Available online at www.sciencedirect.com
SciVerse ScienceDirect
Journal of the Chinese Medical Association 75 (2012) 335–340

www.jcma-online.com

Original Article

# Opposite associations of osteoprotegerin and ZBTB40 polymorphisms with bone mineral density of the hip in postmenopausal Taiwanese women

Tai-Huang Chao [a], Hsing-Ning Yu [a], Chi-Chuan Huang [a], Wen-Sheng Liu [b], Ko-Hsiu Lu [c,d,*]

[a] *Department of Orthopedics, Zuoying Armed Forces General Hospital, Kaohsiung, Taiwan, ROC*
[b] *Asia-Pacific Biotech Developing, Inc., Kaohsiung, Taiwan, ROC*
[c] *Department of Orthopedics, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC*
[d] *School of Medicine, Chung Shan Medical University, Taichung, Taiwan, ROC*

Received August 22, 2011; accepted March 5, 2012

**Abstract**

*Background*: An elevated annual incidence rate of hip fracture has been reported among elderly Taiwanese. Moreover, bone mineral density (BMD) is the single most reliable predictor of fragility fractures. We aimed to identify the association between gene sequence variants and hip BMD in postmenopausal Taiwanese women.
*Methods*: We prospectively analyzed data from 163 postmenopausal Taiwanese women to test an association between rs7524102, rs6696981, or rs6993813 single-nucleotide polymorphisms (SNPs) and hip BMD.
*Results*: Our study showed that rs6993813 (osteoprotegerin gene) and rs6696981 (ZBTB40 gene) SNPs have an opposite association with hip BMD. For rs6993813 genotypic frequencies, the adjusted odds ratio for hip osteoporosis was 9.53 for individuals with T/T minor allele homozygotes, compared with that of participants with C/C wild-type homozygotes. Hip BMD also had an association with rs6993813 SNPs, especially in T/T minor allele homozygotes. For rs6696981 SNPs, hip BMD in G/T heterozygotes and at least one mutated T allele was higher than that in wild-type G/G homozygotes.
*Conclusion*: The gene sequence variant rs6993813 reduced hip BMD and increased the risk of hip osteoporosis, whereas rs6696981 increased hip BMD in postmenopausal Taiwanese women. This indicated that the two SNPs may provide some explanation for the high risk of hip fracture in this population.


*Keywords:* bone mineral density; hip; osteoprotegerin; single-nucleotide polymorphism; ZBTB40

## 1. Introduction

Osteoporosis is a common disease that predisposes people to fragility fractures at the spine, hip, forearm, or other skeletal sites.[1] Vertebral fractures are the most common type of osteoporotic fractures and are associated with significant morbidity and 1.2–2.3 times increased relative mortality rates. However, hip fractures can lead to an excess mortality rate of 5–20%, mostly attributable to medical complications,[2] morbidity that severely restricts patients, and the substantial economic costs to society.[3] Although elderly women of European descent are at the highest risk,[4] this disorder affects the elderly of both sexes and all racial groups. Similarly, the aging of the Taiwanese population has caused a marked increase in the prevalence of age-related osteoporotic fractures, especially fractures of the hip.[5,6]

The incidence of hip fracture in the elderly apparently occurs at a substantially reduced frequency in the ethnic Chinese population as compared to that of some Caucasian populations, based on hospital discharge records.[7–9] However, hip fracture incidence is relatively high in one of the major

* Corresponding author. Dr. Ko-Hsiu Lu, Department of Orthopedics, Chung Shan Medical University Hospital, 110, Section 1, Chien-Kuo N. Road, Taichung 402, Taiwan, ROC.
*E-mail address:* cshy307@csh.org.tw (K.-H. Lu).

1726-4901/$ - see front matter 
http://dx.doi.org/10.1016/j.jcma.2012.05.004

cities of Taiwan.[10] According to the national health insurance database of Taiwan, a high annual incidence rate of hip fracture among elderly Taiwanese has been reported.[11] Unlike Chinese, the incidence of hip fracture in elderly Taiwanese women is similar to that of US white women, but the incidence in elderly Taiwanese men is higher than that of US white men.

Since the World Health Organization published diagnostic criteria for osteoporosis in 1994,[12] the measurement of bone mineral density (BMD) at multiple skeletal sites is used in the clinical diagnosis of osteoporosis[13] and the assessment of fracture risk.[14] It is well known that low BMD is an important risk factor for fracture,[15] and the risk of osteoporotic fracture is a complex disease regulated by both genetic and environmental factors. Although individual polymorphisms in several candidate genes accounting for only a small portion of the genetic contribution to BMD regulation has been reported,[16,17] there is abundant evidence for a genetic contribution to variation in BMD, with heritability estimates of up to 80%.[18,19] Numerous candidate genes have an association with BMD and with osteoporotic fractures.[20,21] Several quantitative trait loci have also yielded a linkage with BMD.[22]

To clear up the question as to why the ethnic Taiwanese population has higher hip fracture rates, we require an explanation, and there may be a secular effect making the differences more profound. Although several mechanisms have been suggested for the higher incidence rates of hip fracture in Taiwan,[11] we hypothesized that a genetic component with the heritability of hip fracture in the elderly Taiwanese plays an important role in the pathogenesis of osteoporosis. Because osteoporosis is a highly heritable and multifactorial trait, the aim of this study is to identify genetic loci that are associated with low BMD and high fragility fractures of the hip. Sequence variants in the genomic regions rs7524102 and rs6696981, located on the 1p36 (the zinc finger and BTB domain containing 40 gene, *ZBTB40*), and rs6993813 on 8q24 (osteoprotegerin gene, *OPG*), have recently been reported to be significantly associated with hip BMD and osteoporotic fractures.[23−29] Thus, we evaluated the effect of rs7524102, rs6696981, and rs6993813 single-nucleotide polymorphisms (SNPs) on hip BMD in postmenopausal Taiwanese women.

## 2. Methods

### 2.1. Participants and samples collection

A total of 163 postmenopausal Taiwanese women were recruited into this study (Table 1). Their body weight (kg), height (cm), body mass index (BMI), and hip BMD were measured. The women were divided into two groups: the first with a *T*-score of greater than −2.5 standard deviation (SD) (normal and osteopenia), the second with *T*-score less than or equal to −2.5 SD (osteoporosis) groups in accordance with the World Health Organization's published diagnostic criteria for osteoporosis.[12] A questionnaire was administered to collect information regarding age, smoking status, caffeine consumption, and calcium tablet intake. This study was conducted in accordance with the principles in the Declaration of Helsinki, and was approved by the Institutional Review Board of Zuoying Armed Forces General Hospital, Taiwan. All participants gave informed consent for the use of their data and blood specimens. Venous blood specimens obtained from each participant were placed in tubes containing EDTA, and were immediately centrifuged and stored at −80°C for genomic DNA extraction.

### 2.2. Bone mineral density measurements

Using Hologic QDR 2000 (Hologic Corp., Waltham MA, USA), dual-energy X-ray absorptiometry measurements at the hip, total hip measurements were obtained in all patients. Standardized BMD was calculated and corrected for sex, age, and weight in each participating individual.

### 2.3. DNA extraction

Following the manufacturer's protocol, DNA extraction was performed using the DNeasyTM Kit (Qiagen, Valencia, CA, USA). Briefly, the blood was digested with 0.5 mg/mL proteinase K in 400 μL cell-lysis solution for 24 hours at 55°C until the blood was completely lysed. After adding 200 μL absolute ethanol to the lysed sample, the mixture was transferred into the DNeasy mini-column and centrifuged for 1 minute at 8000 rpm. The DNeasy mini-column was washed with 500 μL washing buffer and centrifuged for 1 minute at 8000 rpm. Finally, the DNA was eluted in a clean 1.5-mL microcentrifuge tube.

### 2.4. SNP selection and genotyping

DNA from venous blood was extracted and amplified by allele-specific polymerase chain reaction using allele-specific sense and antisense primers (Table 2).[30] Genotyping was completed on each participant for three tagging SNPs from two chromosomal regions: 1p36 (*ZBTB40*; rs7524102, rs6696981) and 8q24 (*OPG*; rs6993813).

### 2.5. Statistical analysis

All data were expressed as mean ± SD. Linear regression analysis was used for the correlation between age, height, weight or BMI, and BMD of the hip. The Student *t*-test and analysis of variance were used to analyze the differences for continuous variables between groups two and three, respectively. Chi-square test was used to test the differences for categorical variables. Hardy−Weinberg equilibrium was assessed using a goodness-of-fit chi-square test for biallelic markers. The odds ratios (ORs) with 95% confidence intervals (CIs) of the association between genotype frequencies and hip osteoporosis risk were estimated by logistic regression models, after controlling for other covariates. Statistical significance was set at $p < 0.05$.

Table 1
Distributions of demographical characteristics of the popkulation.

| Demographic variables | Total ($n = 163$) | $T$-score $>-2.5$ ($n = 90$) | $T$-score $\leq-2.5$ ($n = 73$) | $p$ |
|---|---|---|---|---|
| Age (y) | 61.47 ± 8.46 | 57.97 ± 5.97 | 65.78 ± 9.10 | <0.001[a] |
| Height (cm) | 155.74 ± 9.80 | 158.41 ± 5.32 | 153.83 ± 4.35 | <0.001[a] |
| Weight (kg) | 58.52 ± 12.59 | 61.79 ± 10.06 | 53.11 ± 8.06 | <0.001[a] |
| BMI | 23.60 ± 3.40 | 24.56 ± 3.30 | 22.43 ± 3.17 | <0.001[a] |
| Smoking | | | | |
| No | $n = 162$ | $n = 90$ | $n = 72$ | 0.265[b] |
| Yes | $n = 1$ | $n = 0$ | $n = 1$ | |
| Caffeine | | | | |
| No | $n = 135$ | $n = 73$ | $n = 62$ | 0.520[b] |
| Yes | $n = 28$ | $n = 17$ | $n = 11$ | |
| Calcium | | | | |
| No | $n = 103$ | $n = 64$ | $n = 39$ | 0.020[b] |
| Yes | $n = 60$ | $n = 26$ | $n = 34$ | |

Values are mean ± SD.
BMI = body mass index.
[a] Student's $t$-test was used to analyze differences in age, height, and weight between $T$-score $>-2.5$ and $T$-score $\leq-2.5$ groups.
[b] Chi-square test was used to test differences in smoking status, caffeine consumption, or calcium intake between "yes" and "no" groups.

## 3. Results

### 3.1. Characteristics of study population

The demographic data of study participants included 90 women with $T$-score $>-2.5$ SD (73 normal and 17 osteopenia), and 73 women with $T$-score $\leq-2.5$ SD. Age, body weight, height, and BMI all showed significant differences between $T$-score $\leq-2.5$ and $T$-score $>-2.5$ groups ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). Significant differences were found between the numbers in calcium tablet intake and non-intake groups ($p = 0.019$), but were not observed between that in either the smoking and nonsmoking groups or the caffeine consumption and non-caffeine consumption groups. Furthermore, declines in BMD correlated significantly with decreased body height ($Y = -19.980 + 0.116X$, $r^2 = 0.228$, $p < 0.001$) and decreased body weight ($Y = -5.873 + 0.070X$, $r^2 = 0.176$, $p < 0.001$), but also with increased age ($Y = 3.661 - 0.089X$, $r^2 = 0.252$, $p < 0.001$) using linear regression.

Table 2
Sequences of sense and antisense primers used in SNPs and the expected sizes of resultant allele-specific PCR products.

| SNP | Primer sequence | Allele-specific PCR products (bp) | Genotype |
|---|---|---|---|
| rs7524102 | | | AA |
| F | 5′-GAGGCAGCAAAGAACAGAGG-3′ | | AG |
| R | 5′-AGACGCTCTGCAAGTTCACA-3′ | 222 | GG |
| A-R | 5′-AGGAGAAAT TTGAGATGCTA-3′ | 81 | |
| G-R | 5′-AGGAGAAAT TTGAGATGCTG-3′ | 81 | |
| rs6696981 | | | GG |
| F | 5′-GAGCAT TTAGCCAGGC ACTC-3′ | | GT |
| R | 5′- CAGGCACATCCACCCTATCT-3′ | 171 | TT |
| G-R | 5′-CCTGGAA GCGGTAGCTG TAG | 142 | |
| T-R | 5′-CCTGGAA GCGGTAGCTG TAT | 142 | |
| rs6993813 | | | CC |
| F | 5′-TCCGGGATAATCTCCCTTTT-3′ | | CT |
| R | 5′-CGGGGTCCACTAACCTAACC-3′ | 255 | TT |
| C-R | 5′-CAAGTTCTGGAGATTAGGGCTC-3′ | 120 | |
| T-R | 5′-CAAGTTCTGGAGATTAGGGCTT-3′ | 120 | |

PCR = polymerase chain reaction; SNP = single-nucleotide polymorphism.

### 3.2. SNPs of rs7524102, rs6696981, and rs6993813

The SNPs from rs7524102, rs6696981, and rs6993813 appeared to vary among individual blood samples. Although the frequency of both rs7524102 and rs6696981 SNPs in this population was in Hardy–Weinberg equilibrium, the rs6993813 SNP did not demonstrate Hardy–Weinberg equilibrium ($p < 0.05$). We still calculated ORs to measure the association, because the Hardy–Weinberg equilibrium test cannot be used to adequately justify the validity of ORs estimates in case-control studies. For the rs7524102 genotype, the frequencies of A allele and G allele in the $T$-score $>-2.5$ group were 76.7% (69/90) and 23.3% (21/90), respectively. In the $T$-score $\leq-2.5$ group, the frequencies of A allele and G allele were 80.14% (58.5/73) and 19.86% (14.5/73), respectively. For rs6696981 genotype, the frequencies of G allele and T allele in the $T$-score $>-2.5$ group were 77.8% (70/90) and 22.2% (20/90), respectively. In the $T$-score $\leq-2.5$ group, the frequencies of G allele and T allele were 84.93% (62/73) and 15.07% (11/73), respectively. For the rs6993813 genotype, the frequencies of C allele and T allele in the $T$-score $>-2.5$ group were 70.0% (63/90) and 30.0% (27/90), respectively. In the $T$-score $\leq-2.5$ group, the frequencies of C allele and T allele were 59.59% (43.5/73) and 40.41% (29.5/73), respectively.

### 3.3. Association between SNPs and osteoporosis

For rs6993813 genotypic frequencies, the adjusted OR (AOR) for hip osteoporosis was 9.53 (95% CI, 1.22–64.27) for individuals with T/T minor allele homozygotes compared with that of patients with C/C wild-type homozygotes, defined as 1 (Table 3). However, there was no significant difference

Table 3
AOR and 95% CIs of *T*-score >−2.5 and *T*-score ≤−2.5 associated with genotypic frequencies.

| Genotype | *T*-score >−2.5 ($n = 90$) (%) | *T*-score ≤−2.5 ($n = 73$) (%) | OR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|
| rs7524102 | | | | |
| AA | 50 (55.56%) | 45 (61.64%) | 1.00 | 1.00 |
| AG | 38 (42.22%) | 27 (36.99%) | 0.79 (0.42−1.49) | 0.87 (0.38−2.00) |
| GG | 2 (2.22%) | 1 (1.37%) | 0.56 (0.05−6.34) | 2.88 (0.13−62.71) |
| AA | 50 (55.56%) | 45 (61.64%) | 1.00 | 1.00 |
| AG or GG | 40 (6.44%) | 28 (38.36%) | 0.78 (0.42−1.46) | 0.91 (0.40−2.06) |
| rs6696981 | | | | |
| GG | 52 (57.78%) | 52 (71.23%) | 1.00 | 1.00 |
| GT | 36 (40.00%) | 20 (27.40%) | 0.56 (0.29−1.08) | 0.47 (0.20−1.12) |
| TT | 2 (2.22%) | 1 (1.37%) | 0.50 (0.04−5.69) | 2.23 (0.10−49.38) |
| GG | 52 (57.78%) | 52 (71.23%) | 1.00 | 1.00 |
| GT or TT | 38 (42.22%) | 21 (28.77%) | 0.55 (0.29−1.07) | 0.50 (0.21−1.18) |
| rs6993813 | | | | |
| CC | 37 (41.11%) | 22 (30.14%) | 1.00 | 1.00 |
| CT | 52 (57.78%) | 43 (58.90%) | 1.39 (0.72−2.70) | 0.86 (0.34−2.17) |
| TT | 1 (1.11%) | 8 (10.96%) | 13.46 (1.58−114.90) | 9.53 (1.22−64.27) |
| CC | 37 (41.11%) | 22 (30.14%) | 1.00 | 1.00 |
| CT or TT | 53 (58.89%) | 51 (69.86%) | 1.62 (0.84−3.11) | 1.02 (0.41−2.52) |

ORs with 95% CI were estimated by logistic regression models. AORs with 95% CI were estimated by multiple logistic regression models, after controlling for age, height, weight, and calcium intake for each estimated variable. AOR = adjusted odds ratio; CI = confidence interval.

between the AORs of patients with C/T heterozygotes and C/C homozygotes. Furthermore, individuals with at least one mutated C allele were classified into the same subgroup, and individuals with homozygous T/T alleles were assigned to another subgroup. For individuals with at least one mutated T allele, the AOR for hip osteoporosis was 1.02 (95% CI, 0.41−2.52), but no significant difference was observed. There were no significant differences in any allele distribution for rs7524102 or rs6696981 SNPs.

### 3.4. Association between SNPs and BMD

Results of hip BMD and genotype distributions of rs7524102, rs6696981, and rs6993813, including differences between polymorphic variants and wild-type homozygotes, are shown in Table 4. Hip BMD had differences between polymorphic variants and wild-type homozygotes in the *OPG* gene (rs6993813, $p < 0.001$). For rs6696981 SNP, hip BMD in G/T heterozygotes ($p < 0.05$) and in G/T and T/T minor allele homozygotes ($p = 0.008$) was significantly higher than that in the wild-type G/G homozygotes. Although hip BMD in T/T minor allele homozygotes also seemed to be higher than G/G homozygotes, it did not show significant difference ($n = 3$). For rs7524102 SNP, no significant differences were observed in any allele distribution.

## 4. Discussion

We investigated the effect of rs7524102, rs6696981, and rs6993813 SNPs on hip BMD in elderly Taiwanese women and, interestingly, found an opposite association of *OPG* and *ZBTB40* polymorphisms with hip BMD. The AOR for hip

Table 4
Hip BMD in relation to the allele distribution of the SNPs.

| Genotype | *n* | Hip BMD | Genotype | *n* | Hip BMD | Genotype | *n* | Hip BMD |
|---|---|---|---|---|---|---|---|---|
| rs7524102 | | | rs6696981 | | | rs6993813 | | |
| AA | 95 | −1.96 ± 1.41 | GG | 104 | −2.03 ± 1.41 | CC | 59 | −1.61 ± 1.42 |
| AG | 65 | −1.61 ± 1.57 | GT | 56 | −1.43 ± 1.55† | CT | 95 | −1.77 ± 1.53† |
| GG | 3 | −0.43 ± 1.96 | TT | 3 | −0.43 ± 1.96 | TT | 9 | −3.27 ± 0.82† |
| F value | | 2.377 | | | 4.302* | | | 5.054** |
| AA | 95 | −1.96 ± 1.41 | GG | 104 | −2.03 ± 1.41 | CC | 59 | −1.61 ± 1.42 |
| AG + GG | 68 | −1.56 ± 1.59 | GT + TT | 59 | −1.38 ± 1.57 | CT + TT | 104 | −1.90 ± 1.54 |
| *P*[a] | | 0.088 | | | 0.008 | | | 0.232 |

Values are mean ± SD.
BMD = bone mineral density; SNP = single-nucleotide polymorphism.
[a] Student's *t*-test was used to analyze the difference of hip BMD between other than the wild type and the wild type.
* $p < 0.05$, analysis of variance with Bonferroni posteriori comparison.
** $p < 0.01$, analysis of variance with Bonferroni posteriori comparison
† Significantly different, at $p < 0.05$, when compared to the wild type.

osteoporosis was 9.53 for individuals with T/T minor allele homozygotes of rs6993813, compared to individuals with C/C wild-type homozygotes. Also, BMD of the hip had differences between polymorphic variants and wild-type homozygotes in the rs6993813 SNP. In contrast, hip BMD in G/T heterozygotes and in G/T and T/T minor allele homozygotes of the rs6696981 SNP was significantly higher than that in wild-type G/G homozygotes. Thus, the minor allele homozygotes of rs6993813 had a strong effect on decreasing hip BMD and the development of hip osteoporosis, while at least one mutated T allele of the rs6696981 SNP possessed a protective effect by increasing BMD of the hip, and this effect was independent of hip osteoporosis.

Osteoporosis increases in incidence with age and is an important health challenge to hundreds of millions of elderly individuals worldwide; not surprisingly, reduced BMD was directly associated with increased age in these elderly Taiwanese women. Consistent with significant differences in body height and weight between low BMD and high BMD individuals,[29,31] BMD correlated significantly with body height, weight, and BMI in the study. Because osteoporosis is a common disease with a heritable component characterized by decreasing BMD and increasing risk of fragility fractures,[18,19] polymorphisms in several candidate genes have been reported to be associated with bone mass or osteoporotic fractures.[24] Despite the focus on BMD because of its high heritability,[18,19] medical and environmental factors such as diet and specific nutrients contribute to interact with genetic polymorphisms to regulate bone mass. With respect to significant differences in calcium tablet intake or not between *T*-score $\leq -2.5$ and *T*-score $> -2.5$ groups, the women who had been diagnosed as osteoporotic presumably should have had an increased calcium tablet intake in an effort to decrease bone fragility and susceptibility to fracture. Nevertheless, calcium tablet supplementation does not appear to possess a significantly increasing effect on BMD postmenopausally.[32]

Both BMD and osteoporotic fractures have high genetic determinations.[33,34] BMD is the single best predictor of fragility fractures,[14] and is currently the predominant study phenotype for osteoporosis.[13] The *OPG* gene has been previously shown to be important to the biologic characteristics of bone through the regulation of osteoclastogenesis,[23,35] and Wnt signaling controls the process of bone resorption by negatively regulating *TNFRSF11B* expression in osteoblasts.[36] In addition to being associated with osteoporotic fractures, rs7524102 and rs6696981 on the 1p36 (*ZBTB40*), and rs6993813 on 8q24 (*OPG*) have been reported to be the most important quantitative trait loci for BMD of the hip,[23–29] although the SNPs tested are different. Furthermore, 98% of Taiwan's population is made up of Han Chinese, while 2% are Taiwanese aborigines according to official governmental statistics. However, the composite category of "Taiwanese population" is often characterized by many Taiwanese to include a significant population of at least four constituent ethnic groups: the Hoklo (70%), the Hakka (15%), Mainlander (13%), and Taiwanese aborigines (2%).[37,38] For these reasons, we focus here on three SNPs of rs7524102, rs6696981, and rs6993813. Consequently, hip BMD had a positive association with the rs6993813 SNP in Taiwanese women, but had a negative association with the rs6696981 SNP. Compared to individuals with C/C wild-type homozygotes, individuals with T/T minor allele homozygotes of rs6993813 had a 9.53-fold risk of developing hip osteoporosis as well. Two rs7524102 and rs6696981 SNPs in linkage disequilibrium on the 1p36 (*ZBTB40*) tend to be inherited together, and both show an association with hip BMD,[24] whereas we could not find that the rs7524102 SNP has a similar effect on hip BMD or osteoporosis in the study. This may be one of the reasons that the ethnic Taiwanese population has higher hip fracture rates.

Our study had several limitations. First, it is difficult to identify the underlying genes of a complex polygenic trait such as BMD by linkage studies, and it requires large numbers of patients due to the limited power.[39] Intriguingly, we investigated an opposite association of rs6696981 and rs6993813 SNPs with BMD of the hip and the minor allele homozygotes of rs6993813 with an AOR of 9.53 for hip osteoporosis. Second, the statistical power to identify linkage signals for stature may miss some false negative results due to the small sample size.[40] This may be the reason why the rs6696981 SNP possesses a protective effect by increasing the BMD of the hip, although this effect is independent of hip osteoporosis. However, this result should be validated in a substantially larger cohort. Third, the disease–genotype association produced by case-control studies may be spurious when the genotype distribution of healthy controls deviates from Hardy–Weinberg equilibrium, as in the case of the rs6993813 SNP. Even so, the Hardy–Weinberg equilibrium may be affected by many factors, including sample size, selection bias, and laboratory errors.[41] Moreover, a standard adjustment of ORs for allele frequencies still does not exist.[42] Accordingly, we clarified this issue, including study design and limitation, and still calculated AORs to measure the association in order to avoid missing the ultimate finding.

In conclusion, our study has provided strong evidence that polymorphic variants in the *OPG* gene (rs6993813) and *ZBTB40* gene (rs6696981) have an opposite association with hip BMD in elderly Taiwanese women. The rs6993813 SNP with T/T homozygotes has strong decreasing effects on hip BMD and the risk to develop hip osteoporosis. The rs6696981 SNP with G/T and T/T genotypes may be considered a factor affecting decreased susceptibility to hip osteoporosis. These advances in knowledge about the genetic basis of osteoporosis have noteworthy strengths and provide us with new genetic markers with which to assess fracture risk and to prevent future fractures in postmenopausal Taiwanese women. This observation, of course, needs to be replicated in other dependent or independent populations, especially in postmenopausal Chinese women.

## Acknowledgments

We thank Shun-Fa Yang, PhD, from the Institute of Medicine, Chung Shan Medical University for advice regarding statistics.

## References


1. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. *Lancet* 2002;**359**:1761−7.
2. Melton 3rd LJ. Adverse outcomes of osteoporotic fractures in the general population. *J Bone Miner Res* 2003;**18**:1139−41.
3. Cooper C, Campion G, Melton 3rd LJ. Hip fractures in the elderly: a world-wide projection. *Osteoporos Int* 1992;**2**:285−9.
4. Kanis JA, Melton 3rd LJ, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res* 1994;**9**:1137−41.
5. Shaw CK. An epidemiologic study of osteoporosis in Taiwan. *Ann Epidemiol* 1993;**3**:264−71.
6. Yang NP, Deng CY, Chou YJ, Chen PQ, Lin CH, Chou P, et al. Estimated prevalence of osteoporosis from a Nationwide Health Insurance database in Taiwan. *Health Policy* 2006;**75**:329−37.
7. Ho SC, Bacon WE, Harris T, Looker A, Maggi S. Hip fracture rates in Hong Kong and the United States, 1988 through 1989. *Am J Public Health* 1993;**83**:694−7.
8. Lau EM, Lee JK, Suriwongpaisal P, Saw SM, Das De S, Khir A, et al. The incidence of hip fracture in four Asian countries: the Asian Osteoporosis Study (AOS). *Osteoporos Int* 2001;**12**:239−43.
9. Xu L, Lu A, Zhao X, Chen X, Cummings SR. Very low rates of hip fracture in Beijing, People's Republic of China the Beijing Osteoporosis Project. *Am J Epidemiol* 1996;**144**:901−7.
10. Huang KY, Chang JK, Ling SY, Endo N, Takahashi HE. Epidemiology of cervical and trochanteric fractures of the proximal femur in 1996 in Kaohsiung City, Taiwan. *J Bone Miner Metab* 2000;**18**:89−95.
11. Chie WC, Yang RS, Liu JP, Tsai KS. High incidence rate of hip fracture in Taiwan: estimated from a nationwide health insurance database. *Osteoporos Int* 2004;**15**:998−1002.
12. World Health Organization. *Assessment of fracture risk and its application to screening for postmenopausal osteoporosis*. Technical Report Series. Switzerland: World Health Organization; 1994. report 843.
13. Kanis JA, McCloskey EV, Johansson H, Oden A, Melton 3rd LJ, Khaltaev N. A reference standard for the description of osteoporosis. *Bone* 2008;**42**:467−75.
14. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. *J Bone Miner Res* 2005;**20**:1185−94.
15. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* 1995;**332**:767−73.
16. Deng HW, Mahaney MC, Williams JT, Li J, Conway T, Davies KM, et al. Relevance of the genes for bone mass variation to susceptibility to osteoporotic fractures and its implications to gene search for complex human diseases. *Genet Epidemiol* 2002;**22**:12−25.
17. Andrew T, Antoniades L, Scurrah KJ, Macgregor AJ, Spector TD. Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. *J Bone Miner Res* 2005;**20**:67−74.
18. Nguyen TV, Blangero J, Eisman JA. Genetic epidemiological approaches to the search for osteoporosis genes. *J Bone Miner Res* 2000;**15**:392−401.
19. Peacock M, Turner CH, Econs MJ, Foroud T. Genetics of osteoporosis. *Endocr Rev* 2002;**23**:303−26.
20. Albagha OM, Ralston SH. Genetics and osteoporosis. *Rheum Dis Clin North Am* 2006;**32**:659−80.
21. Lei SF, Jiang H, Deng FY, Deng HW. Searching for genes underlying susceptibility to osteoporotic fracture: current progress and future prospect. *Osteoporos Int* 2007;**18**:1157−75.
22. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. Meta-analysis of genome-wide linkage studies for bone mineral density. *J Hum Genet* 2006;**51**:480−6.
23. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008;**371**:1505−12.
24. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, et al. Multiple genetic loci for bone mineral density and fractures. *N Engl J Med* 2008;**358**:2355−65.
25. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, et al. New sequence variants associated with bone mineral density. *Nat Genet* 2009;**41**:15−7.
26. Rivadeneira F, Styrkársdottir U, Estrada K, Halldórsson BV, Hsu YH, Richards JB, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat Genet* 2009;**41**:1199−206.
27. Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M, et al. Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. *Am J Hum Genet* 2003;**72**:144−55.
28. Styrkarsdottir U, Halldorsson BV, Gudbjartsson DF, Tang NL, Koh JM, Xiao SM, et al. European bone mineral density loci are also associated with BMD in East-Asian populations. *PLoS ONE* 2010;**5**:e13217.
29. Liu JM, Zhang MJ, Zhao L, Cui B, Li ZB, Zhao HY, et al. Analysis of recently identified osteoporosis susceptibility genes in Han Chinese women. *J Clin Endocrinol Metab* 2010;**95**:E112−20.
30. Ugozzoli L, Wallace RB. Allele-specific polymerase chain reaction. *Methods: A Companion Methods Enzymol* 1991;**2**:42−8.
31. Huang QY, Li GH, Kung AW. The −9247 T/C polymorphism in the SOST upstream regulatory region that potentially affects C/EBPalpha and FOXA1 binding is associated with osteoporosis. *Bone* 2009;**45**:289−94.
32. Shea B, Wells G, Cranney A, Zytaruk N, Robinson V, Griffith L, et al. Meta-analyses of therapies for postmenopausal osteoporosis: VII. Meta-analysis of calcium supplementation for the prevention of postmenopausal osteoporosis. *Endocr Rev* 2002;**23**:552−9.
33. Michaelsson K, Melhus H, Ferm H, Ahlbom A, Pedersen NL. Genetic liability to fractures in the elderly. *Arch Intern Med* 2005;**165**:1825−30.
34. Yang TL, Chen XD, Guo Y, Lei SF, Wang JT, Zhou Q, et al. Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. *Am J Hum Genet* 2008;**83**:663−74.
35. Langdahl BL, Carstens M, Stenkjaer L, Eriksen EF. Polymorphisms in the osteoprotegerin gene are associated with osteoporotic fractures. *J Bone Miner Res* 2002;**17**:1245−55.
36. Glass 2nd DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005;**8**:751−64.
37. Copper J. *Taiwan: nation state or province?*. 4th ed. Boulder, CO: Westview Press; 2003.
38. Hsiao AC. *Contemporary Taiwanese cultural nationalism*. London: Routledge Press; 2004.
39. Ioannidis JP, Ng MY, Sham PC, Zintzaras E, Lewis CM, Deng HW, et al. Meta-analysis of genome-wide scans provides evidence for sex- and site-specific regulation of bone mass. *J Bone Miner Res* 2007;**22**:173−83.
40. Ioannidis JP. Why most published research findings are false. *PLoS Med* 2005;**2**:e124.
41. Zou GY, Donner A. The merits of testing Hardy−Weinberg equilibrium in the analysis of unmatched case-control data: a cautionary note. *Ann Hum Genet* 2006;**70**:923−33.
42. Zintzaras E. Variance estimation of allele-based odds ratio in the absence of Hardy−Weinberg equilibrium. *Eur J Epidemiol* 2008;**23**:323−6.