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Original Article

Estrogen receptor α (*ESR1*) IVS1-397T>C polymorphism lowers risk of fracture

Jian Wang, Gang Feng, Hang Li, Weixu Li, Zhijun Pan, Jianwei Wang

The Department of Orthopedics, The Second Hospital Affiliated to Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

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Abstract: Background: Genetic factors are reported to affect fracture incidence. Many groups have explored the correlation of fracture risk with *ESR1* IVS1-397T>C. The observed associations, however, are largely inconsistent. This meta-analysis of data from early-released studies was performed in an effort to determine the role of IVS1-397T>C in fracture. Methods: Relevant studies were searched through Pubmed, Embase, ScienceDirect, and Wiley Online Library databases. 16 studies meeting all selection criteria were finally identified. We calculated ORs with 95% CIs to assess risk of fracture. Subgroup analyses were performed by subtype, ethnicity and gender. Results: Data on 2916 cases and 19170 controls were analyzed in the meta-analysis. Overall, we found moderately decreased risk in association with IVS1-397 CC genotype (OR = 0.82, 95% CI = 0.73-0.92; OR = 0.84, 95% CI = 0.76-0.94). The decrease persisted in both hip fracture (OR = 0.82, 95% CI = 0.71-0.94; OR = 0.83, 95% CI = 0.73-0.94) and vertebral fracture (OR = 0.67, 95% CI = 0.50-0.91; OR = 0.78, 95% CI = 0.64-0.97; OR = 0.82, 95% CI = 0.68-0.98) when data were stratified by subtype. We also found a significant trend of decreasing risk in relation to the CC genotype in Caucasian, male and female. All fixed-effects meta-analysis results were homogeneous. Conclusion: The meta-analysis demonstrates that risk of fracture seems likely to be decreased due to IVS1-397 CC or CT genotype.

Keywords: *ESR1*, polymorphism, fracture

Introduction

Fracture preferentially occurring among the elderly people arises predominantly from osteoporosis, a prevalent skeletal disorder with two characteristics: progressive microarchitectural deterioration and low bone mass [1]. Many people are being debilitated by the pain due to the age-related bone disease [2]. The etiology of osteoporosis is complex and there is limited knowledge in its pathogenesis. Several lines of evidence have shown that genetic factors affect risk of fracture by interplaying with exogenous substances [3, 4]. Therefore, investigating the role of predisposing genes and their interactions with common confounders may shed light on the molecular mechanisms underlying the disease.

A candidate gene widely focused has been the *ESR1*, an isoform of estrogen receptor known to be associated with bone-related diseases, such as articular cartilage. The well-characterized *ESR1* serves as an important regulator of signal transduction, and elevated serum *ESR1* levels have been detected in chondrocytes and bone cells [5]. The plasma level of estradiol could predict bone mineral density and incidence of osteoporotic fracture; in addition, lack of estrogen would result in bone loss in postmenopausal women as well as in men [6-8]. Epidemiology studies have reported that postmenopausal women who received estrogen replacement therapy are less likely to develop fractures [9-11], indicating *ESR1* is a possible determinant of fracture risk.

The *ESR1* is high polymorphic, and in the promoter region, there lies a T to C substitution polymorphism (IVS1-397T>C, rs2234693) extensively studied in fracture community. The published genetic association studies, however, have yielded inconsistent conclusions, partially because of the non-homogeneous populations and the limited number of subjects analyzed in each study. Another important

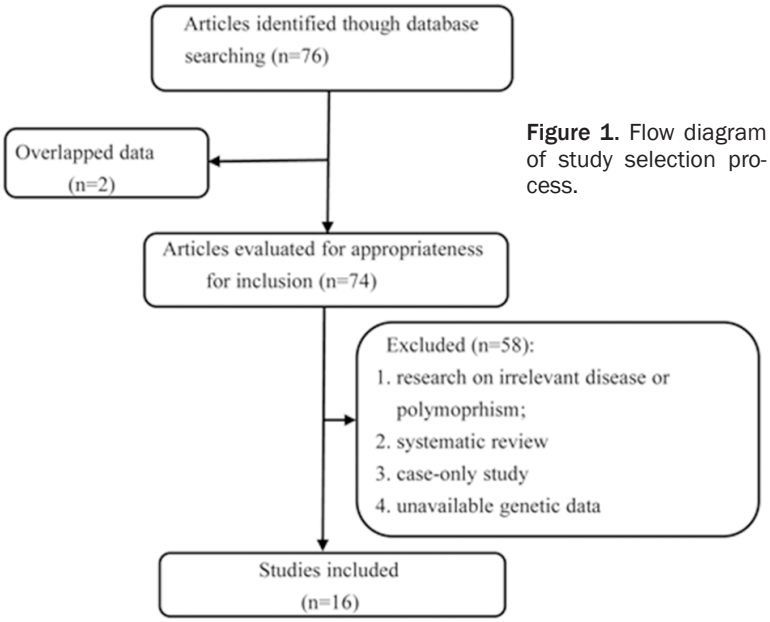

Figure 1. Flow diagram of study selection process.

impetus promoting us to perform the meta-analysis is the less reliable results revealed in previous meta-analyses, as they failed to include all usable data [12, 13].

The aim of this investigation was to comprehensively evaluate the association of IVS1-397T>C in intron 1 of the *ESR1* gene with risk of fracture.

Materials and methods

Search strategy

To identify all studies that explored the association of *ESR1* IVS1-397T>C with fracture risk, we undertook literature searches of multiple online databases (Pubmed, Embase, ScienceDirect, and Wiley Online Library), by combining the following keywords: estrogen receptor α, *ESR1*, fracture, polymorphism and polymorphisms, without implementing any language restrictions. The references cited in each identified systematic review or meta-analysis were subsequently screened to obtain additional usable data. The last search was updated on January 10, 2014. We also contacted the corresponding author of a large-scale case-control study, where the original data were not reported in the article [14]. Finally, no reply was received from the authors.

Inclusion criteria

Studies meeting all inclusion requirements are included in the meta-analysis: a) a case-control, nested case-control or cohort study for human subjects; b) risk of fracture associated with *ESR1* IVS1-397T>C was evaluated; c) genotype frequencies were reported in detail. In the condition that the same case population was investigated in two or more publications by the same group of authors, we selected the publication containing more participants.

Data abstraction

Data were extracted in duplicate by two investigators. The characteristics collected for the human studies included first author's name, year of publication, country of origin, ethnicity of participants (Asian or Caucasian), fracture type (hip or vertebral), genotyping method, gender of participants, count of genotyped cases and controls, and frequency of IVS1-397 TT, CT, CC. The extracted data were first checked by other investigators and then by an expert in this field to ensure the accuracy. Disparities were handled via discussion.

Quality assessment

We selected Newcastle-Ottawa scale (NOS) for nonrandomised studies [40] to assess the methodological quality of all eligible studies. The NOS identifies high quality with a star and a total of three aspects were evaluated, including selection, outcome and comparability, with each aspect containing a maximum of four stars, three stars and two stars, respectively. A study with at least six stars was considered as a high quality study; otherwise, the study was classified into "low quality study".

Statistical analysis

Statistical data were analyzed using the STATA software (version 12.0, Stata Corporation, College Station, Texas). All tests were two-sided and a *P* value less than 0.05 was taken as the significance level.

Overall analysis, along with subgroup analyses by subtype, ethnicity and gender was carried out to evaluate risk of fracture [odds ratio and 95% confident interval (OR and

Table 1. Characteristics summarized for the studies included in the meta-analysis

Authors	Year	Population	Fracture type	Case	Control	Gender	Method	Quality※	HWE
Wang et al.	2012	Asian	Hip	128	128	M	PCR-RFLP	7	0.171
Wei et al.	2012	Asian	Hip/Vertebral	120	120	F/M	PCR-RFLP	6	0.712
Harslof et al.	2010	Caucasian	Hip	291	281	F/M	PCR-RFLP	7	0.310
Massart et al.	2009	Caucasian	Hip	102	725	F	PCR-RFLP	7	0.659
Valero et al.	2008	Caucasian	Hip	498	356	F	Taqman	8	0.343
Dincel et al.	2008	Caucasian	Hip	19	20	F/M	AS-PCR	6	0.456
Lian et al.	2007	Caucasian	Hip	567	4113	F	PCR-RFLP	7	0.533
Kjaergaard et al.	2007	Caucasian	Hip	273	8970	F/M	PCR-RFLP	7	0.814
van Meurs et al.	2003	Caucasian	Vertebral/non-Vertebral	360	2866	F/M	PCR-RFLP	8	0.806
Kobayashi et al.	2002	Asian	Vertebral	48	149	F	PCR	7	0.827
Albagha et al.	2001	Caucasian	Any	66	138	F	PCR	7	0.359
Efstathiadou et al.	2001	Caucasian	Any	17	108	F	PCR	8	0.055
Salmen et al.	2000	Caucasian	Any	28	303	F	PCR	7	0.701
Aerssens et al.	2000	Caucasian	Hip	135	239	F	PCR-RFLP	8	0.409
Becherini et al.	2000	Caucasian	Vertebral	122	488	F	PCR	7	0.626
Vandevyver et al.	1999	Caucasian	Any	142	166	F	PCR	7	0.424

Abbreviation: M, Male; F, female; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; AS-PCR, allele-specific-PCR, ※number of stars, HWE, Hardy-Weinberg equilibrium.

95% CI)] in association with IVS1-397 polymorphism. The pooled ORs were calculated assuming the homozygous model (CC vs TT), the heterozygous model (CT vs TT), the dominant model (CC/CT vs TT), and the recessive model (CC vs CT/TT).

To decide whether the fixed-effects model (Mantel-Haenszel method) or the random-effects model (DerSimonian-Laird method) was used in evaluating the values for each study, we tested the heterogeneity by the χ^2-based Q-test, supplemented with the I^2 metric [15]. It was indicative of absence of heterogeneity when $P > 0.05$ and $I^2 < 50\%$. Under such a condition, we chose the former model; otherwise, the latter model was selected.

To test reliability of the combined meta-analysis estimations, we carried out sensitivity analysis and publication bias tests [16]. We also examined Hardy-Weinberg equilibrium in the control groups using the χ^2 test for goodness of fitness.

Results

Studies included in the meta-analysis

A total of 16 studies [17-32], providing 2916 fracture cases 19170 healthy controls, were included in the meta-analysis. Searches of the online databases yielded 76 publications. We excluded 60 publications due to a variety of reasons, including research on irrelevant disease or polymorphism, systematic review, case-only study, unavailable genetic data, or overlapped data. The details of study selection are presented in **Figure 1**.

We identified 13 Caucasian studies (2620 cases and 18773 controls) and 3 Asian studies (296 cases and 397 controls). In the subgroup analysis by subtype, we included 9 datasets for hip fracture and 4 for vertebral fracture. In addition, there were 12 datasets on fracture in women and 3 in men. All studies used polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism to genotype *ESR1* IVS1-397T>C with the exception of Valero et al. [21], who applied Taqman in genotype determination. We observed no departure of genotype frequencies from those expected under Hardy-Weinberg equilibrium. The characteristics of the included studies are summarized in **Table 1**.

Meta-analysis results

Figures 2, **3** display the association of fracture risk with IVS1-397T>C polymorphism under the homozygous model and the recessive model, respectively. The fixed effects meta-analysis revealed that individuals with the CC genotype,

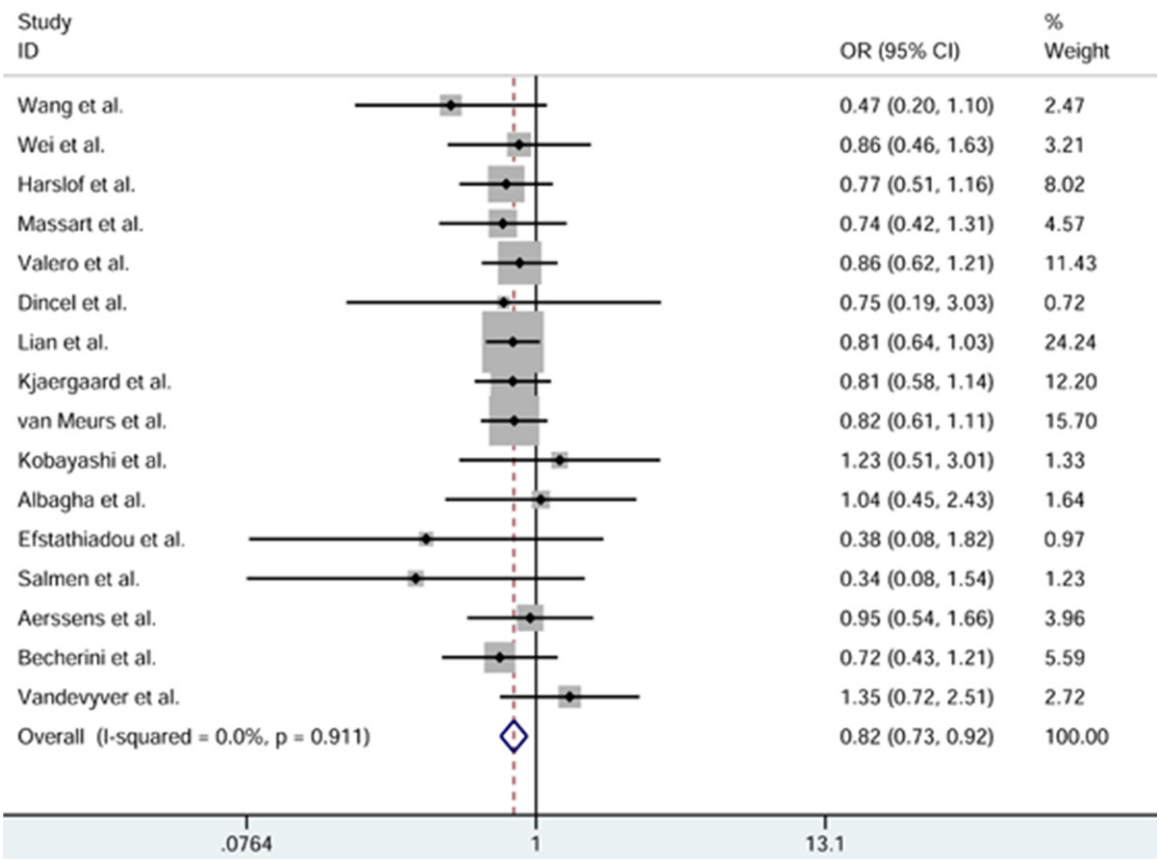

Figure 2. Forest plot of overall risk of fracture in association with *ESR1* IVS1-397T>C (the homozygous model). The symbol filled diamond indicates pooled OR and its 95% CI.

as compared to those with the TT genotype, were 18% less likely to develop fracture (OR = 0.82, 95% CI = 0.73-0.92). Slightly lower risk was observed under the recessive model (OR = 0.84, 95% CI = 0.76-0.94), as shown in **Table 2**.

We then stratified the data by ethnicity and found moderately decreased risk in Caucasian under the same genetic models (OR = 0.82, 95% CI = 0.73-0.93, OR = 0.84, 95% CI = 0.75-0.94, **Table 2**).

In the subgroup analysis by fracture type, the association seemed to be less pronounced among the individuals with hip fracture (OR = 0.82, 95% CI = 0.71-0.94; OR = 0.83, 95% CI = 0.73-0.94), and more pronounced in the individuals with vertebral fracture (OR = 0.67, 95% CI = 0.50-0.91; OR = 0.78, 95% CI = 0.64-0.97; OR = 0.82, 95% CI = 0.68-0.98), as shown in **Table 2**.

The significant associations persisted when stratifying the datasets according to gender. The association appeared to be stronger in men (OR = 0.64, 95% CI = 0.42-0.98), as shown in **Table 2**.

Heterogeneity test and sensitivity analysis

We did not detect any significant heterogeneity across the studies ($P > 0.05$, $I^2 < 50\%$, **Table 2**) and thus only the fixed effects model was used to evaluate the values. For the sake of checking whether the single datasets exert substantial influence on the pooled results, we performed the leave-one-out sensitivity analysis. No significant disparity was found between the primary ORs and recalculated values, suggesting the effect estimations were robust (figure not shown).

Publication bias

We tested the publication bias for all genetic models and none of the funnel plots showed evidence of obvious asymmetry. Nor did the statistical data provided by the Egger's test

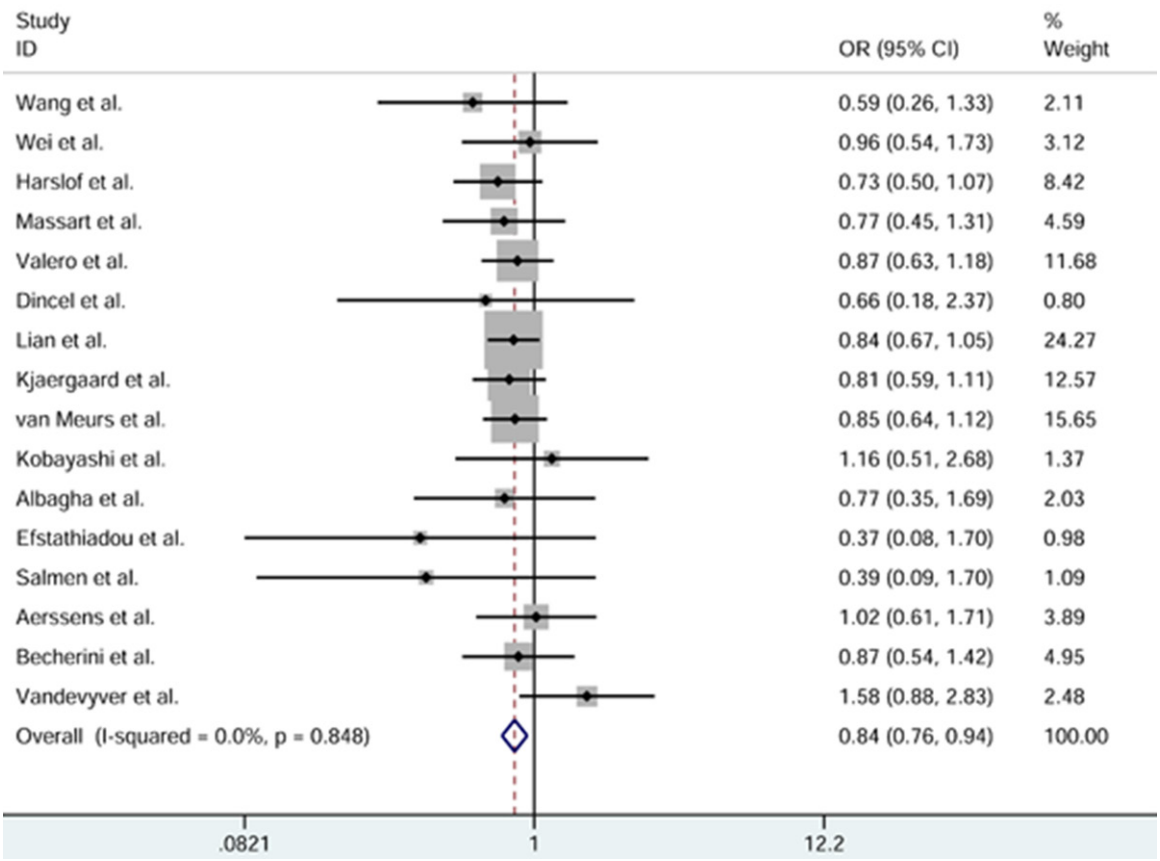

Figure 3. Forest plot of overall risk of fracture in association with *ESR1* IVS1-397T>C (the recessive model). The symbol filled diamond indicates pooled OR and its 95% CI.

show significant bias in this study. The funnel plot constructed for the homozygous model is shown in **Figure 4** (Begg: P = 0.163; Egger: P = 0.403).

Discussion

Single genetic association studies characterized by a relatively small sample, ethnic differences, and heterogeneous populations may not be sufficient to determine the role of single-nucleotide polymorphisms (SNP) in the progression of common diseases, such as cancer. Meta-analysis, an analytical tool that could overcome all aforementioned difficulties and maximize the detection power by combining different independent studies, is extensively used to identify SNP-disease associations in recent years.

In the current investigation, we performed a meta-analysis to evaluate the association between *ESR1* IVS1-397T>C and fracture risk based on data on 2916 fracture cases 19170 controls subjects provided by 16 studies. We found individuals carrying the CC genotype, compared to those carrying the TT genotype, were 18% less likely to develop fracture. 16% deceased risk attributable to the same genotype was also found in overall analysis. We observed such a decrease in all subsequent subgroup analyses. However, is seems that the risk of vertebral fracture was lower than that of hip fracture for individuals with the CC genotype (0.67 vs 0.82). We also noted that men harboring the IVS1-397CC in comparison to women, had less risk of developing fracture (0.64 vs 0.86). Taken together, the carriage of IVS1-397CC was associated with declined risk of fracture.

These observations seem to be biologically reasonable. It is known that decline in bone mass is a major cause of fracture. Estrogen is an essential mediator of bone mass, and estrogen deficiency will result in decreased bone mass and subsequent bone loss. Moreover, absence of estrogen leads to increases in osteoclast

Table 2. The association of *ESR1* IVS1-397T>C with fracture risk

Variables	Case/control	Homozygous (CC vs TT)		Heterozygous (CT vs TT)		Dominant (CC/CT vs TT)		Recessive (CC vs CT/TT)	
		OR (95% CI)	P_{Het}/I^2	OR (95% CI)	P_{Het}/I^2	OR (95% CI)	P_{Het}/I^2	OR (95% CI)	P_{Het}/I^2
All	2916/19170	0.82 (0.73, 0.92)	0.911/0.0	0.93 (0.86, 1.00)	0.997/0.0	0.93 (0.87, 1.00)	0.998/0.0	0.84 (0.76, 0.94)	0.848/0.0
Fracture type									
Hip	2073/14952	0.82 (0.71, 0.94)	0.945/0.0	0.93 (0.85, 1.03)	0.995/0.0	0.93 (0.86, 1.01)	0.993/0.0	0.83 (0.73, 0.94)	0.944/0.0
Vertebral	382/1789	0.67 (0.50, 0.91)	0.463/0.0	0.78 (0.64, 0.97)	0.679/0.0	0.82 (0.68, 0.98)	0.672/0.0	0.79 (0.59, 1.06)	0.658/0.0
Ethnicity									
Asian	296/397	0.79 (0.51, 1.23)	0.291/0.189	0.86 (0.65, 1.14)	0.561/0.0	0.88 (0.69, 1.13)	0.508/0.0	0.89 (0.59, 1.34)	0.483/0.0
Caucasian	2620/18773	0.82 (0.73, 0.93)	0.925/0.0	0.93 (0.86, 1.01)	0.997/0.0	0.93 (0.87, 1.00)	0.998/0.0	0.84 (0.75, 0.94)	0.783/0.0
Gender									
M	285/4217	0.66 (0.42, 1.03)	0.394/0.0	0.91 (0.71, 1.18)	0.331/0.096	0.89 (0.71, 1.13)	0.389/0.0	0.64 (0.42, 0.98)	0.840
F	2132/11947	0.84 (0.73, 0.96)	0.793/0.0	0.93 (0.85, 1.02)	0.988/0.0	0.93 (0.86, 1.01)	0.990/0.0	0.86 (0.76, 0.98)	0.678

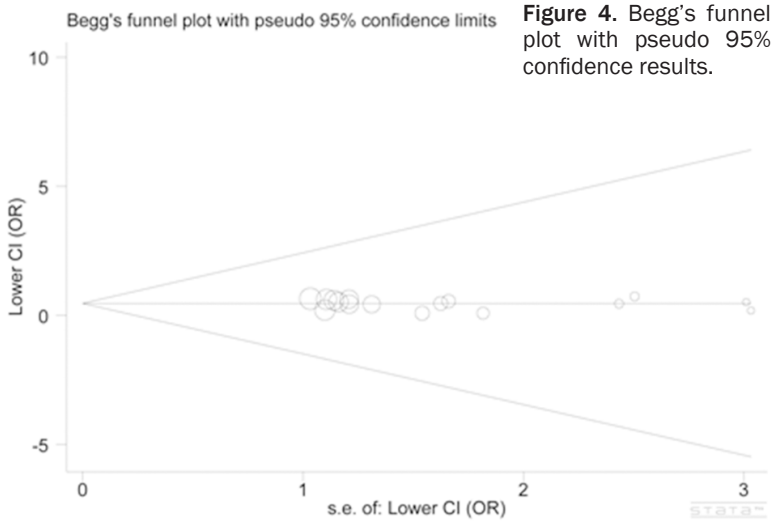

Figure 4. Begg's funnel plot with pseudo 95% confidence results.

recruitment and upregulation of the bone-resorbing activity of mature osteoclast [33, 34]. Nuclear *ESR1* nevertheless could decrease the resorbtion [33]. Bone mass varies widely among individuals and it is the heritability that has been presumed to play a major role. A twins study along with a family aggregation study carried out nearly twenty years ago demonstrated that a vast majority of the variability in bone mass should attribute to genetic factors [35, 36]. Therefore, the presence of *ESR1* IVS1-397T>C polymorphism may have a key role in protection against the loss of bone mass, consequently decreasing the likelihood of fracture. Interestingly, various lines of evidence derived from recent genome-wide association studies or large-scale meta-analysis have shown that the polymorphisms at *ESR1* exert slight effects on the variance in bone mineral density at the individual level [37-39]. In this sense, IVS1-397T>C may merely play a minor role in fracture risk. Notably, the wide disparities reported in previous studies highlights the necessity to further elucidate the role of *ESR1* polymorphisms on the development of fracture.

The first group investigating the effects of IVS1-397T>C of *ESR1* on fracture risk by means of meta-analysis is Ioannidis et al., who summarized a limited number of subjects (655 cases and 1574 controls), showing no evidence for any effect of IVS1-397T>C genotypes [12]. Recently, Tang et al. evaluated the association of IVS1-397T>C with hip fracture risk among 8 datasets, and observed statistically significant associations similar to our study [13]. We identified a total of 16 studies, and comprehensive assessed the effects IVS1-397T>C has on fracture. The inclusion of all available data, including those analyzed in previous studies and the new information reported in subsequently released studies, helps to ensure the reliability of our results.

In interpreting the observations, some limitations should be considered. First, fracture, like other diseases, is a heterogeneous disease which seems unlikely to be caused by a single polymorphism. Although we have detected significant contribution of *ESR1* IVS1-397T>C alone to genetic risk of fracture, the risk may be further affected after combining with many known modifying genes and environmental factors. For example, we only found moderately declined risk irrespective of any confounding factors. Nonetheless, the genetic risk was more notable when fracture type and gender were considered. Second, minor heterogeneity was observed in our study, and the obtained results may to some extent be affected. Third, we failed to identify any association for Asian. One possible reason may relate to the inadequate sample.

To the best of our knowledge, this is the largest study examining the association between *ESR1* IVS1-397T>C and fracture risk. The results showed a declined overall risk in relation to the promoter polymorphism. Similar declined risk was found in all subgroups with the possible exception of Asian. Larger genetic analyses including multiple genes may provide more compelling evidence for the association under investigation.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jianwei Wang, The Department of Orthopedics, The Second Hospital Affiliated to Zhejiang University School of Medicine,

Hangzhou 310009, Zhejiang, China. E-mail: wang35k@126.com

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