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ORIGINAL RESEARCH

# OPG/RANK/RANKL Single-Nucleotide Polymorphisms in Rheumatoid Arthritis: Associations with Disease Susceptibility, Bone Mineral Density, and Clinical Manifestations in a Chinese Han Population

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**Objective:** The osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/receptor activator of nuclear factor-κB ligand (RANKL) system plays a pivotal role in the balance between osteoblasts and osteoclasts and is closely related to the pathogenesis of rheumatoid arthritis (RA). The present study aimed to clarify the associations of OPG/RANK/RANKL gene single-nucleotide polymorphisms (SNPs) with disease susceptibility, bone mineral density (BMD), and clinical manifestations in RA patients.

**Methods:** A case-control study including 319 RA patients and 330 healthy controls was conducted. All subjects were genotyped for rs4355801 and rs1023968 in OPG, rs10805033 in RANK, and rs9533155 and rs875625 in RANKL. BMD and clinical manifestations were recorded.

**Results:** An association was found between OPG rs4355801 and risk of RA. In recessive models, the GG genotype of rs4355801 was associated with an increased risk of RA compared with the AA/AG genotypes (OR=1.679, 95% CI: 1.062–2.655, p=0.025). A correlation between RANKL rs9533155 and BMD was found in RA patients. Patients with the GG genotype (n=108) in RANKL rs9533155 had the more decreased BMD values at lumbar level 2 (t=3.424, p=0.009), lumbar level 3 (t=3.171, p=0.019), lumbar level 4 (t=4.187, p=0.001), and total lumbar levels 2–4 (t=2.989, p=0.021) compared with CC+GC genotypes. No associations were found between the OPG, RANK, and RANKL SNPs and clinical manifestations of RA (all p>0.05). Logistic regression analysis indicated that older age (OR=1.057, 95% CI: 1.017–1.099, p=0.005), higher HAQ (OR=2.786, 95% CI: 1.329–5.841, p=0.007), and GG genotype of rs9533155 (OR=3.242, 95% CI: 1.254–8.376, p=0.015) were risk factors of lumbar osteoporosis onset in RA patients.

**Conclusion:** In summary, OPG rs4355801 is associated with susceptibility to RA and RANKL rs9533155 GG genotype potentially contributes to decreased BMD in RA. Studies with larger sample sizes are needed to confirm the present findings.

**Keywords:** OPG/RANK/RANKL, rheumatoid arthritis, single-nucleotide polymorphism, bone mineral density

## Introduction

Rheumatoid arthritis (RA), a systemic autoimmune disease with unknown etiology, is characterized by continuous symmetrical synovitis in joints that eventually leads to bone erosion, cartilage damage, and joint destruction. The prevalence rates of RA vary in different regions of the world and approximately 0.25% to 1% of the total global population are affected.[1] Skeletal complications, such as focal erosion of marginal and subchondral bone, periarticular osteoporosis, and generalized bone loss with reduced bone mass, are the most common manifestations of RA and are significant causes of disability in patients.[2]

Osteoclasts are one of the main effector cells involved in the bone remodeling process. The osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/receptor activator of nuclear factor-κB ligand (RANKL) system is commonly recognized to mediate the function of osteoclasts.[3] RANKL, mainly secreted by fibroblast-like synoviocytes in RA patients, binds to its receptor, RANK, predominantly expressed on the surface of osteoclasts. The interaction between RANKL and RANK promotes

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osteoclast differentiation, maturation, activation, and survival, thus enhancing bone resorption and bone loss. OPG acts as a decoy receptor for binding to RANKL, thereby inhibiting activation of RANK-RANKL signaling and regulating osteoclastic bone resorption. Numerous studies have shown that abundant generation and activation of osteoclasts play a pivotal role in joint cartilage and bone destruction in RA[4] and that the OPG/RANKL ratio may be a protective predictor of bone loss in RA.[5]

Although the etiology and pathogenesis of RA are complex and remain to be completely elucidated, virus infection, obesity, low socioeconomic status, and changes in the lung, gut, and oral microbiome have been implicated in the disease.[6] Genetic predisposition has been demonstrated to play a key role in RA, with first-degree relatives of RA patients carrying a 2–5 times higher risk of RA development compared with the general population.[7] A study confirmed that several single-nucleotide polymorphisms (SNPs) located in the RANK, RANKL, and OPG genes were associated with the presence of anti-citrullinated peptide (anti-CCP) antibodies, as RA-specific autoantibodies, and bone erosion in RA patients.[8]

Considering the pivotal role of the OPG/RANK/RANKL system in the modulation of bone metabolism and its involvement in RA, we hypothesized that SNPs in the RANK, RANKL, and OPG genes may influence RA susceptibility. Elucidation of the correlations between these SNPs and RA may provide new indicators for evaluation of individuals at higher risk of developing RA and its complications such as osteoporosis. Thus, we conducted the present study to clarify the associations between OPG, RANK, and RANKL SNPs and RA susceptibility. The possible correlations of the SNPs with bone mineral density (BMD) and disease activity in RA patients were also assessed.

# Subjects, Materials, and Methods

## Study Population

A total of 319 RA patients (57 men, 262 women; mean age: 54.08±12.82 years) from the Han Chinese population and 330 healthy controls from June 2021 to February 2024 were enrolled in the study. All RA patients were from the Department of Rheumatology and Immunology at Maanshan People's Hospital in China, and were diagnosed according to the criteria published by the American College of Rheumatology/European League against Rheumatism Collaborative Initiative for RA.[9] Patients of other nationalities and complicated with thyroid disease, parathyroid gland disease, other endocrine disorders, serious liver or kidney disease, radiological abnormalities (scoliosis, platyspondyly, others), and tumors were excluded, as these diseases may interfere with bone metabolism and affect results. The 330 healthy controls (66 men, 264 women; mean age: 55.05±12.99 years), matched for age and sex, were recruited from the Health Examination Center of Maanshan People's Hospital.

## Ethical Approval

This study was approved by the Ethics Committee of Maanshan People's Hospital (Grant No. 2021–05-006). All subjects gave written informed consent before enrollment. This work was conducted in accordance with the World Medical Association Declaration of Helsinki.

## Clinical Assessment

Patients were questioned about age, sex, and disease duration. The tender joint and swollen joint counts were recorded to calculate the disease activity score for 28 joints (DAS28). Functional status was evaluated by rheumatologists, using a health assessment questionnaire (HAQ) and a visual assessment score (VAS).[10] Erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), rheumatoid factor (RF), and anti-CCP antibody levels in the peripheral blood of the RA patients were measured by Clinical Examination Center of Ma'anshan People's Hospital and recorded by rheumatologists.

## Measurement of BMD

Dual-energy X-ray absorptiometry (Lunar Prodigy DF +303033; GE Healthcare, Madison, WI, USA) was used to measure the BMD in lumbar levels 2–4 and the proximal femur, including the femoral neck, Ward's triangle, greater trochanter, and total hip. BMD values were automatically calculated from the bone area ($cm^2$) and bone mineral content (g) and expressed as absolute values in $g/cm^2$.

## SNP Screening

The NCBI Genome Database (http://www.ncbi.nlm.nih.gov) was used to screen for genetic signatures indicating SNP loci. The identified loci also had to meet the following two conditions: (a) minor allele frequency ≥5% in the Chinese Han population and (b) linkage disequilibrium coefficient $r^2 \geq 0.8$. Relevant literatures were also reviewed, and only the locus that have not been reported before were selected. A total of five SNPs were finally included in the study: rs4355801 and rs1023968 in OPG; rs10805033 in RANK; and rs9533155 and rs875625 in RANKL.

## DNA Extraction and Genotyping

Whole blood (5mL) was drawn from an arm vein into a sterile tube containing ethylene diamine tetraacetic acid (EDTA) and stored at - 80 °C until genotype analysis was performed. Genomic DNA was extracted from 200 μL of whole blood in accordance with the manufacturer's instructions (Qiagen, Hilden, Germany). The extracted DNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). We designed primers independently and commissioned Shanghai Biowing Applied Biotechnology Company (http://www.biowing.com.cn) to genotype the SNPs using ligase detection reactions.[11] The primer sequences are shown in Table 1.

## Statistical Analysis

Statistical analyses were performed using SPSS software (version 23.0). Hardy–Weinberg equilibrium (HWE) tests were conducted online (http://analysis.bio-x.cn[a]). Data were checked for normality using Kolmogorov–Smirnov test. Normal data was presented as mean and standard deviation, and non-normal data was presented as median and interquartile range. Categorical variables were presented as percentage. The distribution of genotype and allele frequencies between case and control groups was analyzed using chi-square test. The two-tailed independent samples *t*-test (for normally distributed data) or Kruskal–Wallis test (for skewed distributed data) were performed to evaluate the diversity of parameters between different groups. The correlation of variables with osteoporosis was analyzed by binomial logistic regression analysis. All p-values were two-sided. Values of $p<0.05$ were considered statistically significant.

# Results

## Basic Characteristics

A total of 649 subjects were enrolled in the study, comprising 319 RA patients and 330 healthy controls. The mean ages of the RA patients and healthy controls were 54.08±12.82 years and 55.05±12.99 years, respectively. Women accounted for 82.1% of the RA patients and 80.0% of the healthy controls. There were no significant differences in age and sex between the RA patients and healthy controls (both $p>0.05$). The clinical and laboratory data for the RA patients are shown in Table 2. The characteristics of the five SNPs are shown in Table 3. The HWE test results confirmed that the genotype distributions of the five SNPs conformed to HWE in the control group ($p>0.05$) (Table 3).

**Table 1** Primer Sequences for RT-PCR

| SNP | Primers |
|---|---|
| rs4355801 | 5'-TAAACAGGTGTACAGGTCTCAATAA-3' |
| | 5'-TGGGTGGTAGGTGTCAGGGAAAGTC-3' |
| rs1023968 | 5'-ACAAAATTGTATGACTCCAAATCAC-3' |
| | 5'-CCTTGTATTTTGAATCGTAGTTTTG-3' |
| rs10805033 | 5'-AGTGCCCAGTGCTCCCTACATCTCT-3' |
| | 5'-ATAGCTGTGACTTTGAACCAAGACT-3' |
| rs9533155 | 5'-CTTTCCTGACTGTTGGGTGAGCCCT-3' |
| | 5'-CTCGGATGCTTGCTTCTGGCTACAC-3' |
| rs875625 | 5'-TGCTGTTAATATCCTCCGTTTATGC-3' |
| | 5'-GGAACTTGAACCAAGGTGAAAGCCT-3' |

**Abbreviations**: SNP, single-nucleotide polymorphism; RT-PCR, reverse transcription polymerase chain reaction.

**Table 2** Demographic Characteristics and Clinical Features of RA Patients and Healthy Controls

| Parameter | RA Patients (n=319) | Healthy Controls (n=330) | $t/\chi^2$ | p |
|---|---|---|---|---|
| Age (years) | 54.08±12.82 | 55.05±12.99 | 0.960 | 0.337 |
| Female, n (%) | 262(82.1) | 264(80.0) | 0.480 | 0.488 |
| Disease duration (years) | 5(2, 13) | NA | NA | NA |
| DAS28 | 5.85±1.39 | NA | NA | NA |
| The tender joint counts | 6.86±4.63 | NA | NA | NA |
| The swollen joint counts | 11.28±6.87 | NA | NA | NA |
| VAS | 50.83±18.86 | NA | NA | NA |
| HAQ score | 1.48±0.71 | NA | NA | NA |
| ESR(mm/h) | 55.61±32.36 | NA | NA | NA |
| CRP(mg/L) | 38.26±30.94 | NA | NA | NA |
| RF(IU/mL) | 101.03±74.43 | NA | NA | NA |
| Anti-CCP(RU/mL) | 518.39±453.99 | NA | NA | NA |

**Notes**: Data are presented as mean±SD, n (%) or median with the 25th-75th percentile. RA, rheumatoid arthritis; DAS28, disease activity score for 28 joints.
**Abbreviations**: VAS, visual assessment score; HAQ, health assessment questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; anti-CCP, anti-citrullinated peptide antibody.

**Table 3** Characteristics of OPG/RANK/RANKL Gene SNPs

| SNP | Chr | Chr.Position | Allele | p value for HWE Test |
|---|---|---|---|---|
| rs4355801 | 8 | 118911634 | A/G | 0.397 |
| rs1023968 | 8 | 118953299 | T/A | 0.154 |
| rs10805033 | 4 | 62325344 | T/C | 0.079 |
| rs9533155 | 13 | 42573485 | C/G | 0.492 |
| rs875625 | 13 | 42599964 | G/A | 0.367 |

**Abbreviations**: SNP, single-nucleotide polymorphism; Chr, chromosome; HWE, Hardy-Weinberg equilibrium.

## Associations of OPG, RANK, and RANKL SNPs With RA Susceptibility

The distribution frequencies of the alleles and genotypes for the five SNPs are shown in Table 4. The G allele frequency of OPG rs4355801 was higher in the RA patients than in the healthy controls, but the difference did not reach statistical significance (p=0.073). However, a significant difference was noted in the genotype distribution of rs4355801 between the RA patients and healthy controls ($p<0.05$). Specifically, the GG genotype was associated with an increased risk of RA compared with the AA/AG genotypes in recessive models (OR=1.679, 95% CI: 1.062–2.655, p=0.025). No significant differences in the distribution frequencies of the alleles and genotypes were observed for the other four SNPs between the RA patients and healthy controls (all $p>0.05$).

## Associations of OPG, RANK, and RANKL SNPs With BMD in RA Patients

A total of 310 RA patients completed the BMD evaluations. Patients with the GG genotype (n=108) in RANKL rs9533155 had the more decreased BMD values at lumbar level 2 (t=3.424, p=0.009), lumbar level 3 (t=3.171, p=0.019), lumbar level 4 (t=4.187, p=0.001), and total lumbar levels 2–4 (t=2.989, p=0.021) compared with CC+GC genotypes (Table 5). No significant differences were observed at the femoral neck, Ward's triangle, greater trochanter and total hip. The other four SNPs showed no significant differences (all $p>0.05$).

## Associations of OPG, RANK, and RANKL SNPs With Clinical Manifestations in RA Patients

We further explored whether the five SNPs were associated with clinical and serological characteristics in the RA patients. There were no associations between any of the five SNPs and clinical manifestations in the RA patients,

**Table 4** The Distribution of OPG, RANK, and RANKL SNPs Genotypes and Alleles in RA Patients and Controls

| SNP | Analyze Model | Control(n(%)) | RA Patients(n(%)) | $\chi^2$ | p | OR(95% CI) |
|---|---|---|---|---|---|---|
| rs4355801 | Genotype | | | | | |
| | AA | 175(53.0) | 159(49.8) | 5.008 | 0.082 | |
| | GG | 35(10.6) | 53(16.6) | | | |
| | AG | 120(36.4) | 107(33.5) | | | |
| | Allele | | | | | |
| | A | 470(71.2) | 425(66.6) | 3.203 | 0.073 | 0.807(0.637–1.021) |
| | G | 190(28.8) | 213(33.4) | | | |
| | Dominant model | | | | | |
| | AA | 175(53.0) | 159(49.8) | 0.660 | 0.417 | 0.880(0.647–1.198) |
| | GG+AG | 155(47.0) | 160(50.2) | | | |
| | Recessive model | | | | | |
| | GG | 35(10.6) | 53(16.6) | 4.996 | 0.025* | 1.679(1.062–2.655) |
| | AA+AG | 295(89.4) | 266(83.4) | | | |
| rs1023968 | Genotype | | | | | |
| | TT | 206(62.4) | 200(70.9) | 0.413 | 0.814 | |
| | AA | 27(8.2) | 22(16.7) | | | |
| | TA | 97(29.4) | 97(12.4) | | | |
| | Allele | | | | | |
| | T | 509(77.1) | 491(77.9) | 0.113 | 0.737 | 1.046(0.806–1.357) |
| | A | 151(22.9) | 141(22.1) | | | |
| | Dominant model | | | | | |
| | TT | 206(62.4) | 200(62.7) | 0.005 | 0.943 | 1.012(0.736–1.390) |
| | AA+TA | 124(37.6) | 119(37.3) | | | |
| | Recessive model | | | | | |
| | AA | 27(8.2) | 22(6.9) | 0.384 | 0.536 | 0.831(0.463–1.492) |
| | TT+TA | 303(91.8) | 297(93.1) | | | |
| rs10805033 | Genotype | | | | | |
| | TT | 269(81.5) | 264(82.8) | 0.227 | 0.893 | |
| | CC | 11(3.3) | 9(2.8) | | | |
| | TC | 50(15.2) | 46(14.4) | | | |
| | Allele | | | | | |
| | T | 588(89.1) | 574(90.0) | 0.266 | 0.606 | 1.098(0.769–1.568) |
| | C | 72(10.9) | 64(10.0) | | | |
| | Dominant model | | | | | |
| | TT | 269(81.5) | 264(82.8) | 0.171 | 0.679 | 1.088(0.728–1.627) |
| | CC+TC | 61(18.5) | 55(17.2) | | | |
| | Recessive model | | | | | |
| | CC | 11(3.3) | 9(2.8) | 0.142 | 0.706 | 0.842(0.344–2.060) |
| | TT+TC | 319(96.7) | 310(97.2) | | | |
| rs9533155 | Genotype | | | | | |
| | GG | 122(37.0) | 113(35.4) | 0.234 | 0.890 | |
| | CC | 64(19.4) | 61(19.1) | | | |
| | GC | 144(43.6) | 145(45.5) | | | |
| | Allele | | | | | |
| | G | 388(58.8) | 371(58.2) | 0.054 | 0.816 | 0.974(0.781–1.215) |
| | C | 272(41.2) | 267(41.8) | | | |
| | Dominant model | | | | | |
| | GG | 122(37.0) | 113(35.4) | 0.168 | 0.682 | 0.935(0.679–1.288) |
| | CC+GC | 208(63.0) | 206(64.6) | | | |
| | Recessive model | | | | | |

(*Continued*)

**Table 4** (Continued).

| SNP | Analyze Model | Control(n(%)) | RA Patients(n(%)) | $\chi^2$ | p | OR(95% CI) |
|---|---|---|---|---|---|---|
| | CC | 64(19.4) | 61(19.1) | 0.008 | 0.930 | 0.983(0.665–1.452) |
| | GG+GC | 266(80.6) | 258(80.9) | | | |
| rs875625 | Genotype | | | | | |
| | GG | 108(32.7) | 103(32.3) | 0.106 | 0.949 | |
| | AA | 76(23.0) | 71(22.3) | | | |
| | GA | 146(45.2) | 145(45.5) | | | |
| | Allele | | | | | |
| | G | 362(54.8) | 351(55.0) | 0.004 | 0.952 | 1.007(0.809–1.253) |
| | A | 298(45.2) | 287(45.0) | | | |
| | Dominant model | | | | | |
| | GG | 108(32.7) | 103(32.3) | 0.014 | 0.905 | 0.980(0.706–1.361) |
| | AA+GA | 222(67.3) | 216(67.7) | | | |
| | Recessive model | | | | | |
| | AA | 76(23.0) | 71(22.3) | 0.055 | 0.814 | 0.957(0.662–1.382) |
| | GG+GA | 254(77.0) | 248(77.7) | | | |

**Notes**: Data are presented as n (%).*p<0.05.
**Abbreviations**: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

**Table 5** Associations of rs9533155 With BMD in RA Patients

| BMD(g/cm$^2$) | Genotypes | | t | p |
|---|---|---|---|---|
| | GG(108) | CC+GC(202) | | |
| Femoral neck | 0.85±0.22 | 0.86±0.18 | 0.474 | 0.636 |
| Wards triangle | 0.72±0.27 | 0.73±0.27 | 0.583 | 0.955 |
| Greater trochanter | 0.71±0.22 | 0.73±0.20 | 0.720 | 0.472 |
| Total hip | 0.88±0.20 | 0.90±0.17 | 1.019 | 0.309 |
| Lumbar 2 | 0.93±0.21 | 1.00±0.19 | 3.424 | 0.001* |
| Lumbar 3 | 0.99±0.23 | 1.08±0.21 | 3.171 | 0.002* |
| Lumbar 4 | 0.99±0.25 | 1.10±0.19 | 4.187 | <0.001* |
| Total lumbar 2–4 | 0.93±0.28 | 1.03±0.26 | 2.989 | 0.003* |

**Notes**: Data are presented as mean±SD. *p<0.05.
**Abbreviation**: BMD, bone mineral density.

including age, sex, disease duration, DAS28 score, tender joint count, swollen joint count, VAS, HAQ score, ESR, and CRP, RF, and anti-CCP antibody levels.

## Logistic Regression Analysis of Risk Factors for Onset of Osteoporosis in the Lumbar Spine in RA Patients

The onset of osteoporosis in the lumbar spine in RA patients was taken as a dependent variable, and age, gender, disease duration, DAS28, the he tender joint counts, the swollen joint counts, VAS, HAQ score, ESR, CRP, RF level, anti-CCP level and genotypes of rs9533155 were taken as independent variables. The results of binary logistic regression analysis indicated that older age (OR=1.057, 95% CI: 1.017–1.099, p=0.005), higher HAQ (OR=2.786, 95% CI: 1.329–5.841, p=0.007), GG genotype of rs9533155 (OR=3.242, 95% CI: 1.254–8.376, p=0.015) were risk factors of lumbar osteoporosis onset in RA patients (Table 6).

**Table 6** Logistic Regression Analysis of Risk Factors for Onset of Osteoporosis in the Lumbar Spine in RA Patients

| Variables | OR | 95% CI | p |
|---|---|---|---|
| Age (years) | 1.057 | 1.017–1.099 | 0.005* |
| Gender | 3.265 | 0.862–12.362 | 0.082 |
| Disease duration (years) | 1.012 | 0.970–1.056 | 0.583 |
| DAS28 | 1.287 | 0.940–1.762 | 0.115 |
| The tender joint counts | 1.163 | 0.803–1.063 | 0.267 |
| The swollen joint counts | 0.924 | 0.951–1.423 | 0.142 |
| VAS | 1.075 | 0.852–1.232 | 0.059 |
| HAQ score | 2.786 | 1.329–5.841 | 0.007* |
| ESR(mm/h) | 0.989 | 0.972–1.007 | 0.227 |
| CRP(mg/L) | 1.017 | 0.931–1.034 | 0.056 |
| RF(IU/mL) | 0.999 | 0.992–1.005 | 0.688 |
| Anti-CCP(RU/mL) | 0.895 | 0.999–1.001 | 0.895 |
| Genotypes of rs9533155(GG) | 3.242 | 1.254–8.376 | 0.015* |

**Notes**: *p<0.05.
**Abbreviations**: RA, rheumatoid arthritis; DAS28, disease activity score for 28 joints; VAS, visual assessment score; HAQ, health assessment questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; anti-CCP, anti-citrullinated peptide antibody.

## Discussion

In the present study, we mainly focused on clarifying the relationships between several SNPs in OPG/RANK/RANKL system genes and susceptibility, clinical manifestations, and BMD values in RA patients. A significant difference was noted in the genotype distribution of rs4355801 in OPG between RA patients and healthy controls. Patients with the GG genotype of rs4355801 had a higher risk of RA development than patients with the AA/GA genotypes. Meanwhile, the genotype distribution of rs9533155 in RANKL was associated with BMD in RA patients. Specifically, compared to the other two genotypes, patients with the rs9533155 GG genotype showed a greater decrease in BMD values, especially in the lumbar spine. The GG genotype of rs9533155 is a risk factor for the development of lumbar osteoporosis in RA patients. No correlations were found between any of the five SNPs and clinical manifestations in RA patients.

Abnormal bone metabolism is an important feature of RA, and the OPG/RANK/RANKL system, as the most important system for regulating bone metabolism and osteoclastogenesis,[12] is deeply involved in the pathogenesis of RA. A study by Xu S et al showed that RA patients have higher concentrations of RANKL and lower concentrations of OPG in their serum compared with the normal population.[13] In addition, high RANKL/OPG ratios have been observed in the synovial fluid and serum of patients with RA,[14] and these ratios may be able to predict the progression of joint and bone destruction in RA patients.[15,16] Meanwhile, the bone destruction effects of various inflammatory factors in RA, such as IL-6 and TNF-α, depend on the OPG/RANK/RANKL system.[17] For example, IL-6 can promote RANKL expression and inhibit OPG expression by activating the JAK/STAT pathway, thereby promoting osteoclast differentiation.[18]

Although the pathogenesis of RA is not fully understood, genetic variations have been shown to contribute to RA susceptibility.[19] Studies have demonstrated that several SNPs in the OPG/RANK/RANKL system genes are involved in RA. A meta-analysis conducted by Yang H et al[20] published in 2019, found that rs2277438 in RANKL increases the risk of RA. Assmann G et al[21] confirmed that the minor allele of rs35211496 in RANK may have a protective effect on RA, while the minor allele of rs2277438 in RANKL may increase RA susceptibility. Another study by Cai Y et al suggested that rs3102735 in OPG may be associated with RA susceptibility and that its G allele may be a risk factor for RA development,[22] which was inconsistent with the findings of the above study.[21] In the present study, the frequency of the G allele of rs4355801 in OPG was 34.2% in the RA patients, which was higher than the 29.5% observed in the healthy controls, but the difference did not reach statistical significance. The distributions of the AA, GG, and GA genotypes also did not differ significantly between the RA patients and healthy controls, although the GG genotype was a risk factor for RA susceptibility compared with the GA/AA genotypes in recessive models (OR=1.679; 95% CI: 1.062–2.655;

p=0.025). These results suggest that the G allele of rs4355801 may be a risk factor for RA, although confirmation of this finding requires further studies with larger sample sizes. The other four SNPs, namely rs1023968 in OPG, rs10805033 in RANK, and rs9533155 and rs875625 in RANKL, were not found to be related to RA susceptibility.

Osteoporosis, the most important cause of hip fracture and thoracolumbar vertebral compression fracture, arises through major abnormal bone metabolism in RA patients. Osteoporosis is common in RA patients, with up to 30% of patients affected, and they have double the risk of osteoporotic fractures compared with the general population.[23] Generalized osteoporosis (axial and peripheral), juxta-articular osteoporosis, adjacent to synovial membranes, and marginal and subchondral erosions comprise the main clinical patterns of bone mass loss in RA patients. Multiple factors contribute to the presence of osteoporosis in RA patients, including higher disease activity, presence of anti-CCP antibodies, elevated RF levels, use of certain prescribed drugs, especially glucocorticoids, female sex, older age, and menopause. The OPG/RANK/RANKL system is another key regulator in RA. For example, Xu S et al[24] reported that the OPG/RANKL ratio in peripheral blood was a protective factor for the occurrence of osteoporosis in RA patients.

Genetic variations affecting the OPG/RANK/RANKL system for bone remodeling are strongly associated with osteoporosis in both healthy controls and RA subjects. A study by Mydlárová MM et al[25] showed that rs2073618 and rs9525641 in OPG were associated with the manifestation of osteoporosis in Roma and non-Roma women. They recorded a higher frequency of the CC genotype in the osteoporotic group (34.286%) compared with the control group (27.885%). Another SNP in OPG, rs2073618, was also correlated with osteoporosis, with a lower CG genotype frequency in the osteoporosis group (36.8%) than in the control group (47%). Rs3018362 of RANK gene seemed to be associated with osteoporosis of the lumbar spine in a Mexican population,[26] reported by Casas-Avila L et al in 2019, although further studies were needed to confirm these findings.

A few studies have confirmed relationships between SNPs in the OPG/RANK/RANKL system and osteoporosis in RA patients. In a Japanese study involving 2282 RA patients, patients who were homozygous for the major allele of rs6993813 in OPG had a higher risk of hip fracture, while no association was found for rs3018362 in RANK.[27] Nava-Valdivia CA et al[28] reported that rs2073618 in OPG was not associated with low BMD in Mexican-Mestizo female patients with RA. We found a significant correlation between rs9533155 in RANKL and BMD in RA patients. Patients with the GG genotype of rs9533155 had significantly lower BMD values in the lumbar spine, and the GG genotype of rs9533155 was a independent risk factor for osteoporosis in the lumbar spine of RA patients. This result seems to suggest that the G allele of rs9533155 is associated with decreased BMD in lumbar. However, BMD in pelvis is not related to this SNP, and the specific reason is unknown. In addition, unsurprisingly, the study showed that older age and higher HAQ scores are risk factors for osteoporosis in the lumbar spine of RA patients. Overall, such data remain relatively scarce and more research is needed.

Although our study did not find correlations between all five SNPs and clinical manifestations in RA patients, the findings confirmed that certain SNPs in the OPG/RANK/RANKL system genes can affect the disease characteristics in RA patients. Arida A et al[29] found that RA patients with the CC genotype of rs2073618 in OPG had increased rates of RF positivity compared with those having the CG/GG genotypes. Trends toward higher DAS28 scores and rates of carotid atherosclerotic plaque formation were also observed in RA patients with the CC genotype versus the CG/GG genotypes. Another study showed that one SNP in RANK (rs8086340) and three SNPs in RANKL (rs7984870, rs7325635, rs1054016) were significantly associated with the presence of anti-CCP antibodies, a specific marker of RA that is positively correlated with bone erosion.[8] The study further found that rs2073618 in OPG and rs7325635 in RANKL were significantly associated with bone erosion in RA. Such findings can provide indicators to monitor the prognosis of RA patients.

Several potential limitations of the present study should not be ignored. First, the population size was limited, and multicenter studies with large sample sizes may be needed to validate the findings. Second, the exact mechanism underlying the association of the SNP in RANKL and BMD in RA patients remains to be elucidated. Third, the functional role of the OPG/RANK/RANKL system was not examined, and bone erosion was not investigated in the study.

## Conclusions

In summary, our study showed GG genotype of OPG rs4355801 was associated with a higher susceptibility to RA. We also revealed that rs9533155 GG genotype was associated with decreased BMD values in the lumbar spine in RA

patients. These findings are beneficial for screening of populations at high risk of RA and evaluating the risk of osteoporosis in RA patients.

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## Disclosure

The authors have declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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