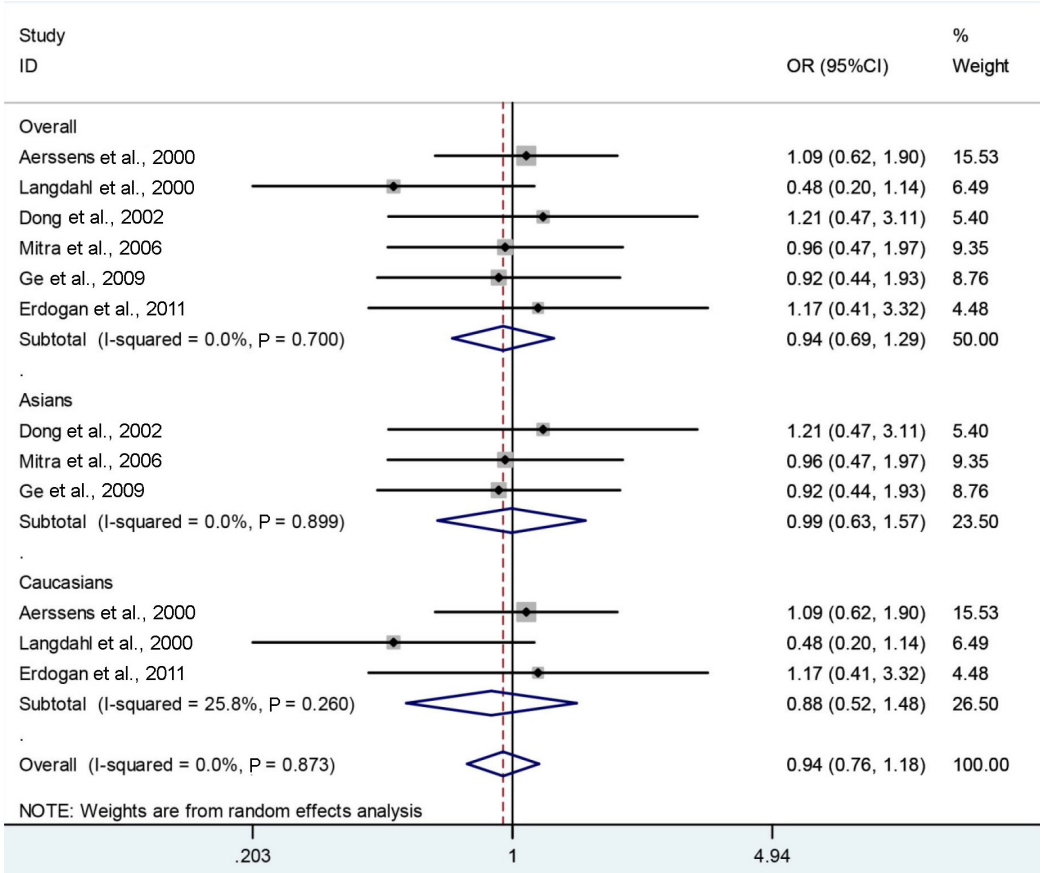

Figure 2. Forest plots of *Pvu*II polymorphism in fracture *vs* normal control and subgroup analyses.

Table 2. Summary ORs and 95%CI of *Pvu*II polymorphism with fracture risk.

Subgroup	Genetic model	Sample size		Type of model	Test of heterogeneity		Test of association		Test of publication bias	
		Case	Control		I^2	P	OR	95% CI	z	P
Overall	PP *vs* pp	592	705	Random	82.7%	0.00	1.17	0.44-3.15	0.00	1.00
	PP *vs* Pp			Fixed	0.0%	0.70	0.94	0.69-1.28	0.00	1.00
	Dominant model			Fixed	42.9%	0.12	0.99	0.74-1.32	0.00	1.00
	Recessive model			Random	84.4%	0.00	1.22	0.56-2.68	0.00	1.00
Asians	PP *vs* pp	331	356	Random	62.7%	0.07	1.17	0.54-2.57	0.00	1.00
	PP *vs* Pp			Fixed	0.0%	0.90	0.99	0.63-1.56	0.00	1.00
	Dominant model			Fixed	0.0%	0.41	0.97	0.64-1.48	0.00	1.00
	Recessive model			Random	77.1%	0.01	1.20	0.59-2.43	0.00	1.00
Caucasians	PP *vs* pp	261	349	Random	91.8%	0.00	1.16	0.08-17.06	0.00	1.00
	PP *vs* Pp			Fixed	25.8%	0.26	0.90	0.59-1.37	0.00	1.00
	Dominant model			Random	71.5%	0.03	1.15	0.50-2.64	0.00	1.00
	Recessive model			Random	92.0%	0.00	1.30	0.11-14.94	0.00	1.00

Publication bias

Publication bias of the literature was assessed by Begg's funnel plot (Table 2). A funnel plot was used to measure the asymmetry. The results of the Begg's funnel plot test are shown in Table 2. The results revealed no publication bias (all $P > 0.05$).

DISCUSSION

Estrogen functions as a potent regulator of sexual dimorphism of the skeleton, peak bone mass, and bone remodeling balance in adults. A gene that is a candidate as an estrogen receptor is the *ESR1* gene. Because estrogens have important effects on bone mass and bone remodeling, numerous studies have evaluated the role of *ESR1* gene *Pvu*II polymorphism in the genetic regulation of fracture. Some studies have reported that the *Pvu*II polymorphism is associated with the risk of osteoporotic fracture, while other studies failed to confirm this association. For the above conclusion based on a small number of samples, Ioannidis et al. (2002) performed a meta-analysis and found that the *Pvu*II polymorphism was not associated with fracture risk. However, estrogen deficiency plays a major role in the pathogenesis of post-menopausal osteoporosis. The PP genotype of the *Pvu*II polymorphism was a relatively hormone-insensitive genotype, and post-menopausal women with the p allele may have an increased risk of fracture resulting from estrogen deficiency (Salmén et al., 2000). To resolve these issues, we conducted this meta-analysis of published studies.

This is the first systematic study to examine the association between *Pvu*II polymorphism and fracture risk in post-menopausal women through a meta-analysis. We examined the association between *Pvu*II polymorphism and the risk of fracture by examining published studies. A total of 6 case-control studies were included and comprised a total of 592 patients and 705 healthy controls. We found no significant association between *Pvu*II polymorphism and fracture risk. Because the result may have been affected by ethnicity, we performed a race-related subgroup analysis, and no significant association was found in both the Caucasian and Asian populations. Further sensitivity analysis confirmed a significant association between *Pvu*II polymorphism and fracture risk. No evidence showed publication bias in this meta-analysis for fracture. As the eligible study number was limited in the meta-analysis, these results require further investigation.

Fracture occurs because of the joint effect of multiple genes and gene-environment interactions. Potential function of *Pvu*II polymorphism may be affected via gene-gene interactions. The *Pvu*II and *Xba*I polymorphisms in intron I are in linkage disequilibrium, which is strongly associated with a low number of TA repeats. The length of TA repeats may influence gene transcription and decrease bone mineral density values by affecting promoter usage (Becherini et al., 2000). One study could not be included in our meta-analysis, and further studies of gene-gene interactions should be taken into consideration to assess fracture risk.

There were some limitations to our meta-analysis. First, because of incomplete raw data or publication limitations, some relevant studies could not be included in this study. Second, our systematic review was based on unadjusted data, as the genotype information stratified for the main confounding variables was not available in the original papers and the confounding factors addressed in different studies were variable. Finally, the genotype information stratified for the main confounding variables was not available in the original papers, such as age, gender, and exposure. These confounding factors may have caused serious confounding bias.

In conclusion, our meta-analysis revealed no association between the *ESR1* gene *Pvu*II polymorphism and fracture risk. As few studies are available in this field and current evidence remains limited. Further large-scale studies with adequate methodological quality are needed to confirm our conclusions.

Conflicts of interest

The authors declare no conflict of interest.

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