Comprehensive Association Analysis of 10 Single Nucleotide Polymorphisms Associated With Osteoporosis among a Taiwanese Population

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ABSTRACT  In the present study, we tested for an association between single nucleotide polymorphisms (SNPs) and bone mineral density (BMD) of the hip and lumbar spine in a Taiwanese population by analyzing 252 healthy persons (non-osteoporosis) and 193 persons with osteoporosis. We found that age; body mass index; family history; and consumption of coffee or vitamin D were associated with osteoporosis in our Taiwanese population. Our results also indicated that osteoprotegerin (OPG) SNP (rs6993813 T→C) and receptor activator of nuclear factor kappa-B ligand (RANKL) SNP (rs9594738 C→T) were significantly associated with BMD in our Taiwanese population. Additionally, we propose that the mean threshold value of integration of 7 wild-type SNPs (rs7524102 and rs6696981 of 1p36, rs11898505 of 2p16, rs9479055 of 6q25, rs326340 and rs1289759 of 3q13, and rs9594738 of 13q14) can be used for both a Taiwanese reference for bone density testing and to achieve the effect of grading.

INTRODUCTION  Osteoporosis, which literally means “porous bones,” is a multifactorial skeletal bone disease characterized by microarchitectural deterioration and low bone mineral density (BMD) of bone tissue. In the United States alone, the direct medical costs for osteoporosis were estimated to be about $13.8 billion in 1995, of which $11 billion (80.4 %) were attributed to the treatment of women (Ray et al. 1997). To date, the expenditure of this disease in the United States is $17 billion every year (Richards et al. 2008), and the annual cost of fractures is expected to increase another 50% by 2025 (Burge et al. 2007). However, osteoporosis is a worldwide public health issue that is present not only in Western countries but also in Asia (Delmas 2002). According to the World Health Organization (WHO), osteoporosis is defined in those whose BMD value is more than 2.5 SD below the mean BMD of the ethnicity-matched and young adult population (Murphy et al. 2003). Based on this WHO definition, many women over the age of 80 years suffer from osteoporosis (Boonen 2010). Osteoporosis can cause symptoms such as fracture of the hip, spine, and wrist (Huang et al. 2003), with hip fracture often resulting in high mortality.

Recently, some studies have examined the effectiveness of available treatments for osteoporosis, including calcium, vitamin D, hormone replacement therapy, selective estrogen receptor modulators (SERMs), bisphosphonates, parathyroid hormone (PTH), and calcitonin (Altkorn & Vokes 2001; Delmas 2002). However, these medicines are not omnipotent for every patient. In clinical practice, treatments of osteoporosis need to be evaluated on a case-by-case basis. Therefore, the identification of effective diagnostic markers in advance of os-
teoporosis merits further investigation. Os-
teoporosis is associated with environmental fac-
tors and with many genes (Rivadeneira et al.
2009). Some studies have indicated that genetic
factors could be used for evaluating population
variation of BMD (Brown et al. 2005; Crawford
et al. 2010). There is abundant evidence for a
genetic contribution to variation in BMD, with
heritability estimates between 0.6 and 0.8 (Pea-
cock et al. 2002). Moreover, BMD may be regar-
ded as a trait that is polygenic in nature. Sev-
eral studies of susceptibility genes and genome-
wide linkages have estimated that multiple ge-
etic loci are involved in BMD (Cheung et al.
2010; Hsu et al. 2010; Zhang et al. 2010). In
populations of European ancestry, numerous
candidate genes related to osteoporosis have
been analyzed, including estrogen receptor 1
(ESR1), receptor activator of nuclear factor
(RANK), major histocompatibility complex
(MHC), and osteoprotegerin (OPG) (Liu et al.
2010; Styrrkarsdottir et al. 2008). However, there
is some evidence indicating that genes regulat-
ing BMD differ between various gender and
skeletal sites (Kaufman et al. 2008).

With the rapid advancement in single nucle-	otide polymorphism (SNPs) identifications and
the development of databases, such as the ge-
nome-wide association study and International
HapMap Project, genetic studies of osteoporo-
sis are greatly facilitated (Koller et al. 2010).
Although many osteoporosis-associated genes
have been reported in the past 15 years (Li et al.
2010), only a few studies have directly exam-
ined the association between SNPs and osteo-
porosis in Taiwanese (Chao et al. 2010; Chen
et al. 2001; Lin et al. 2008; Tsai et al. 2003). In
the present study, we tested for an association
between SNPs and BMD of the hip and lumbar
spine by performing an analysis on a Taiwanes-
e population containing 252 healthy persons
(non-osteoporotic) and 193 persons with osteo-
porosis (including postmenopausal Taiwanese women and osteoporotic men). Using a double-
blind test, we evaluated the feasibility of using
the analyzed SNPs for determining osteoporo-
sis in Taiwanese.

MATERIALS AND METHODS

Sample Collection

In this study, the Taiwanese population liv-
ing on the area of Taiwan was divided into 2
groups: one group contained 252 healthy per-
sons (non-osteoporotic) and another group had
193 osteoporotic persons (including postmeno-
pausal Taiwanese women and osteoporotic men).
Standardized BMD was calculated and correc-
ted for sex, age, and weight. All blood samples
were obtained from these 2 populations and kept
at 4 °C. All of these study protocols were ap-
proved from The Institutional Review Board of
Kaohsiung Veterans General Hospital (IRB No.
VGHKS97-CT9-09).

Bone Mass Measurement

The BMDs of the lumbar spine and hip (a
total hip or femoral neck measurement) of the
study participants were measured by a dual en-
ergy X-ray absorptiometry machine (DELPHI
QDR series, Hologic, USA.) (Flicker et al.
1995). A normal BMD is more than -1 standard
deviation compared to a control matched for age,
sex, and race; the BMD of a person with osteo-
penia is between -1 standard deviation and -2.5
standard deviation; and the BMD in an os-
teoporotic person is less than -2.5 standard de-

DNA Extraction

DNA extraction was performed using the QIA amp DNA Blood Mini Kit (QIAGEN® Hil-
den’ Germany) according to the manufacturer’s
recommendations. The blood was digested with
0.5 mg/mL proteinase K in 400 µL cell lysis
solution for 24 h at 55 °C until the blood was
completely lysed. Then, 200 µL absolute ethanol
was added to the lysed sample. The mixture was
transferred into the DNeasy mini column and
centrifuged for 1 min at 8,000 rpm. The DNeasy
mini column was washed with 500 µL washing
buffer and centrifuged for 1 min at 8,000 rpm.
Finally, the DNA was eluted into a clean 1.5-

Genotyping

Genotyping was conducted by the Allele-Spe-
cific PCR method (AS-PCR). Ten SNPs from 8
chromosomal regions were used in association
studies with spinal BMD (Table 1). The SNPs
was performed by As-PCR and used in this study
were as follows: allelic SNPs on lp36 (rs7524102,
rs6696981), one SNP on lp21 (major histocom-
patibility complex, MHC) (rs3130340), one SNP on 2p16 (MutS homolog 6, MSH6) (rs3130340), one SNP on 3q13 (Intraflagellar transport protein 57 homolog, IFT57) (rs3130340), one SNP on 6q25 (estrogen receptor 1, ESR1) (rs9479055 C, rs326340 T, and rs1289759 C), one SNP on 8q24 (osteoprotegerin, OPG) (rs6993813), one SNP on 13q14 (receptor activator of nuclear factor kappa-B ligand, RANKL) (rs9594738), and one SNP on 18q21 (receptor activator of nuclear factor kappa-B, RANK) (rs3018362). These SNPs were selected based on previous genome-wide association studies (Lei et al. 2007).

<table>
<thead>
<tr>
<th>Chromosomal regions</th>
<th>SNP and allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36</td>
<td>rs7524102 G</td>
</tr>
<tr>
<td>6p21(MHC)</td>
<td>rs3130340 C</td>
</tr>
<tr>
<td>2p16(SPTBN1)</td>
<td>rs11898505 G</td>
</tr>
<tr>
<td>6q25(ESR1)</td>
<td>rs9479055 C</td>
</tr>
<tr>
<td>3q13(IFT57)</td>
<td>rs326340 T</td>
</tr>
<tr>
<td>8q24(OPG)</td>
<td>rs6993813 T</td>
</tr>
<tr>
<td>13q14(RANKL)</td>
<td>rs9594738 C</td>
</tr>
<tr>
<td>18q21(RANK)</td>
<td>rs3018362 G</td>
</tr>
</tbody>
</table>

Double-blind Test

Using the double-blind test, we analyzed another 10 volunteers not including in the previous two groups. According to the previous integration of SNP sites, we scored the SNPs of these 10 volunteers. We used the integration of SNP sites to distinguish normal, osteopenia, and osteoporosis, respectively. These 10 volunteers were also measured at the lumbar spine and at the hip (a total hip or femoral neck measurement) by DXA. We followed the same definitions that the authors used in the previous study (Kanis et al. 1994) to identify normal, osteopenia, and osteoporosis with the BMD of these 10 volunteers. Then, we compared the score and the BMD to analyze the difference.

Statistical Analysis

The results of the genetic tests were expressed by genotype, and divided into several categories according to wild/mutant status for the SNPs determined. A 95% confidence interval for the odds ratio for subjects carrying a haplotype either above or below 1.0, or $p < 0.05$, was defined as constituting statistical significance. The effect of the haplotypes of each gene on the BMD was evaluated by percentages of DXA measurements at the lumbar spine and at the hip in the subjects carrying the individual haplotypes. Data were compared with an analysis of variance (ANOVA). When the ANOVA results were statistically significant, multiple comparisons were performed using the Scheffé method. All data were analyzed using SPSS version 10.0 software (SPSS for Windows Inc., Chicago, IL, USA) and $p$ values <0.05 were defined as constituting statistical significance for every analysis.

RESULTS

Analysis of 10 SNPs Associated with Osteoporosis

According to BMD, our study sample was composed of 102 osteoporosis participants, 91 osteopenia participants, and 252 healthy participants. The data, evaluated by the SPSS software for analysis of variance (one-way ANOVA) (Table 2), were divided into 3 groups according to BMD analysis of 10 SNPs ANOVA (Table 3). The results of rs6993813 (T→C, $p = 0.049$) and rs9594738 (C→T, $p = 0.033$) showed significant association with BMD. OPG (rs6993813 T→C) and RANKL (rs9594738 C→T) were the 2 genes that were significantly associated with BMD among the 10 SNPs. These results show that family history affects BMD in a Taiwanese population. In addition to family history, our results showed that BMD changed with age, body mass index (BMI), coffee consumption, calcium supplements, vitamin D, and bisphosphonate.

Correlation of Integrated SNP Sites for Osteoporosis Analysis

For 7 SNPs (rs7524102, rs6696981, rs11898505, rs9479055, rs326340, rs1269759, rs9594738), the control group displayed the normal allele (wild type). For 3 SNPs (rs3130340, rs6993813, rs3018362) the control group displayed the mutant allele. We integrated the SNP sites into 3 groups: total SNPs, wild type, and mutant. We analyzed the median of the 3 groups (Table 4) and the wild type showed a significant difference when the value was 3.481 ($p = 0.002$) (Table 5). We used BMDs to evaluate the correlation of the key point for ag-
### Table 2: Demographic data

<table>
<thead>
<tr>
<th></th>
<th>Healthy individuals</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.36±6.236</td>
<td>62.53±7.967</td>
<td>65.17±8.217</td>
<td>50.021</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>24.4 ±3.07</td>
<td>24.1 ±3.30</td>
<td>22.6 ±3.43</td>
<td>11.444</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postmenopausal age</td>
<td>48.85±5.682</td>
<td>48.96±5.267</td>
<td>47.78±6.673</td>
<td>1.416</td>
<td>0.244</td>
</tr>
<tr>
<td>Lumbar BMD</td>
<td>0.03±1.077</td>
<td>-1.13±0.899</td>
<td>-2.28±0.981</td>
<td>194.071</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip BMD</td>
<td>-0.46±0.867</td>
<td>-1.79±0.427</td>
<td>-2.98±0.673</td>
<td>429.304</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip &amp; Neck bone</td>
<td>0.79±0.108</td>
<td>0.66±0.060</td>
<td>0.55±0.072</td>
<td>258.428</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fracture</td>
<td>7.48±3.542</td>
<td>12.62±6.643</td>
<td>19.51±8.636</td>
<td>160.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip fracture</td>
<td>0.52±0.892</td>
<td>1.86±2.159</td>
<td>5.32±4.661</td>
<td>131.503</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3: SNP analysis of osteoporosis by one-way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Healthy individuals</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q36 rs7524102 (G→A)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.514</td>
<td>0.599</td>
</tr>
<tr>
<td>1q36 rs6696981 (G→T)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.514</td>
<td>0.599</td>
</tr>
<tr>
<td>6p21 (MHC) rs3130340 (C→T)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.661</td>
<td>0.517</td>
</tr>
<tr>
<td>2p16 (MSH6) rs11898505 (G→A)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.639</td>
<td>0.528</td>
</tr>
<tr>
<td>6q25 (ESR1) rs9479055 (C→G)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.284</td>
<td>0.753</td>
</tr>
<tr>
<td>3q13 (IFT57) rs326340 (T→G)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>2.002</td>
<td>0.136</td>
</tr>
<tr>
<td>3q13 (IFT57) rs1269759 (C→T)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.939</td>
<td>0.392</td>
</tr>
<tr>
<td>8q24 (OPG) rs6993813 (T→C)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>3.035</td>
<td>0.049*</td>
</tr>
<tr>
<td>13q14 (RANKL) rs9594738 (C→T)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>5.802</td>
<td>0.003*</td>
</tr>
<tr>
<td>18q21 (RANK) rs3018362 (G→A)</td>
<td>252</td>
<td>91</td>
<td>102</td>
<td>0.268</td>
<td>0.765</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level

### Table 4: Integration of SNP sites for osteoporosis analyzed

<table>
<thead>
<tr>
<th></th>
<th>Numbers</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>445</td>
<td>1.0</td>
<td>13.0</td>
<td>7.8</td>
<td>2.106</td>
</tr>
<tr>
<td>Wild type</td>
<td>445</td>
<td>1.0</td>
<td>8.0</td>
<td>3.481</td>
<td>1.468</td>
</tr>
<tr>
<td>Mutant</td>
<td>445</td>
<td>0.0</td>
<td>6.0</td>
<td>4.32</td>
<td>1.229</td>
</tr>
</tbody>
</table>

The numbers were aggregated such that the wild type is homologous for zero units and heterologous for one unit, and the mutant is homologous for 2 units.

*Includes the total SNP sites analyzed.

### Table 5: Analyzed results of key point for aggregated numbers in total, wild-type, and mutant SNP sites

<table>
<thead>
<tr>
<th></th>
<th>Numbers</th>
<th>Mean</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>445</td>
<td>7.8</td>
<td>0.197</td>
<td>-0.053 to 0.256</td>
</tr>
<tr>
<td>Wild type</td>
<td>238</td>
<td>7.8</td>
<td>0.384</td>
<td>-0.096 to 0.251</td>
</tr>
<tr>
<td>Mutant</td>
<td>207</td>
<td>7.8</td>
<td>0.384</td>
<td>-0.096 to 0.251</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level
aggregated numbers with osteoporosis. The results showed a significant difference only between NS and OS (p = 0.0106, Table 6). As a consequence, neither the difference of osteoporosis and osteopenia nor the difference of osteoporosis and healthy individuals could be distinguished.

**Double-blind Test**

According to the correlation of integrated SNP sites for osteoporosis analysis, we randomly choose 10 volunteers to genotype the 7 wild-type SNPs associated with osteoporosis. Then, we compared the score from the integration of the 7 SNPs to BMDs measured by DXA (Table 7). Using the integrated score, we were able to identify 8 volunteers with osteopenia/osteoporosis and 2 volunteers with normal phenotype. The conformity showed 100% match between the integrated score and BMD. These results demonstrate that the evaluation score could accurately identify the osteopenia/osteoporosis from normal phenotype by genotyping the 7 wild-type SNPs.

**Table 6: Correlation of analyzed results of key point for aggregated numbers with osteoporosis**

<table>
<thead>
<tr>
<th>Numbers</th>
<th>Total</th>
<th>Wild type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals, osteopenia and osteoporosis correlation</td>
<td>0.065</td>
<td>0.123**</td>
<td>-0.036</td>
</tr>
<tr>
<td>sig. (2-tailed)</td>
<td>0.173</td>
<td>0.010</td>
<td>0.452</td>
</tr>
<tr>
<td>Healthy individuals and osteopenia correlation</td>
<td>0.051</td>
<td>0.097</td>
<td>-0.025</td>
</tr>
<tr>
<td>sig. (2-tailed)</td>
<td>0.351</td>
<td>0.072</td>
<td>0.650</td>
</tr>
<tr>
<td>Healthy individuals and osteoporosis correlation</td>
<td>0.065</td>
<td>0.128*</td>
<td>-0.037</td>
</tr>
<tr>
<td>sig. (2-tailed)</td>
<td>0.223</td>
<td>0.016</td>
<td>0.491</td>
</tr>
<tr>
<td>Osteopenia and osteoporosis correlation</td>
<td>0.018</td>
<td>0.032</td>
<td>-0.015</td>
</tr>
<tr>
<td>sig. (2-tailed)</td>
<td>0.808</td>
<td>0.654</td>
<td>0.841</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

**DISCUSSION**

Osteoporosis is considered to be associated with diet habits, such as coffee consumption (Barrett-Connor et al. 1994; Hallstrom et al. 2006) and cola intake (Tucker et al. 2006). Age and BMI have also been identified as risk factors for osteoporosis (van der Voort et al. 2000). Fox et al. (1998) have reported that a positive family history is a potential risk factor for osteoporotic fractures. By analyzing environmental and medical factors, we found that age, BMI, family history, and consumption of coffee were associated with osteoporosis in our Taiwanese study population. Overall, our results also support the above-mentioned hypothesis that family history, age, BMI, and coffee are associated with osteoporosis in Taiwanese. In addition, our results showed that BMD changed with calcium supplements, vitamin D, and bisphosphonate.

In this study, we also identified genetic associations with osteoporosis. We tested 10 SNPs from 8 chromosomal regions. Two SNPs (rs6993813 of OPG and rs9594738 of RANKL) were significantly associated with osteoporosis in this Taiwanese population. We found that the key point of integration of 7 wild-type SNPs (rs7524102 and rs6696981 of 1p36, rs11898505 of MSH6, rs9479055 of ESR1, rs326340 and rs1289759 of IFT57, and rs9594738 of RANKL) is 3.48 (p = 0.002). Using BMD as a reference to determine the key point, we found significant differences between healthy individuals, osteopenia, and osteoporosis (p = 0.011), and healthy individuals and osteopenia (p = 0.0106). Using a double-blind test, we succeeded in distinguishing between normal and osteopenia/osteoporosis in 10 volunteers with 100% accuracy by following the key point of 7 wild-type SNPs.

Geographic ancestry is an important factor when evaluating the genetic risk factors of complicated diseases, especially osteoporosis (Styrkarsdottir et al. 2010). Several susceptibility genes for osteoporosis, including 1p36, MHC, MSH6, and ESR1, have recently been revealed and analyzed mainly in populations of European descent (Styrkarsdottir et al. 2008; Styrkarsdottir et al. 2009; Zhang et al. 2010). However, none of these SNPs had been fully investigated in Taiwanese populations. In contrast, a SNP in 1p36, ESR1, which had previously been shown to be associated with BMD in a Han Chinese population (Liu et al. 2010), did not show strong evidence of association in our study. Taiwanese population is composed of three subgroups, and three subgroups immigrated from China to Taiwan Island at different times; the Minnan (70% of the population; about 300–400 years ago), the Hakka (13%; about 200 years ago), and the Mainlanders (14%; about 50 years ago) (Yang et al. 2006). Rosenberg et al. (2010)
Table 7: Double-blind test

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>BMI</th>
<th>Menopause</th>
<th>Lumbar BMD</th>
<th>Hip &amp; Neck Bone Fracture</th>
<th>rs94</th>
<th>rs12</th>
<th>rs31</th>
<th>rs66</th>
<th>rs95</th>
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<table>
<thead>
<tr>
<th>Score</th>
<th>BMD</th>
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*Score is derived from the integration of the 7 wild-type SNPs. The groups are separated using the value of 3.481, such that a score higher than 3.481 is designated as OS/OA; and a score lower than 3.481 is determined to be NS.

*BMD values greater than -1 are defined as control groups (NS); values between -2.5 and -1 are defined as osteopenia (OS); and values less than -2.5 are defined as osteoporosis (OA).

*Conformity represents a comparison between the BMD to the score integrated by the 7 wild-type SNPs. If the situation matched with each other, the conformity would be designated as “+”. If the situation did not match with each other, the conformity would be designated as “-”.

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suggest that if two populations are separated by a barrier to gene flow, a marker allele might be found to be associated with a disease in one population, but might not in another population. This theory partially explains why a SNP in 1p36, ESR1 are associated with BMD in a Han Chinese population but not in Taiwanese. That is the reason the previous study of Han Chinese population cannot be directly applied to Taiwanese. Our results showed similar findings to those from other studies evaluating different ethnic populations (Liu et al. 2010; Styrkarsdottir et al. 2009), in that we did not find any significant association between BMD and rs11898505 of MSH6 or rs3130340 of MHC in our Taiwanese study population. Our results indicate that these variants represent osteoporosis susceptibility genes of Taiwanese that display different association profiles from either Han Chinese or European populations.

A variety of studies have suggested that polymorphisms in RANKL, RANK, and OPG may modulate bone density and turnover (Paternoster et al. 2010; Roshandel et al. 2010; Styrkarsdottir et al. 2008). The RANKL/RANK/OPG signaling system plays a critical role in bone remodeling, and an imbalance of the RANKL/RANK/OPG system may cause osteoporosis (Sasaki et al. 2001; von Tirpitz et al. 2003). RANKL binds to RANK, increasing production, activation, and survival of osteoclasts (Hsu et al. 1999), but these effects of RANKL are blocked by OPG (Simonet et al. 1997). Recently, some studies found that SNPs located near RANKL, RANK, and OPG were associated with BMD in genome-wide association studies (Richards et al. 2008; Styrkarsdottir et al. 2008; Styrkarsdottir et al. 2009). In contrast to the previously mentioned study in Han Chinese population (Liu et al. 2010), rs3018362 of RANK is not significantly associated with BMD in our Taiwanese sample. Similar to previous studies, our results also indicated that OPG SNP (rs6993813 T→C) and RANKL SNP (rs9594738 C→T) were significantly associated with BMD in Taiwanese. These finding implicate OPG and RANKL as loci containing variations associated with BMD and provides further insight into the mechanism by which the RANK/RANKL/OPG pathway may affect the skeletal system. Our present study did not focus on the SNP interaction. However, the SNP interaction studies become important (Lin et al. 2009; Meyers et al. 2010; Yang et al. 2011; Yen et al. 2008). Therefore, it is warranted that future studies could further explore and investigate the SNP interaction among these 10 SNPs in our study.

Several studies of susceptibility genes and genome-wide linkage have proven that multiple genetic loci are involved in modulation of BMD and the risk of osteoporosis (Zhang et al. 2010). Because many osteoporosis-associated genes are often located in different chromosomal regions, all the additive or synergistic influences among these candidate SNPs should be considered simultaneously instead of evaluating individually (Lin et al. 2008). Therefore, we divided 10 candidate SNPs into 3 groups (total, wild type, and mutant). We identified the key point of integration of 7 wild-type SNPs. Furthermore we employed the key point of 7 SNPs to perform a double-blind test of BMD on 10 Taiwanese volunteers. Using SNPs to detect osteopenia or osteoporosis, we were able to identify with 100% accuracy the OS patients that had been confirmed via the conventional DXA measurement method. This result reveals that the 7 wild-type SNPs may be useful in the clinical setting, not only in academic research. We suggest these 7 wild-type SNPs may serve as references for bone density testing of Taiwanese.

CONCLUSION

With the increase of the quality of medical care, the number of elderly persons is also growing. Although osteoporosis is not a lethal disease, the risk of fracture accompanying osteoporosis can be fatal to older persons. Therefore, osteoporosis becomes a major issue of modern medicine. Osteoporosis is a disease in which screening of asymptomatic individuals by BMD testing should be beneficial because osteoporosis has a long preclinical course before the onset of fracture. How this individualized practice of screening should be achieved still remains controversial, such as the issue of when and how often to test for BMD. How to detect or even predict the risk of osteoporosis as early as possible is an urgent issue. In this research, we proposed that the mean value of 3.48 in the integration of 7 wild-type SNPs can be used as a Taiwanese reference for bone density testing, and to achieve the effect of grading. The results of our present study may provide valuable mark-
ers in examining multiple genetic factors that cooperatively determine the phenotypic characteristics of osteoporosis.

REFERENCES


Roshandel D, Holliday KL, Pye SR, Boonen S, Borghs H et


