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# Association of genetic polymorphisms of RANK, RANKL and OPG with bone mineral density in Chinese peri- and postmenopausal women

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ABSTRACT

**Objectives:** To explore the influence of 14 single nucleotide polymorphisms (SNPs) in receptor activator of nuclear factor-kappa B (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) on bone mineral density (BMD) in a Chinese female population.

**Design and methods:** A cross-sectional study was conducted in 108 perimenopausal and 127 postmenopausal women aged 43–65 years. All participants underwent lumbar spinal and nondominant femoral BMD evaluation by dual energy X-ray absorptiometry. Fourteen RANK, RANKL and OPG genotypes were determined by chip-based MALDI-TOF mass spectrometry. The differences between the BMDs of the RANK genotypes were analyzed.

**Results:** Five SNPs (rs6993813, rs4355801, rs1032129 and rs2073618 in OPG and rs3018362 in RANK) were significantly associated with BMD or with BMD adjusted for body weight or years since menopause, mostly at the femoral neck but also partly at the total hip ($p < 0.05$). The risk allele frequencies observed in our sample were different from those found in Europeans but the effects of these risk alleles on BMD values had the same direction in our cohort as in Europeans, except for rs3018362 with G as the risk allele, which was contrary to other studies. None of the SNPs in RANKL were associated with BMD at any anatomical site.

**Conclusions:** Our findings suggest that OPG and RANK but not RANKL genetic polymorphisms influence BMD mainly in the femoral neck in peri- and postmenopausal Chinese women. This contributes to the understanding of the role of genetic variation in this pathway in determining bone health.



## Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength that predisposes the afflicted person to an increased risk of fracture [1]. Bone strength is influenced by a number of factors and approximately 70% of bone strength variation can be predicted by bone mineral density (BMD). Although bone remodeling is affected by many environmental and hormonal factors, family and twin studies have confirmed that approximately 70% of BMD variability is genetically determined [2].

Over the last decades, numerous candidate genes have been investigated and linked with bone density or fracture risk [3]. In an attempt to identify the genes that are involved in the regulation of bone health-related phenotypes, genetic linkage analyses [4,5], candidate gene association studies [6] and, recently, genome-wide association studies (GWASs) [7,8] have been used to implicate several loci and candidate genes such as osteoprotegerin (OPG), receptor activator of nuclear factor-kappa B (NF-κB) (RANK), and RANK ligand (RANKL) [7–9], all of which have a critical role in bone remodeling and have shown highly suggestive associations with BMD/osteoporosis, enabling a prioritization of possible osteoporosis candidate genes from the many proposed in recent years [10]. Regardless, the majority of genes that contribute to genetic susceptibility to osteoporosis remain to be elucidated.

A number of studies suggest that polymorphisms in RANKL, RANK, and OPG may influence bone density but the results are not entirely consistent [11,12], which may be due to ethnic differences or differential associations of the polymorphisms with BMD from different bone sites [11,13]. In the largest meta-analysis to date, which included 17 GWASs and 32,961 individuals of European and East Asian ancestry, 56 loci were identified to be associated with lumbar spine and femoral neck BMD at genome-wide significance [14]. The strongest connections were seen for members of three key biological pathways: the

*Abbreviations:* SNP, single nucleotide polymorphism; NF-κB, nuclear factor-kappa B; RANK, receptor activator of nuclear factor-kappa B; RANKL, receptor activator of nuclear factor-kappa B ligand; OPG, osteoprotegerin; BMD, bone mineral density; GWAS, genome-wide association study; DXA, dual energy X-ray absorptiometry; CV, coefficient of variation; WHO, World Health Organization; MALDI-TOF, matrix assisted laser desorption ionization-time of flight; PCR, polymerase chain reaction; BMI, body mass index; SD, standard deviation; ANOVA, one-way analysis of variance; GLM, generalized linear model; YSM, years since menopause; SSCP, single-strand confirmation polymorphism.

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RANK-RANKL-OPG, the mesenchymal stem cell differentiation, and the Wnt signaling pathways. While genetic variations of these essential pathways seem to influence BMD and bone turnover, these results have still to be confirmed in different populations.

To our knowledge, the association of the RANK and RANKL genes with the osteoporotic phenotype has not been widely studied and the RANKL, RANK, and OPG genes have been analyzed mainly in populations of European ancestry. Because most previous GWAS results were derived from Europeans, more studies are needed to confirm susceptibility loci, especially in different ethnic groups.

Osteoporosis and osteopenia are common in peri- and postmenopausal women due to estrogen deficiency. The aim of this study was to determine whether genetic polymorphisms in RANKL, RANK, and OPG were associated with BMD variations in Chinese peri- and postmenopausal women. In this study, we selected 14 SNPs in RANKL (6 SNPs), RANK (3 SNPs), and OPG (5 SNPs) based on several factors, including functional significance, previous association studies, and allele frequencies [8]. The rs1805034 SNP was selected because this site showed different single-strand conformation polymorphism (SSCP) migration patterns and to be associated with calcaneal BMD in Korean postmenopausal women [15]. In a recent study aimed at identifying sequence variants associated with BMD and fracture in 5861 Icelandic subjects, rs3018362, which is located within the same linkage disequilibrium block on RANK, showed a consistent association with hip BMD, while rs6993813 and rs6469804 of OPG were shown to be associated with hip and lumbar spine BMD, which is why we chose to study these SNPs [8]. The rs12458117 SNP was selected because of its significant association with the BMD of the lumbar spine and proximal femur sites in Korean postmenopausal women and as a possible genetic factor for low BMD [16]. rs2073618 was selected because it is the only nonsynonymous polymorphism in the signal peptide involved in the cellular secretion of OPG and its association with BMD [17,18]. rs3102735 is associated with the stress strain index [19,20] and rs1032129 is associated with low BMD at different skeletal sites [21]. rs4355801 was selected because of its significant association with cortical volumetric BMD [22]. rs9533155 (−693G>C) and rs9533156 of RANKL were selected because of their associations with lumbar spine BMD [23] and because rs9533155 is associated with bone loss in the hip and femoral neck [24]. The rs12585014, rs7988338, and rs2148073 polymorphisms of RANKL were selected because they are significantly associated with the femoral neck compression strength index, a novel phenotypic parameter that integrates bone density, bone size, and body size, and has significant potential to improve hip fracture risk assessment [25]. The characteristics of these SNPs are shown in Table 1.

## Materials and methods

### Subjects

As osteoporosis predominantly affects postmenopausal women, the present study was performed only in peri- and postmenopausal females. Two hundred and thirty-five community-dwelling peri- and postmenopausal Chinese Han female volunteers, aged 43–65 years, were recruited. Each patient was examined clinically and routine biochemical tests were performed. Calcium intake and physical activity were calculated and recorded. Women were excluded if they were suffering from diseases such as thyroid and parathyroid diseases, renal failure, autoimmune diseases (e.g., systemic lupus erythematosus and rheumatoid arthritis), nephrotic syndrome, or if they had a past or current history of any cancer. Women who had taken drugs that may affect bone metabolism for more than 6 months or within the previous 12 months were also excluded. Calcium intake was calculated on the basis of the number of portions of dairy products and calcium supplements the women consumed daily. Physical activity was assessed as the time the women spent on physical activities at home and at work. The study was approved by the Ethics Committee on Human Research of Beijing Friendship Hospital of Capital Medical University and informed consent was obtained from all of the patients participating in the study.

### Measurement of BMD

BMD ($g/cm^2$) of the lumbar spine (L2–L4), nondominant femoral neck, and total hip were measured by dual energy X-ray absorptiometry (DXA) (Hologic Discovery W, Bedford, MA, USA). The precision of this technique, presented as the coefficient of variation (CV), was 0.83% for the lumbar spine, 1.13% for the femoral neck, and 1.22% for the total hip locations. Subjects were divided into normal and osteoporosis/osteopenia groups (T score less than −1 at the lumbar and/or femoral sites) according to World Health Organization (WHO) criteria [26].

### Genotyping

Fasting venous blood samples were obtained from all study participants. Genomic DNA was extracted from peripheral blood leukocytes using a commercial blood DNA extraction kit (Tiangen Biotechn, Beijing, China) and stored at −20 °C until used for genotype testing. SNP genotyping of OPG, RANKL, and RANK was performed by Shanghai Benegene Biotechnology (Shanghai, China) using a MassARRAY system

**Table 1**
The basic characteristic of all SNPs for OPG, RANK and RANKL.

| SNP | Variant[a] | MAF in normal group | MAF in low BMD group | Location | HWE P |
|---|---|---|---|---|---|
| rs4355801 | A>G | 0.31 | 0.30 | Upstream of OPG | 0.10 |
| rs1032129 | C>A | 0.45 | 0.42 | Intron of OPG | 0.18 |
| rs6993813 | C>T | 0.45 | 0.36 | Upstream of OPG | 0.19 |
| rs3102735 | T>C | 0.45 | 0.14 | Downstream of OPG | 0.10 |
| rs2073618 | G>C | 0.45 | 0.31 | Exon of OPG | 0.04 |
| rs6469804 | A>G | 0.45 | 0.24 | Downstream of OPG | 0.10 |
| rs1805034 | T>C | 0.45 | 0.34 | Exon of RANK | 0.30 |
| rs12458117 | G>A | 0.45 | 0.27 | Intron of RANK | 0.18 |
| rs3018362 | A>G | 0.45 | 0.24 | Downstream of RANK | 0.52 |
| rs9533155 | C>G | 0.45 | 0.38 | Intron of RANKL | 0.76 |
| rs12585014 | G>A | 0.45 | 0.40 | Intron of RANKL | 0.31 |
| rs7988338 | G>A | 0.45 | 0.41 | Intron of RANKL | 0.21 |
| rs9533156 | T>C | 0.45 | 0.40 | Intron of RANKL | 0.76 |
| rs2148073 | C>G | 0.45 | 0.39 | Intron of RANKL | 0.22 |

MAF: minor allele frequencies.
[a] The second allele is the minor allele.

(Sequenom, San Diego, CA, USA) by means of the matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry method according to the manufacturer's instructions. Primers were also obtained from Shanghai Benegene Biotechnology; these sequences are shown in Table 2. Briefly, the DNA sample was diluted to 5 ng/μL, and 1 μL of DNA was combined with 0.95 μL of deionized water, 0.625 μL of polymerase chain reaction (PCR) buffer, 1 μL of PCR primers and 0.1 μL of 5 units/μL HotStar Taq (Qiagen, Hilden, Germany). The reaction was incubated at 94 °C for 15 min followed by 45 cycles at 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 1 min, and a final incubation at 72 °C for 3 min. After PCR amplification, the remaining dNTPs were dephosphorylated by adding shrimp alkaline phosphatase (Sequenom) at 37 °C for 40 min; the enzyme was deactivated by incubating at 85 °C for 5 min. Then the single primer extension over the SNP was combined with iPLEX enzyme (Sequenom) and extension primer at 94 °C for 30 s and 94 °C for 5 s, followed by 5 cycles of 52 °C for 5 s and 80 °C for 5 s for a total of 40 cycles, and 72 °C for 3 min. The completed genotyping reactions were spotted onto a 384 well spectroCHIP (Sequenom) plate using the MassARRAY Nanodispenser (Sequenom) and analyzed by the MALDI-TOF mass spectrometer. Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 and analyzed using the MassARRAY Typer software version 3.4 (Sequenom). All SNPs were re-genotyped by direct sequencing in a subset of 120 individuals to check for genotyping discrepancies. Genotyping error rates were ≤0.01 for all SNPs.

### *Statistical analysis*

The chi-squared test was used to determine whether the observed genotype frequencies were compatible with the Hardy–Weinberg equilibrium (HWE) and to test for differences in genotype frequencies between normal individuals and those with a low BMD (i.e., the osteoporosis/osteopenia group). Haploview software version 4.1 [27] was used to analyze the association between haplotypes and the disease. Comparisons of continuous measurements such as age, body mass index (BMI) and BMD that showed a normal distribution were expressed as mean ± standard deviation (SD) and were performed using one-way analysis of variance (ANOVA), while the Bonferroni post hoc test correction was used to correct for multiple comparisons. A generalized linear model (GLM) was used to analyze the relationships between the SNPs and BMD adjusted for years since menopause (YSM) and weight, which were identified as significant variables from the stepwise linear regression model. The initial stepwise model included all potential confounding factors, such as BMI ($kg/m^2$), age (year), the family history of osteoporosis (yes/no), education level (less than middle school/at or greater than middle school), YSM (year) and age at menopause (year). The independent sample *t*-test was used to analyze data in terms of genetic models: (A) dominant or (B) recessive allele. All statistical analyses were performed using SPSS software package (SPSS Inc. Chicago, IL, USA) version 11.5. Each SNP was tested separately. All tests were two-sided and the significance level was set at 0.05. With an expected SD of 0.15 $g/cm^2$ for BMD in the study population, 150 women needed to be studied, providing >80% power to detect a 10% difference in BMD for each polymorphism at a level of a = 0.05.

## Results

### *Demographic characteristics and BMDs of the study population*

Of the 235 women who were enrolled in the study, 127 were postmenopausal and 108 were perimenopausal. Perimenopause was defined as less than 12 consecutive months of amenorrhea and follicle-stimulating hormone levels greater than 40 IU/L. Of the 235 subjects, 4% had a history of low trauma fracture and 7.2% had a family history of osteoporosis in first- and second-degree relatives. None of the subjects had smoked or drank alcohol. A total of 6 women (2.6%) were osteoporotic, 62 (26.4%) were osteopenic and 167 (71.0%) had normal BMD. The characteristics of the subjects are presented in Table 3. For each genotype, the differences in age, weight, height, BMI, calcium intake and physical exercise between genotype subgroups were compared in the total group and in the postmenopausal and perimenopausal subgroups. In the postmenopausal women group, the difference in age at menopause and YSM were also tested. No statistically significant differences were found (data not shown).

### *Genotype distribution in normal individuals and those with a low BMD*

Only 229 and 226 subjects were successfully genotyped for rs12458117 and rs7988338, respectively, while all 235 subjects were successfully genotyped for the other 12 SNPs in the RANK, RANKL, and OPG genes. All these SNPs were compatible with the Hardy–Weinberg equilibrium ($p > 0.05$) (Table 1). In perimenopausal women, the TT genotype of rs1805034 was present in 63.6% of women in the osteoporosis/osteopenia group and only in 39.5% of those in the normal group (Table 4). Thus, the rs1805034 genotype distribution was significantly different in women with a low BMD at lumbar and/or femoral sites ($T < -1.0$) and in normal individuals ($p = 0.042$) with an OR (95% CI) of 0.374 (0.142–0.986). This difference seen in the perimenopausal women was not observed in the postmenopausal and total women groups. No association with osteoporosis/osteopenia was observed for allele and genotype frequency for other SNPs in either total subjects including peri- and postmenopausal women or perimenopausal and postmenopausal women separately (Tables 1 and 4). Haploview software analysis identified four blocks (Fig. 1) and no association with osteoporosis/osteopenia was observed

**Table 2**
The primer sequences used for SNP analysis.

| SNP_ID | Forward | Reverse |
|---|---|---|
| rs9533156 | ACGTTGGATGACACGCCCCTTTACCCTTTT | ACGTTGGATGGCAGTAGAGAGCCTATAGAC |
| rs2148073 | ACGTTGGATGATCGCAACTTGTACTCCACG | ACGTTGGATGAGAGAGGCGAAAGGGTATG |
| rs12585014 | ACGTTGGATGGGTCAGGTATCACCCAAAGG | ACGTTGGATGTCTCTGTAAACAAGCTGCTG |
| rs9533155 | ACGTTGGATGACTGTATCATCAGCTTCGTG | ACGTTGGATGCGTGTAGCCAGAAGCAAGCA |
| rs4355801 | ACGTTGGATGACACTGTGCCTAGTCTAAGC | ACGTTGGATGAGCAGCTGACTTTCCCTGAC |
| rs6993813 | ACGTTGGATGTTTCCCTTGGGTGTGTAATC | ACGTTGGATGGCAGAATAATAACCCCCAAAG |
| rs3102735 | ACGTTGGATGTTGCTCTAGGGTTCGCTGTC | ACGTTGGATGGGGACCACACTTTACAAGTC |
| rs1805034 | ACGTTGGATGAGAGTAGAACATCATGGGAC | ACGTTGGATGCCATTTGGTGGTTTTCTAGC |
| rs3018362 | ACGTTGGATGGAGATCATCTTACCTACACC | ACGTTGGATGTCCCTGCAGGTCCTATATAC |
| rs2073618 | ACGTTGGATGTCCAAGCCCCTGAGGTTTC | ACGTTGGATGCCAGGGACTTACCACGAGC |
| rs6469804 | ACGTTGGATGACAAGAGGAGGAATGAGGAC | ACGTTGGATGGCAGAAGGCCTTTGTTTATG |
| rs7988338 | ACGTTGGATGAGACATGCAATTAGGAAGAC | ACGTTGGATGGAGCTATCCTAAGCTGAGAG |
| rs12458117 | ACGTTGGATGCAGCTGAATATTTCATTCTC | ACGTTGGATGCCCCCAATCCAGTGTAGAAA |
| rs1032129 | ACGTTGGATGCAGCAGGAAGAGCCAATAAC | ACGTTGGATGGAGTCTCATATAGAAGGCAG |

**Table 3**
Characteristics of the study population (n = 235).

| | Total | Perimenopausal (n = 108) | Postmenopausal (n = 127) |
|---|---|---|---|
| Age (years) | 51.5 ± 3.3 | 50.1 ± 2.7 | 52.8 ± 3.2 |
| Age at menopause (years) | 49.2 ± 3.2 | | 49.2 ± 3.2 |
| Years since menopause | | | 3.6 ± 3.2 |
| Weight (kg) | 61.3 ± 8.9 | 60.9 ± 9.1 | 61.7 ± 8.8 |
| Height (cm) | 160.4 ± 5.4 | 160.3 ± 5.3 | 160.4 ± 5.4 |
| Parity | 1.1 ± 0.4 | 1.1 ± 0.4 | 1.1 ± 0.5 |
| BMI ($kg/m^2$) | 23.8 ± 3.2 | 23.7 ± 3.4 | 23.9 ± 3.0 |
| Calcium intake (mg/dL) | 1023.4 ± 33.5 | 997.2 ± 41.2 | 1045.7 ± 31.6 |
| Physical exercise (h/week) | 3.2 ± 2.3 | 3.1 ± 2.2 | 3.3 ± 2.1 |
| BMD neck ($g/cm^2$) | 0.779 ± 0.112 | 0.793 ± 0.106 | 0.766 ± 0.116 |
| BMD hip ($g/cm^2$) | 0.891 ± 0.129 | 0.910 ± 0.116 | 0.875 ± 0.178 |
| BMD lumbar spine ($g/cm^2$) | 0.969 ± 0.143 | 1.001 ± 0.122 | 0.941 ± 0.153 |

Results are expressed as mean ± SD for continuous variables and number of subjects (%-age) for categorical variables.

for haplotype frequency for all the three polymorphisms in total subjects (data not shown).

*RANK, RANKL, and OPG polymorphisms and BMD*

In the present study, statistical analysis showed an association with BMD for the following SNPs: rs6993813 (OPG), rs2073618 (OPG), rs4355801 (OPG), rs1032129 (OPG), and rs3018362 (RANK). For the rs6993813 (OPG), the highest BMD at all anatomical sites was observed in TT homozygotes and statistical significance was reached when testing for genetic models of recessive alleles (i.e., when a group of the CC genotype was compared with a group of the TC and TT genotypes), according to BMD at the total hip and femoral neck and adjusted BMD at all three sites ($p < 0.05$; Tables 5 and 6). Furthermore, statistical significance was reached when testing for genetic models of dominant alleles (i.e., when a group of the TT genotype was compared with a group of the TC and CC genotypes) according to femoral neck BMD ($p < 0.05$; Tables 5 and 6).

For the rs2073618 polymorphism of OPG, the highest BMD at all anatomical sites was observed in CC homozygotes and statistical significance was reached when testing for genetic models of dominant alleles according to femoral neck adjusted BMD ($p < 0.05$; Table 6).

For the rs4355801 polymorphism of OPG, the highest BMD at all anatomical sites was observed in GG homozygotes and statistical significance was reached when testing for genetic models of dominant alleles according to femoral neck BMD and adjusted BMD ($p < 0.05$; Tables 5 and 6).

For the rs1032129 polymorphism of OPG, the highest BMD at all anatomical sites was observed in AA homozygotes and statistical significance was reached only at the femoral neck when testing for genetic models of dominant alleles according to adjusted BMD ($p < 0.05$; Tables 5 and 6).

For rs3018362 of RANK, statistical significance was reached only at the femoral neck when testing for genetic models of dominant alleles according to adjusted BMD at the femoral neck ($p < 0.05$; Tables 5 and 6).

For rs3102375 and rs6469804 of OPG, rs12458117 of RANK and all the SNPs of the RANKL gene, differences between the three genotypes in lumbar spine, femoral neck or hip BMD were not detected with statistical significance when tested by ANOVA regardless of whether the BMD was adjusted for body weight or YSM. Statistical significance was

**Table 4**
Genotype distribution in normal individuals and those with a low BMD.

| SNP | Total | | | | Perimenopausal | | | Postmenopausal | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Genotype | Normal | Low BMD | p | Normal | Low BMD | p | Normal | Low BMD | p |
| rs4355801 | AA | 76 (0.466) | 31 (0.431) | | 44 (0.512) | 13 (0.591) | | 32 (0.416) | 18 (0.360) | |
| | AG + GG | 87 (0.534) | 41 (0.569) | 0.6 | 42 (0.488) | 9 (0.409) | 0.5 | 45 (0.584) | 32 (0.640) | 0.5 |
| rs9533155 | CG + GG | 117 (0.718) | 45 (0.625) | | 64 (0.744) | 13 (0.591) | | 53 (0.688) | 32 (0.640) | |
| | CC | 46 (0.282) | 27 (0.375) | 0.2 | 22 (0.256) | 9 (0.409) | 0.2 | 24 (0.312) | 18 (0.360) | 0.6 |
| rs1032129 | CC | 48 (0.294) | 20 (0.278) | | 27 (0.314) | 7 (0.318) | | 21 (0.273) | 13 (0.260) | |
| | AC + AA | 115 (0.706) | 52 (0.722) | 0.8 | 59 (0.686) | 15 (0.682) | 1 | 56 (0.727) | 37 (0.740) | 0.9 |
| rs6993813 | CC | 54 (0.331) | 27 (0.375) | | 27 (0.314) | 10 (0.455) | | 27 (0.351) | 17 (0.340) | |
| | TT + CT | 109 (0.669) | 45 (0.625) | 0.5 | 59 (0.686) | 12 (0.545) | 0.2 | 50 (0.649) | 33 (0.660) | 0.9 |
| rs1805034 | TT | 66 (0.405) | 32 (0.444) | | 34 (0.395) | 14 (0.636) | | 32 (0.416) | 18 (0.360) | |
| | CC + CT | 97 (0.595) | 40 (0.556) | 0.6 | 52 (0.605) | 8 (0.364) | 0 | 45 (0.584) | 32 (0.640) | 0.5 |
| rs12585014 | AA + AG | 89 (0.546) | 46 (0.639) | | 46 (0.535) | 13 (0.591) | | 43 (0.558) | 33 (0.660) | |
| | GG | 74 (0.454) | 26 (0.361) | 0.2 | 40 (0.465) | 9 (0.409) | 0.6 | 34 (0.442) | 17 (0.340) | 0.3 |
| rs7988338 | AA + AG | 84 (0.532) | 44 (0.647) | | 43 (0.518) | 12 (0.571) | | 41 (0.547) | 32 (0.681) | |
| | GG | 74 (0.468) | 24 (0.353) | 0.1 | 40 (0.482) | 9 (0.429) | 0.7 | 34 (0.453) | 15 (0.319) | 0.1 |
| rs9533156 | CC | 42 (0.258) | 26 (0.361) | | 21 (0.244) | 3 (0.136) | | 23 (0.299) | 18 (0.360) | |
| | TT + CT | 121 (0.742) | 46 (0.639) | 0.1 | 65 (0.756) | 19 (0.864) | 0.3 | 54 (0.701) | 32 (0.640) | 0.5 |
| rs12458117 | AG + AA | 93 (0.581) | 32 (0.464) | | 49 (0.590) | 13 (0.591) | | 44 (0.571) | 19 (0.404) | |
| | GG | 67 (0.419) | 37 (0.536) | 0.1 | 34 (0.410) | 9 (0.409) | 1 | 33 (0.429) | 28 (0.596) | 0.1 |
| rs3102735 | TT | 121 (0.742) | 54 (0.750) | | 68 (0.791) | 15 (0.682) | | 53 (0.688) | 39 (0.780) | |
| | CC + CT | 42 (0.258) | 18 (0.250) | 0.9 | 18 (0.209) | 7 (0.318) | 0.3 | 24 (0.312) | 11 (0.220) | 0.3 |
| rs2073618 | GG | 77 (0.472) | 30 (0.417) | | 45 (0.523) | 12 (0.545) | | 32 (0.416) | 18 (0.360) | |
| | CC + CG | 86 (0.528) | 42 (0.583) | 0.4 | 41 (0.477) | 10 (0.455) | 0.9 | 45 (0.584) | 32 (0.640) | 0.5 |
| rs2148073 | CC | 74 (0.454) | 28 (0.389) | | 40 (0.465) | 9 (0.409) | | 34 (0.442) | 19 (0.380) | |
| | GG + CG | 89 (0.546) | 44 (0.611) | 0.4 | 46 (0.535) | 13 (0.591) | 0.6 | 43 (0.558) | 31 (0.620) | 0.5 |
| rs3018362 | AA | 79 (0.485) | 42 (0.583) | | 43 (0.500) | 16 (0.727) | | 36 (0.468) | 26 (0.520) | |
| | AG + GG | 84 (0.515) | 30 (0.417) | 0.2 | 43 (0.500) | 6 (0.273) | 0.1 | 41 (0.532) | 24 (0.480) | 0.6 |
| rs6469804 | AA | 92 (0.564) | 39 (0.542) | | 49 (0.570) | 15 (0.682) | | 43 (0.558) | 24 (0.480) | |
| | AG + GG | 71 (0.436) | 33 (0.458) | 0.7 | 37 (0.430) | 7 (0.318) | 0.3 | 34 (0.442) | 26 (0.520) | 0.4 |

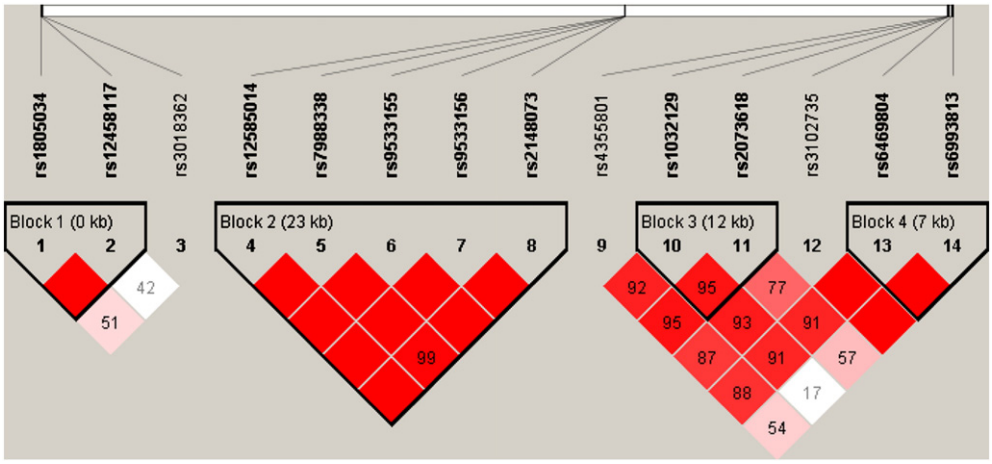


**Fig. 1.** The haplotype frequencies of RANK, RANKL and OPG in Chinese perimenopausal and postmenopausal women.

not reached when testing for genetic models of dominant and recessive alleles (Tables 5 and 6).

## Discussion

In our cross-sectional study, we confirmed that OPG and RANK, which have been associated with lower BMD and fracture risk in Europeans, are also associated with reduced BMD in a Han Chinese female population. We analyzed the quantitative phenotype – BMD – because it was considered to be a more powerful technique than a comparison between a disease group and a control group classified according to a quantitative variable [28]. In the stepwise linear regression model, as YSM and weight were identified as significant variables from all potential confounding factors, including BMI ($kg/m^2$), age (years), family history of osteoporosis (yes/no), education level (less than middle school/at or greater than middle school), YSM (years) and age at menopause (years), we also analyzed the relationship between SNPs and adjusted BMD.

The RANKL/RANK/OPG signaling pathway has a critical role in bone remodeling regulation [29]. This pathway has been extensively studied since the discovery of OPG and the subsequent discovery that it interacts with RANKL and RANK. They are important candidates in osteoporosis development.

OPG acts as a soluble decoy receptor for RANKL and behaves as an inhibitor of osteoclastogenesis [30]. A variety of studies have been performed on OPG polymorphisms, mostly focusing on the A163-G, T245-G, and G1181-C variants (Lys3Asn, rs2073618) [17,18]. The newly detected SNPs in European populations are rs4355801, rs6993813, rs1032129, rs3102735, and rs6469804 [7–9,19–21]. The OPG gene is of particular interest since not only is it expressed in bone and found to be involved in bone diseases such as Paget's and osteoporosis, but it also is expressed in other tissues, such as the kidneys, lungs, and brain, and has been linked to other diseases such as vascular disease [31]. Polymorphisms within the OPG gene are associated with plaque instability [32], showing that the RANKL, RANK, and OPG system might link osteoporosis with an increased risk of vascular disease.

In the present study, we found that rs6993813 was significantly associated with BMD at the femoral neck and adjusted BMD at the total hip and femoral neck and that rs4355801 and rs1032129 were significantly associated with BMD at the femoral neck. The rs2073618 polymorphism is the only non-synonymous polymorphism in the signal peptide that is involved in the cellular secretion of OPG, and its association with BMD was also confirmed in our results, which is in accordance with several earlier studies [18,33] and provides further confirmatory evidence for this association. The observed risk alleles of rs6993813, rs4355801, rs1032129 and rs2073618 in the present study were C, A, C and G, respectively, and the frequencies of the risk alleles were 60.4%, 69.1%, 55.7% and 69.6%, respectively, which were different from those reported in European populations (49.6%, 67%, 38.3% and 52.2%, respectively) [8], but compared well with those observed in Han Chinese women in Shanghai [34]. The frequencies of these risk alleles were 54.4%, 56.9%, 37.3% and 49.9% for American and 70.5%, 84.4%, 34.4% and 60.7% for African-American subjects, respectively. These observations agree with the results of previous studies [8]. However, no associations between rs6469804 or rs3102375 and BMD were detected.

RANK is the only receptor for RANKL and it is capable of initiating osteoclastogenic signal transduction upon binding of RANKL. RANK is essential for osteoclastogenesis and RANK-deficient mice exhibit severe osteopetrosis. The roles of RANK in controlling bone remodeling have been well documented and some studies have revealed an association between RANK and BMD [8,11,16,35].

The SNP rs1805034 was reported to be in association with BMD in the RANK gene in Korean postmenopausal women [15]. The same SNP was shown to be associated with hip BMD of men but not with that of women in the Chinese population [35]. In the subgroup of perimenopausal women of our study, a significant difference was found between TT and TC/CC, which suggests that this SNP could be more important in relation to the peak bone mass attained in young adulthood than in regulating the extent of bone loss after the menopause. However, these differences were not considered to be relevant as no influence on BMD was found in total, peri-, or postmenopausal women (data not shown).

The SNP rs3018362 was associated with BMD in recent GWASs [22]. We also confirmed its association with adjusted BMDs at the femoral neck when testing for genetic models of dominant alleles (i.e., homozygotes of the risk allele G had a significantly lower BMD than heterozygotes and non-carriers of the risk allele) (Tables 5 and 6). Our results suggest that the rs3018362 SNP is an important determinant of BMD at the femoral neck, independent of YSM and body weight. We did not find this association in the lumbar spine or total hip BMD. Thus, the RANK polymorphism appears to influence mainly femoral neck BMD. Nevertheless, we found that the proportion of BMD variance explained by rs3018362 was 5.5%. The modest effect of the RANK gene on BMD may be related to the fact that a variety of different genes, each contributing to a small degree, are implicated in the development of osteoporosis. It should also be noted that the risk allele frequencies observed in our sample were different from those found in Europeans [8], but similar to those found in Chinese people [34]. These differences are most likely to be an ethnic phenomenon. However, the effects of these risk alleles on BMD values had the same direction in our cohort as in Europeans, except for rs3018362 with G as the risk allele, which was contrary to other studies [34]. However, no associations were observed between rs12458117 and BMD in peri- and postmenopausal women in our study. RANKL plays a key role in osteoclast differentiation [29]. Furthermore, a human anti-RANKL antibody (denosumab) has been shown to be able to prevent bone loss in patients with osteoporosis [36]. Thus, it was proposed that

**Table 5**
Lumbar and femoral BMD in Chinese peri- and postmenopausal women according to the 14 RANK, RANKL and OPG gene polymorphisms studied.

| | | | | | | | p (dominant) | p (recessive) |
|---|---|---|---|---|---|---|---|---|
| *OPG* | | | | | | | | |
| rs3102735 | TT (n = 175) | TC (n = 52) | CC (n = 8) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.977 ± 0.144 | 0.939 ± 0.144 | 0.980 ± 0.081 | 1.000 | 0.295 | 1.000 | 0.136 | 0.814 |
| Total hip (g/cm$^2$) | 0.899 ± 0.126 | 0.870 ± 0.146 | 0.866 ± 0.071 | 1.000 | 0.484 | 1.000 | 0.131 | 0.570 |
| Neck (g/cm$^2$) | 0.782 ± 0.111 | 0.769 ± 0.118 | 0.754 ± 0.074 | 1.000 | 1.000 | 1.000 | 0.367 | 0.527 |
| rs2073618 | GG (n = 107) | GC (n = 113) | CC (n = 15) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.975 ± 0.137 | 0.953 ± 0.146 | 1.037 ± 0.144 | 0.345 | 0.734 | 0.095 | 0.507 | 0.054 |
| Total hip (g/cm$^2$) | 0.886 ± 0.113 | 0.890 ± 0.143 | 0.935 ± 0.126 | 0.502 | 1.000 | 0.608 | 0.583 | 0.170 |
| Neck (g/cm$^2$) | 0.765 ± 0.099 | 0.784 ± 0.122 | 0.827 ± 0.108 | 0.133 | 0.612 | 0.486 | 0.094 | 0.081 |
| rs1032129 | CC (n = 68) | AC (n = 126) | AA (n = 41) | CC vs AA | CC vs CA | AA vs CA | | |
| L2–L4 (g/cm$^2$) | 0.971 ± 0.143 | 0.959 ± 0.147 | 0.994 ± 0.129 | 1.000 | 1.000 | 0.513 | 0.870 | 0.206 |
| Total hip (g/cm$^2$) | 0.880 ± 0.118 | 0.889 ± 0.141 | 0.915 ± 0.107 | 0.530 | 1.000 | 0.813 | 0.412 | 0.195 |
| Neck (g/cm$^2$) | 0.757 ± 0.990 | 0.784 ± 0.116 | 0.797 ± 0.116 | 0.211 | 0.312 | 1.000 | 0.058 | 0.247 |
| rs4355801 | AA (n = 107) | AG (n = 111) | GG (n = 17) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.970 ± 0.137 | 0.958 ± 0.147 | 1.032 ± 0.143 | 0.295 | 1.000 | 0.141 | 0.893 | 0.059 |
| Total hip (g/cm$^2$) | 0.882 ± 0.107 | 0.894 ± 0.147 | 0.936 ± 0.127 | 0.328 | 1.000 | 0.639 | 0.295 | 0.141 |
| Neck (g/cm$^2$) | 0.762 ± 0.097 | 0.788 ± 0.123 | 0.821 ± 0.111 | 0.135 | 0.266 | 0.776 | 0.036 | 0.107 |
| rs6993813 | CC (n = 81) | TC (n = 122) | TT (n = 32) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.968 ± 0.132 | 0.958 ± 0.144 | 1.010 ± 0.159 | 0.479 | 0.208 | 1.000 | 0.957 | 0.079 |
| Total hip (g/cm$^2$) | 0.875 ± 0.095 | 0.891 ± 0.149 | 0.934 ± 0.117 | 0.084 | 0.269 | 1.000 | 0.163 | 0.043 |
| Neck (g/cm$^2$) | 0.761 ± 0.087 | 0.780 ± 0.124 | 0.819 ± 0.109 | 0.037 | 0.230 | 0.683 | 0.049 | 0.027 |
| rs6469804 | AA (n = 131) | AG (n = 95) | GG (n = 9) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.979 ± 0.140 | 0.948 ± 0.148 | 1.037 ± 0.144 | 0.714 | 0.221 | 0.316 | 0.213 | 0.144 |
| Total hip (g/cm$^2$) | 0.892 ± 0.115 | 0.885 ± 0.146 | 0.946 ± 0.134 | 0.678 | 1.000 | 0.530 | 0.919 | 0.195 |
| Neck (g/cm$^2$) | 0.772 ± 0.103 | 0.781 ± 0.121 | 0.845 ± 0.126 | 0.174 | 1.000 | 0.294 | 0.334 | 0.067 |
| *Rank* | | | | | | | | |
| rs12458117 | AA (n = 18) | AG (n = 107) | GG (n = 104) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.979 ± 0.144 | 0.977 ± 0.148 | 0.965 ± 0.134 | 1.000 | 1.000 | 1.000 | 0.541 | 0.813 |
| Total hip (g/cm$^2$) | 0.919 ± 0.162 | 0.887 ± 0.121 | 0.896 ± 0.131 | 0.998 | 1.000 | 1.000 | 0.802 | 0.385 |
| Neck (g/cm$^2$) | 0.782 ± 0.096 | 0.783 ± 0.105 | 0.777 ± 0.121 | 1.000 | 1.000 | 1.000 | 0.702 | 0.947 |
| rs1805034 | CC (n = 24) | CT (n = 113) | TT (n = 98) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.912 ± 0.123 | 0.980 ± 0.137 | 0.970 ± 0.152 | 0.224 | 1.000 | 0.105 | 0.912 | 0.040 |
| Total hip (g/cm$^2$) | 0.858 ± 0.133 | 0.888 ± 0.127 | 0.903 ± 0.130 | 0.394 | 1.000 | 0.904 | 0.249 | 0.187 |
| Neck (g/cm$^2$) | 0.754 ± 0.137 | 0.782 ± 0.108 | 0.781 ± 0.109 | 0.874 | 1.000 | 0.806 | 0.789 | 0.255 |
| rs3018362 | AA (n = 121) | AG (n = 98) | GG (n = 16) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (kg/m$^2$) | 0.957 ± 0.154 | 0.990 ± 0.123 | 0.931 ± 0.159 | 1.000 | 0.273 | 0.380 | 0.189 | 0.272 |
| Total hip (g/cm$^2$) | 0.885 ± 0.137 | 0.901 ± 0.121 | 0.878 ± 0.115 | 1.000 | 1.000 | 1.000 | 0.446 | 0.663 |
| Neck (g/cm$^2$) | 0.766 ± 0.108 | 0.799 ± 0.114 | 0.747 ± 0.116 | 1.000 | 0.085 | 0.249 | 0.076 | 0.246 |
| *RANKL* | | | | | | | | |
| rs9533155 | CC (n = 73) | GC (n = 118) | GG (n = 44) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.956 ± 0.151 | 0.973 ± 0.137 | 0.978 ± 0.146 | 1.000 | 1.000 | 1.000 | 0.371 | 0.621 |
| Total hip (g/cm$^2$) | 0.873 ± 0.118 | 0.897 ± 0.136 | 0.906 ± 0.127 | 0.531 | 1.000 | 0.642 | 0.146 | 0.392 |
| Neck (g/cm$^2$) | 0.763 ± 0.112 | 0.784 ± 0.112 | 0.788 ± 0.110 | 0.776 | 1.000 | 0.624 | 0.166 | 0.551 |
| rs9533156 | TT (n = 68) | CT (n = 119) | CC (n = 48) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.951 ± 0.153 | 0.973 ± 0.133 | 0.983 ± 0.152 | 0.698 | 0.969 | 1.000 | 0.232 | 0.423 |
| Total hip (g/cm$^2$) | 0.871 ± 0.121 | 0.897 ± 0.135 | 0.906 ± 0.125 | 0.461 | 0.581 | 1.000 | 0.129 | 0.376 |
| Neck (g/cm$^2$) | 0.760 ± 0.113 | 0.786 ± 0.113 | 0.788 ± 0.106 | 0.540 | 0.379 | 1.000 | 0.097 | 0.517 |
| rs12585014 | GG (n = 100) | AG (n = 101) | AA (n = 34) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.977 ± 0.141 | 0.966 ± 0.140 | 0.954 ± 0.156 | 1.000 | 1.000 | 1.000 | 0.808 | 0.526 |
| Total hip (g/cm$^2$) | 0.904 ± 0.126 | 0.885 ± 0.134 | 0.872 ± 0.122 | 0.624 | 1.000 | 0.865 | 0.363 | 0.346 |
| Neck (g/cm$^2$) | 0.786 ± 0.110 | 0.775 ± 0.113 | 0.768 ± 0.115 | 1.000 | 1.000 | 1.000 | 0.656 | 0.556 |
| rs7988338 | GG (n = 98) | AG (n = 95) | AA (n = 33) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.983 ± 0.136 | 0.967 ± 0.143 | 0.952 ± 0.158 | 0.826 | 1.000 | 1.000 | 0.575 | 0.386 |
| Total hip (g/cm$^2$) | 0.909 ± 0.123 | 0.886 ± 0.138 | 0.872 ± 0.124 | 0.457 | 1.000 | 0.664 | 0.267 | 0.285 |
| Neck (g/cm$^2$) | 0.789 ± 0.110 | 0.777 ± 0.113 | 0.766 ± 0.116 | 0.929 | 1.000 | 1.000 | 0.625 | 0.410 |
| rs2148073 | CC (n = 102) | GC (n = 99) | GG (n = 34) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.976 ± 0.141 | 0.966 ± 0.141 | 0.954 ± 0.156 | 1.000 | 1.000 | 1.000 | 0.859 | 0.526 |
| Total hip (g/cm$^2$) | 0.902 ± 0.126 | 0.887 ± 0.135 | 0.872 ± 0.122 | 0.724 | 1.000 | 1.000 | 0.472 | 0.346 |
| Neck (g/cm$^2$) | 0.784 ± 0.110 | 0.776 ± 0.114 | 0.768 ± 0.115 | 1.000 | 1.000 | 1.000 | 0.784 | 0.556 |

RANKL may also be an important candidate gene of osteoporosis. However, the RANKL SNPs have yielded inconsistent results in previous studies. In this study, rs9533155, rs12585014, rs7988338, rs2148073, and rs9533156 were selected because of their association with lumbar spine BMD [23], bone loss in the hip and femoral neck [24], and femoral neck compression strength index [25]. However, the results of the European populations [8] could not be replicated in the present study. A Korean study and a Chinese study [34] also failed to reveal an association between RANKL and BMD. The reason for this discrepancy is unclear but it may be due to ethnic differences.

BMD is a complex phenotype influenced by genetic factors (including many different candidate genes) and environmental factors (including age, menopausal status, YSM, ethnic background, calcium intake, and smoking) that may be different between studies. In this study, we did not observe any interaction between calcium intake and physical exercise and the effect of RANK, OPG and RANKL polymorphisms on femoral neck,

**Table 6**
The adjusted lumbar and femoral BMD in Chinese peri- and postmenopausal women according to the 14 RANK, RANKL, and OPG gene polymorphisms studied.

| | | | | p | | | p (dominant) | p (recessive) |
|---|---|---|---|---|---|---|---|---|
| *OPG* | | | | | | | | |
| rs3102735 | TT (n = 175) | TC (n = 52) | CC (n = 8) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.974 ± 0.010 | 0.954 ± 0.019 | 0.954 ± 0.048 | 1.000 | 1.000 | 1.000 | 0.314 | 0.746 |
| Total hip (g/cm$^2$) | 0.895 ± 0.009 | 0.885 ± 0.017 | 0.837 ± 0.042 | 0.538 | 1.000 | 0.881 | 0.349 | 0.192 |
| Neck (g/cm$^2$) | 0.779 ± 0.008 | 0.783 ± 0.014 | 0.729 ± 0.036 | 0.522 | 1.000 | 0.508 | 0.793 | 0.166 |
| rs2073618 | GG (n = 107) | GC (n = 113) | CC (n = 15) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.970 ± 0.013 | 0.959 ± 0.013 | 1.028 ± 0.034 | 0.354 | 1.000 | 0.190 | 0.887 | 0.077 |
| Total hip (g/cm$^2$) | 0.882 ± 0.011 | 0.896 ± 0.011 | 0.927 ± 0.031 | 0.504 | 1.000 | 1.000 | 0.258 | 0.228 |
| Neck (g/cm$^2$) | 0.761 ± 0.010 | 0.789 ± 0.010 | 0.820 ± 0.026 | 0.115 | 0.136 | 0.829 | 0.019 | 0.105 |
| rs1032129 | CC (n = 68) | CA (n = 126) | AA (n = 41) | CC vs AA | CC vs CA | AA vs CA | | |
| L2–L4 (g/cm$^2$) | 0.967 ± 0.016 | 0.961 ± 0.012 | 0.997 ± 0.021 | 0.734 | 1.000 | 0.381 | 0.875 | 0.132 |
| Total hip (g/cm$^2$) | 0.876 ± 0.014 | 0.891 ± 0.011 | 0.916 ± 0.019 | 0.258 | 1.000 | 0.731 | 0.207 | 0.138 |
| Neck (g/cm$^2$) | 0.753 ± 0.012 | 0.786 ± 0.009 | 0.798 ± 0.016 | 0.079 | 0.094 | 1.000 | 0.014 | 0.185 |
| rs4355801 | AA (n = 107) | AG (n = 111) | GG (n = 17) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.966 ± 0.013 | 0.964 ± 0.013 | 1.014 ± 0.033 | 0.530 | 1.000 | 0.471 | 0.799 | 0.151 |
| Total hip (g/cm$^2$) | 0.879 ± 0.011 | 0.899 ± 0.011 | 0.918 ± 0.029 | 0.620 | 0.647 | 1.000 | 0.146 | 0.328 |
| Neck (g/cm$^2$) | 0.760 ± 0.010 | 0.792 ± 0.010 | 0.805 ± 0.025 | 0.268 | 0.059 | 1.000 | 0.011 | 0.258 |
| rs6993813 | CC (n = 81) | CT (n = 122) | TT (n = 32) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.967 ± 0.015 | 0.958 ± 0.012 | 1.013 ± 0.024 | 0.259 | 0.119 | 1.000 | 0.883 | 0.043 |
| Total hip (g/cm$^2$) | 0.875 ± 0.013 | 0.891 ± 0.011 | 0.935 ± 0.021 | 0.044 | 0.169 | 1.000 | 0.126 | 0.024 |
| Neck (g/cm$^2$) | 0.761 ± 0.011 | 0.780 ± 0.009 | 0.820 ± 0.018 | 0.017 | 0.140 | 0.568 | 0.052 | 0.014 |
| rs6469804 | AA (n = 131) | AG (n = 95) | GG (n = 9) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.974 ± 0.012 | 0.956 ± 0.014 | 1.028 ± 0.044 | 0.712 | 0.921 | 0.356 | 0.497 | 0.173 |
| Total hip (g/cm$^2$) | 0.887 ± 0.010 | 0.892 ± 0.012 | 0.939 ± 0.040 | 0.605 | 1.000 | 0.776 | 0.540 | 0.214 |
| Neck (g/cm$^2$) | 0.768 ± 0.009 | 0.787 ± 0.010 | 0.840 ± 0.034 | 0.125 | 0.471 | 0.429 | 0.073 | 0.067 |
| *RANK* | | | | | | | | |
| rs12458117 | GG (n = 104) | AG (n = 107) | AA (n = 18) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.968 ± 0.013 | 0.974 ± 0.013 | 0.983 ± 0.032 | 1.000 | 1.000 | 1.000 | 0.541 | 0.718 |
| Total hip (g/cm$^2$) | 0.898 ± 0.012 | 0.885 ± 0.012 | 0.915 ± 0.029 | 1.000 | 1.000 | 1.000 | 0.802 | 0.44 |
| Neck (g/cm$^2$) | 0.779 ± 0.010 | 0.776 ± 0.025 | 0.782 ± 0.010 | 1.000 | 1.000 | 1.000 | 0.609 | 0.876 |
| rs1805034 | TT (n = 98) | CT (n = 113) | CC (n = 24) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.969 ± 0.014 | 0.977 ± 0.013 | 0.929 ± 0.027 | 0.578 | 1.000 | 0.358 | 0.912 | 0.131 |
| Total hip (g/cm$^2$) | 0.901 ± 0.012 | 0.887 ± 0.011 | 0.874 ± 0.024 | 0.978 | 1.000 | 1.000 | 0.249 | 0.45 |
| Neck (g/cm$^2$) | 0.779 ± 0.010 | 0.781 ± 0.010 | 0.767 ± 0.021 | 1.000 | 1.000 | 1.000 | 0.520 | 0.572 |
| rs3018362 | AA (n = 121) | AG (n = 98) | GG (n = 16) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (kg/m$^2$) | 0.954 ± 0.012 | 0.993 ± 0.013 | 0.931 ± 0.033 | 1.000 | 0.091 | 0.249 | 0.079 | 0.241 |
| Total hip (g/cm$^2$) | 0.883 ± 0.011 | 0.904 ± 0.012 | 0.875 ± 0.030 | 1.000 | 0.554 | 1.000 | 0.263 | 0.572 |
| Neck (g/cm$^2$) | 0.764 ± 0.009 | 0.802 ± 0.010 | 0.745 ± 0.025 | 1.000 | 0.019 | 0.111 | 0.026 | 0.171 |
| *RANKL* | | | | | | | | |
| rs9533155 | CC (n = 73) | GC (n = 118) | GG (n = 44) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.959 ± 0.016 | 0.974 ± 0.012 | 0.972 ± 0.020 | 1.000 | 1.000 | 1.000 | 0.446 | 0.854 |
| Total hip (g/cm$^2$) | 0.877 ± 0.014 | 0.897 ± 0.011 | 0.900 ± 0.018 | 0.943 | 1.000 | 0.804 | 0.221 | 0.592 |
| Neck (g/cm$^2$) | 0.767 ± 0.012 | 0.784 ± 0.009 | 0.782 ± 0.015 | 1.000 | 1.000 | 0.802 | 0.257 | 0.803 |
| rs9533156 | TT (n = 68) | CT (n = 119) | CC (n = 48) | TT vs CC | TT vs TC | CC vs TC | | |
| L2–L4 (g/cm$^2$) | 0.956 ± 0.016 | 0.973 ± 0.012 | 0.977 ± 0.019 | 1.000 | 1.000 | 1.000 | 0.346 | 0.645 |
| Total hip (g/cm$^2$) | 0.877 ± 0.014 | 0.896 ± 0.011 | 0.899 ± 0.017 | 0.968 | 0.906 | 1.000 | 0.250 | 0.594 |
| Neck (g/cm$^2$) | 0.765 ± 0.012 | 0.785 ± 0.009 | 0.782 ± 0.015 | 1.000 | 0.616 | 1.000 | 0.197 | 0.785 |
| rs12585014 | GG (n = 100) | AG (n = 101) | AA (n = 34) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.971 ± 0.013 | 0.969 ± 0.013 | 0.961 ± 0.023 | 1.000 | 1.000 | 1.000 | 0.808 | 0.731 |
| Total hip (g/cm$^2$) | 0.899 ± 0.012 | 0.887 ± 0.012 | 0.881 ± 0.020 | 1.000 | 1.000 | 1.000 | 0.363 | 0.587 |
| Neck (g/cm$^2$) | 0.782 ± 0.010 | 0.776 ± 0.010 | 0.776 ± 0.018 | 1.000 | 1.000 | 1.000 | 0.656 | 0.888 |
| rs 7988338 | GG (n = 98) | AG (n = 95) | AA (n = 33) | AA vs GG | AA vs AG | GG vs AG | | |
| L2–L4 (g/cm$^2$) | 0.978 ± 0.014 | 0.970 ± 0.014 | 0.961 ± 0.023 | 1.000 | 1.000 | 1.000 | 0.575 | 0.619 |
| Total hip (g/cm$^2$) | 0.904 ± 0.012 | 0.887 ± 0.012 | 0.883 ± 0.021 | 1.000 | 1.000 | 0.985 | 0.267 | 0.590 |
| Neck (g/cm$^2$) | 0.784 ± 0.011 | 0.778 ± 0.011 | 0.776 ± 0.018 | 1.000 | 1.000 | 1.000 | 0.625 | 0.802 |
| rs 2148073 | CC (n = 102) | GC (n = 99) | GG (n = 34) | GG vs CC | GG vs GC | CC vs GC | | |
| L2–L4 (g/cm$^2$) | 0.970 ± 0.013 | 0.969 ± 0.014 | 0.961 ± 0.023 | 1.000 | 1.000 | 1.000 | 0.859 | 0.731 |
| Total hip (g/cm$^2$) | 0.898 ± 0.012 | 0.888 ± 0.012 | 0.881 ± 0.020 | 1.000 | 1.000 | 1.000 | 0.472 | 0.587 |
| Neck (g/cm$^2$) | 0.781 ± 0.010 | 0.777 ± 0.010 | 0.776 ± 0.018 | 1.000 | 1.000 | 1.000 | 0.784 | 0.888 |

lumbar spinal or hip BMD. Furthermore, there was no difference in calcium intake and physical exercise between the genotypes of each separate SNP (data not shown). In addition, in our population with a mean age of 51.5 ± 3.3 years, the association of these 14 SNPs with BMD was independent of age.

In a recently published meta-analysis pooling five large scale GWASs, OPG, RANKL, and RANK were confirmed to be associated with BMD at a genome-wide significance level [9]. Because most previous GWAS results were derived from Europeans, more studies are needed to confirm susceptibility loci, especially in different ethnic groups. In our study, we confirmed that of the 14 SNPs investigated in the present study, 5 SNPs (rs6993813, rs4355801, rs1032129 and rs2073618 of OPG and rs3018362 of RANK) were significantly associated with BMD at the femoral neck or total hip.

There are some limitations to this study. As already stated, our study population had a low prevalence of osteoporosis and since the study sample was homogeneous with respect to ethnic background, the results are not necessarily applicable to other populations. In addition,

BMD is only one of several parameters related to bone quality and risk of fracture and no data on fragility fractures, bone turnover, and the quality of bone were available for analysis. Furthermore, a relatively small sample size allowed only a limited power to detect individual effects and interactions. The use of a cross-sectional design makes it difficult to draw conclusions about the rate of bone loss over time according to genotype. Finally, we did not analyze the mechanisms by which these 5 SNPs affect BMD and their functions. Further functional studies on the polymorphisms are needed to determine whether these polymorphisms influence the secretory kinetics of OPG and/or RANKL.

Despite these limitations, our study confirms that the OPG and RANK genes contribute to variations in BMD among Chinese peri- and postmenopausal women, as has been shown by previous studies in other populations. Follow-up studies performed with multiple and large sample sets are needed before the effect of these variants can be fully and accurately evaluated.

## Conclusions

In summary, our study has provided the associations between several SNPs in OPG and RANK and BMD in Chinese peri- and postmenopausal women. These findings add to our knowledge of the possible influence of genetic variation in the RANK/RANKL/OPG signaling pathway on bone and suggest that SNPs in OPG and RANK affect BMD in Chinese women. To gain further insight into the genetic background underlying osteoporosis, more studies into RANK and OPG candidate genes are warranted. These findings need to be confirmed in larger cohorts and the number of the studied SNPs should be increased. If they are confirmed, fine mapping and functional studies will be needed to identify the causal variants and to determine their effects on osteoporosis at the molecular, cellular, and disease levels.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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