The relationship between the g.27450A>T genetic variant of OPG gene and osteoporosis in Chinese postmenopausal women

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Abstract

The objective of this study is to evaluate the relationship between the g.27450A–T genetic variant of osteoprotegerin (OPG) gene and osteoporosis in Chinese postmenopausal women. A total of 886 subjects were enrolled in this study. The femoral neck hip, lumbar spine (L2–4), and total hip bone mineral density (BMD) were detected by dual-energy X-ray absorptiometry (DEXA). The genotyping of the g.27450A–T genetic variant of OPG gene was investigated by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and DNA sequencing methods. Significant differences in the femoral neck hip, lumbar spine (L2–4), and total hip BMD among different genotypes were found, and the subjects with AA genotype were significantly higher than those of AT and TT genotypes (P < 0.05). The allele-A could be a decreased risk factor for osteoporosis. Results from this study support that the g.27450A–T genetic variant of OPG gene has potential relationship with BMD and osteoporosis in Chinese postmenopausal women.

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1. Introduction

Osteoporosis is a complex disease and polygenic health problem, particularly in postmenopausal women. It is characterized by low bone mineral density (BMD), micro-architecture deterioration of bone tissue and a consequent increase of fracture risk [1–9]. Previous studies indicate that low BMD is a major risk factor for osteoporosis and is highly heritable [9–12]. Multiple association studies have pointed to candidate genes that might be involved in the pathogenesis of osteoporosis [9,13,14], and genetic factors play key function [8, 9, 15–21]. Osteoprotegerin (OPG) gene is a member of the tumor necrosis factor receptor superfamily and plays a key role in bone remodeling [9, 11,22]. It is located on chromosome eight and consists of five exons [6, 23]. Recently, OPG gene is considered to be one of the most important genes for influencing the development of BMD and osteoporosis [9,20, 21,24–31]. The potential association of genetic variants of OPG gene (such as A163G, T245G, T950C and G1181C, C21775T, G23276A, T23367C and C27406T) with BMD and osteoporosis has been reported [6,11,20–22,25,29,32–41], results from these observations indicated that these genetic variants may affect BMD and osteoporosis [20,21, 24–31,37]. However, no related reports have been achieved to analyze the potential association between the g.27450A–T genetic variant of OPG gene and BMD and osteoporosis. Considering the importance of the OPG gene on the development of BMD and osteoporosis, in this study, we aimed to evaluate the potential association of this genetic variant with its influence on BMD and osteoporosis.

2. Materials and methods

2.1. Study population

A total of 886 unrelated Chinese postmenopausal women, consisting of 441 subjects with osteoporosis and 445 age-matched healthy controls were recruited in this study from the Affiliated Hospital of Putian University (Putian, China) between January 2009 and December 2013. Their ages ranged from 45 to 88 years old (mean 63.8 ± 7.5 years). Age, height, weight and body mass index (BMI) of participants were recorded. Subjects with present or past history of diseases and taking drugs to prevent osteoporosis that might affect bone metabolism were excluded. All subjects are genetically unrelated Chinese Han ethnicity and live in Putian City, Fujian Province of China. The protocol of this study was approved by the ethics committee of the Affiliated Hospital of Putian University. Informed written consent forms were obtained from all participants.

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2.2. Measurement of BMD

BMD was measured at the femoral neck, lumbar spine (L2–4), and total hip. Dual-energy X-ray absorptiometry (DEXA) (Lunar Expert 1313, Lunar Corp., USA) was utilized for measurement. The value of BMD (g/cm²) was automatically calculated from bone mineral content (g) and bone area (cm²).

2.3. Genotyping

The peripheral venous blood was collected from all subjects. Genomic DNA was extracted from peripheral venous blood using the Axygen DNA Isolation Kit (Axygen, CA). Using the Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA), we designed the specific polymerase chain reaction (PCR) primers (F: 5'-GAAATTCT GCCAGAACCTTTGCC-3'; R: 5'-TTCAATGCTTCTGCTCCACTTTCC-3'). The PCR reaction was carried out in a total volume of a 20 μl solution, containing 50 ng template DNA, 1× buffer (100 mmol Tris–HCl, pH 8.3; 500 mmol KCl), 0.25 μmol primers, 2.0 mmol MgCl₂, 0.25 mmol dNTPs (Bioteke Corporation, Beijing, China), and 0.5 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were performed in an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 32 s, 63.9 °C for 32 s and 72 °C for 32 s, and then a final extension at 72 °C for 5 min. The PCR-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing methods were utilized to detect the genotype of the g.27450A→T genetic variant of OPG gene. Following the supplier's manual, the amplified PCR products (5 μl) were digested with 5 units of Maell restriction enzyme (MBI Fermentas, St. Leon-Rot, Germany) at 37 °C for 10 h. The digested products were separated by electrophoresis containing ethidium bromide and visualized under UV light.

2.4. Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS 15.0; SPSS Inc., Chicago, IL, USA). The Hardy–Weinberg equilibrium (HWE) for genotypic distributions was calculated by the chi-square (χ²) test. The multiple regression analyses was utilized to evaluate the effect of the g.27450A→T genetic variant of OPG gene on BMD and osteoporosis. All data were performed as mean ± SD (standard deviation of the mean). The BMD value was adjusted by age, weight and height. A P value less than 0.05 was considered as the statistically significant level.

3. Results

3.1. Identification and genotyping of OPG genetic variant

In total, 886 subjects were enrolled in this case–control study. There were no differences in the age, height, weight, BMI (all P values > 0.05). Using the PCR-RFLP and DNA sequencing methods, we identified the g.27450A→T genetic variant of OPG gene. Our sequence analyses indicate that this genetic variant is caused by an A → T mutation. It is a synonymous mutation in exon5 at 27450 position of OPG gene (p.Leucine) Leu295Leu, reference sequences GenBank IDs: NG_012202.1, NM_002546.3, NP_002537.3. The PCR amplified products were digested with Maell restriction enzyme and divided into three genotypes: AA (166 and 54 bp), AT (220,166 and 54 bp) and TT (220 bp).

3.2. Allelic and genotypic frequencies

We detected all three possible genotypes of the g.27450A→T genetic variant of OPG gene in the studied populations. The distributions of allele and genotype were corresponded to the HWE (P > 0.05, Table 1). Table 1 shows the allelic and genotypic frequencies in osteoporosis cases and healthy controls. The allele-A and genotype-AA were predominant in the studied populations. The allele frequencies in osteoporosis cases (A, 63.83%; T, 36.17%) were significantly different from healthy controls (A, 71.24%; T, 28.76%; χ² = 11.0781, P = 0.0009, Table 1). The genotype frequencies in osteoporosis cases were not consistent with healthy controls, the differences being statistically significant (χ² = 11.2059, P = 0.0037, Table 1).

3.3. Association between OGG1 genetic variant and BMD

Table 2 shows the age, weight, BMI, height, femoral neck hip BMD value, spine BMD value, and total hip BMD value in each genotype. We found significant differences in the femoral neck hip, lumbar spine (L2−4), and total hip BMD among different genotypes in the studied subjects, subjects with the AA genotype showed significantly higher BMD value than those of the AT and TT genotypes (all P values < 0.05, Table 2).

4. Discussion

Osteoporosis is an important health disease with a strong genetic component. It is generally accepted that the genetic factors have been considered to play key functions in the development of osteoporosis [8, 10–12,15–22]. Several published studies have reported that OPG gene is an important candidate gene for influencing BMD and osteoporosis [9, 20,21,24–31,37]. Analyses of the potential association between OPG single nucleotide polymorphisms (SNPs) and BMD and osteoporosis have given various results [6,11,20–22,25,29,32–37]. However, the findings from these observations still remain conflicting rather than conclusive for the development of BMD and osteoporosis. In the current study, we detected the g.27450A→T genetic variant of OPG gene using the PCR-RFLP and DNA sequencing methods, and then assessed the potential influence of this SNP with BMD and osteoporosis by association analysis. We found significant differences in the frequencies of allele and genotype between osteoporosis patients and healthy controls (Table 1). Our data have demonstrated that this SNP was significantly associated with BMD and osteoporosis, individuals with the AA genotype had significantly higher BMD value compared to those of the AT and TT genotypes (P < 0.05, Table 2). The allele-A and genotype-AA could be decreased risk factors for the development of osteoporosis in Chinese postmenopausal women (Table 2). Results from this study provided more evidence to reveal the role of OPG gene in the development of osteoporosis and genetic variants of OPG gene could be useful molecular biomarkers for evaluating the risk of osteoporosis. To date, there are several similar studies demonstrating

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>The genotype and allele frequencies of the g.27450A→T genetic variant in the studied populations.</td>
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<tr>
<td>Groups</td>
</tr>
<tr>
<td>Case group (n = 441)</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>AT</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>Control group (n = 445)</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>AT</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>Total (n = 886)</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>AT</td>
</tr>
<tr>
<td>TT</td>
</tr>
</tbody>
</table>

| χ² | P |
| 11.0781 | 0.0009 |
the potential association of other OPG SNPs with BMD and osteoporosis (for example, A163G, T245G, 1950C and G1181C, C2177T, G2276A, T23367C and C27406T) which were consistent with our findings that the genetic variants of OPG gene may contribute to genetic effects on BMD and osteoporosis [20,21,24–31]. Although the g.27450A>C genetic variant is a synonymous mutation and does not result into amino acid replacement, it might be linked to other known non-synonymous genetic variants, such as lysine (Lys)3 asparagine (Asn), isoleucine (Ile)184 methionine (Met), threonine (Thr) 154Met and Valine (Val) 281Met, which have been proven to be significantly associated with the risk of BMD and osteoporosis and influence the function of OPG protein [20,21,22,32]. Thus, the g.27450A>C genetic variant of OPG gene might play similar functions on the development of osteoporosis. In order to clarify the molecular mechanisms and pathophysiology underlying the association, further functional studies on larger different populations should be undertaken to replicate these results and to investigate other genetic variants spanning the whole OPG gene region.

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References


<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AT</th>
<th>TT</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Number (%)</td>
<td>420 (47.41)</td>
<td>357 (40.29)</td>
<td>105 (12.30)</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.5 ± 8.9</td>
<td>63.3 ± 9.3</td>
<td>63.9 ± 8.5</td>
<td>0.367</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162 ± 6.8</td>
<td>164 ± 7.2</td>
<td>165 ± 6.5</td>
<td>0.335</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.0 ± 5.8</td>
<td>61.8 ± 6.5</td>
<td>62.5 ± 7.2</td>
<td>0.245</td>
</tr>
<tr>
<td>BMI</td>
<td>23.0 ± 3.33</td>
<td>23.3 ± 3.45</td>
<td>23.7 ± 3.52</td>
<td>0.426</td>
</tr>
<tr>
<td>Femoral neck hip BMD (g/cm²)</td>
<td>0.767 ± 0.211</td>
<td>0.881 ± 0.125</td>
<td>0.839 ± 0.235</td>
<td>0.021</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>0.867 ± 0.142</td>
<td>0.850 ± 0.116</td>
<td>0.840 ± 0.116</td>
<td>0.017</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>0.835 ± 0.108</td>
<td>0.835 ± 0.165</td>
<td>0.821 ± 0.112</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Note: Data are shown as mean ± standard deviation (SD); BMI, body mass index; BMD, bone mineral density (BMD values adjusted by age, height and weight).

