Original Article

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

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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
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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 comprehen-
sively 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 associa-
tion of ESR1 IVS1-397T>C with fracture risk,
we undertook literature searches of multiple
online databases (Pubmed, Embase, Science-
Direct, and Wiley Online Library), by combining
the following keywords: estrogen receptor α,
ESR1, fracture, polymorphism and polymor-
phisms, without implementing any language
restrictions. The references cited in each iden-
tified 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 cor-
responding author of a large-scale case-control
study, where the original data were not report-
ed 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-con-
trol, nested case-control or
cohort study for human sub-
jects; b) risk of fracture asso-
ciated with ESR1 IVS1-
397T>C was evaluated; c)
genotype frequencies were
reported in detail. In the con-
dition 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 duplic-
ate 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 geno-
typed 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, includ-
ing 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 ana-
lyses by subtype, ethnicity and gender was
carried out to evaluate risk of fracture [odds ratio and 95% confident interval (OR and
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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 χ²-based Q-test, supplemented with the I² metric [15]. It was indicative of absence of heterogeneity when P > 0.05 and I² < 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 χ² test for goodness of fitness.

Results

Studies included in the meta-analysis

A total of 16 studies [17-32], providing 2916 fracture cases and 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,
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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, I² < 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...
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, it 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

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case/control</th>
<th>Homozygous (CC vs TT)</th>
<th>OR (95% CI)</th>
<th>P_ref/I²</th>
<th>Heterozygous (CT vs TT)</th>
<th>OR (95% CI)</th>
<th>P_ref/I²</th>
<th>Dominant (CC/CT vs TT)</th>
<th>OR (95% CI)</th>
<th>P_ref/I²</th>
<th>Recessive (CC vs CT/TT)</th>
<th>OR (95% CI)</th>
<th>P_ref/I²</th>
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</thead>
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<tr>
<td>All</td>
<td>2916/19170</td>
<td>0.82 (0.73, 0.92)</td>
<td>0.911/0.0</td>
<td>0.93 (0.86, 1.00)</td>
<td>0.997/0.0</td>
<td>0.93 (0.87, 1.00)</td>
<td>0.998/0.0</td>
<td>0.84 (0.76, 0.94)</td>
<td>0.848/0.0</td>
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<tr>
<td>Fracture type</td>
<td></td>
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<tr>
<td>Hip</td>
<td>2073/14952</td>
<td>0.82 (0.71, 0.94)</td>
<td>0.945/0.0</td>
<td>0.93 (0.85, 1.03)</td>
<td>0.995/0.0</td>
<td>0.93 (0.86, 1.01)</td>
<td>0.993/0.0</td>
<td>0.83 (0.73, 0.94)</td>
<td>0.944/0.0</td>
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<tr>
<td>Vertebral</td>
<td>382/1789</td>
<td>0.67 (0.50, 0.91)</td>
<td>0.463/0.0</td>
<td>0.78 (0.64, 0.97)</td>
<td>0.679/0.0</td>
<td>0.82 (0.68, 0.98)</td>
<td>0.672/0.0</td>
<td>0.79 (0.59, 1.06)</td>
<td>0.658/0.0</td>
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<td>Ethnicity</td>
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<tr>
<td>Asian</td>
<td>296/397</td>
<td>0.79 (0.51, 1.23)</td>
<td>0.291/0.189</td>
<td>0.86 (0.65, 1.14)</td>
<td>0.561/0.0</td>
<td>0.88 (0.69, 1.13)</td>
<td>0.508/0.0</td>
<td>0.89 (0.59, 1.34)</td>
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<tr>
<td>Caucasian</td>
<td>2620/18773</td>
<td>0.82 (0.73, 0.93)</td>
<td>0.925/0.0</td>
<td>0.93 (0.86, 1.01)</td>
<td>0.997/0.0</td>
<td>0.93 (0.87, 1.00)</td>
<td>0.998/0.0</td>
<td>0.84 (0.75, 0.94)</td>
<td>0.783/0.0</td>
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<tr>
<td>M</td>
<td>285/4217</td>
<td>0.66 (0.42, 1.03)</td>
<td>0.394/0.0</td>
<td>0.91 (0.71, 1.18)</td>
<td>0.331/0.096</td>
<td>0.89 (0.71, 1.13)</td>
<td>0.389/0.0</td>
<td>0.64 (0.42, 0.98)</td>
<td>0.840</td>
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<tr>
<td>F</td>
<td>2132/11947</td>
<td>0.84 (0.73, 0.96)</td>
<td>0.793/0.0</td>
<td>0.93 (0.85, 1.02)</td>
<td>0.988/0.0</td>
<td>0.93 (0.86, 1.01)</td>
<td>0.990/0.0</td>
<td>0.86 (0.76, 0.98)</td>
<td>0.678</td>
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</table>
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Figure 4. Begg’s funnel plot with pseudo 95% confidence limits.

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.

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