The genetic association between osteoprotegerin (OPG) gene polymorphisms and bone mineral density (BMD) in postmenopausal women

A meta-analysis

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Abstract

Background: Osteoporosis is a common skeletal disorder in eldest people, especially in postmenopausal women. The osteoprotegerin (OPG) gene has been reported to be associated with the BMD and pathogenesis of osteoporosis. However, the results were inconsistent and inconclusive in previous studies.

Methods: A meta-analysis was performed to investigate the effect of four common OPG gene polymorphisms (A163G, G1181C, T245G, and T950C) on BMD in postmenopausal women.

Results: A total of 23 eligible studies with 12,973 postmenopausal women were enrolled in present study. Individuals who with AA genotype of A163G were found to have slightly higher femoral hip (P = .03, SMD = 0.49, [95% CI] = [0.06, 0.91]) and total hip BMD (P = .002, SMD = −0.25, [95% CI] = [−0.42, −0.09]) than those with AG genotype. Subjects with GG genotype of G1181C was found to have lower BMD than those with CC or GC genotypes in lumbar spine (GG vs GC: P = .002, SMD = −0.85, [95% CI] = [−1.29, −0.41]; GG vs CC: P = .02, SMD = −0.21, [−0.39, −0.03]) and total hip BMD (GG vs GC: P = .002, SMD = −0.25, [95% CI] = [−0.42, −0.09]; GG vs CC: P = .01, SMD = −0.15, [95% CI] = [−0.26, −0.03]). In addition, the subjects with GC genotype of G1181C was detected to have lower BMD than those with CC genotype in lumbar spine BMD (P < .05). Furthermore, individuals with TT genotype of T950C were shown to have significant lower lumbar spine BMD compared with those with genotype CC in Caucasian (P < .05). The lumbar spine BMD was lower for subjects with TC genotype of T950C than those with CC genotype in both Caucasian and Asian populations (P < .05). In contrast to A163G, G1181C, and T950C, no association was detected between T245G polymorphism and BMD (P > .05).

Conclusion: The present meta-analysis demonstrated the OPG A163G, G1181C, and T950G, but not T245G, might influence the BMD in postmenopausal women.

Abbreviations: BMD = bone mineral density, BMI = Body Mass Index, CNKI = Chinese National Knowledge Infrastructure, CTR = calcitonin receptor, LD = linkage disequilibrium, NOS = Newcastle-Ottawa Scale, OPG = osteoprotegerin, RANK = receptor activator of nuclear factor-B, RANKL = receptor activator of nuclear factor-B ligand, SD = standard deviation, TGBF1 = transforming growth factor b1, TNFRS11B = tumor necrosis factor receptor superfamily member 1b, VDR = vitamin D receptor.

Keywords: bone mineral density (BMD), meta-analysis, Osteoprotegerin (OPG) gene, postmenopausal women

1. Introduction

Osteoporosis, characterized by low bone mineral density (BMD), microarchitectural deterioration, and increased bone fragility and fracture risk, is a systemic skeletal disease, especially in the postmenopausal women.1–4 Multiple factors including metabolic factors, environmental factors such as exercise, smoking and diet, and genetic factors were reported to have affected on BMD.5–7 Studies from twins and families have shown that BMD in key skeletal sites such as spine and hip was genetically determined.8,9 A number of susceptible genes such as vitamin D receptor (VDR),10 transforming growth factor b1 (TGBF1),11 calcitonin receptor (CTR),12 and osteoprotegerin gene (OPG), also known as tumor necrosis factor receptor superfamily member 11b (TNFRS11B),13,14 have been identified to be involved in the pathogenesis of osteoporosis. Osteoprotegerin, a member of the tumor necrosis factor receptor superfamily, is one of the most important candidate genes in the control of bone resorption.13,16 Growing evidence has indicated that OPG gene plays an important role in influencing the etiology of osteoporosis.17,18 Several polymorphisms including A163G, G1181C, T245G, T950C, A19163G, and G27563A in OPG gene have
been shown to influence the BMD and development of osteoporosis.\textsuperscript{13–21} The A163G polymorphism, located at the OPG promoter region, was shown to regulate OPG gene expression and may contribute to the genetic regulation of bone mass.\textsuperscript{22} However, the association between the A163G polymorphism and BMD is very contradictory. Although Geng et al\textsuperscript{23} has reported a significant association of A163G polymorphism with lumbar spine and femoral neck BMD, most other studies have shown no association between A163G polymorphism and lumbar spine, femoral hip, and top hip BMD.\textsuperscript{24–26} For G1181C, the first single nucleotide polymorphism (SNP) described in the OPG gene, was shown involved in cellular secretion of OPG.\textsuperscript{27} In previous association studies, genotype of G1181C was related to peripheral BMD in Slovak,\textsuperscript{28} Spanish,\textsuperscript{26} Korean,\textsuperscript{29} American,\textsuperscript{30} and Chinese populations.\textsuperscript{31} However, these positive results cannot be replicated in several other populations such as in Finland,\textsuperscript{32} Australian,\textsuperscript{33} and Irish.\textsuperscript{34} For another 2 common polymorphisms (T245G and T950C), significant associations were observed between genotypes of T245G and T950C and BMD in Japanese\textsuperscript{35} and Finnish populations,\textsuperscript{12} but not in Chinese,\textsuperscript{36} Korean,\textsuperscript{29} and Slovak populations.\textsuperscript{24} These inconsistent in different populations may due to the different ethnic backgrounds, as well as the relatively small number of subjects included in previous studies. Meta-analysis is an effective tool to compensate the limitations by combined all publications and improves statistical power to obtain potential effects of individual studies with small or moderate sizes of subjects. In order to obtain a more precise effect of OPG gene polymorphisms in postmenopausal women, a meta-analysis was performed to assess the association between OPG gene polymorphisms and BMD.

2. Materials and methods

2.1. Patient and public involvement

There was no patient and public involvement in present meta-analysis. Ethical approval is not necessary for a meta-analysis.

2.2. Literature search

An exhaustive literature search for studies on the association of OPG polymorphisms and BMD in postmenopausal women was conducted in the following databases: PubMed, Embase, Cochrane Library, and Chinese National Knowledge Infrastructure (CNKI) using the keywords “osteoporosis” or “OPG” or “tumor necrosis factor receptor superfamily member 1b” or “TNFRS11B” and “polymorphism” or “variation” or “single nucleotide polymorphisms” or “SNP” and “bone density” or “BMD” and “postmenopausal women”. No language was restricted. The last search date was June 1, 2018. All available publications from the database have screened the title firstly. Then the abstracts were checked in case of the titles fulfilled our criteria. Meanwhile, other potentially relevant literatures were identified by searching cross-references within available studies.

2.3. Inclusion and exclusion criteria

Inclusion criteria:

1) number of subjects and genotypes, means, and standard deviation (SD) of BMD were available;
2) all subjects must be postmenopausal women;

Exclusion criteria:

1) repeated studies, letters, dissertations, abstracts or reviews;
2) the outcome was not BMD;
3) only haplotype data;
4) publications that violating the inclusion criteria.

2.4. Data extraction and quality assessment

Two independent authors (YPP and XWS) extracted the information and assessed the quality of each study. The following terms were extracted: the first author, year of published, ethnicity, age in cases, minor allele frequency of OPG polymorphisms, Body Mass Index (BMI) in postmenopausal women, number of subjects in each genotype, and the BMD data for each genotype. All discrepancies were resolved by a consensus achieved by discussion. The study quality was evaluated by the Newcastle-Ottawa Scale (NOS).\textsuperscript{37} Total score ranged from 0 (lowest quality) to 8 (highest quality). A study with a score of 6 or higher was considered to be of high quality.

2.5. Statistical analysis

The standard deviation (SD) of BMD difference between genotypes of A163G, G1181C, T950C, and T245G (A163G: AA vs GG, AA vs AG, AG vs GG; G1181C: GG vs CC, GG vs GC, GC vs CC; T950C: TT vs CC, TT vs TC, TC vs CC; and, T245G: TT vs GG, TT vs TG, TG vs GG) were calculated. Variation and heterogeneity were evaluated using a chi-square-based Cochran Q test and Higgins I-squared statistic ($I^2 = 100 \times (Q–df)/Q$). If significant heterogeneity was observed across studies ($P<.05$ or $I^2 > 50$), the random effect model was used for meta-analysis. Otherwise, the fixed effect model was used. Egger test was used to access publication bias. $P<.05$ indicated a statistical difference. Statistical analyses were performed with the STATA 12.0 software (StataCorp, College Station, TX, USA) and Revman 5 (Cochrane Collaboration, London, UK).

3. Results

3.1. Characteristics of the eligible studies

A total of 2183 publications were originally retrieved from databases. After screened the titles, abstracts and contexts, 1957 were excluded for duplicated studies, 147 were excluded for not related to the association between OPG gene polymorphisms and BMD, 10 were excluded for not related to the association between OPG gene polymorphisms and BMD in postmenopausal women, 47 were excluded for being review, letters, and short communications. Finally, 22 eligible records were selected for data extraction and assessment.\textsuperscript{23–26,28–36,38–46} Fig. 1. Among these publications, 1 paper by Chen et al\textsuperscript{40} contained 2 independent studies. Therefore, there were 23 papers that encompassed 12,973 cases in the present meta-analysis. Two studies referred to the same subjects in Chinese population.\textsuperscript{36,43} 12 groups were conducted in Asian,\textsuperscript{23–29,31–34,36,38,40–44} and 10 groups were in Caucasian.\textsuperscript{24–26,28,30,32,33,39,45,46} For A163G, we enrolled 9 studies consisted of 2933 cases. For G1181C, 14 publications met the inclusion criteria, comprising 11,235 cases. For T950C, 10 publications including 3028 cases were enrolled. The
3.2. Meta-analyses for OPG SNPs and lumbar spine BMD

The 13 publications have shown the association between G1181C and lumbar spine BMD. The pooled results revealed that subjects with the GG genotype were found to have significantly lower BMD values than that with the GC and CC genotypes (GG vs GC: \( P = .0002, \text{SMD} = -0.85, [95\% \text{ CI}] = [1.29, -0.41] \); GG vs CC: \( P = 0.02, \text{SMD} = -0.21, [-0.39, -0.03] \)) (Fig. 2A and B). And individuals with GC genotype have significantly lower BMD values than that with the CC genotype (GG vs CC: \( P = 0.02, \text{SMD} = -0.21, [-0.39, -0.03] \)). Subgroup analysis stratified by ethnicity, shown that the mean BMD in subjects with TT and TC genotypes were significantly lower than those with CC genotype in Caucasian (TT vs CC: \( P = .0002, \text{SMD} = -0.39, [95\% \text{ CI}] = [-0.63, -0.14] \) and TC vs CC: \( P = .0002, \text{SMD} = -0.39, [95\% \text{ CI}] = [-0.63, -0.14] \)). Furthermore, we noticed that subjects with the TC genotype had a slightly lower BMD than

As for T950C polymorphism, 9 publications have shown the association between T950C and lumbar spine BMD. Significant lower BMD values were found in subjects with TC genotype compared with those with CC genotype (\( P = .0004, \text{SMD} = -0.25, [95\% \text{ CI}] = [-0.38, -0.11] \)) (Fig. 2D). Subgroup analysis stratified by ethnicity, shown that the mean BMD in subjects with TT and TC genotypes were significantly lower than those with CC genotype in Caucasian (TT vs CC: \( P = .0002, \text{SMD} = -0.39, [95\% \text{ CI}] = [-0.63, -0.14] \) and TT vs TC: \( P = .0002, \text{SMD} = -0.39, [95\% \text{ CI}] = [-0.63, -0.14] \)). Furthermore, we noticed that subjects with the TC genotype had a slightly lower BMD than
those with CC genotype in Asian (P = 0.01, SMD = -0.37, [95% CI] = [-0.65, -0.09]) (Table 4). In contrast to G1181C and T950C results, no association was observed between the A163G and T245G polymorphisms and lumbar spine BMD (P > 0.05) (Tables 2 and 5).

3.3. Meta-analyses for OPG, SNPs, and femoral hip BMD

The 7 publications have shown the association between A163G and Femoral hip BMD. A slightly higher femoral hip BMD was found in subjects with AA genotype compared to those with AG genotype (P = 0.03, SMD = 0.49, [95% CI] = [0.06, 0.91])(Fig. 3). However, this significant difference didn’t exist in ethnicity-specific meta-analysis (P > 0.05) (Table 2). In addition, 12 publications have reported the association between G1181C and femoral hip BMD (Table 3). The individuals with G1181C GG genotype had significantly lower femoral hip BMD compared to those with CC genotype in Caucasian (P = 0.001, SMD = -0.10, [95% CI] = [-0.15, -0.04]). No association was found between T950C, T245G and femoral hip BMD (P > 0.05) (Tables 4 and 5).

3.4. Meta-analyses for OPG SNPs and total hip BMD

The 7 publications have shown the association between A163G and Total hip BMD. A slightly higher total hip BMD was found in subjects with AA genotype compared to those with AG genotype (P = 0.02, SMD = -0.25, [95% CI] = [-0.42, -0.09]) (Fig. 4A). However, this significant difference didn’t exist in ethnicity-specific meta-analysis (P > 0.05) (Table 2). As for G1181C, GG genotype were found to have significantly lower BMD values than that with the GC and CC genotypes (GG vs GC: P = 0.006, SMD = -0.10, [95% CI] = [-0.16, -0.04]) and Asian (P = 0.03, SMD = -0.85, [95% CI] = [-1.64, -0.06]), and that with the GC genotypes in Caucasian (P = 0.002, SMD = -0.08, [95% CI] = [-0.13, -0.03]). In addition, The GC genotype were found to have significantly lower BMD values than that with the CC genotypes in Asian (P = 0.008, SMD = -0.43, [95% CI] = [-0.75, -0.12]) (Table 3). Furthermore, a slightly lower total hip BMD was detected in subjects with T950C TT genotype compared to those with TC genotype in Caucasian (P = 0.04, SMD = -0.16, [95% CI] = [-0.32, -0.00]) (Table 4). No association was found between T245G and total hip BMD (P > 0.05) (Table 5).

Table 1

| First author | Year | Ethnicity | Number | Age | BMI (kg/m²) | BMD type | SNPs (MAF) | NOS scores |
|--------------|------|-----------|--------|-----|-------------|----------|------------|------------|
| Peng et al.  | 2018 | Medicine  | 97:51  |     |             |          |            |            |

NOS = Newcastle-Ottawa Scale, BMI = Body Mass Index, lumbar spine = LS, Femoral hip = FH, Total hip = TH, SNPs = SNPs = single nucleotide polymorphism, MAF = minor allele frequency, NA = not available.
Figure 2. Association between genotypes of G1181C and T950C and lumbar spine BMD. A: G1181C, GG vs CC; B: G1181C, GG vs GC; C: G1181C, GC vs CC; D: T950C, TC vs CC.
### Table 2
The association between osteoprotegerin gene (OPG) A163G and BMD in postmenopausal women.

| BMD/Polymorphism | Genotype | Population | Number of studies | SMD  | 95% CI       | P value | Model | Test of heterogeneity |
|------------------|----------|------------|-------------------|------|-------------|---------|-------|-----------------------|
| Lumbar spine L1–L4 | AA vs GG | Overall    | 6                 | 0.93 | [0.94, 0.72] | .18     | R     | <.00001               |
|                  |          | European   | 4                 | 0.22 | [0.06, 0.83] | .12     | F     | .44                   |
|                  |          | Asian      | 2                 | 2.18 | [1.25, 0.49] | .32     | R     | <.00001               |
|                  | AA vs AG | Overall    | 8                 | 0.04 | [0.04, 0.73] | .08     | R     | <.00001               |
|                  |          | European   | 5                 | 0.00 | [0.16, 0.83] | .52     | F     | .57                   |
|                  |          | Asian      | 3                 | 0.05 | [0.15, 2.24] | .09     | R     | <.00001               |
|                  | AG vs GG | Overall    | 6                 | 0.55 | [0.39, 1.41] | .25     | R     | <.00001               |
|                  |          | European   | 4                 | 0.30 | [0.06, 0.67] | .10     | F     | .49                   |
|                  |          | Asian      | 2                 | 0.94 | [0.00, 0.85] | .44     | R     | <.00001               |
| Femoral hip      | AA vs GG | Overall    | 6                 | 0.55 | [0.39, 1.41] | .25     | R     | <.00001               |
|                  |          | European   | 4                 | 0.22 | [0.05, 0.77] | .62     | F     | .25                   |
|                  |          | Asian      | 2                 | 2.75 | [1.25, 7.66] | .27     | R     | <.00001               |
|                  | AA vs AG | Overall    | 7                 | 0.49 | [0.06, 0.81] | .03     | R     | <.00001               |
|                  |          | European   | 4                 | 0.03 | [0.00, 0.14] | .53     | F     | .89                   |
|                  |          | Asian      | 3                 | 1.23 | [0.17, 2.64] | .09     | R     | <.00001               |
|                  | AG vs GG | Overall    | 6                 | 0.32 | [0.04, 1.39] | .55     | R     | <.00001               |
|                  |          | European   | 4                 | 0.00 | [0.41, 0.22] | .55     | F     | .48                   |
|                  |          | Asian      | 2                 | 0.22 | [0.01, 0.43] | .25     | R     | <.00001               |
|                  | Total    | AA vs GG   | 6                 | 0.87 | [0.57, 2.32] | .24     | R     | <.00001               |
|                  |          | European   | 4                 | 0.02 | [0.26, 0.02] | .36     | F     | .32                   |
|                  |          | Asian      | 2                 | 2.76 | [0.51, 1.76] | .27     | R     | <.00001               |
|                  | AA vs AG | Overall    | 7                 | 0.03 | [0.12, 1.10] | .02     | R     | <.00001               |
|                  |          | European   | 4                 | 0.00 | [0.41, 0.11] | .08     | F     | .68                   |
|                  |          | Asian      | 3                 | 1.69 | [0.14, 3.25] | .03     | R     | <.00001               |
|                  | AG vs GG | Overall    | 6                 | 0.37 | [0.49, 1.34] | .45     | R     | <.00001               |
|                  |          | European   | 4                 | 0.02 | [0.30, 0.34] | .90     | F     | .41                   |
|                  |          | Asian      | 2                 | 1.71 | [0.97, 3.20] | .29     | R     | <.00001               |

- R = random model; F = fixed model. SMD = standard mean difference. CI = confidence intervals. BMD = bone mineral density.
- P value: chi-square-based Cochran Q test; F: Higgins I-squared statistic.

### Table 3
The association between osteoprotegerin gene (OPG) G1181C and BMD in postmenopausal women.

| BMD/Polymorphism | Genotype | Population | Number of studies | SMD  | 95% CI       | P value | Model | Test of heterogeneity |
|------------------|----------|------------|-------------------|------|-------------|---------|-------|-----------------------|
| Lumbar spine L1–L4 | GG vs CC | Overall    | 13                | 0.95 | [1.29, 0.41] | .002    | R     | <.00001               |
|                  |          | European   | 9                 | 0.22 | [0.42, 0.02] | .03     | R     | <.00001               |
|                  |          | Asian      | 4                 | 2.63 | [5.34, 0.08] | .06     | R     | <.00001               |
|                  | GG vs GC | Overall    | 12                | 0.21 | [0.39, 0.03] | .02     | R     | <.00001               |
|                  |          | European   | 9                 | 0.07 | [0.17, 0.03] | .18     | R     | .03                   |
|                  |          | Asian      | 3                 | 0.72 | [1.68, 0.25] | .15     | R     | <.00001               |
|                  | GC vs CC | Overall    | 12                | 0.64 | [0.08, 0.31] | .0002   | R     | <.00001               |
|                  |          | European   | 9                 | 0.16 | [0.32, 0.00] | .06     | R     | <.00001               |
|                  |          | Asian      | 3                 | 2.69 | [5.06, 0.31] | .03     | R     | <.00001               |
| Femoral hip      | GG vs CC | Overall    | 13                | 0.95 | [1.29, 0.41] | .002    | F     | .49                   |
|                  |          | European   | 8                 | 0.06 | [0.12, 0.00] | .05     | F     | .93                   |
|                  |          | Asian      | 3                 | 0.86 | [2.28, 0.56] | .24     | R     | <.00001               |
|                  | GC vs CC | Overall    | 11                | 0.19 | [0.45, 0.07] | .15     | R     | <.00001               |
|                  |          | European   | 8                 | 0.03 | [0.09, 0.03] | .34     | F     | .35                   |
|                  |          | Asian      | 3                 | 0.54 | [3.07, 1.39] | .67     | R     | <.00001               |
|                  | Total    | GG vs CC   | 10                | 0.25 | [0.42, 0.09] | .002    | R     | <.00001               |
|                  |          | European   | 8                 | 0.10 | [0.16, 0.04] | .0006   | F     | .49                   |
|                  |          | Asian      | 2                 | 0.85 | [1.64, 0.06] | .03     | R     | .93                   |
|                  | GG vs GC | Overall    | 10                | 0.15 | [0.26, 0.03] | .01     | R     | <.00001               |
|                  |          | European   | 8                 | 0.08 | [0.13, 0.03] | .002    | F     | .61                   |
|                  |          | Asian      | 2                 | 0.38 | [1.07, 0.31] | .26     | R     | <.00001               |
|                  | GC vs CC | Overall    | 10                | 0.08 | [0.18, 0.02] | .13     | R     | .02                   |
|                  |          | European   | 10                | 0.04 | [0.13, 0.05] | .38     | F     | .09                   |
|                  |          | Asian      | 2                 | 0.43 | [0.75, 0.12] | .008    | F     | .60                   |

- R = random model; F = fixed model. SMD = standard mean difference. CI = confidence intervals. BMD = bone mineral density.
- P value: chi-square-based Cochran Q test; F: Higgins I-squared statistic.
The association between osteoprotegerin gene (OPG) T950C and BMD in postmenopausal women.

| BMD/Polyorphism | Genotype | Population | Number of studies | SMD [95% CI] | P value | Model | Test of heterogeneity |
|-----------------|----------|------------|-------------------|--------------|---------|-------|-----------------------|
| Lumbar spine L1-L4 | TT vs CC | Overall | 9 | -0.35 [-0.78, 0.07] | .10 R | <.0001 | 88 |
|                 |          | European  | 3 | -0.37 [-0.65, -0.09] | .01 F | .60 | 0 |
|                 |          | Asian     | 6 | -0.33 [-0.04, 0.29] | .30 R | <.0001 | 92 |
|                 | TT vs TC | Overall | 8 | -0.16 [-0.75, 0.44] | .60 R | <.0001 | 96 |
|                 |          | European  | 3 | 0.20 [-0.25, 0.65] | .39 R | .003 | 83 |
|                 |          | Asian     | 5 | -0.37 [-1.07, 0.34] | .30 R | <.0001 | 95 |
|                 | TC vs CC | Overall | 8 | -0.25 [-0.36, -0.11] | 0.0004 F | .53 | 0 |
|                 |          | European  | 3 | -0.39 [-0.63, -0.14] | .002 F | .28 | 21 |
|                 |          | Asian     | 5 | -0.18 [-0.35, -0.02] | .03 F | .78 | 0 |
| Femoral hip     | TT vs CC | Overall | 8 | -0.21 [-0.65, 0.23] | .36 R | <.0001 | 92 |
|                 |          | European  | 2 | -0.15 [-0.32, 0.02] | .08 F | .33 | 0 |
|                 |          | Asian     | 6 | -0.18 [-0.82, 0.47] | .59 R | <.0001 | 93 |
|                 | TT vs TC | Overall | 7 | -0.16 [-0.39, 0.08] | .19 R | .0001 | 78 |
|                 |          | European  | 2 | -0.12 [-0.27, 0.02] | .10 F | .70 | 0 |
|                 |          | Asian     | 5 | -0.15 [-0.51, 0.22] | .43 R | .0002 | 82 |
|                 | TC vs CC | Overall | 7 | -0.14 [-0.57, 0.29] | .52 R | <.0001 | 91 |
|                 |          | European  | 2 | -0.02 [-0.16, 0.12] | .78 F | .38 | 0 |
|                 |          | Asian     | 5 | -0.15 [-0.70, 0.50] | .66 R | <.0001 | 89 |
| Total hip       | TT vs CC | Overall | 7 | -0.35 [-1.08, 0.38] | .35 R | <.0001 | 96 |
|                 |          | European  | 2 | -0.18 [-0.48, 0.11] | .22 R | .16 | 50 |
|                 |          | Asian     | 5 | -0.39 [-1.43, 0.66] | .47 R | <.0001 | 96 |
|                 | TT vs TC | Overall | 7 | -0.37 [-1.04, 0.29] | .27 R | <.0001 | 97 |
|                 |          | European  | 2 | -0.16 [-0.30, -0.01] | .04 F | .49 | 0 |
|                 |          | Asian     | 5 | -0.44 [-1.37, 0.49] | .36 R | <.0001 | 97 |
|                 | TC vs CC | Overall | 7 | -0.10 [-0.38, 0.17] | .46 R | .0002 | 77 |
|                 |          | European  | 2 | 0.03 [-0.12, 0.18] | .73 F | .30 | 6 |
|                 |          | Asian     | 5 | -0.14 [-0.52, 0.24] | .47 R | .02 | 66 |

R = random model, F = fixed model, SMD = standard mean difference, CI = confidence intervals, BMD = bone mineral density.

P value: chi-square-based Cochran Q test; I²: Higgins I-squared statistic.

3.5. Test of heterogeneity

Significant between-study heterogeneity were detected in all the meta-analysis of A163G, G1181C, T950C, and T245G polymorphisms (Table 2–5). Therefore, subgroup analysis stratified by ethnicity was conducted. Notable, most of the between-study heterogeneity in Caucasian disappeared (except for G1181C, T950C (TT vs TC) and T245G in lumbar spine BMD, T950C (TT vs CC) in total hip BMD). The significant heterogeneity in A163G were contributed mainly by Gen et al. Removal of this study from meta-analysis gave 0% to 32% (P > .05) heterogeneity. The significant heterogeneity in T245G were contributed mainly by Yamada et al. Removal of these studies from meta-analysis gave 0% to 36% (P > .05) heterogeneity. In addition, the significant heterogeneity in T950C were contributed mainly by Yamada et al. Removal of these studies from meta-analysis gave 0% to 27% (P > .05) heterogeneity. In addition, Hardy-Weinberg equilibrium fitness by using the chi-square goodness-of-fit test for all the genotypes of A163G, G1181C, T245G, and T950C were performed out. The data in Kim et al for G1181C, and Chen et al for T950C were not in Hardy-Weinberg equilibrium (Table S1, http://links.lww.com/MDC/703). After excluding the study conducted by Kim et al the pooled SMDs of G1181C have no significant change. After excluded the study conducted by Chen et al The combined SMDs changed slightly, which may indicate the state of Hardy-Weinberg equilibrium fitness has no effect on the association between OPG polymorphisms (A163G, G1181C, T245G, and T950C) SNPs and BMD in postmenopausal women.

Figure 3. Association between AA genotype of A163G and femoral hip BMD compared with AG genotype.
3.6. Publication bias

The results of Egger regression test for A163G have shown slight publication bias of individuals with AG genotype of A163G compared to those with GG genotype at lumbar spine, femoral hip and total hip BMD \((P < .05)(\text{Table 6}).\) And the funnel plots of A163G, T950C, and T245G showed no apparent evidence of publication bias was found for another comparison of A163G, G1181C, T950C, and T245G (Table 6).

4. Discussion

In present study, we investigated the effect of OPG polymorphisms (A163G, G1181C, T245G, and T950C) on the BMD in postmenopausal women and detected the A163G may be associated with the femoral hip and total hip BMD, G1181C and T950C may be associated with the lumbar spine and total hip BMD. In addition, T245G has no effect on BMD in postmenopausal women.

Osteoprotegerin (OPG) has been discovered in regulating osteoclastogenesis in 1997.\(^{[47,48]}\) Together with receptor activator of nuclear factor-B ligand (RANKL),\(^{[49]}\) receptor activator of nuclear factor-B (RANK),\(^{[50]}\) OPG plays a key role in osteoclastogenesis. Transgenic mice (OPG \((-/-)\)) exhibited a decreased total bone density and developed severe osteoporosis,\(^{[51]}\) whereas mice over-expressing OPG develop an osteopetrotic phenotype.\(^{[52]}\) OPG is 1 member of the TNF and TNF receptor superfamily, encoded in humans by the \(TNFRSF11B\) gene that is located at 8q24.12.\(^{[53]}\) Many genetic polymorphisms, such as A163G, T245G, T950C and G1181C, A27450T, and G19074A, have been investigated to be associated with BMD and osteoporosis.\(^{[54,55]}\)

The A163G polymorphism located in the promoter region of OPG gene and was identified by Kusk.\(^{[56]}\) Although no recognition sites of the known transcription factors have been found, there is a possibility that the OPG polymorphism is in linkage disequilibrium (LD) with nearby genetic variations that are associated with BMD.\(^{[57]}\) In previous studies, the G allele of A163G polymorphism
was shown to be a risk factor for low BMD. Among the included studies, only Geng et al have reported that the AA genotype of A163G was associated with the lumbar spine, femoral hip and total hip BMD in Chinese. However, our combined results showed that the subjects with AA genotype of A163G have significant higher femoral hip and total hip BMD in postmenopausal women. It seemed the AA genotype of A163G has more effect on the femoral hip and total hip BMD, but not lumbar spine BMD in postmenopausal women. The results were slightly different from the previous meta-analysis conducted by Lee et al, which may mainly due to the 6 more included publications in our study. Notable, the difference of femoral hip and total hip BMD in postmenopausal women disappeared in Caucasian and Asian populations, which may due to the limited number of studies in ethnic subgroup analysis.

The G1181C polymorphism has been firstly discovered in Irish postmenopausal women in 2002. Most subsequent researches have indicated the G1181C was associated with BMD. Zhao et al reported that the individuals with 1181G allele have lower lumbar spine BMD and 2.7 fold risk of osteoporosis than those with 1181C. Similar results were observed in 6640 American postmenopausal women. However, no association was also reported between G1181C and BMD in Maltese and Australian postmenopausal women. Lee et al have investigated the association between G1181C and BMD using a meta-analysis with 3 studies and found the GG genotype of G1181C might have a significantly lower lumbar, femoral neck, and total hip BMD than subjects with the CC genotype. However, the results changed in subgroup analysis stratified by ethnicity. Notable, subjects such as premenopausal women, postmenopausal women, and males with osteoporosis were all included in study conducted by Lee et al, while, no subgroup analysis were performed. In addition, most studies conducted in Chinese population were not included in Lee et al for the language limited and earlier publication years, which might partly influence the final results. Subsequently, Zhang et al performed a meta-analysis on the association between G1181C and BMD with 9 studies and found that GG and GC genotypes of G1181C seems to have significantly lower mean lumbar BMD than subjects with the CC genotype in Asian population, GG and GC genotypes of G1181C may have significantly lower mean femoral neck BMD than subjects with the CC genotype in Caucasian population. In present study, we included 13 studies in analyzing the association between G1181C and BMD. More complicated results were detected. GG genotype of G1181C seems to have significantly lower lumbar, femoral neck and total hip BMD than subjects with the CC genotype in Caucasian population. In addition, GC genotype of G1181C seems to have significantly lower lumbar and total hip BMD than subjects with the CC genotype in Asian population. The inconsistent in these 3 meta-analyses might mainly due to the limited number of included studies and subjects. To identify the results, more studies larger number of individuals was needed in the future.

For T950C, we observed that subjects with TT genotype of T950C seem to have significantly lower lumbar BMD than those with the CC genotype in Caucasian population, TC genotype of T950C seems to have significantly lower lumbar BMD than subjects with the CC genotype both in Caucasian and Asian populations, which were significantly different from the results reported by Lee et al these difference may mainly due to the larger number of subjects in present study.

### Table 5

| BMD/Polymorphism | Genotype | Population | Number of studies | SMD | 95% CI | P value | Model | Test of heterogeneity | P value | F (%) |
|------------------|----------|------------|-------------------|-----|--------|---------|-------|-----------------------|---------|-------|
| Lumbar spine L1-L4 | TT vs GG | Overall | 5 | 2.01 | [-3.13, 7.16] | .44 | R | <.00001 | 99 |
|                   |          | European | 2 | 0.07 | [-1.16, 1.30] | .91 | R | .14 | 53 |
|                   |          | Asian | 3 | 3.36 | [-4.74, 11.46] | .42 | R | <.00001 | 99 |
|                   | TT vs TG | Overall | 9 | 0.01 | [-0.42, 0.44] | .97 | R | <.00001 | 92 |
|                   |          | European | 4 | 0.06 | [-0.27, 0.39] | .72 | R | .04 | 64 |
|                   |          | Asian | 5 | -0.05 | [-0.78, 0.69] | .90 | R | <.00001 | 95 |
|                   | TG vs GG | Overall | 5 | 1.22 | [-1.37, 3.81] | .36 | R | <.00001 | 98 |
|                   |          | European | 2 | 0.32 | [-0.96, 1.60] | .63 | R | .14 | 53 |
|                   |          | Asian | 3 | 1.87 | [-2.22, 5.95] | .37 | R | <.00001 | 99 |
| Femoral hip | TT vs GG | Overall | 4 | 3.52 | [-4.19, 11.22] | .37 | R | <.00001 | 98 |
|                   |          | European | 2 | 0.23 | [-0.58, 1.03] | .58 | F | .38 | 0 |
|                   |          | Asian | 2 | 6.92 | [-8.33, 22.17] | .37 | R | <.00001 | 99 |
|                   | TT vs TG | Overall | 6 | -0.04 | [-0.61, 0.53] | .89 | R | <.00001 | 94 |
|                   |          | European | 3 | -0.06 | [-0.46, 0.34] | .78 | R | .03 | 73 |
|                   |          | Asian | 3 | -0.06 | [-1.32, 1.21] | .93 | R | <.00001 | 98 |
|                   | TG vs GG | Overall | 4 | 2.07 | [-1.70, 5.84] | .28 | R | <.00001 | 98 |
|                   |          | European | 2 | 0.42 | [-0.42, 1.25] | .33 | F | .54 | 0 |
|                   |          | Asian | 2 | 3.80 | [-4.17, 11.77] | .35 | R | <.00001 | 98 |
| Total hip | TT vs GG | Overall | 3 | 5.03 | [-4.93, 14.99] | .32 | R | <.00001 | 99 |
|                   |          | European | 1 | 0.35 | [-0.64, 1.34] | .49 | - | - | - |
|                   |          | Asian | 2 | 7.37 | [-8.20, 22.94] | .35 | R | <.00001 | 99 |
|                   | TT vs TG | Overall | 7 | 0.12 | [-0.17, 0.42] | .42 | R | <.00001 | 81 |
|                   |          | European | 3 | -0.05 | [-0.33, 0.23] | .71 | F | .16 | 46 |
|                   |          | Asian | 4 | 0.24 | [-0.23, 0.72] | .32 | R | <.00001 | 87 |
|                   | TG vs GG | Overall | 3 | 2.93 | [-1.84, 7.71] | .23 | R | <.00001 | 99 |
|                   |          | European | 1 | 0.56 | [-0.46, 1.59] | .28 | - | - | - |
|                   |          | Asian | 2 | 4.12 | [-3.38, 12.13] | .31 | R | <.00001 | 99 |

R = random model, F = fixed model; SMD = standard mean difference; CI = confidence intervals; BMD = bone mineral density. P value: chi-square-based Cochran Q test; I²: Higgins I-squared statistic.
### Table 1

| Gene     | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A163G    | 0.309   | 0.056   | 0.020   | 0.020   | 0.257   | 0.054   | 0.020   | 0.020   | 0.257   |
| BMD      | 44.633  | 4.633   | 3.264   | 3.264   | 2.796   | 2.264   | 2.264   | 2.264   | 2.264   |
| FH       | 6.157   | 6.157   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   |
| LS       | 16.117  | 16.117  | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   |
| FN       | 22.078  | 22.078  | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   |
| TH       | 3.296   | 3.296   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   | 3.630   |

Several limitations should be considered in present study. First, although more number of studies has been enrolled in present study, the number of studies included in this meta-analysis was relatively small and insufficient to detect associations with small effects, especially in terms of subgroup analysis stratified by ethnicity. Second, the interaction between gene polymorphisms and metabolic factors and environmental factors, as well as other genes and the OPG gene may also be risk factors for low BMD in postmenopausal women. Thirdly, several polymorphisms in OPG gene has been shown to be in linkage disequilibrium, which may indicate not only polymorphisms in OPG gene, but also the haplotypes containing OPG polymorphisms were associated with the risk of BMD in postmenopausal women. However, the polymorphisms contained in haplotypes in each study were not consistent. We failed to performed a linkage analysis of OPG haplotypes and BMD risk.

## 5. Conclusions

The present study demonstrates that A163G might be associated with the femoral hip and total hip BMD, G1181C and T950C might be associated with the lumbar spine and total hip BMD. And, T245G has no effect on BMD in postmenopausal women.

### Author contributions

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### References

[1] Siaw EA, Vanderford V. Osteoporosis and bone mineral density. Radiol Technol 2001;72:383-6.
[2] Zaidi P, Hanif M, Murad R, et al. Concordance of bone mineral density (BMD) and biochemical parameters for the diagnosis of osteoporosis in postmenopausal women studied at Khan, Karachi, Pakistan. Int J BioBiotechnol 2010;7:437–8.
[3] Yun KJ, Bo HK, Kim JI. The diagnosis of osteoporosis. J Korean Med Assoc 2016;59:842–8.
[4] Brugue MBMS. Polish guidelines for the diagnosis and management of osteoporosis: a review of 2013 update. Pol Arch Med Wewn 2013;124:255–63.
[5] Binici DN, Gunes N. Risk factors leading to reduced bone mineral density in hemodialysis patients with metabolic syndrome. Ren Fail 2010;32:469–74.
[6] Ng MY, Sham PC, Paterson AD, et al. Effect of environmental factors and gender on the heritability of bone mineral density and bone size. Ann Hum Genet 2012;76:428–38.
[7] Kaufman JM, Ostertag A, Saint-Pierre A, et al. Genome-wide linkage screen of bone mineral density (BMD) in European pedigrees ascertained through a male relative with low BMD values: evidence for quantitative trait loci on 17q21-23, 11q12-13, 13q12-14, and 22q11. J Clin Endocrinol Metab 2008;93:3753–62.
[8] Peacock M, Turner C, Econs M, et al. Genetics of osteoporosis. Endocr Rev 2002;23:303–26.
[9] Ralston SH. Genetic control of susceptibility to osteoporosis. J Clin Endocrinol Metab 2002;87:2460–6.
[10] Cantocetina T, Cetina Manzanilla JA, González HL, et al. VDR polymorphisms are associated with bone mineral density in postmenopausal Mayan-Mestizo women. Ann Hum Biol 2014;41:460–6.
[11] Krela Kaźmierczak I, Michalak M, Wawrzyniak A, et al. The c.297>T>C polymorphism of the transforming growth factor beta-1 (TGFB1) gene, bone mineral density and the occurrence of low-energy fractures in patients with inflammatory bowel disease. Mol Biol Rep; 2017:1-7.
[12] Bandräš E, Pombo I, Gonzál M I, et al. Association between bone mineral density and polymorphisms of the VDR, ERα,p COL1A1 and CTR genes in Spanish postmenopausal women. J Endocrinol Invest; 2005; 8 (6):312–21.

[13] Wang F, Gao Y, Li F, et al. Association Analysis between g.18873C>T and g.27522G>A gene polymorphisms of OPG and bone mineral density in Chinese postmenopausal women. Biomed Res Int 2015;2015:108283.

[14] Myliárova Blášáčková M, Poračová J, Mydlár J, et al. Relationship between A163G osteoprotegerin gene polymorphism and other osteoporosis parameters in Roma and non-Roma postmenopausal women in western Slovakia. J Clin Lab Anal 2017;31:1–11.

[15] Riggs L, Baron R, Boyle W, et al. Proposed standard nomenclature for postmenopausal women. Curr Biochem 2013;46:1493–501.

[16] Rojano-Mejía D, Coral-Vázquez RM, Espinosa LC, et al. TNFRSF1B, gene haplotype and its association with bone mineral density variations in postmenopausal Mexican-Mestizo women. Maturitas 2012;71:49–54.

[17] Chen LX, Miao YD, Liu J, et al. Association between T245G polymorphisms in the osteoprotegerin gene and bone mineral density in elderly individuals. J Clin Rehabil Tissue Eng Res 2011;15:2069–73.

[18] Cheng Q, Zhu HM, Miao YX, et al. Effect of osteoprotegerin gene polymorphism on bone mass in postmenopausal women. Natl Med J China 2004;84:274–7.

[19] Liu J, Miao Y, Zhang Y. The study of the association between T950C gene polymorphism in the promoter region of osteoprotegerin and bone mineral density in postmenopausal women. Chin J Osteoporos 2010;16:184–6.

[20] Wu Z, Feng J, Liu M, et al. The relationship between body fat ratio combined with G209-A and T245-G polymorphism of osteoprotegerin promoter region and osteoporosis. Chin J Genetol 2007;24:2409–12.

[21] Yu LY, Zhou XY, Xing XP, et al. Association between osteoprotegerin gene polymorphisms and bone mineral density of pre-and postmenopausal Han women from Beijing areas. Chin J Clin Rehabil Tissue Eng Res 2006;10:204–7.

[22] Canto-Cetina T, Polanco Reyes L, González Herrera L, et al. Polymorphism of LRP5, but not of TNFRSF1B, is associated with a decrease in bone mineral density in postmenopausal maya-mestizo women. Am J Hum Biol 2013;25:713–7.

[23] Szemiráková A, Bablik M, Drews K, et al. The genetic variants of RANKL/RANK/OPG signal trial in postmenopausal women with osteoporosis and osteopenia. Arch Perinat Med 2011;17:72–80.

[24] Taboulet J, Frenkian M, Frondel J, et al. Calcitonin receptor polymorphism is associated with a decreased fracture risk in postmenopausal women. Hum Mol Genet 1997;8:2129–33.

[25] Liu KB, Harrop J, Reddy M, et al. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic cell lineages. Gene 1998;225:85–91.

[26] Zheng H, Wang C, He JW, et al. Osteoprotegerin and RANKL gene polymorphisms in early-onset osteoporosis and its association with bone mineral density in Chinese postmenopausal women. Mol Genet Metabol 2003;80:344–50.

[27] Krajcovicova V, Omelka R, Darusova J, et al. The effect of A163G polymorphism in the osteoprotegerin gene on osteoporosis related traits in Slovak postmenopausal women 2015;7:231–9.

[28] Lee YH, Woo JH, Choi SJ, et al. Associations between osteoprotegerin polymorphisms and bone mineral density: a meta-analysis. Mol Biol Rep 2010;37:227–34.

[29] Zhang H, Gan L, Guo Y, et al. Associations between osteoprotegerin gene G1181C polymorphism and bone mineral density in postmenopausal women: a meta-analysis. Chin J Tissue Eng Res 2013;17:609–700.