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台灣女性 *SPP1* 基因多型性與骨密度低下之關聯研究

Secreted Phosphoprotein-1 (*SPP1*) Polymorphisms Are Associated with a Decreased Risk of Low Bone Mineral Density in Taiwanese Women

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本論文係 毛健麟 君（學號 R98846001）在國立臺灣大學流行病學與預防醫學研究所完成之碩士學位論文，於民國 100 年 10 月 28 日承下列考試委員審查通過及口試及格，特此證明。

口試委員：程蘊菁 （簽名）

（指導教授）

丘政民　　簡國泰

李文宗　　李建璋

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摘要

背景：Secreted phosphoprotein-1 (*SPP1*) 骨橋蛋白基因，藉由和破骨細胞膜表面的 vitronectin 受體結合，參與破骨細胞接合骨質的破骨作用中。最近的統合分析發現，*SPP1* 基因多型性與骨密度及骨折的風險有關。

方法：這是一篇橫斷式研究。 1319 位健康台灣女性，年齡在 40 至 55 歲，於 2009 年 10 月至 2010 年 8 月間，從美兆健康體檢中心被招募。高與低骨密度比較，高骨密度定義為三等分的第一組，而第二組加上第三組則為低骨密度。本研究探討三種常見的(對偶基因頻率> 5%) haplotype-tagging 單核苷酸多型性(htSNP)來分析 *SPP1* 基因多型性與低骨密度風險的關聯。我們還評估了更年期，年齡，身體質量指數和相關的生物標誌物如何影響 *SPP1* 多型性與低骨密度風險之間的相關

結果：研究結果顯示帶有 rs4754 變異的對偶基因會降低低骨密度風險[2 vs. 0 copies: adjust odds ratio（AOR）= 0.54，95％CI= 0.36 - 0.81]，與非攜帶者相比，攜帶兩個變異的婦女有較高的骨密度。在單倍體分析方面，在校正偽陽性率後，攜帶 HAP1 TGC 兩個變異的婦女在高鹼性磷酸酶 [AOR= 0.30，95％CI= 0.15 - 0.64] 和或低尿酸 [AOR= 0.33，95％CI= 0.16 - 0.68] 時有保護的效果。 *SPP1* 基因和低

骨密度風險，無論是在單核苷酸多型性或單倍體水平沒有顯著作用。在本研究中，停經與否並不會顯著的修飾 *SPP1* 基因與低骨密度的關係。

結論：在中年亞裔女性身上，*SPP1* 基因多型性與保護骨密度低下有顯著的相關。

關鍵詞：骨質疏鬆症、骨質密度、骨橋蛋白、單倍型、單核苷酸多型性、單倍型

ABSTRACT

Background. Secreted phosphoprotein-1 (*SPP1)* is involved in the anchoring of osteoclasts to the mineral of bone matrix by binding with vitronectin receptor. A recent meta-analysis found *SPP1* genetic polymorphisms were associated with bone mineral density (BMD) and fracture risk.

Methods. This is a cross-sectional study. A total of 1,319 healthy Taiwanese women aged 40 to 55 years old were recruited from MJ health screening center from October 2009 to August 2010. High versus low bone mineral density (BMD) was defined as the 1st tertile versus 2nd plus 3rd tertiles of BMD. Three common (allele frequency>5%) haplotype-tagging single nucleotide polymorphisms (htSNPs) were selected to examine the association between sequence variants of *SPP1* and BMD.

Results. Women carrying two copies of variant rs4754 had a significantly decreased risk of low BMD [adjusted OR (AOR) = 0.54, 95% CI = 0.36 - 0.81] as compared with non-carriers. Women carrying two copies of minor haplotype TGC had a decreased risk of low BMD among those with high alkaline phosphatase [AOR = 0.30, 95% CI = 0.15 - 0.64] or with low uric acid [AOR = 0.33, 95% CI = 0.16 - 0.68]. These associations remained significantly associated with low BMD after controlling for FDR.

Menopausal status did not significant modify the association between *SPP1* polymorphisms and low BMD.

Conclusion . Genetic polymorphisms of *SPP1* were significantly associated with decreased risk of low BMD in middle-aged Asian women.

Keywords: *SPP1*, osteopontin, osteoporosis, bone mineral density, single nucleotide polymorphism, haplotypes

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Chapter 1. INTRODUCTION

1.1 Osteoporosis (OP) and bone mineral density (BMD)

Osteoporosis (OP) has become a global health issue as the rapid growth of aging population [1]. OP is characterized by low bone mass and has been prone to fractures, which is associated with increased mortality in the elderly [2]. About 21% of women aged 50 to 84 years has OP, which is three times higher than that in men [3]. The prevalence of OP in postmenopausal American women varies by Race/Ethnicity (Non-Hispanic white: 19%; Non-Hispanic black: 7%; Mexican American:16% [4]). Among postmenopausal women, average bone mineral density (BMD) was highest among African American followed by Hispanic, native Americans, and Asian [5]. In Taiwan, the prevalence of OP was 10% and 7% based on BMD at spine and femoral neck, respectively [6]. Compared with Caucasian women, Taiwanese women have a lower BMD but a lower incidence of osteoporosis [6]. This might be attributible to different ethnicity, life style and genetic profiles. Because OP is a “silent disease” until a sudden strain, twist, fall, or fracture, identification of low BMD at an early age will be helpful in preventing OP, fall, and fracture in late life.

1.2 Secreted phosphoprotein-1 (SPP1) gene

Secreted phosphoprotein-1 (*SPP1),* also known as osteopontin (OPN), is located on chromosome 4q21–q25. It is a glycoprotein related to bone formation and anchoring of osteoclasts to the bone remodeling matrix by binding with vitronectin receptor, which located in the osteoclast plasma membrane [7,8]. In animal study, *SPP1* gene knockout mice are resistant to bone resorption as compared to wildtype mice [9]. In addition, SPP1 exists in osteoblasts and mineralized bone matrix and intramembranous ossification [7] which enhances osteoblastic differentiation and proliferation [10,11]. SPP1 is thought to be a molecule for induce both bone formation and resorption [8]. A Chinese study found that the heritability of BMD was quite high (0.6 to 0.9, depend on body site and sex) [12]. Therefore, *SPP1* may play an important role in the pathogenesis of OP.

1.3 Associations between SPP1 polymorphisms and OP or BMD

Few epidemiologic studies have explored the association between *SPP1* polymorphisms and OP or BMD. A Caucasian study reported that two SNPs (rs11730582 and rs4754) were not associated with hip or spine BMD [13]. Another

study found the increased plasma SPP1 level, which has been related to low BMD [14]. A recent meta-analysis study, included 5 genome-wide association studies (GWASs) on BMD and fracture, found that polymorphisms of 9 genes (*ESR1*, *LRP4*, *ITGA1*, *LRP5*, *SOST*, *SPP1*, *TNFRSF11A*, *TNFRSF11B*, and *TNFSF11*) were significantly associated with BMD level [15]. Among them, (*SPP1*, *SOST*, *LRP5*, and *TNFRSF11A*) have been related to elevated risk of fracture [15].

1.4 Aims

SPP1 gene plays a role in regulating osteoclast [9] that may affect BMD level or OP risk. Few epidemiologic studies have related *SPP1* polymorphisms to BMD or OP and no study has been done in Asian populations. Therefore, this study aimed to explored this association. A systematic approach was used to select haplotype-tagging SNPs (htSNPs) in *SPP1* to catch sufficient genetic information in *CLU* and to identify SNPs representative for Asian population. We also evaluated how menopausal status, body mass index (BMI), serum alkaline phosphatase (ALP) level and other biomarkers modified the association of *SPP1* polymorphisms with BMD.

Chapter 2. MATERIALS AND METHODS

2.1 Study population

This is a cross-sectional study. A total of 1,577 healthy Taiwanese women, aged 40 to 55 years old, were recruited from MJ health screening center from October 2009 to August 2010. The outcome of this study is BMD (g/cm^2). The study protocol has been approved by the institutional review board of MJ health screening center and National Taiwan University. Informed consent was obtained from each participant.

A questionnaire was administered to collect information on demography, lifestyle (e.g., smoking status, alcohol consumption, and exercise), and disease history, etc. Blood sample was collected in an 8 ml EDTA tube from each participant. Genomic DNA was extracted by using QuickGene-Mini80 kit (Fujifilm, Tokyo, Japan). A total of 1,319 participants were included for data analyses after exclusion of participants whose BMD was measured at sites other than spine (n=85), lack of blood sample (n=113), without genotyping data (n=1), and having steroid or hormone therapy (n=59).

2.2 Bone mineral density measurement

The BMD (g/cm^2) of the lumbar spine (L1-4) was measured by a dual-energy X-ray absorptiometry densitometer (DXA, General Electric Lunar Health Care, DPX-L, USA.). Calibration of BMD measurement was performed daily. The long-term coefficient of variation in BMD was around 1%.

2.3 SNP selection and genotyping assays

Common (frequency ≥ 0.05) SNPs in *SPP1* were identified from genotyping data of Han Chinese in Beijing (CHB) of the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov). Haplotype block was defined by Haploview (http://www.broadinstitute.org/haploview/haploview) using modified Gabriel algorithm [16,17] Three htSNPs, rs11730582 (5'UTR), rs6839524 (intron), rs4754 (exon) were selected from these common SNPs using tagSNP program [18]. TaqMan Assay was used to determine genotypes using HT7900 (Applied Biosystems Inc., CA, USA). Genotyping success rate was greater than 95% for all SNPs. Quality control samples were replicates of 5% study participants and the concordance rate was 100%.

2.4 Statistical analyses

Hardy-Weinberg equilibrium (HWE) test was performed for each SNP to check genotyping error. The expectation-maximization algorithm was used to estimate haplotype frequencies in the haplotype block using tagSNP program [18]. BMD was tertiled into T1, T2, and T3. High BMD was defined as T3; low BMD was defined as T1 plus T2 with a BMD cut-off point of 1.27 g/cm^2 (Table 1).

Logistic regression model was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for low BMD in participants carrying either 1 or 2 versus 0 copies of minor allele of each SNP and each multilocus haplotype. Age, menopausal status (yes/no), BMI (kg/m^2), ALP (IU/L), creatinine (IU/L), uric acid (UA, mg/dl), and low-density lipoprotein (LDL, mg/dl) were adjusted in the models.

Stratified analyses were performed by menopausal status, BMI (< 18.5, 18.5 ~ <24, ≥ 24 kg/m^2), ALP (≤57, >57 IU/L), creatinine (≤0.79 >0.79 IU/L), UA (≤4.5, >4.5 mg/dl), and LDL (≤106, >106 mg/dl). Because this population is relatively healthy, for the biochemical value above, less than 10% of participants had abnormal values. To prevent sparse cell after stratification, median was used as the cutoff point for these factors. Under additive model with an alpha of 0.05, 880 participants with low BMD (comparison group) and 427 participants with high BMD (reference group) had > 90%

power to detect an OR>1.4 with MAF of 30% and an OR>2.0 with MAF of 40%. Type I error was controlled by using false discovery rate (FDR) which were formally described by Benjamini and Hochberg (1995) (*m*, the total number of hypotheses tested; *k,* order of the p values in increasing order and denote them by $p_{(1)}$ *to* $p_{(m)}$) [19]. For a given α, find the largest *k* so that p value ≤α**k*/*m,* which indicates those p values remained significant after controlling for FDR. Statistical analyses were performed by using SAS 9.2 (SAS Institute, Cary, NC) and all statistical tests were two-sided.

Chapter 3. RESULTS

3.1 Characteristics of the study population

This study included 1,319 participants. As compared to women with high BMD, women with low BMD, were older (46.8 vs. 45.6 years old), had a lower BMI (22.0 vs. 23.3 kg/m^2), lower ALP (55.6 vs. 61.6 IU/L), lower creatinine (0.79 vs. 0.81 mg/dl), lower systolic blood pressure (108.9 vs. 111.0 mmHg), lower UA (4.5 vs. 4.7 mg/dl), and higher LDL (110.2 vs. 105.4 mg/dl, Table 2). In addition, as compared with women with high BMD, more women with low BMD had already menopaused (26.0% vs. 10.5%), and fewer of them had hyperglycemia (25.0% vs. 31.2%). The distributions of alcohol consumption, cigarette smoking, regular exercise (≥30 mins/day), history of hypertension and hyperlipidemia were similar between women with low and high BMD. The distribution of BMD was shown in Figure 1.

3.2 SPP1 polymorphisms and BMD

Three *SPP1* htSNPs were genotyped. The minor allele frequencies (MAFs) of the three SNPs ranged from 31% to 42%, which were similar to the MAFs of CHB data of

International HapMap Project (Table 3). All *SPP1* SNPs were in HWE for both participants with low or high BMD or the whole population (Table 3).

The distribution of BMD ± 1 standard error (SE) in the different *SPP1* genotype groups was showed in Figure 6 to 8. In rs11730582 genotype groups, women who carry T allele had lower BMD compared with non-carriers, but we could not see a clear trend in this figure. In rs6839524 genotype groups, women who carry G allele had lower BMD compared with non-carriers, and we could see a trend in figure7. In rs4754 genotype groups, women who carry T allele had higher BMD compared with non-carriers, and we could see a clear trend in figure 8.

Women carrying two copies of variant rs4754 had a significantly decreased risk of low BMD [CC vs. TT: adjusted OR (AOR) = 0.51, 95% CI = 0.33 - 0.78, Table 4] as compared with non-carriers. This association remained significant after controlling for FDR (Table 4).

3.3 SPP1 haplotypes and BMD

Three common (frequency ≥ 5%) htSNPs spanning *SPP1* formed one block, which was determined by modified Gabriel et al. algorithm (Figure 1) [16,17]. Participants

carrying two copies of the minor HAP1 TGC had a decreased risk of low BMD (AOR = 0.53, 95% CI = 0.32 - 0.89, Table 5). This association did not reach statistical significance after controlling for FDR. HAP2 to HAP4 were not associated with low BMD.

3.4 Effect modification by potential confounders

Several potential confounders (e.g., menopausal status, BMI, ALP, creatinine, UA, and LDL) were identified in this study. Although these factors did not significantly modify the association between *SPP1* polymorphisms and low BMD, stratified analysis showed significant association in specific subgroups.

Women carrying two copies of variant rs4754 (TT genotype) had a significantly decreased risk of low BMD in women who had not menopaused [AOR = 0.57, 95% CI = 0.36 - 0.92, Table 6], had high BMI (≥24 kg/m^2) [AOR = 0.38, 95% CI = 0.15 - 0.96, Table 7], had high serum ALP (> 57 IU/L) [AOR = 0.35, 95% CI = 0.18 - 0.67, Table 8], had high serum creatinine (> 0.79 IU/L) [AOR = 0.33, 95% CI = 0.18 - 0.61, Table 9], had low serum UA (≤ 4.5 mg/dl) [AOR = 0.31, 95% CI = 0.17 - 0.57, Table 10], or had low serum LDL (≤ 106 mg/dl) [AOR = 0.46, 95% CI = 0.26 - 0.83,

Table 11]. After correction for multiple tests by controlling for FDR, rs4754 remained significantly associated with low BMD in women with high serum ALP, high creatinine, or low UA.

For haplotype analysis, women carrying two copies of the minor HAP1 TGC had a decreased risk of low BMD in those with high BMI [AOR = 0.18, 95% CI = 0.05 - 0.72, Table 13], with high serum ALP [AOR = 0.30, 95% CI = 0.15 - 0.64, Table 14], with high serum creatinine [AOR = 0.35, 95% CI = 0.17 - 0.73, Table 15], with low serum UA [AOR = 0.33, 95% CI = 0.16 - 0.68, Table 16], with low serum LDL [AOR = 0.42, 95% CI = 0.21 - 0.83, Table 17]. After controlling for FDR, haplotype TGC remained significantly associated with low BMD in women with high serum ALP or low UA.

Chapter 4. DISCUSSIONS

4.1 Main findings

This study found homozygsity of rs4754 (TT) and TGC haplotype were associated with low BMD, which was inconsistent with the only previous study (null finding) [13].

Rs4754 is a synonymous SNP. Although its polymorphism do not lead to change of amino acid, this SNP may affect BMD via its influence on translation efficiency. C allele is the major allele of rs4754 in Chinese (C=0.66, T=0.34) but a minor allele in Caucasian (C=0.23, T=0.77, http://hapmap.ncbi.nlm.nih.gov). This may explain why *SPP1* was not identified in previous GWASs for BMD or osteoporosis until the meta-analysis [15], probably due to low statistical power. We found rs11730582, a promoter SNP, did not associated with low BMD, which is consistent with the only previous study as well [13]. The intronic SNP rs6839524 has not been explored previously and was not associated with low BMD. Among 3 htSNPs, rs4754 was the only one significantly associated with low BMD. Although these htSNPs were in one haplotype block i.e., high LD, the pairwise r^2 between any two SNPs were low (0.01~0.33, Fig.3). This may explains no association between *SPP1* rs11730582 or rs6839524 and low BMD.

Women carrying two copies of haplotype TGC had a 0.53-fold decreased risk of low BMD (Table 5) but this association did not maintain after controlling for FDR.

4.2 Postulated Mechanism of SPP1 and low BMD

SPP1 plays a role in a wide spectrum of physiologic and pathologic processes [20-23]. It mediates the attachment of osteoclasts to bone matrix and then regulates bone resorption and normal bone development [23]. The polymorphisms of *SPP1* gene may affect SPP1 structure or decrease serum SPP1 level. Change or reduce SPP1 protein may affect bone formation and resorption, as well as osteoclastic process. The downgrading of osteoclastic process may slow BMD decline and thus preventing OP. SPP1 also plays an important role in regulating immune response [14], extracellular matrix organization [24], angiogenesis [25], multiple sclerosis [26], insulin resistance [27]. Therefore, polymorphisms of *SPP1* may block or reduce the inflammation response and thus lead to lower risk of low BMD. In addition, the association between *SPP1* polymorphisms and BMD might be affected by some other factors as detailed below.

Menopause. Sex hormone plays an important role in maintaining bone strength [28]. For many women, bone density decreases fast in the first few years after menopause [29]. In this study found variant of rs4754 protected from low BMD only in pre-menopausal women. This indicated that the change of sex hormone might affect the association of genetic polymorphisms with BMD level.

BMI. The impact of overweight (BMI$\geqq$24 kg/m^2) on BMD is complex [30]. After stratified by BMI groups (low, normal, high) the protective effect of rs4754 on low BMD was only observed in high BMI group, which is consistent with a Poland study include young women (age: 23~59) [31].

ALP. ALP has been a useful marker of bone turnover [32]. Elevation of serum ALP representing a upgraded bone metabolism [33]. In addition, SPP1 has been reported that increases ALP activity [34]. Women with higher ALP may reflect higher metabolism of bone and higher SPP1 level. This may explain the significant protective effects of *SPP1* polymorphisms on low BMD in women with high serum ALP. Even though total ALP is a less specific marker than bone ALP [35], bone ALP data were not available in this population.

Creatinine. Serum creatinine is an indicator of renal function [36] and a breakdown product of creatine phosphate in muscle [37]. SNP rs4754 and haplotype TGC were associated with decreased risk of low BMD in women with high serum creatinine. This might be a result of high muscle mass [37] in this study population and may explain protective effect of *SPP1* gene polymorphism on low BMD in the high creatinine group.

Uric acid. UA is an important endogenous antioxidant that is capable of scavenging radicals and blocking the formation of strong oxidant [38,39]. Oxidative stress has been related to decreased osteoblastogenesis and bone formation [40,41], Therefore, this may explain the protective effect of SNP rs4754 and haplotype TGC on low BMD in low UA group.

LDL. Serum LDL increases oxidation of lipids, which may affect bone cells indirectly via the induction of an inflammatory response and production of cytokines [42]. Becuase of these role, LDL has been identified as a risk factor for BMD, in a previous study [43]. This may explain the protective effect of rs4754 on low BMD in women with low LDL

4.3 Strengths and Limitations

This study has several strengths. First, the association between the 3 SNPs (rs11730582, rs6839524, rs4754) of *SPP1* and low BMD has not been explored in Asian previously. Importantly, the selections of a set of representative htSNPs captured the majority of genetic information of *SPP1* ($r^2 = 0.78$) and are representative for Asian. Second, haplotypes provided a stronger statistical power then SNP alone to detect the association between *SPP1* polymorphisms and BMD. This is because these htSNPs are in highly linkage disequilibrium [16] and thus tend to inherit together over generations. In addition, this study found the association between *SPP1* polymorphisms and low BMD in different subgroups (e.g., high serum ALP, high serum creatinine, low serum UA, low serum LDL), which were not explored previously probably due to lack of these information in past studies. Therefore, our findings for the first time identified specific subgroups that are susceptible to low BMD.

This study had some limitations. Because this population is relatively healthy, OP prevalence is low (0.38%) and few women with abnormal biochemical values. Therefore, we tertiled BMD level and used medium as the cutoff points for some biochemical values (e.g., serum ALP, creatinine, UA, and LDL). Thus, our findings

may underestimate the associations observed. However, these cutoff points may be better for predicting low BMD visit at in an early age. Second, this study used the cross-sectional design and thus the causal inferences is not applicable.

Chapter 5. CONCLUSIONS

SPP1 plays a role in bone remodeling. rs4754 and haplotype TGC in *SPP1* were significantly associated with reduced risk of low BMD, the latter has not been explored previously. Serum ALP, creatinine, LDL, and UA may regulate the bone remodeling and thus affect the above association. Future longitudinal studies are warranted to explore these associations.

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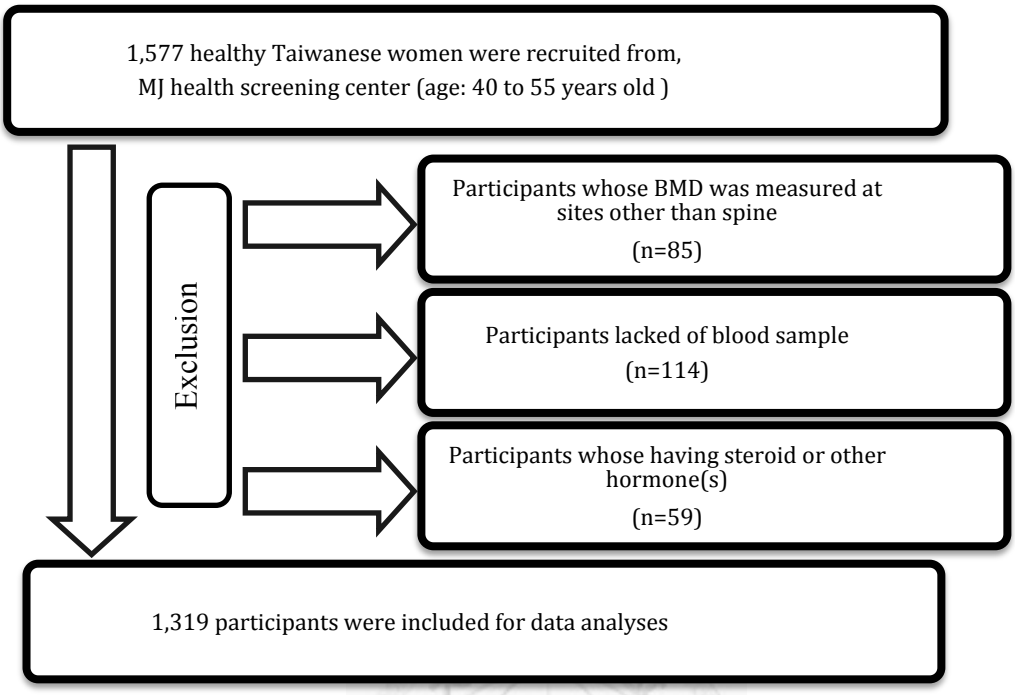

Figure 1. The flowchart of participant recruitment

Abbreviation: BMD, Bone mineral density.

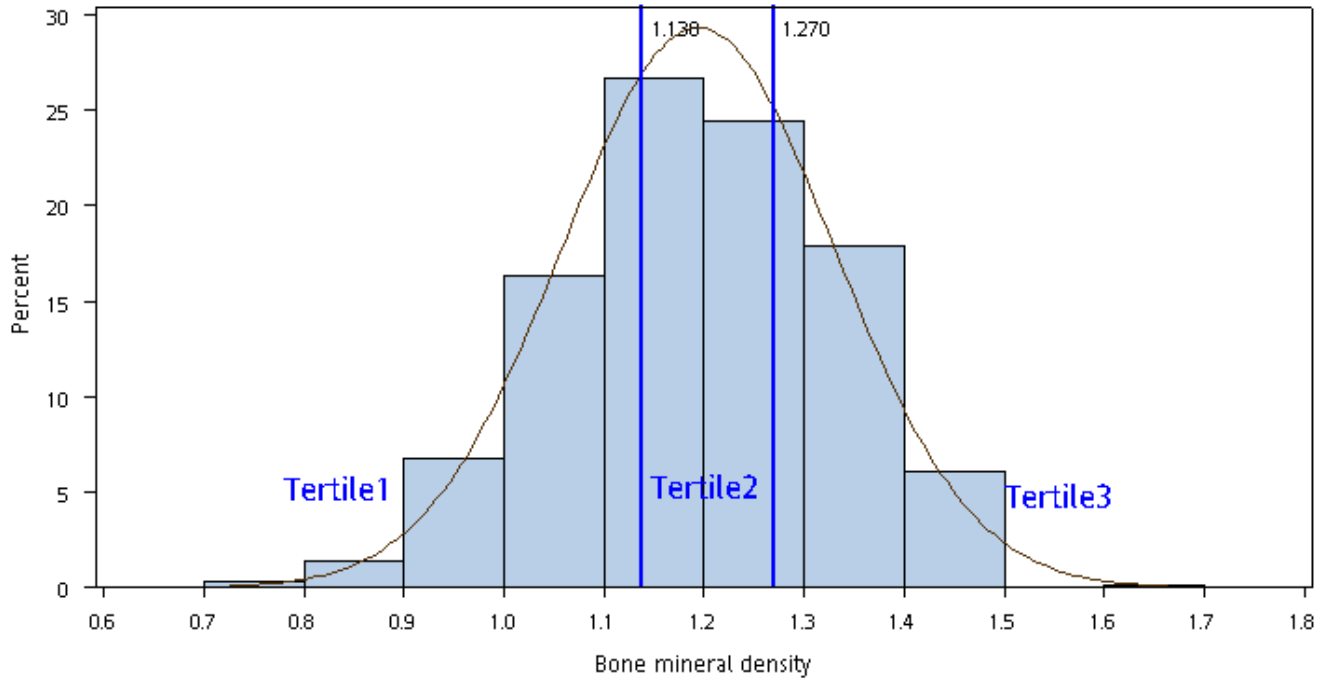

Figure 2. Distribution of BMD

Abbreviations: BMD, Bone mineral density.

BMD was tertiled into T1, T2, and T3. High BMD was defined as T3; low BMD was defined as T1 plus T2.

Hap1:	T	G	C
Hap2:	C	C	C
Hap3:	T	C	T
Hap4:	C	C	T

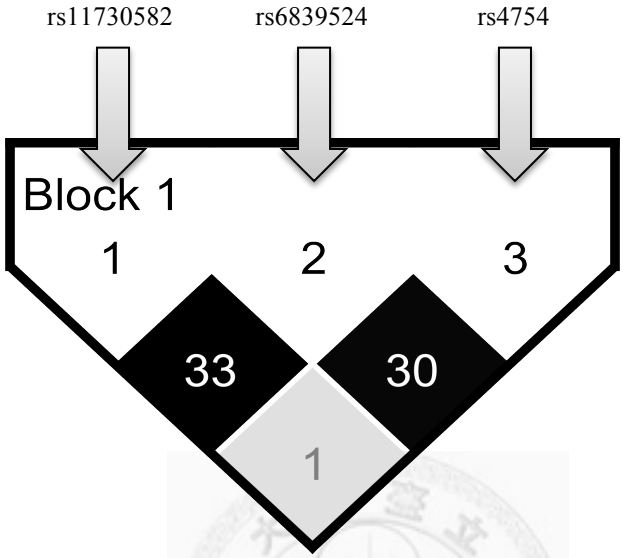

Figure 3. Linkage disequilibrium (LD) plot of *SPP1* gene

This plot was generated by Haploview program using the modified Gabriel et al. algorithm using data from this study. Three common haplotype (frequency ≥ 0.05) were identified and formed one block. The SNP name, e.g., rs11730582, rs6839524, and rs4754, indicated three htSNP genotyped in this study. The level of pair-wise D', which indicated the distance between two SNPs, was shown in the linkage disequilibrium structure in gray scale. The darker a square, the higher the distance is between the two SNPs concerned, which could be defined as a single block. The numbers in cells indicate pairwise r^2, which indicated the strength of linkage disequilibrium between two SNPs.

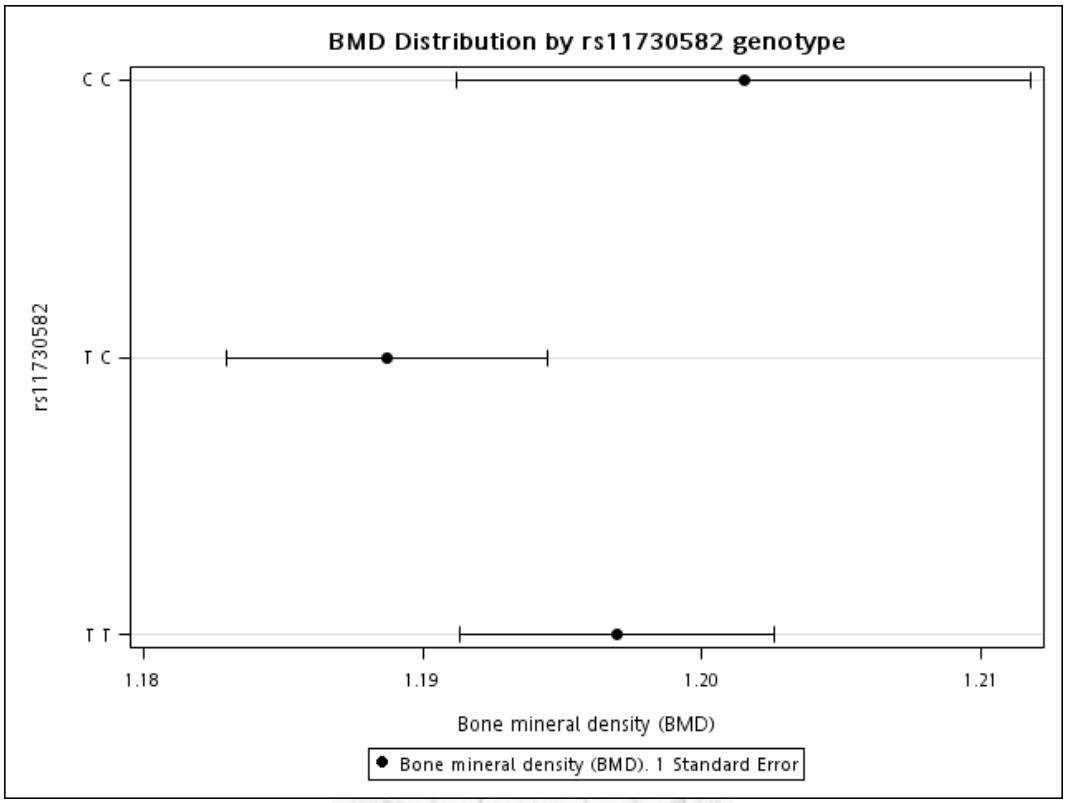

Figure 4. Mean bone mineral density (BMD) e 1 standard error (SE) in the different rs11730582 genotype groups.

Abbreviations: BMD, bone mineral density.

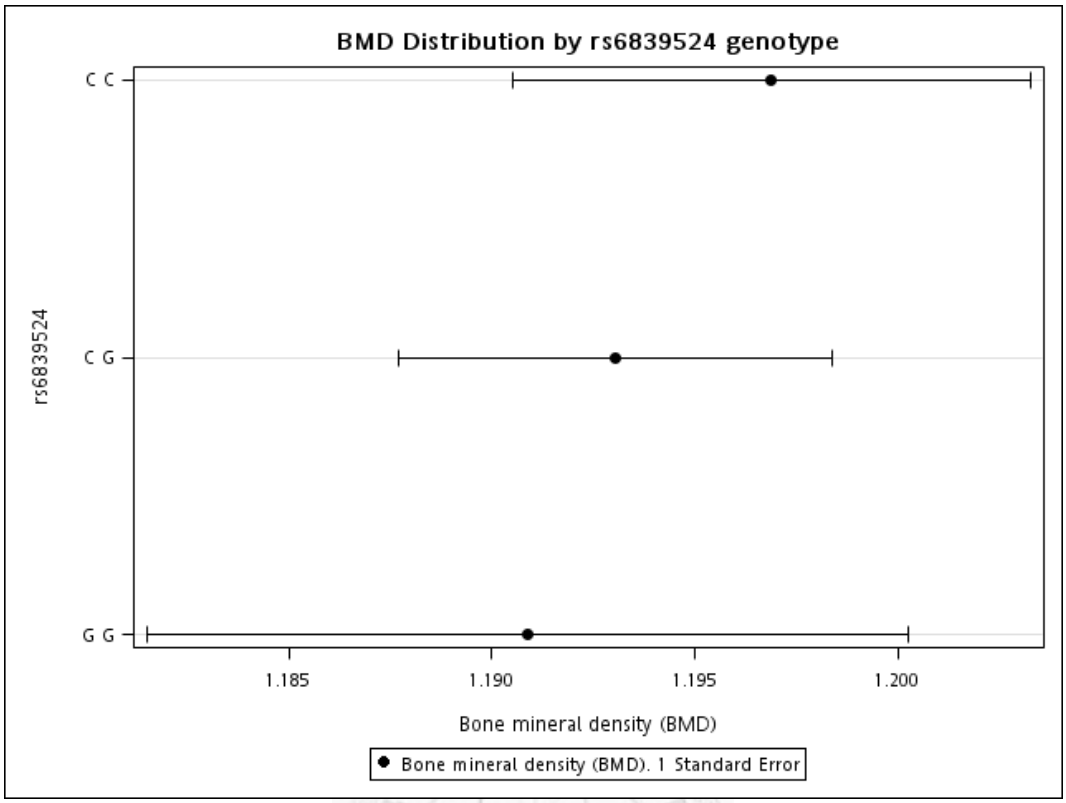

Figure 5. Mean bone mineral density (BMD) ± 1 standard error (SE) in the different rs6839524 genotype groups.

Abbreviations: BMD, bone mineral density.

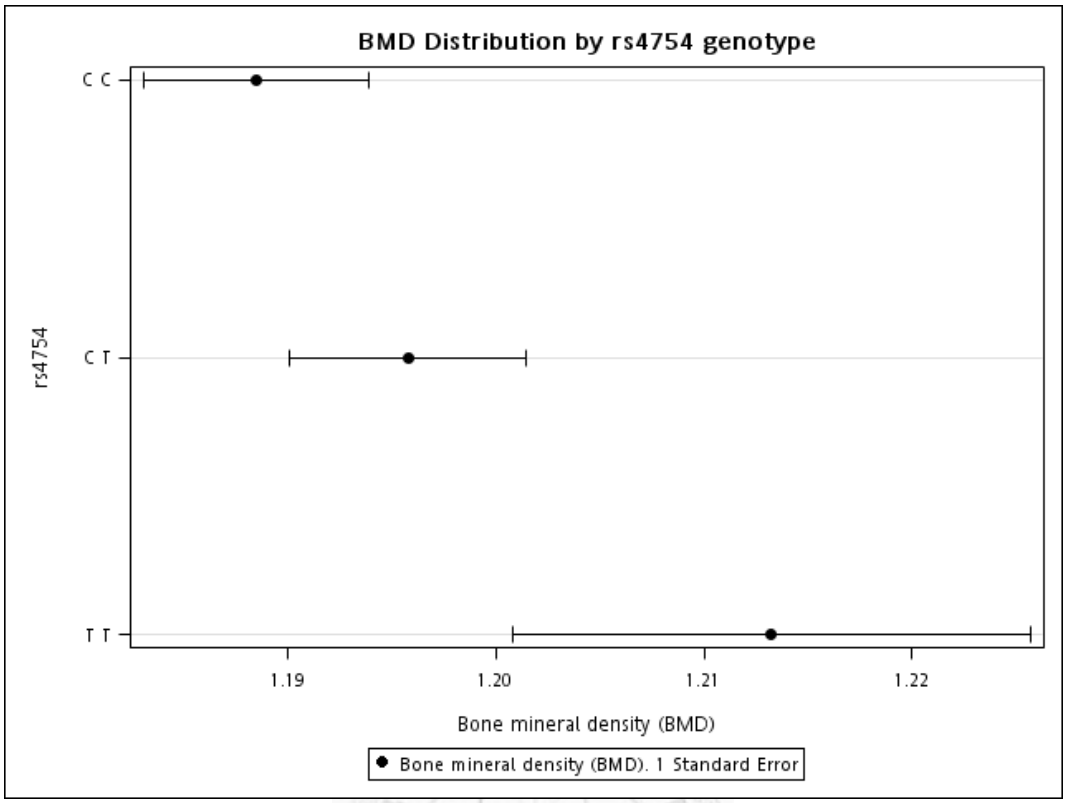

Figure 6. Mean bone mineral density (BMD) ± 1 standard error (SE) in the different rs4754 genotype groups.

Abbreviations: BMD, bone mineral density.

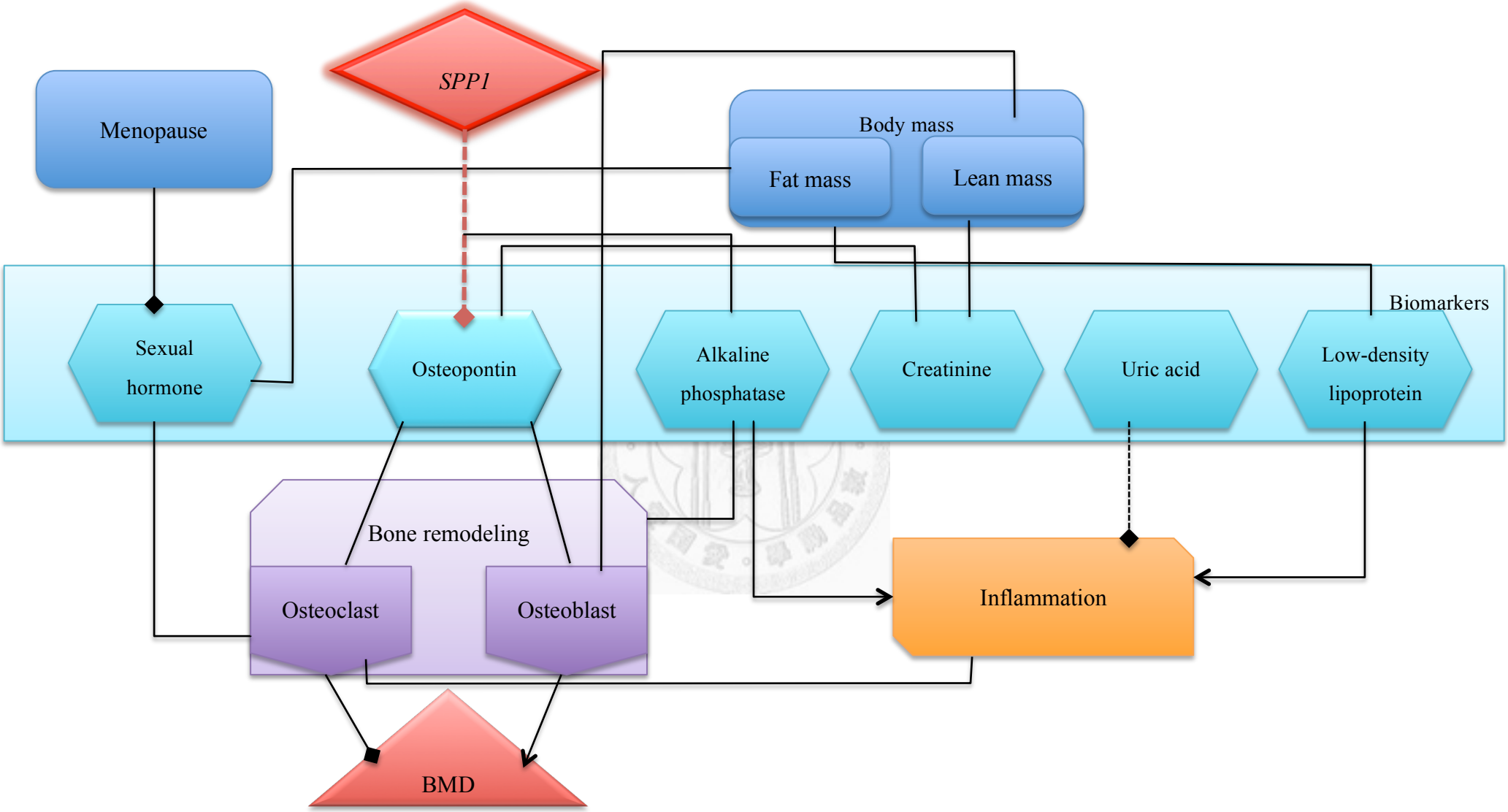

Figure 7. Postulated mechanisms of *SPP1* and BMD

Abbreviations: *SPP1*, secreted phosphoprotein-1; OPN, osteoporosis; LDL, low-density lipoprotein; ALP, alkaline phosphatase; BMI, body mass index; BMD, bone mineral density.

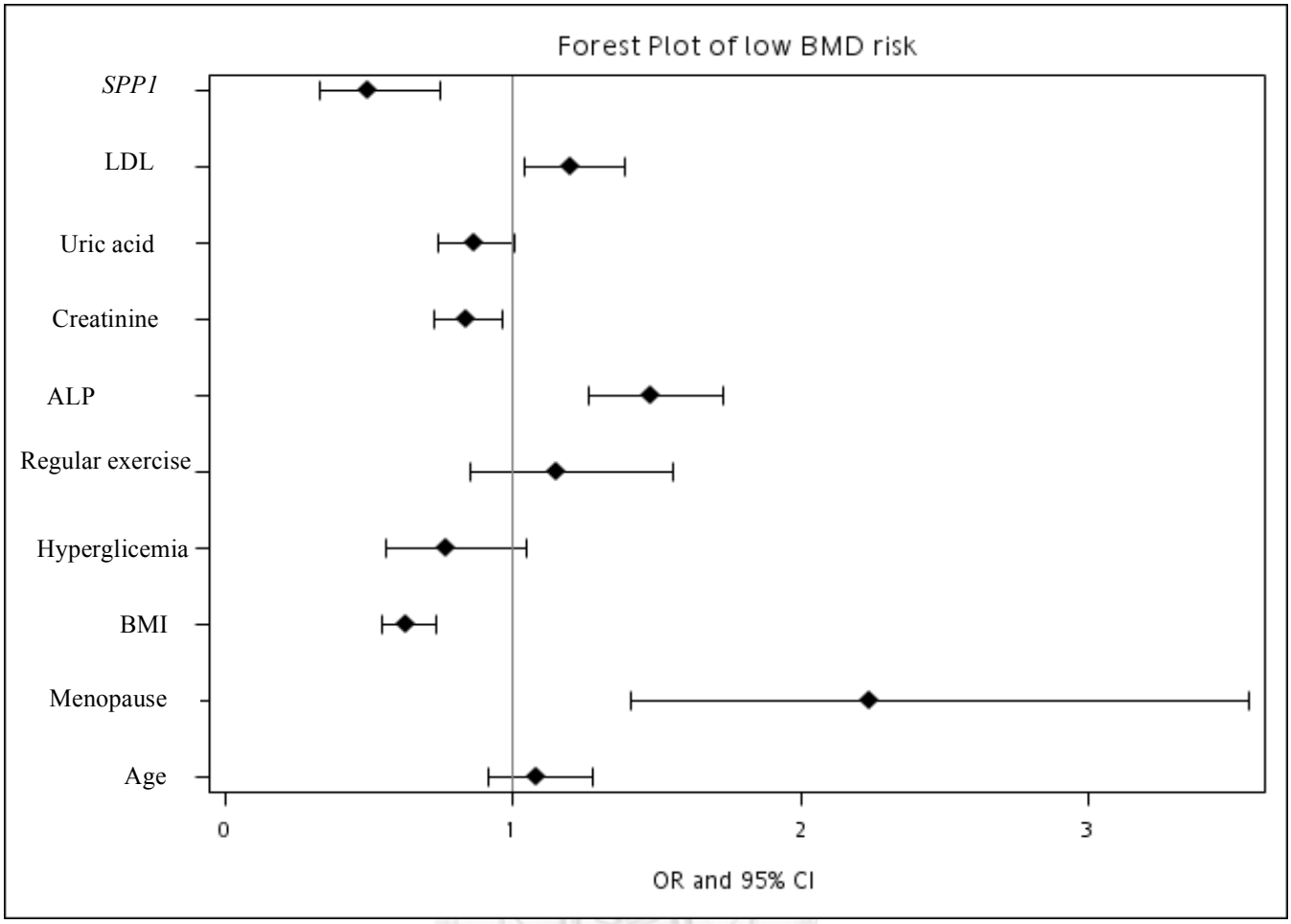

Figure 8. Forest plot of low BMD risk for different covariates

Abbreviations: *SPP1*, secreted phosphoprotein-1; LDL, low-density lipoprotein; ALP, alkaline phosphatase; BMI, body mass index; BMD, bone mineral density. OR, odds ratio; 95% CI, 95% confidence interval.

Table 1. Definition of low and high BMD groups

Tertile	Low BMD (<1.27 g/cm^2)		High BMD (≥1.27 g/cm^2)
	T_1 (n=434)	T_2 (n=454)	T_3 (n=431)
BMD, g/cm^2 (Mean ± SD)	1.04 ± 0.07	1.20 ± 0.04	1.34 ± 0.06
	1.12 ± 0.10		

Abbreviations: BMD, bone mineral density; T1, T2, and T3 denote tertiles of BMD

Table 2. Characteristics of the study population

Variables	Low BMD (< 1.27 g/cm^2) n=888	high BMD (≥ 1.27 g/cm^2) n=431	p-value
	Mean ± SE		
Age	46.8 ± 0.2	45.6 ± 0.2	< 0.001
BMI, kg/m^2	22.0 ± 0.1	23.3 ± 0.2	< 0.001
Alkaline phosphatase, IU/L	61.5 ± 0.62	55.5 ± 0.72	< 0.001
Creatinine, mg/dl	0.80 ± 0.003	0.81 ± 0.005	0.002
Low density lipoprotein, mg/dl	110.3 ± 1.29	105.3 ± 0.98	0.004
Systolic blood pressure, mmHg	108.9 ± 0.50	111.0 ± 0.74	0.02
Fasting glucose, mg/dl	98.1 ± 0.58	99.4 ± 0.81	0.21
Uric acid, mg/dl	4.5 ±0.03	4.7 ± 0.05	< 0.001
Triglyceride, mg/dl	91.9 ± 1.8	94.1 ± 2.7	0.53
	n (%)		
Post-menopause	227 (25.8)	45 (10.6)	< 0.001
Cigarette smoking	82 (9.7)	46 (11.1)	0.45
Alcohol consumption	55 (6.9)	28 (6.6)	0.84
Hyperglycemia	230 (25)	135 (31.2)	0.04
Hypertension	133 (15.0)	71 (16.5)	0.48
Hyperlipidemia	146 (16.4)	75 (17.7)	0.66
Regular exercise	97 (25.0)	210 (26.8)	0.52

Abbreviations: BMD, bone mineral density; BMI, body mass index; hyperglycemia, fasting glucose > 100 mg/dl or using drug management; hypertension, systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg or had medication for controlling blood pressure; hyperlipidemia: low density lipoprotein < 50 mg/dL or triglyceride > 150 mg/dl; regular exercise: walking or hiking ≧30 mins/day.

Table 3. Characteristics of *SPP1* haplotype-tagging SNPs

rs no.	Nucleotide change	Location	Low BMD (< 1.27 g/cm^2)		High BMD (≥ 1.27 g/cm^2)		Total	
			MAF	HWE *p*	MAF	HWE *p*	MAF	HWE *p*
rs11730582	T→C	5'UTR	0.33	0.55	0.32	0.26	0.33	0.25
rs6839524	C→G	intron	0.42	0.96	0.41	0.29	0.42	0.57
rs4754	C→T	exon	0.29	0.77	0.34	0.16	0.31	0.49

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density; HWE, Hardy–Weinberg equilibrium; UTR, untranslated;

MAF, minor allele frequency

Table 4. *SPP1* SNPs and the risk of low BMD

SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	398/207	1.00	389/177	1.21 (0.92-1.60)	0.17	103/48	1.20 (0.78-1.86)	0.41	1.13 (0.93-1.37)	0.20
rs6839524	298/156	1.00	434/198	1.32 (0.98-1.77)	0.07	158/78	0.93 (0.64-1.35)	0.70	1.00 (0.83-1.20)	0.92
rs4754	443/195	1.00	372/180	1.05 (0.80-1.39)	0.71	75/57	**0.51 (0.33-0.79)***	**0.003**	**0.82 (0.67-0.99)**	**0.04**

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

*Association remained significant after controlling for FDR.

Table 5. *SPP1* haplotypes and the risk of low BMD

Haplotype	FAC (%)	Co-dominant model								Additive model	
		0 copies		1 copy			2 copies				
		BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	41.3	526/242	1.00	319/153	1.04 (0.79-1.37)	0.83	43/36	**0.51 (0.30-0.86)**	**0.01**	0.85 (0.68-1.05)	0.12
Hap2:CCC	26.1	300/159	1.00	436/195	1.31 (0.98-1.74)	0.04	152/77	0.94 (0.65-1.36)	0.62	1.01 (0.84-1.22)	0.94
Hap3:TCT	24.0	856/419	1.00	32/12	1.15 (0.52-2.57)	0.74	0/0	NA		1.15 (0.49-2.71)	0.74
Hap4:CCT	6.4	881/427	1.00	7/4	0.78 (0.16-3.78)	0.75	0/0	NA		0.76 (0.15-3.96)	0.75

Abbreviation: NA, not applicable; FAC, frequency among controls; BMD, bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

Table 6. *SPP1* SNPs and the risk of low BMD by menopausal status

SNP	Pre-menopause									
	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	291/183	1.00	285/158	1.22 (0.91-1.64)	0.18	76/39	1.42 (0.88-2.30)	0.15	1.20 (0.98-1.49)	0.09
rs6839524	221/132	1.00	319/174	1.20 (0.88-1.65)	0.26	112/74	0.78 (0.52-1.16)	0.22	0.92 (0.76-1.12)	0.41
rs4754	323/178	1.00	271/156	1.08 (0.81-1.46)	0.59	58/46	**0.58 (0.36-0.93)**	**0.02**	0.86 (0.70-1.07)	0.18
Post-menopause										
SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	103/20	1.00	99/16	1.04 (0.42-2.58)	0.94	25/9	0.42 (0.13-1.35)	0.15	0.71 (0.40-1.27)	0.25
rs6839524	71/21	1.00	111/20	2.43 (0.98-5.98)	0.05	45/4	3.40 (0.89-12.92)	0.07	**2.00 (1.06-3.78)**	**0.03**
rs4754	117/16	1.00	94/20	0.98 (0.39-2.46)	0.96	16/9	0.35 (0.11-1.18)	0.09	0.65 (0.36-1.19)	0.16

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density.

All models were adjusted for age, BMI, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

Table 7. *SPP1* SNPs and the risk of low BMD by BMI

SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Low BMI (< 18.5 kg/m^2)										
rs11730582	28/5	1.00	23/7	0.35 (0.05-2.68)	0.31	4/2	0.14 (0.003-6.82)	0.32	0.36 (0.08-1.63)	0.19
rs6839524	21/8	1.00	23/4	2.69 (0.19-37.52)	0.46	11/2	4.13 (0.25-67.06)	0.32	2.11 (0.53-8.36)	0.29
rs4754	23/5	1.00	22/5	1.51 (0.13-17.16)	0.70	10/3	0.40 (0.03-4.59)	0.46	0.67 (0.19-2.31)	0.52
Normal BMI (18.5 - < 24 kg/m^2**)**										
rs11730582	299/132	1.00	276/117	1.14 (0.82-1.58)	0.45	81/29	1.49 (0.87-2.55)	0.15	1.19 (0.94-1.50)	0.15
rs6839524	220/96	1.00	314/126	1.14 (0.80-1.63)	0.46	122/56	0.77 (0.50-1.19)	0.24	0.91 (0.73-1.13)	0.38
rs4754	333/137	1.00	273/106	1.25 (0.89-1.74)	0.20	50/35	0.59 (0.35-1.00)	0.05	0.91 (0.72-1.15)	0.44
High BMI (≧24 kg/m^2**)**										
rs11730582	71/70	1.00	87/51	**1.82 (1.06-3.12)**	**0.03**	18/17	1.05 (0.45-2.44)	0.91	1.24 (0.85-1.81)	0.26
rs6839524	55/51	1.00	96/67	1.54 (0.88-2.71)	0.13	25/20	0.95 (0.43-2.10)	0.89	1.07 (0.73-1.57)	0.72
rs4754	86/52	1.00	76/68	0.73 (0.43-1.26)	0.26	14/18	**0.38 (0.15-0.96)**	**0.04**	**0.66 (0.44-0.99)**	**0.04**

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density; BMI, body mass index.

All models were adjusted for age, menopausal status, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

Table 8. *SPP1* SNPs and the risk of low BMD by serum ALP level (low and high)

SNP	Low ALP (≤ 57 IU/L)									
	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	181/115	1.00	183/111	1.07 (0.74-1.56)	0.73	52/30	1.16 (0.65-2.07)	0.61	1.08 (0.83-1.40)	0.59
rs6839524	152/92	1.00	193/121	1.10 (0.74-1.62)	0.65	71/43	0.86 (0.52-1.45)	0.58	0.96 (0.75-1.23)	0.72
rs4754	205/113	1.00	168/111	1.01 (0.69-1.48)	0.95	43/32	0.66 (0.37-1.19)	0.17	0.87 (0.67-1.14)	0.32
	High ALP (> 57 IU/L)									
SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	217/92	1.00	204/65	1.45 (0.95-2.20)	0.08	51/18	1.06 (0.55-2.05)	0.86	1.17 (0.87-1.57)	0.31
rs6839524	144/63	1.00	241/77	**1.76 (1.12-2.75)**	**0.01**	87/35	1.03 (0.60-1.78)	0.91	1.09 (0.83-1.44)	0.54
rs4754	238/82	1.00	203/69	1.19 (0.78-1.82)	0.43	31/24	**0.35 (0.18-0.67)***	**0.0016**	0.75 (0.55-1.01)	0.06

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density; ALP, alkaline phosphatase.

All models were adjusted for age, menopausal status, BMI, serum creatinine, UA, LDL, hyperglycemia, and regular exercise.

*Association remained significant after controlling for FDR.

Table 9. *SPP1* SNPs and the risk of low BMD by serum creatinine level (low and high)

SNP	Low creatinine (≤ 0.79 IU/L)									
	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	213/94	1.00	213/78	1.18 (0.78-1.77)	0.43	75/18	1.49 (0.75-2.94)	0.25	1.20 (0.88-1.61)	0.21
rs6839524	175/63	1.00	225/86	1.06 (0.68-1.64)	0.80	83/41	**0.58 (0.34-0.99)**	**0.05**	0.79 (0.60-1.03)	0.08
rs4754	229/96	1.00	208/72	**1.73 (1.14-2.64)**	**0.01**	46/22	0.91 (0.47-1.75)	0.77	1.19 (0.88-1.61)	0.26
High creatinine (> 0.79 IU/L)										
SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	185/113	1.00	174/98	1.27 (0.86-1.87)	0.22	46/30	1.03 (0.57-1.86)	0.92	1.09 (0.84-1.42)	0.52
rs6839524	121/92	1.00	209/112	**1.56 (1.04-2.33)**	**0.03**	75/37	1.41 (0.83-2.40)	0.21	1.24 (0.96-1.62)	0.10
rs4754	214/99	1.00	163/108	0.70 (0.48-1.03)	0.07	28/34	**0.33 (0.18-0.61)***	**0.0004**	**0.62 (0.47-0.81)***	**0.0005**

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum ALP, UA, LDL, hyperglycemia, and regular exercise.

*Association remained significant after controlling for false FDR.

Table 10. *SPP1* SNPs and the risk of low BMD by serum UA level (low and high)

SNP	Low UA (≤ 4.5 mg/dl)									
	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	215/102	1.00	211/82	1.42 (0.95-2.11)	0.09	64/23	1.57 (0.85-2.91)	0.15	1.31 (0.99-1.72)	0.06
rs6839524	167/80	1.00	220/82	1.33 (0.87-2.04)	0.19	103/45	0.99 (0.60-1.63)	0.97	1.02 (0.80-1.32)	0.86
rs4754	261/97	1.00	191/78	1.07 (0.71-1.60)	0.76	38/32	**0.31 (0.17-0.57)***	**0.0002**	**0.69 (0.52-0.91)**	**0.008**
	High UA (> 4.5 mg/dl)									
SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	183/105	1.00	176/94	1.09 (0.74-1.62)	0.66	39/25	0.93 (0.49-1.77)	0.83	1.01 (0.76-1.34)	0.95
rs6839524	129/75	1.00	214/116	1.30 (0.86-1.96)	0.22	55/33	0.85 (0.47-1.52)	0.58	0.99 (0.75-1.32)	0.96
rs4754	182/98	1.00	180/102	1.06 (0.72-1.58)	0.76	36/24	0.82 (0.43-1.56)	0.55	0.96 (0.72-1.28)	0.78

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density; UA, uric acid.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, LDL, hyperglycemia, and regular exercise.

*Association remained significant after controlling for FDR.

Table 11. *SPP1* SNPs and the risk of low BMD by serum LDL level (low and high)

SNP	Low LDL (≤ 106 mg/dl)									
	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	179/112	1.00	197/105	1.24 (0.85-1.81)	0.26	49/26	1.40 (0.74-2.65)	0.30	1.21 (0.91-1.59)	0.19
rs6839524	146/94	1.00	205/108	1.42 (0.94-2.13)	0.09	74/41	0.99 (0.58-1.68)	0.96	1.06 (0.81-1.37)	0.68
rs4754	219/111	1.00	166/98	1.05 (0.71-1.56)	0.79	40/34	**0.47 (0.26-0.86)**	**0.01**	0.79 (0.60-1.03)	0.08
	High LDL (> 106 mg/dl)									
SNP	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
rs11730582	219/93	1.00	190/70	1.15 (0.76-1.75)	0.50	54/22	0.96 (0.52-1.78)	0.90	1.03 (0.78-1.36)	0.84
rs6839524	150/61	1.00	229/87	1.27 (0.82-1.97)	0.29	84/37	0.93 (0.54-1.61)	0.80	1.00 (0.76-1.31)	0.97
rs4754	224/83	1.00	205/80	1.04 (0.69-1.57)	0.86	34/22	0.52 (0.27-1.01)	0.05	0.82 (0.61-1.11)	0.20

Abbreviations: SNP, single nucleotide polymorphism; BMD, bone mineral density; LDL, low-density lipoprotein.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, UA, hyperglycemia, and regular exercise.

Table 12. *SPP1* haplotypes and the risk of low BMD by menopausal status

Pre-menopause										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	384/215	1.00	232/135	1.04 (0.77-1.40)	0.82	36/30	0.58 (0.33-1.02)	0.06	0.88 (0.70-1.10)	0.01
Hap2:CCC	223/136	1.00	321/171	1.25 (0.91-1.71)	0.17	108/73	0.77 (0.52-1.15)	0.21	0.92 (0.76-1.13)	0.93
Hap3:TCT	629/372	1.00	23/8	1.44 (0.55-3.76)	0.45	0/0	NA		1.44 (0.55-3.76)	0.50
Hap4:CCT	648/376	1.00	4/4	0.63 (0.11-3.69)	0.61	0/0	NA		0.63 (0.11-3.69)	0.72
Post-menopause										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	138/25	1.00	82/15	1.10 (0.44-2.77)	0.83	7/5	0.30 (0.07-1.37)	0.12	0.73 (0.37-1.41)	0.89
Hap2:CCC	72/21	1.00	112/20	2.46 (0.99-6.08)	0.05	43/4	3.27 (0.85-12.54)	0.08	1.99 (1.05-3.77)	0.96
Hap3:TCT	218/42	1.00	9/3	0.35 (0.03-8.83)	0.39	0/0	NA		0.35 (0.03-3.83)	0.55
Hap4:CCT	224/44	1.00	3/1	NA	NA	0/0	NA	NA	NA	NA

Abbreviation: NA, not applicable; BMD, bone mineral density.

All models were adjusted for age, BMI, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

Table 13. *SPP1* haplotypes and the risk of low BMD by BMI

Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	OR	BMD Low/High	OR (95% CI)	p-value	BMD Low/High	OR (95% CI)	p-value	OR (95% CI)	p-value
Low BMI(< 18.5 kg/m^2)										
Hap1:TGC	29/7	1.00	20/5	1.25 (0.11-13.91)	0.86	6/2	0.97 (0.06-15.89)	0.98	1.01 (0.26-3.99)	0.98
Hap2:CCC	22/8	1.00	22/4	2.43 (0.19-30.85)	0.49	11/2	3.93 (0.25-62.53)	0.33	2.05 (0.53-7.87)	0.30
Hap3:TCT	51/13	1.00	4/1	NA		0/0	NA		NA	
Hap4:CCT	54/13	1.00	1/1	NA		0/0	NA		NA	
Normal BMI (18.5 - < 24 kg/m^2)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	394/164	1.00	229/92	1.11 (0.79-1.56)	0.55	33/22	0.66 (0.36-1.21)	0.18	0.93 (0.73-1.20)	0.59
Hap2:CCC	222/98	1.00	317/124	1.20 (0.84-1.71)	0.31	117/56	0.75 (0.49-1.17)	0.21	0.91 (0.73-1.13)	0.38
Hap3:TCT	633/269	1.00	23/9	0.88 (0.34-2.28)	0.79	0/0	NA		0.88 (0.34-2.28)	0.79
Hap4:CCT	650/276	1.00	6/2	1.60 (0.15-17.19)	0.70	0/0	NA		NA	
High BMI (≧ 24 kg/m^2)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	101/69	1.00	71/57	0.87 (0.51-1.49)	0.62	4/12	**0.18 (0.05-0.72)**	0.02	0.66 (0.42-1.01)	0.06
Hap2:CCC	55/52	1.00	97/67	1.57 (0.89-2.77)	0.21	24/19	1.00 (0.45-2.23)	1.00	1.11 (0.75-1.63)	0.61
Hap3:TCT	170/134	1.00	6/4	3.70 (0.39-35.45)	0.26	0/0	NA		3.70 (0.39-35.45)	0.26
Hap4:CCT	175/135	1.00	1/3	NA		0/0	NA		NA	

Abbreviation: NA, not applicable; BMD, bone mineral density; BMI, body mass index.

All models were adjusted for age, menopausal status, serum ALP, creatinine, UA, LDL, hyperglycemia, and regular exercise.

Table 14. *SPP1* haplotypes and the risk of low BMD by ALP level (low and high)

Low ALP (≤ 57 IU/L)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	238/146	1.00	154/91	1.13(0.77-1.65)	0.54	24/19	0.79 (0.38-1.64)	0.53	0.99 (0.75-1.33)	0.98
Hap2:CCC	155/95	1.00	190/118	1.16 (0.79-1.73)	0.45	71/43	0.89 (0.53-1.49)	0.67	0.98 (0.76-1.26)	0.87
Hap3:TCT	406/249	1.00	10/7	0.45 (0.12-1.62)	0.22	0/0	NA		0.45 (0.12-1.62)	0.22
Hap4:CCT	414/253	1.00	2/3	0.16 (0.01-4.73)	0.29	0/0	NA		0.16 (0.01-4.73)	0.29
High ALP (> 57 IU/L)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	287/95	1.00	166/63	1.02 (0.66-1.58)	0.93	19/17	**0.30 (0.15-0.64)***	**0.0016**	**0.71 (0.52-0.98)**	0.03
Hap2:CCC	145/63	1.00	246/78	**1.77 (1.13-2.77)**	0.01	81/34	0.98 (0.56-1.70)	0.93	1.07 (0.81-1.43)	0.62
Hap3:TCT	450/169	1.00	22/6	2.38 (0.65-8.68)	0.19	0/0	NA		2.38 (0.65-8.68)	0.19
Hap4:CCT	467/174	1.00	5/1	1.26 (0.11-13.99)	0.85	0/0	NA		1.26 (0.11-13.99)	0.85

Abbreviation: NA, not applicable BMD, bone mineral density; ALP, alkaline phosphatase.

All models were adjusted for age, menopausal status, BMI, serum creatinine, UA, serum LDL, hyperglycemia and regular exercise.

*Association remained significant after controlling for FDR.

Table 15. *SPP1* haplotypes and the risk of low BMD by creatinine level (low and high)

Low creatinine (≤ 0.79 IU/L)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	277/112	1.00	180/64	1.43(0.94-2.19)	0.10	26/14	0.82 (0.37-1.84)	0.63	1.14 (0.82-1.57)	0.44
Hap2:CCC	178/64	1.00	224/86	1.07 (0.69-1.66)	0.77	81/40	**0.58 (0.34-0.99)**	**0.04**	0.79 (0.60-1.04)	0.09
Hap3:TCT	464/183	1.00	19/7	0.95 (0.32-2.87)	0.93	0/0	NA		0.95 (0.32-2.87)	0.93
Hap4:CCT	479/187	1.00	4/3	0.47 (0.05-4.33)	0.50	0/0	NA		0.47 (0.05-4.33)	0.50
High creatinine (> 0.79 IU/L)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	248/129	1.00	140/90	0.79 (0.53-1.16)	0.23	17/22	**0.35 (0.17-0.73)**	**0.0051**	**0.68 (0.50-0.90)**	**0.008**
Hap2:CCC	121/94	1.00	213/110	**1.66 (1.11-2.48)**	**0.01**	71/37	1.37 (0.81-2.34)	0.24	1.25 (0.96-1.62)	0.10
Hap3:TCT	392/235	1.00	13/6	1.33 (0.34-5.25)	0.68	0/0	NA		1.33 (0.34-5.25)	0.68
Hap4:CCT	401/239	1.00	4/2	1.26 (0.10-15.68)	0.86	0/0	NA	NA	1.26 (0.10-15.68)	0.86

Abbreviation: NA, not applicable BMD, bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum ALP, UA, LDL, hyperglycemia, and regular exercise.

Table 16. *SPP1* haplotypes and the risk of low BMD by UA level (low and high)

Low UA (≤ 4.5 mg/dl)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	310/116	1.00	159/71	0.88 (0.58-1.32)	0.53	21/20	**0.33 (0.16-0.68)***	**0.0027**	0.69 (0.51-0.93)	0.01
Hap2:CCC	170/81	1.00	221/82	1.38 (0.90-2.12)	0.14	99/44	0.96 (0.58-1.58)	0.86	1.01 (0.79-1.30)	0.93
Hap3:TCT	472/202	1.00	18/5	1.56 (0.43-5.69)	0.50	0/0	NA		1.56 (0.43-5.69)	0.50
Hap4:CCT	485/205	1.00	5/2	1.58 (0.13-19.73)	0.72	0/0	NA		1.58 (0.13-19.73)	0.72
High UA (> 4.5 mg/dl)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	215/125	1.00	161/83	1.18 (0.80-1.76)	0.40	22/16	0.78 (0.36-1.69)	0.53	1.02 (0.75-1.39)	0.89
Hap2:CCC	129/77	1.00	216/114	1.36 (0.90-2.06)	0.15	53/33	0.86 (0.48-1.53)	0.60	1.01 (0.76-1.34)	0.96
Hap3:TCT	384/216	1.00	14/8	0.70 (0.21-2.30)	0.55	0/0	NA		0.70 (0.21-2.30)	0.55
Hap4:CCT	395/221	1.00	3/3	0.33 (0.03-3.61)	0.37	0/0	NA		0.33 (0.03-3.61)	0.37

Abbreviation: NA, not applicable BMD, bone mineral density; UA, uric acid.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, LDL, hyperglycemia, and regular exercise.

* Association remained significant after controlling for FDR.

Table 17. *SPP1* haplotypes and the risk of low BMD by serum LDL level (low and high)

Low LDL (≤ 106 mg/dl)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	259/133	1.00	143/87	0.93 (0.62-1.38)	0.71	23/23	**0.42 (0.21-0.83)**	**0.01**	0.75 (0.56-1.00)	0.05
Hap2:CCC	147/95	1.00	205/108	1.42 (0.95-2.13)	0.09	73/40	1.00 (0.59-1.70)	0.99	1.06 (0.82-1.38)	0.65
Hap3:TCT	410/236	1.00	15/7	1.54 (0.40-5.93)	0.53	0/0	NA		1.54 (0.40-5.93)	0.53
Hap4:CCT	422/240	1.00	3/3	0.58 (0.06-6.01)	0.64	0/0	NA		0.58 (0.06-6.01)	0.64
High LDL (> 106 mg/dl)										
Haplotype	Co-dominant model								Additive model	
	0 copies		1 copy			2 copies				
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	BMD Low/High	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Hap1:TGC	266/107	1.00	177/65	1.15 (0.76-1.75)	0.51	20/13	0.65 (0.28-1.50)	0.31	0.97 (0.70-1.34)	0.85
Hap2:CCC	151/63	1.00	233/85	1.38 (0.89-2.14)	0.15	79/37	0.90 (0.52-1.56)	0.70	0.99 (0.75-1.31)	0.97
Hap3:TCT	447/179	1.00	18/6	1.02 (0.33-3.18)	0.98	0/0	NA		1.02 (0.33-3.18)	0.98
Hap4:CCT	460/183	1.00	5/2	0.87 (0.08-9.46)	0.91	0/0	NA	NA	0.87 (0.08-9.46)	0.91

Abbreviation: NA, not applicable BMD, bone mineral density; LDL, low-density lipoproteind.

All models were adjusted for age, menopausal status, BMI, serum ALP, creatinine, UA, hyperglycemia, and regular exercise.

Table 18. Interaction between rs4754 and serum biomarkers on low BMD risk

Serum biomarkers	rs4754					$P_{interaction}$*
	0 or 1 copy		2 copies			
	BMD Low/High	AOR	BMD Low/High	AOR (95% CI)	p-value	
Alkaline phosphatase						
Low (≤ 57 IU/L)	373/224	1.00	43/32	0.66 (0.38-1.15)	0.14	0.09
High (> 57 IU/L)	441/151	1.00	31/24	**0.32 (0.17-0.60)**	**0.0004**	
Creatinine						
Low (≤ 0.79 IU/L)	437/168	1.00	46/22	0.71 (0.38-1.34)	0.29	0.17
High (>0.79 IU/L)	377/207	1.00	28/34	**0.39 (0.22-0.70)**	**0.0017**	
Uric acid						
Low (≤ 4.5 mg/dl)	452/175	1.00	38/32	**0.30 (0.17-0.55)**	**<0.0001**	**0.04**
High (> 4.5 mg/dl)	362/200	1.00	36/24	0.80 (0.43-1.47)	0.46	

Abbreviation: bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum creatinine, UA, LDL, hyperglycemia, and regular exercise.

*$p_{interaction}$ was obtained by using the recessive model.

Table 19. Interaction between HAP1 and serum biomarkers on low BMD risk

Serum biomerkers	HAP1 (TGC)					$P_{interaction}$*
	0 or 1 copy		2 copies			
	BMD Low/High	OR	BMD Low/High	OR (95% CI)	p-value	
Alkaline phosphatase						
Low (≤ 57 IU/L)	392/237	1.00	24/19	0.76 (0.37-1.55)	0.45	0.06
High (> 57 IU/L)	453/158	1.00	19/17	**0.30 (0.15-0.62)**	**0.0012**	
Uric acid						
Low (≤ 4.5 mg/dl)	469/187	1.00	21/20	**0.34 (0.17-0.70)**	**0.0033**	0.22
High (> 4.5 mg/dl)	376/208	1.00	22/16	0.73 (0.34-1.55)	0.41	

Abbreviation: bone mineral density.

All models were adjusted for age, menopausal status, BMI, serum creatinine, UA, LDL, hyperglycemia, and regular exercise.

*$p_{interaction}$ was obtained by using the recessive model.

Table 20. Previous studies on *SPP1* polymorphisms and BMD

Study	Journal	Sample Size	Outcome (Y)	Gene/SNP (X)	Results	Limitations
Taylor et al. The CARDIA study.	Hum Genet (2005).	741 participants.	coronary calcification Hip and spine bone mineral density (BMD).	*MGP*: T-138C (rs1800802) ***SPP1***: T-443C (**rs11730582**) Asp94Asp (**rs4754**).	None significant association when adjusted for smoking status, log homeostatic model assessment, education, systolic blood pressure, total cholesterol, and BMI.	CARDIA participants are relatively young and healthy. No Asian data
Richards et al. Collaborative meta-analysis.	Ann Intern Med. (2009).	Large-scale meta-analysis of genome-wide association data.	BMD: 19,195 participants. Fracture: 5,974 participants.	150 candidate genes and 36,016 SNPs were identified.	SNPs from *ESR1, LRP4, ITGA1, LRP5, SOST, **SPP1**, TNFRSF11A, TNFRSF11B,* and *TNFSF11* were associated with BMD. SNPs from the *LRP5, SOST, **SPP1***, and *TNFRSF11A* were significantly associated with fracture risk.	Only common polymorphisms in linkage disequilibrium with SNPs in HapMap could be assessed.