

## Original Article

# IL6-174G/C polymorphism and fracture risk

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Received August 24, 2014; Accepted October 3, 2014; Epub October 15, 2014; Published October 30, 2014

**Abstract:** Several studies have examined the associations of polymorphism in interleukin-6 (IL6) with fracture risk. However, the results were conflicting. Thus, a meta-analysis was conducted to determine the relationship between IL6-174C/G polymorphism and risk of fracture. Databases including PubMed, EMBASE, Wanfang were searched. Summary odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated using random-effects models. Six studies were included in this meta-analysis. IL6-174C/G polymorphism was associated with a significantly increased risk of fracture (OR=1.32; 95% CI, 1.10-1.58;  $I^2=11\%$ ; Figure 1). In the subgroup analysis according to gender, women (RR=1.26; 95% CI, 1.09-1.46;  $I^2=0\%$ ) was significantly associated with risk of fracture. In the age subgroup analysis, old population (RR=1.27; 95% CI, 1.11-1.48;  $I^2=0\%$ ) showed increased fracture risk. However, young population did show increased risk of fracture (RR=1.95; 95% CI, 0.70-5.47;  $I^2=51\%$ ). Postmenopausal women also showed an increased fracture risks (RR=1.24; 95% CI, 1.08-1.44;  $I^2=0\%$ ). This meta-analysis suggested that IL6-174C/G polymorphism contributed the development of fracture.

**Keywords:** Fracture, interleukin-6, meta-analysis, polymorphism

### Introduction

Fracture is a global public health concern. In the United States, 25% to 30% of hip fractures occur in men [1]. In 2004, femoral neck fractures occurred in 93,000 men older than 65 years compared to 236,000 in women. While hip fracture rates have decreased since 1995, the number of fractures continues to rise as the population ages, and it is estimated the number of hip fractures worldwide will increase from the 1.26 million in 1990 to 2.6 million in 2025 and 4.5 million in the 2050 [2].

Interleukin-6 (IL6) is a pleiotropic inflammatory cytokine that is important for immune responses, cell survival, proliferation and apoptosis [3]. Increased levels of IL-6 within the bone micro-environment promote the differentiation of osteoclast precursor cells into mature osteoclasts, which increase bone resorption [4]. It has been demonstrated that the levels of IL-6 increase during menopause in response to decreasing estradiol levels and osteoporosis in mice due to ovariectomy has been causally linked to IL-6 [5].

The best characterized genetic variants of IL6 is a G-to-C substitution at position -174, upstream of the transcription start site, which has been reported to influence IL6 levels in vitro and in vivo [6]. Therefore, we speculated that IL6-174C/G polymorphism could influence fracture risk. Some reports have established the association of IL6-174C/G polymorphism with fracture [7-12]. The IL6-174C/G polymorphism has been found to be associated with the development of fracture in some studies but with inconsistent results. In this study, we conducted a meta-analysis to estimate the association of IL6-174C/G polymorphism with risk of fracture.

### Methods

#### Publication search

Online electronic databases (PubMed, EMBASE, and Wanfang database) was searched using the search terms: ("Interleukin-6" or IL6) and (polymorphism or variant or variation) and ("fracture" or "bone fracture"). Additional studies were identified by a hand search from reference of original studies or review articles on this topic. There was no language restriction.

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

First author/Year	Ethnicity	Gender	Age	Menses status	No. of subjects	Quality score
Nordstrom/2004	Caucasian	Female	75	Postmenopausal	964	9
Moffett/2004	Caucasian	Female	>65	Postmenopausal	3376	9
Dinçel/2008	Caucasian	Mixed	73	NA	42	7
Breuil/2009	Caucasian	Female	70	Postmenopausal	161	8
Korvala/2010	Caucasian	Male	18-27	-	192	8
Yanovich/2012	Caucasian	Mixed	18-32	-	385	7

**Table 2.** Main result and subgroup analyses of this meta-analysis

Characteristics	No. of studies	Test of association			Model	Heterogeneity		
		OR (95% CI)	Z	P Value		$\chi^2$	P Value	I <sup>2</sup> (%)
All studies	6	1.32 (1.10-1.58)	3.05	0.002	R	5.61	0.35	11
Female	3	1.26 (1.09-1.46)	3.05	0.002	R	1.23	0.54	0
Age ≤50 years	2	1.95 (0.70-5.47)	1.28	0.20	R	3.07	0.09	51
Age >50 years	4	1.27 (1.11-1.48)	3.09	0.002	R	1.26	0.74	0
Postmenopausal	3	1.24 (1.08-1.44)	3.05	0.002	R	1.23	0.54	0

OR, odds ratio; CI, confidence intervals; R, random effects model.

### Inclusion and exclusion criteria

The following inclusion criteria were used: (1) the study should have evaluated the association between the *IL6-174C/G* polymorphism and fracture risk; (2) the study should have a casecontrol or cohort design; (3) sufficient data should have been provided in order to calculate odds ratios (OR) and 95% confidence interval (CI). Studies were excluded if any of the following conditions applied: (1) only abstracts or reviews were available, without sufficient data; (2) animal studies; (3) studies were repeated or publications overlapped.

### Data extraction and qualitative assessment

The following data was recorded from each article: first author, years of publication, ethnicity of participants, gender, age, menses, and numbers of subjects. The data were extracted by two of the authors independently. Discrepancies between these two authors were resolved by discussion.

Two reviewers completed the quality assessment independently. The Newcastle-Ottawa Scale (NOS) was used to evaluate the methodological quality, which scored studies by the selection of the study groups, the comparability of the groups, and the ascertainment of the outcome of interest. We considered a study awarded 0-3, 4-6, or 7-9 as a low-, moderate-,

or high-quality study, respectively. Discrepancies were resolved by consensus and discussion.

### Statistical analysis

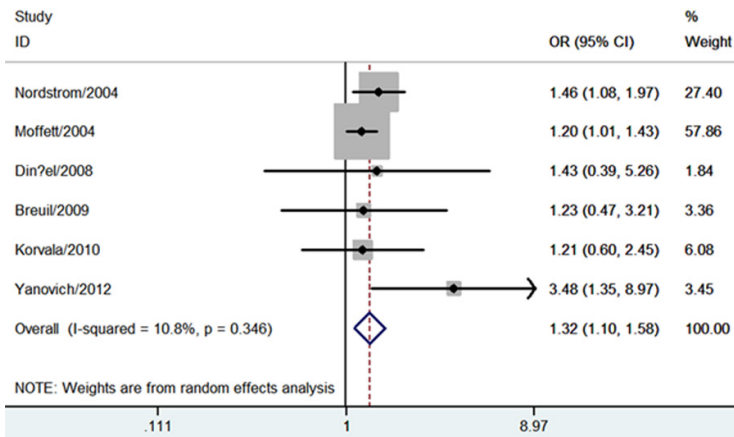
The strength of association between the *IL6-174C/G* polymorphism and fracture risk was assessed by calculating OR with 95% CI. The pooled ORs were performed for allele model since most of the studies reported the results of this genetic model. A statistical test for heterogeneity was performed based on the Q statistic. The  $P > 0.10$  of the Q-test indicated a lack of heterogeneity among studies. The summary OR estimate of each study was calculated by the random-effects model (the DerSimonian and Laird method). Stratified analysis was performed by gender, age, and menses, if possible. Potential publication bias was examined by Egger's test [13]. All statistical tests were performed with the software STATA version 11.0 (Stata Corporation, College station, TX, USA). A  $P$  value  $< 0.05$  was considered statistically significant.

## Results

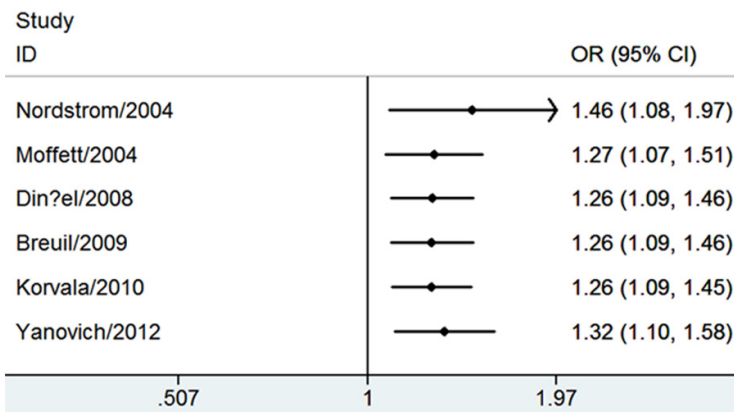
### Study characteristics

A total of 6 articles on *IL6-174C/G* polymorphism and fracture met the study inclusion criteria, and were included in the meta-analysis

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**Figure 1.** Meta-analysis for the association between *IL6*-174C/G polymorphism and fracture risk.



**Figure 2.** Cumulative meta-analysis for the association between *IL6*-174C/G polymorphism and fracture risk.

[7-12]. Finally, 5120 subjects were included in this meta-analysis. All of the included studies were performed in Caucasian populations. All studies were assessed by NOS. The quality scores ranged from 7 to 9, suggesting that the methodological quality was high. The characteristics of each case-control study are presented in **Table 1**.

### Results of meta-analysis

The evaluations of the association between *IL6*-174C/G polymorphism and fracture risk are summarized in **Table 2**. *IL6*-174C/G polymorphism was associated with a significantly increased risk of fracture (OR=1.32; 95% CI, 1.10-1.58;  $I^2=11\%$ ; **Figure 1**). In the subgroup analysis according to gender, women (RR=1.26; 95% CI, 1.09-1.46;  $I^2=0\%$ ) was significantly

associated with risk of fracture. In the age subgroup analysis, old population (RR=1.27; 95% CI, 1.11-1.48;  $I^2=0\%$ ) showed increased fracture risk. However, young population did show increased risk of fracture (RR=1.95; 95% CI, 0.70-5.47;  $I^2=51\%$ ). Postmenopausal women also showed an increased fracture risks (RR=1.24; 95% CI, 1.08-1.44;  $I^2=0\%$ ).

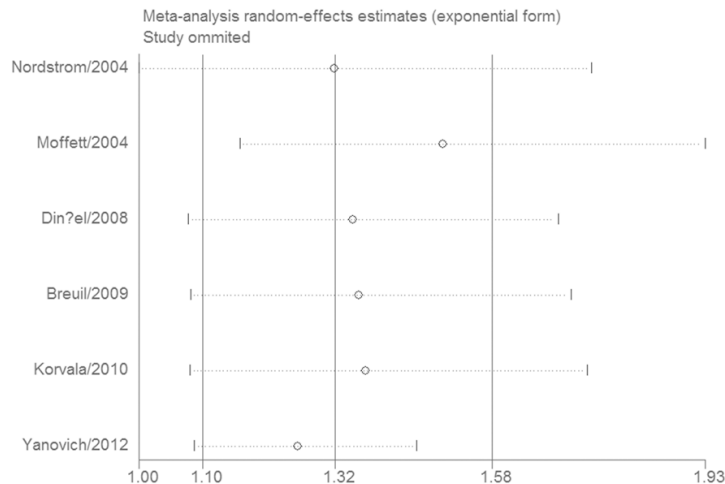
As shown in **Figure 2**, significant associations were evident with each addition of more data over time. The results showed that the pooled ORs tended to be stable. A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (**Figure 3**).

Funnel plot was performed to assess the publication bias of literatures. The shape of the funnel plot showed asymmetry (**Figure 4**). Egger's test found the evidence of publication bias ( $P=0.452$ ).

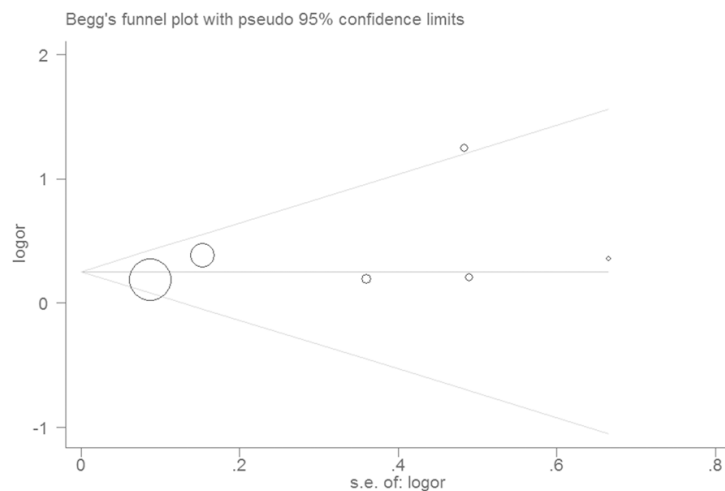
### Discussion

This meta-analysis of six studies systematically evaluated the association between *IL6*-174C/G polymorphism and fracture risk. The results indicated that *IL6*-174C/G polymorphism was a risk factor for developing fracture in the Caucasians. There was no study investigated the association between *IL6*-174C/G polymorphism and fracture risk in Asians. Thus, more studies with Asians are needed in the future. Interestingly, we found that women with *IL6*-174C/G polymorphism were significantly associated with fracture risk. Only one study assessed the association between *IL6*-174C/G polymorphism and fracture risk in male population. Thus, more studies with large sample sizes are needed to further identify the association among male population. Results from this

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**Figure 3.** Sensitivity meta-analysis for the association between *IL6-174C/G* polymorphism and fracture risk.



**Figure 4.** Funnel plot for the association between *IL6-174C/G* polymorphism and fracture risk.

meta-analysis showed that old population with *IL6-174C/G* polymorphism were significantly associated with fracture risk. However, young population with this polymorphism were not significantly associated with fracture risk. We also found that postmenopausal women with this polymorphism had increased fracture risk.

IL-6 is a mediator of estrogen-deficient bone loss. Estrogen represses the induction of IL-6 associated with osteoblast activation, and suppresses the expression of RANKL, and thus helps to maintain BMD [14]. IL-6 is also a candidate cytokine that has been implicated in enhanced osteoclastogenesis, bone resorption, and accelerated bone loss. Previous stud-

ies showed that the -174 C/C genotype is associated with lower biochemical indices of bone resorption and with higher BMD [15]. In addition, The G allele is associated with increased promoter activity and increased IL-6 levels [6]. Thus, *IL6-174C/G* polymorphism might increase fracture risk.

Heterogeneity and publication bias, which may influence the results of meta-analysis, should be addressed in the meta-analysis. We did not detect a significant publication bias in this meta-analysis, suggesting the reliability of our results. Significant heterogeneity was also not observed in this meta-analysis.

Some limitations should be taken into account. First, there was no study investigated the association of *IL6-174C/G* polymorphism with fracture risk in Asians. Therefore, more studies are needed to further identify the association among Asians. Second, lack of the original data of the reviewed studies limited our further evaluation of potential interactions, because the interactions between gene-to-gene and gene-to-environment may modulate lung cancer risk. These gene-to-gene and gene-to-environment interactions should be further evaluated. Third, due to the lack

of sufficient and uniform information in original studies, data were not stratified by other factors.

In summary, results from this meta-analysis indicated that *IL6-174C/G* polymorphism was significantly associated with fracture risk. Further studies considering gene-gene and gene-environment interaction should be conducted to confirm the association.

### Disclosure of conflict of interest

None.

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