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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-

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teoporosis merits further investigation. Osteoporosis is associated with environmental factors 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 (Peacock et al. 2002). Moreover, BMD may be regarded as a trait that is polygenic in nature. Several studies of susceptibility genes and genome-wide linkages have estimated that multiple genetic 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; Styrkarsdottir et al. 2008). However, there is some evidence indicating that genes regulating BMD differ between various gender and skeletal sites (Kaufman et al. 2008).

With the rapid advancement in single nucleotide polymorphism (SNPs) identifications and the development of databases, such as the genome-wide association study and International HapMap Project, genetic studies of osteoporosis 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 examined the association between SNPs and osteoporosis 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 Taiwanese population containing 252 healthy persons (non-osteoporotic) and 193 persons with osteoporosis (including postmenopausal Taiwanese women and osteoporotic men). Using a double-blind test, we evaluated the feasibility of using the analyzed SNPs for determining osteoporosis in Taiwanese.

MATERIALS AND METHODS

Sample Collection

In this study, the Taiwanese population living on the area of Taiwan was divided into 2 groups: one group contained 252 healthy persons (non-osteoporotic) and another group had 193 osteoporotic persons (including postmenopausal Taiwanese women and osteoporotic men). Standardized BMD was calculated and corrected 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 approved 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 energy 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 osteopenia is between -1 standard deviation and -2.5 standard deviation; and the BMD in an osteoporotic person is less than -2.5 standard deviation (Kanis et al. 1994).

DNA Extraction

DNA extraction was performed using the QIA amp DNA Blood Mini Kit (QIAGEN' Hilden' 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-mL microcentrifuge tube.

Genotyping

Genotyping was conducted by the Allele-Specific 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 6p21 (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), tri-allelic SNPs 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 1: Ten SNPs from 8 chromosomal regions

Chromosomal regions	*SNP and allele*	
1p36	rs7524102 G	rs6696981 G
6p21(*MHC*)	rs3130340 C	
2p16(*SPTBN1*)	rs11898505 G	
6q25(*ESR1*)	rs9479055 C	
3q13(*IFT57*)	rs326340 T	rs1289759 C
8q24(*OPG*)	rs6993813 T	
13q14(*RANKL*)	rs9594738 C	
18q21(*RANK*)	rs3018362 G	

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

	Healthy individuals	*Osteopenia*	*Osteoporosis*	*F-value*	*p-value*
	N=252	*N=91*	*N=102*		
Age	57.36±6.236	62.53±7.967	65.17±8.217	50.021	<0.001
BMI	24.4 ±3.07	24.1 ±3.30	22.6 ±3.43	11.444	<0.001
Postmenopausal age	48.85±5.682	48.96±5.267	47.78±6.673	1.416	0.244
Lumbar BMD	0.03±1.077	-1.13±0.899	-2.28±0.981	194.071	<0.001
Hip BMD	-0.46±0.867	-1.79±0.427	-2.98±0.673	429.304	<0.001
Hip & Neck bone	0.79±0.108	0.66±0.060	0.55±0.072	258.428	<0.001
Fracture	7.48±3.542	12.62±6.643	19.51±8.636	160.8	<0.001
Hip fracture	0.52±0.892	1.86±2.159	5.32±4.661	131.503	<0.001
		Case Number			
Smoke	0		1		
Family history	4		15	4.075	0.018
Soda	1		0		
Coffee	16		11	4.396	0.013
Medication					
Calcium supplement	35		48	7.106	0.001
Vitamin D	4		7	8.211	<0.001
Fosamax	10		23	30.838	<0.001
Estrogen	26		27	1.351	0.26
SSRI	4		9	1.001	0.369

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

	Healthy individuals	*Osteopenia*	*Osteoporosis*	*F-value*	*p-value*
1q36					
rs7524102 (G→A)	252	91	102	0.514	0.599
rs6696981 (G→T)				0.305	0.737
6p21 (MHC)					
rs3130340 (C→T)	252	91	102	0.661	0.517
2p16 (MSH6)					
rs11898505 (G→A)	252	91	102	0.639	0.528
6q25 (ESR1)					
rs9479055 (C→G)	252	91	102	0.284	0.753
3q13 (IFT57)					
rs326340 (T→G)				2.002	0.136
rs1269759 (C→T)				0.939	0.392
8q24 (OPG)					
rs6993813 (T→C)	252	91	102	3.035	0.049*
13q14 (RANKL)					
rs9594738 (C→T)	252	91	102	5.802	0.003*
18q21 (RANK)					
rs3018362 (G→A)	252	91	102	0.268	0.765

*Correlation is significant at the 0.05 level

Table 4: Integration of SNP sites for osteoporosis analyzed

	Numbers	*Minimum*	*Maximum*	*Mean*	*Standard deviation*
[a] Total	445	1.0	13	7.8	2.106
[b] Wild type	445	1.0	8.0	3.481	1.468
[c] Mutant	445	0	6.0	4.32	1.229

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.
[a] Includes the total SNP sites analyzed.
[b] Includes 7 SNP sites (rs7524102, rs6696981, rs11898505, rs9479055, rs326340, rs1269759, rs9594738).
[c] Includes 3 SNP sites (rs3130340, rs6993813, rs3018362).

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

	Numbers	*Mean*	*p-value*	*95% CI*
Total	445	7.8	0.197	-0.053 to 0.256
≧ Mean 7.8	238			
< Mean 7.8	207			
Wild type		3.48	0.002*	0.096 to 0.411
≧ Mean 3.48	185			
< Mean 3.48	260			
Mutant		4.32	0.141	-0.269 to 0.039
≧ Mean 4.32	223			
< Mean 4.32	222			

*Correlation is significant at the 0.05 level

gregated 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 phonotype by genotyping the 7 wild-type SNPs.

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

	Numbers	*Total*	*Wild type*	*Mutant*
Healthy individuals, osteopenia and osteoporosis	445			
correlation		0.065	0.123**	-0.036
sig. (2-tailed)		0.173	0.010	0.452
Healthy individuals and osteopenia	343			
correlation		0.051	0.097	-0.025
sig. (2-tailed)		0.351	0.072	0.650
Healthy individuals and osteoporosis	354			
correlation		0.065	0.128*	-0.037
sig. (2-tailed)		0.223	0.016	0.491
Osteopenia and osteoporosis	193			
correlation		0.018	0.032	-0.015
sig. (2-tailed)		0.808	0.654	0.841

*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 ($p = 0.010$), 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

Case no.	*Age*	*BMI*	*Menopausal Age*	*Lumbar BMD*	*Hip BMD*	*Hip & Neck Bone*	*Fracture*	*Hip fracture*	*rs9479055 CA AA =2 AC =1 CC =0*	*rs1289759 CT CC =0 CT =1 TT =2*	*rs7524102 GA AA =2 AG =1 GG =0*	*rs11898505 GA AA =2 AG =1 GG =0*	*rs3130340 TG GG =2 GT =1 TT =0*	*rs6696981 GT GG =0 GT =1 TT =2*	*rs9594738 CT CC =0 CT =1 TT =2*	[a]*Score*	[b]*BMD*	[c]*Conformity*
01	76	21	57	-2.1	-3.7	0.475	19	8.7	0	1	2	1	0	0	0	4 (osteoporosis/osteopenia)	-3.7 (osteoporosis)	+
02	74	23	47	-3.7	3.5	0.496	17	7.1	0	0	2	2	0	0	0	4 (osteoporosis/osteopenia)	-3.5 (osteoporosis)	+
03	65	32	46	-1.6	-2.2	0.629	12	2.3	0	2	2	2	1	0	0	7 (osteoporosis/osteopenia)	-2.2 (osteopenia)	+
04	63	26	54	-3.2	-3	0.547	11	3.1	0	0	2	2	0	0	0	4 (osteoporosis/osteopenia)	-3.2 (osteoporosis)	+
05	72	24	44	-1.5	-1.4	0.708	9	1.4	0	1	1	2	1	1	0	6 (osteoporosis/osteopenia)	-1.5 (osteopenia)	+
06	63	27	43	0.1	-0.5	0.907	5.1	0.1	0	0	1	2	0	0	0	3 (healthy individuals)	-0.5 (healthy individuals)	+
07	64	24	54	-3	-3.6	0.494	14	4.9	0	1	2	2	0	0	0	5 (osteoporosis/osteopenia)	-3.6 (osteoporosis)	+
08	64	24	48	0	-2.7	0.578	10	2.3	0	0	2	2	0	1	0	5 (osteoporosis/osteopenia)	-2.7 (osteoporosis)	+
09	57	23	45	-1.7	-3.2	0.526	9.6	3.1	0	2	2	0	1	0	0	5 (osteoporosis/osteopenia)	-3.2 (osteoporosis)	+
10	68	24	50	0.6	-2.1	0.64	15	2.7	0	0	2	1	0	1	0	4 (osteoporosis/osteopenia)	-2.1 (osteopenia)	+

[a]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.

[b]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).

[c]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 “-”.

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. 2008; 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.

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