J Musculoskelet Neuronal Interact 2007; 7(2):149-154

Original Article

Hylonome

No evidence of association of the osteocalcin gene *Hind*III polymorphism with bone mineral density in Chinese women

D-K. Jiang[1], F-H. Xu[2], M-Y. Liu[1], X-D. Chen[1], M-X. Li[1], Y-J. Liu[2], H. Shen[2], H-W. Deng[1,2,3]

[1]Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan, P.R. China; [2]Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University Medical Center, Omaha, NE 68131, USA; [3]Departments of Basic Medical Sciences, School of Medicine, University of Missouri-Kansas City, Kansas, USA

Abstract

Osteoporosis is a major health problem, mainly characterized by low bone mineral density (BMD). Osteocalcin (also known as BGP, for bone Gla protein) is a significant biomarker of bone turnover and thus the BGP gene has been considered as an important candidate gene for osteoporosis. A few studies on the relationship between variants of the BGP gene and BMD variation, via traditional association and/or linkage methods, have yielded conflicting results. In the present study, we simultaneously tested linkage and/or association of the BGP *Hind*III polymorphism with BMD in a large cohort of pre-menopausal Chinese women. A total of 1,263 subjects from 402 Chinese nuclear families were examined. Each family consists of both parents and at least one daughter aged between 20-45 years. BMDs at the lumbar spine and hip were measured by dual-energy X-ray absorptiometry (DXA). Using the QTDT (quantitative transmission disequilibrium test) program, we did not detect significant evidence of linkage or association between the BGP *Hind*III polymorphisms and the BMD variation at any skeletal site. Our data do not support the BGP gene having a major effect on BMD variation in pre-menopausal Chinese women.

Keywords: Osteoporosis, Bone Mineral Density, Osteocalcin Gene, Association, Linkage

Introduction

Osteoporosis is a major public health problem characterized by fragile bones susceptible to low trauma fractures, particularly in elderly women. In China, approximately 6.97% of the total 1.3 billion population suffer from primary osteoporosis[1]. A major risk factor for osteoporotic fractures is low BMD[2-4], which is under strong genetic control. The heritability of BMD has been estimated ranging from 50% to 80%[5-8]. Given the complex biology of the skeleton, it is likely that BMD is under the control of a large number of genes.

Osteocalcin, also called bone Gla protein (BGP), is the most abundant non-collagenous protein component of bone. It is synthesized exclusively by osteoblasts under transcriptional regulation through a vitamin D-response element, and is well known as a marker for differentiated mature osteoblasts and a determinant of bone calcification process[9-15]. In addition, BGP promotes recruitment and differentiation of osteoclast precursors at the bone surface, suggesting a potential role in bone resorption and remodeling[16-18]. The human BGP gene has been mapped to genomic region 1q25-31. A whole genome linkage screen for quantitative trait loci (QTLs) contributing to normal variation in BMD detected a linkage peak with a maximum LOD score of 3.11 at this region[19]. Recently, a polymorphism was identified in the promoter of BGP gene with the restriction enzyme *Hind*III[20]. Association between the *Hind*III polymorphism and BMD was tested for BMD variation in different racial populations[20-26]. Most of those studies employed population-based association test and the results are largely inconsistent.

In this study, we simultaneously test linkage and/or association of the *Hind*III polymorphism in the BGP gene with BMD variation at the spine and hip in a large sample of Chinese nuclear families.

The authors have no conflict of interest.

Corresponding author: Hong-Wen Deng, Ph. D., Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, P.R. China
E-mail: hwdeng@hunnu.edu.cn

Accepted 20 October 2006

Materials and methods

Subjects

A total of 402 nuclear families comprising 1,263 subjects were enrolled from a local population of Shanghai, a modern city located on the mid-east coast of People's Republic of China. All the study subjects belong to the Chinese Han ethnic group. Each nuclear family is composed of both parents and at least one female child aging between 20 and 45 years. The nuclear families vary in size from 3 to 6 individuals, with a mean of 3.14. The numbers of families with one, two, three, and four daughters are 349, 50, 2, 1, respectively. The exclusion criteria for the subjects included were the same as described by Deng et al.[26]. In brief, individuals who had any diseases or medication known to have potential effects on bone and mineral metabolism were excluded from the study. The study was approved by the Research Administration Department of Hunan Normal University and the 6th People's Hospital of Shanghai, P.R. China. Before entering the projects, informed written consent documents were given by all the participants.

Trait	Fathers (n=387)	Mothers (n=380)	Offspring (n=459)
Age (year)	62.4±6.6	59.1±6.6	31.4±5.8
Height (cm)	166.2±6.0	154.6±5.6	159.9±5.2
Weight (kg)	68.4±10.0	59.2±8.6	55.1±8.0
BMD (g/cm^2)			
Spine L_{1-4}	0.930±0.149	0.813±0.152	0.960±0.102
Femoral neck	0.751±0.115	0.678±0.122	0.776±0.107
Trochanter	0.648±0.100	0.545±0.107	0.665±0.088
Intertrochanteric region	1.024±0.147	0.880±0.161	0.998±0.130
Ward's triangle	0.553±0.139	0.507±0.159	0.711±0.132
Total hip	0.875±0.123	0.749±0.134	0.855±0.108

Note: all data were presented as mean±SD for the raw phenotype values without adjustment. Phenotypes are not available for 15 subjects in the fathers group and 22 subjects in the mothers group.

Table 1. Basic characteristics of fathers, mothers and offspring group.

Measurement

The BMDs (g/cm^2) at lumbar spine (L_{1-4}) and hip (femoral neck, trochanter, intertrochanteric region and ward's triangle) were measured using the Hologic QDR 2000+ dual-energy X-ray absorptiometry (DXA) scanner (Hologic, Waltham, MA, USA). The machine is calibrated daily. The co-efficient of variation (CV) of the BMD measurement obtained by repeated measures on 7 individuals for five times was 0.9%, 0.8%, 1.93%, 1.48%, 1.31% and 3.85% at the lumbar spine, total hip, femoral neck, trochanter, intertrochanteric region and ward's triangle, respectively. Weight and height were measured at the time of BMD measurement.

Genotyping

Genomic DNA was extracted using a standard phenol-chloroform extraction procedure. PCR was performed in a 25 ml reaction mixture with the following final concentrations: 1× PCR buffer, 1.5 mM MgCl2, 12.5 pmol of each primer, 2.5 mM of each dATP, dCTP, dGTP, and dTTP, 100 ng DNA, and 1 U of Taq polymerase (Promega Corporation, Madison, USA). PCR conditions were: 30 cycles for 30 s denaturation at 94°C, 30 s annealing at 59°C, and 60 s elongation at 72°C. After cycling, a final extension at 72°C for 10 minutes was performed. The primers used in the PCR reaction were the same as those described by Gustavsson et al.[22] (forward, 5'-CCG CAG CTC CCA ACC ACA ATA AGC T-3', and reverse, 5'-CAA TAG GGC GAG GAG T-3') to produce a 253 bp fragment. After amplification, the PCR products were digested with restriction endonuclease *Hind*III (Life Technologies, Grand Island, NY, USA) at 37°C for 3 hours and then electrophoresed through a 2% agarose gel (Shanghai Yito Enterprise Company Limited, Shanghai, P.R. China) stained by ethidium bromide. The alleles of BGP gene were designated as the absence (*H*) or presence (*h*) of the *Hind*III restriction site.

Statistical analysis

Regular statistical analyses were performed using SAS program (SAS for personal computer 6.12, SAS Institute Inc., Cary, NC, USA). The contribution of the covariates (age, height and weight) in each BMD variable was assessed by employing simple regression analyses in the offspring group. Agreement of genotype frequencies with Hardy-Weinberg (H-W) ratios was tested using the χ^2 goodness-of-fit test in the parent and offspring groups, respectively. H-W ratios are the ratios of genotypes that involve when mating is random and neither selection nor drift are operating in a large population. For two alleles (H and h) with frequencies p and q, the H-W frequencies for the three genotypes (HH, Hh and hh) are p^2, 2pq and q^2, respectively. The frequencies of alleles and genotypes in the population would remain the same from one generation to next. The χ^2 goodness-of-fit test is used to analyze whether the observed genotype frequencies are different from expected genotype frequencies.

Using the QTDT (quantitative transmission disequilibrium test) program (http://www.sph.umich.edu/csg/abecasis/QTDT), we tested linkage and/or association between the BGP *Hind*III polymorphism and BMD. The association test used in QTDT is based on the method described by Abecasis et al.[27], in which association effects are partitioned into orthogonal between-family and within-family components. The between-family component is sensitive to population structure, while the within-family component is free of confound-

Skeletal site	Age	Height	Weight
Spine L_{1-4}	0.016(<0.005)	0.056(<0.005)	0.141(<0.005)
Femoral neck	-0.000(0.385)	0.025(<0.005)	0.162(<0.005)
Trochanter	0.009(0.023)	0.013(0.008)	0.118(<0.005)
Intertrochanteric region	0.007(0.042)	0.002(0.172)	0.167(<0.005)
Ward's triangle	0.032(<0.005)	0.003(0.125)	0.074(<0.005)
Total hip	0.006(0.058)	0.010(0.018)	0.186(<0.005)

Note: Values are R^2 in simple regression analyses. Numbers in parentheses are *p*-values of the corresponding regression co-efficients.

Table 2. Contribution of covariates to BMD variation at each skeletal site in offspring group.

	Chinese (802)	Japanese (160)	Caucasians (261)
Genotype frequencies			
HH	0.073	0.075	0.061
Hh	0.416	0.306	0.318
hh	0.515	0.619	0.621
Allele frequencies			
H	0.282	0.230	0.220
h	0.718	0.770	0.780
p value of H-W test	0.322	0.514	0.411

Note: Hardy-Weinberg ratio was tested with χ^2 test. The data within the parentheses are the number of the study subjects. The data for Chinese, Japanese, and Caucasians are derived from this study, Dohi et al.[20], and Gustavsson et al.[22], respectively.

Table 3. Genotype and allele frequencies of the BGP *Hind*III polymorphism.

	Population stratification	Within-family association	Total association	Linkage
BMD				
Spine (L_{1-4})	1.000	0.806	0.740	1.000
Femoral neck	0.920	0.327	0.566	1.000
Trochanter	0.439	0.888	0.450	0.130
Intertrochanteric region	0.777	0.240	0.185	1.000
Ward's triangle	0.383	0.624	1.000	1.000
Total hip	0.920	0.399	0.281	1.000

Note: the tests were all conducted by employing the program QTDT, the phenotypic values were adjusted for significant covariates of age, height and weight.

Table 4. P values obtained from QTDT analyses of the BGP *Hind*III and BMD.

ing population-substructure effects and is significant only in the presence of linkage disequilibrium (LD). Hence, a simple test of the within-family association would yield a robust test of association, regardless of the composition of nuclear families. When there is no population stratification/admixture, total association that tests both between- and within-family components could be performed. The linkage test implemented in QTDT is based on allele-sharing probabilities and variance component analysis using the identity-by-decent (IBD) relationships among family members[28]. If both linkage and association are detected in separate analysis, the QTDT provides a test of linkage while simultaneously modeling association to test whether the locus is a functional variant or just in LD with the trait locus[29]. To assess the reliability of the within-family association, permutations (1,000 times simulation) were performed.

In all of the statistical analyses, raw BMD values are adjusted by covariates of age, weight, and height. Sex is not used as a covariate because the phenotypes of parents are excluded in QTDT and all the offspring in our sample are daughters. By performing the Kolmogowv-Smirnov test, we found the BMD data in our samples were generally not significantly deviate from normal distribution.

Results

A total of 1,263 individuals from 402 nuclear families comprising 804 parents and 459 offspring were recruited. Of these, 37 subjects (15 fathers, 22 mothers) did not have phenotypes measured. All the children were females aged 31.4±5.8 (mean±SD) years. The basic characteristics of the daughters are summarized in Table 1.

In offspring group, according to the three BGP genotypes, there were no significant differences ($p>0.05$) in age, height and weight, respectively. But these factors generally have effects on BMD variation at most skeletal sites, with weight being the most important predictor, which explains 14.1% of spine BMD and 18.6% of total hip BMD variations (Table 2).

Due to the failure of PCR amplification or Mendelian inheritance inconsistency, the genotypes of six subjects (two mothers and four daughters) were not available. In the parents' group, the frequencies of genotype HH, Hh, and hh are 7.3%, 41.2%, and 51.5%, respectively. The distribution of genotypes in the parents' group is in Hardy-Weinberg equilibrium ($p = 0.322$). It is noteworthy that the frequency of genotypes and alleles in our sample is similar to those of other ethnicities reported previously[20,22,26], suggesting no significant racial difference at this marker locus (Table 3). In our sample, the heterozygosity of the *Hind*III marker is 41.2%. There are 301 informative nuclear families, each of which has at least one heterozygous parent, for TDT analysis.

In QTDT analyses, four nuclear families were excluded due to the deficiency of the genotype of the offspring. Because the effect of founder's phenotypes were excluded in the analyses,

all the remaining 398 families were included in the analyses although there were 37 parents without phenotype data. Using the QTDT, we did not detect significant population stratification in our samples. Neither did we find significant association (including within family association and total association) nor linkage between the BGP *Hind*III polymorphism and BMD variation at the spine and hip (Table 4).

Discussion

BGP is the most abundant non-collagenous protein component of bone and is involved in bone calcification, resorption and remodeling. The *Hind*III marker used here represents a C→T transition in the promotor region of the BGP gene and is of potential functional importance in the regulation of the BGP gene expression[20].

In this study, we tested linkage and/or association between the BGP *Hind*III polymorphism with spine and hip BMD in 402 nuclear Chinese families. We did not detect any significant evidence of linkage or association between this variant and BMD variation. Our study has fairly high statistical power. For instance, when a marker *per se* is a function mutation or in strong LD with a quantitative trait locus (QTL), our study sample has more than 80% power to detect a QTL that can explain 10% of BMD variation. In addition, this power estimation may be conservative in that we assume that each nuclear family has only one child, whereas among our 402 nuclear families, there are 50 families with two daughters and 3 families with more than two children. Therefore, our results suggest that the BGP *Hind*III polymorphism may not have large effects on BMD variation in pre-menopausal Chinese women.

Our results are consistent with some earlier studies in Asian populations. In postmenopausal Japanese women, Dohi et al.[20] did not find a significant association between lumbar spine BMD and the BGP *Hind*III genotypes. Another study carried out in the Japanese, significant association between lumbar spine and hip BMD and the BGP *Hind*III genotypes found only in postmenopausal women but not in pre-menopausal women[24]. A study in postmenopausal Chinese women in Taiwan failed to detect the significant association either[23]. A recent association study performed between lumbar spine and hip BMD and the BGP *Hind*III genotypes in healthy pre-menopausal and postmenopausal Chinese women also got negative results reported[25]. Their data along with ours do not support that the BGP gene is a QTL for BMD variation in Asian pre-menopausal women and even postmenopausal women. So far, significant association between the BGP gene and BMD variation is observed in most Caucasians. Deng et al.[26] showed that the allele h of the BGP *Hind*III variant was associated with higher hip BMD values in 630 Caucasians from 53 pedigrees. Gustavsson et al.[22] found that the presence of the *H* allele was related to lower BMD at the humerus and the femoral neck in Caucasian adolescent females. Testing a microsatellite marker (D1S3737) near to the BGP gene, Raymond et al.[30] and Andrew et al.[31] also detected a significant association between BMD variation and the BGP gene in Caucasians. It is postulated that ethnic or environmental difference may account for the conflicting results of the studies in Asians as well as Caucasians. One possible reason is the big difference in vitamin K intake between Caucasians and Asians[32]. Vitamin K is essential to activate BGP molecules. The lower vitamin K level in the Caucasians than that in the Asians may be a possible reason to produce the difference in the clinical significance of BGP to maintain BMD[33]. Nevertheless, whether the BGP activity is differentially modulated according to BGP *Hind*III polymorphism and vitamin K levels remains to be investigated. Also, to eventually clarify the role of the BGP gene in BMD variation, further molecular genetic and physiological studies are needed.

The potential limitation of the present study should be mentioned here. First, although our study has rather high statistical power to detect a QTL with relatively large effects, however, for QTL with smaller genetic effect, the statistical power decreases considerably. For example, our sample provides only 25% power to detect a QTL with genetic effect as low as 1%. In order to achieve 80% power to detect a QTL with 1% effect on BMD variation, the number of informative nuclear families (each with only one child) should be greater than 700. Second, we only examined one marker in this study, which may have a potential impact on the results. This is because LD generally exists over a short distance in the genome, while the TDT employed here depends crucially on the LD between the marker and the functional variant inside a gene[34]. If a functional variant is not in strong LD with the marker, a genuine association may not be detected. Thus, further studies by examining denser SNP markers (~ 3kb) spanning the BGP gene locus are necessary to derive a definitive conclusion regarding the association of the BGP gene with BMD variation, with more attention paid on SNPs in coding and promoter regions or SNPs that define "haplotype tags"[35]. Third, despite the large number of nuclear families (402) in our sample, the majority of them have only one child. Hence, only 62 sib pairs from 53 nuclear families are informative for the linkage analysis. The power to detect linkage with this sample is modest. Therefore, negative results of linkage here may not necessarily exclude the possibility of a true linkage.

In summary, the present study did not detect any significant association or linkage between the BGP *Hind*III polymorphism and BMD variation at the spine and hip in a Chinese population. Our data suggested that the BGP *Hind*III polymorphism may not have major effects on BMD variation in pre-menopausal Chinese women.

Acknowledgements

The study was partially supported by Hunan Province Special Professor Start-up Fund (25000612), Chinese National Science Foundation (CNSF) Outstanding Young Scientist Award (30025025), CNSF Grant (30170504), a young investigator award from Huo Ying-Dong Education Foundation, and a Seed Fund and a key project fund from the Ministry of Education of P.R. China (25000106). We thank all the study subjects.

References

1. Liu Z, Piao J, Pang L, Qing X, Nan S, Pan Z, Guo Y, Wang X, Li F, Liu J, Cheng X. The diagnostic criteria for primary osteoporosis and the incidence of osteoporosis in China. J Bone Miner Metab 2002; 20:181-189.
2. Cummings SR, Kelsey JL, Nevitt MC, O'Dowd KJ. Epidemiology of osteoporosis and osteoporotic fractures. Epidemiol Rev 1985; 7:178-208.
3. Melton LJ III, Kan SH, Frye MA, Wahner HW, O'Fallon WM, Riggs BL. Epidemiology of vertebral fractures in women. Am J Epidemiol 1989; 129:1000-1011.
4. Suzuki T. Risk factors for osteoporosis in Asia. J Bone Miner Metab 2001; 19:133-141.
5. Dequeker J, Nijs J, Verstraeten A, Geusens P, Gevers G. Genetic determinants of bone mineral content at the spine and radius: a twin study. Bone 1987; 8:207-209.
6. Sowers MR, Boehnke M, Jannausch ML, Crutchfield M, Corton G, Burns TL. Familiality and partitioning the variability of femoral bone mineral density in women of child-bearing age. Calcif Tissue Int 1992; 50:110-114.
7. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G. Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 1995; 10:2017-2022.
8. Deng HW, Stegman MR, Davies KM, Conway T, Recker RR. Genetic determination of variation and covariation of peak bone mass at the hip and spine. J Clin Densitom 1999; 2:251-263.
9. Price PA, Fraser JD, Metz-Virca G. Molecular cloning of matrix Gla protein: implications for substrate recognition by the vitamin K-dependent gamma-carboxylase. Proc Natl Acad Sci USA 1987; 84:8335-8339.
10. Lian JB, Gundberg CM. Osteocalcin. Biochemical considerations and clinical applications. Clin Orthop 1988; 267-291.
11. Yoon KG, Rutledge SJ, Buenaga RF, Rodan GA. Characterization of the rat osteocalcin gene: stimulation of promoter activity by 1,25-dihydroxyvitamin D3. Biochemistry 1988; 27:8521-8526.
12. Kesterson RA, Stanley L, DeMayo F, Finegold M, Pike JW. The human osteocalcin promoter directs bone-specific vitamin D-regulatable gene expression in transgenic mice. Mol Endocrinol 1993; 7:462-467.
13. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G. Increased bone formation in osteocalcin-deficient mice. Nature 1996; 382:448-452.
14. Sugiyama T, Kawai S. Carboxylation of osteocalcin may be related to bone quality: a possible mechanism of bone fracture prevention by vitamin K. J Bone Miner Metab 2001; 19:146-149.
15. Gutierrez S, Liu J, Javed A, Montecino M, Stein GS, Lian JB, Stein JL. The vitamin D response element in the distal osteocalcin promoter contributes to chromatin organization of the proximal regulatory domain. J Biol Chem 2004; 279:43581-43588.
16. Glowacki J, Rey C, Glimcher MJ, Cox KA, Lian J. A role for osteocalcin in osteoclast differentiation. J Cell Biochem 1991; 45:292-302.
17. Chenu C, Colucci S, Grano M, Zigrino P, Barattolo R, Zambonin G, Baldini N, Vergnaud P, Delmas PD, Zallone AZ. Osteocalcin induces chemotaxis, secretion of matrix proteins, and calcium-mediated intracellular signaling in human osteoclast-like cells. J Cell Biol 1994; 127:1149-1158.
18. Ivaska KK, Hentunen TA, Vaaraniemi J, Ylipahkala H, Pettersson K, Vaananen HK. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption *in vitro*. J Biol Chem 2004; 279:18361-18369.
19. Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P, Curran ME, Rodriguez LA, Conneally PM, Joslyn G, Peacock M, Johnston CC, Foroud T. Genome screen for QTLs contributing to normal variation in bone mineral density and osteoporosis. J Clin Endocrinol Metab 2000; 85:3116-3120.
20. Dohi Y, Iki M, Ohgushi H, Gojo S, Tabata S, Kajita E, Nishino H, Yonemasu K. A novel polymorphism in the promoter region for the human osteocalcin gene: the possibility of a correlation with bone mineral density in postmenopausal Japanese women. J Bone Miner Res 1998; 13:1633-1639.
21. Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M. Genetic markers, bone mineral density, and serum osteocalcin levels. J Bone Miner Res 1999; 14:1411-1419.
22. Gustavsson A, Nordstrom P, Lorentzon R, Lerner UH, Lorentzon M. Osteocalcin gene polymorphism is related to bone density in healthy adolescent females. Osteoporos Int 2000; 11:847-851.
23. Chen HY, Tsai HD, Chen WC, Wu JY, Tsai FJ, Tsai CH. Relation of polymorphism in the promotor region for the human osteocalcin gene to bone mineral density and occurrence of osteoporosis in postmenopausal Chinese women in Taiwan. J Clin Lab Anal 2001; 15:251-255.
24. Yamada Y, Ando F, Niino N, Shimokata H. Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 2003; 88:3372-3378.
25. Mo XY, Cao CK, Xu FH, Liu MY, Li MX, Qin YJ, Zhou Q, Zhang YY, Deng HW. Lack of association between the *Hind*III RFLP of the osteocalcin (BGP) gene and bone mineral density (BMD) in healthy pre- and postmenopausal Chinese women. J Bone Miner Metab 2004; 22:264-269.
26. Deng HW, Shen H, Xu FH, Deng HY, Conway T, Zhang HT, Recker RR. Tests of linkage and/or associ-

ation of genes for vitamin D receptor, osteocalcin, and parathyroid hormone with bone mineral density. J Bone Miner Res 2002; 17:678-686.
27. Abecasis GR, Cardon LR, Cookson WO. A general test of association for quantitative traits in nuclear families. Am J Hum Genet 2000; 66:279-292.
28. Pratt SC, Daly MJ, Kruglyak L. Exact multipoint quantitative-trait linkage analysis in pedigrees by variance components. Am J Hum Genet 2000; 66:1153-1157.
29. Fulker DW, Cherny SS, Sham PC, Hewitt JK. Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet 1999; 64:259-267.
30. Raymond MH, Schutte BC, Torner JC, Burns TL, Willing MC. Osteocalcin: genetic and physical mapping of the human gene BGLAP and its potential role in postmenopausal osteoporosis. Genomics 1999; 60:210-217.
31. Andrew T, Mak YT, Reed P, MacGregor AJ, Spector TD. Linkage and association for bone mineral density and heel ultrasound measurements with a simple tandem repeat polymorphism near the osteocalcin gene in female dizygotic twins. Osteoporos Int 2002; 13:745-754.
32. Kaneki M, Hedges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ, Ishida N, Nakagawa M, Takechi M, Sano Y, Mizuno Y, Hoshino S, Miyao M, Inoue S, Horiki K, Shiraki M, Ouchi Y, Orimo H. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition 2001; 17:315-321.
33. Binkley NC, Suttie JW. Vitamin K nutrition and osteoporosis. J Nutr 1995; 125:1812-1821.
34. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993; 52:506-516.
35. Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA. Haplotype tagging for the identification of common disease genes. Nat Genet 2001; 29:233-237.