Original Article

Genetic and Environmental Factors Affecting Peak Bone Mass in Premenopausal Japanese Women

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Abstract

The purpose of this study was to examine the relationships between peak bone mass and genetic and environmental factors. We measured whole-body bone mineral density (BMD), lumbar spine BMD, and radius BMD with dual-energy X-ray absorptiometry (DXA) and analyzed eight genetic factors: vitamin D receptor (VDR)-3', VDR-5', estrogen receptor (ER), calcitonin receptor (CTR), parathyroid hormone (PTH), osteocalcin (OC), apolipoprotein E (ApoE), and fatty acid binding protein 2 (FABP2) allelic polymorphisms using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs). We also surveyed menstrual history, food intake, and history of physical activity using questionnaires.

After adjusting for age, body mass index (BMI), current smoking status, current Ca intake, alcohol intake, menoxenia, and physical activity, the mean BMD in subjects with the HH/Hh genotype was significantly higher than that of subjects with the hh genotype for whole-body BMD (mean±SD, 1.20±0.10 vs. 1.18±0.09 g/cm^2; HH/Hh vs. hh, p=0.04) and at lumbar spine BMD (mean±SD, 1.18±0.14 vs. 1.14±0.12 g/cm^2; HH/Hh vs. hh, p=0.02) in OC allelic polymorphism. Furthermore, the results of multiple regression analyses taking the 8 genetic factors plus the 7 environmental factors listed above into account showed that the strongest factor contributing to BMD was BMI at any site (whole-body and lumbar BMD p<0.0001, radius BMD p=0.0029). In addition, OC polymorphism (p=0.0099), physical activity (p=0.0245), menoxenia (p=0.0384), and PTH polymorphism (p=0.0425) were independent determinants for whole-body BMD, and OC polymorphism (p=0.0137) and physical activity (p=0.0421) were independent determinants for lumbar BMD and radius BMD, respectively.

Key words: body mass index, menoxenia, osteocalcin gene, peak bone mass, physical activity, polymorphism

Introduction

Osteoporosis is a major public health problem in developed countries at present. The complication of osteoporosis, vertebral compression fractures, and fractures of the femoral neck, affects people's quality of life and increases the cost of health care. A major means of lowering the risk for osteoporosis is to minimize the decline in bone mass in menopausal women, but it is also important to maximize the peak bone mass in early adulthood from the viewpoint of preventive medicine[1].

Peak bone mass is considered to be determined by both genetic and environmental factors. Body mass index (BMI), calcium intake, physical activity, alcohol intake, and smoking have been revealed as environmental factors that influence peak bone mass[1]. Many candidate genes that affect bone mineral density (BMD) have been demonstrated, including vitamin D receptor (VDR)-3'[2], VDR-5'[3], estrogen receptor (ER)[4], calcitonin receptor (CTR)[5], parathyroid hormone (PTH)[6], osteocalcin (OC)[7], apolipoprotein E (ApoE)[8], insulin-like growth factor 1[9], transforming growth factor-β 1[10], collagen type-I α1[11], interleukin-6[12], human leukocyte antigen[13], and peroxisome proliferator-activated receptor γ[14]; however, study results are controversial, and the importance of each genetic factor in the prevention of osteoporosis remains unknown.

The main purpose of this study was to clarify the interaction between genetic and environmental factors affecting peak bone mass. We analyzed the relationship between VDR-3', VDR-5', ER, CTR, PTH, OC, ApoE, and fatty acid binding protein 2 (FABP2) allelic polymorphisms and BMD in the study of premenopausal Japanese women. We also examined the correlation between the BMD and environmental factors, including dietary intake and physical activity.

Materials and Methods

Subjects

One hundred and forty healthy premenopausal women were unrelated volunteers recruited by advertisements in a community newspaper, in Ibaraki Prefecture, Japan. Characteristics of the subjects are shown in Table 1. This protocol was approved by the ethics committee, University of Tsukuba, and written informed consent regarding all the study procedures was obtained from each subject in advance. This report is a part of a 5-year follow-up study of the obtained findings.

Bone mass measurement

The BMD (g/cm^2) was measured in the whole-body, the lumbar spine (L2–L4), and the radius (left hand, one-third distal) by dual-energy X-ray absorptiometry (DXA) using a DCS-3000 (Aloka Co., Tokyo, Japan). The coefficients of variation for whole-body and lumbar BMD measurements in our situation were 0.74% and 0.81%, respectively.

Questionnaires

Participants completed questionnaires that included their medical history, reproductive history, menstrual history (current menstruation and menoxenia), medication use, current smoking habits, alcohol intake (amount per day and number of drinks in one week), current and past food intake, and history of physical activity. We defined menoxenia as having an irregular menstrual cycle, hypermenorrhea, hypomenorrhea, dysmenorrhea, or premenstrual syndrome (PMS) in this study. Subjects reported hours per week spent engaged in physical activities in junior high school, senior high school, and at present. Physical education classes in school were excluded from physical activities. The physical activity rating was defined as positive when the subjects participated in more than an hour of activity in one week. The intake nutrients were estimated by 3-day diet reports, and total calorie intake and an average Ca intake were analyzed with NUT ver 3.0 software (Human Science Laboratory, Shiga, Japan).

DNA extraction and genotyping

Genomic DNA was extracted from the peripheral blood leukocytes of 140 women using the phenol methods. Genotypes of the VDR (BsmI)[15], VDR (FokI)[3], ER (PvuII)[16], CTR (AluI)[17], PTH (BstBI)[6], OC (HindIII)[7], ApoE (HhaI)[18], and FABP2 (HhaI)[19] were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs), as previously described.

Statistical analyses

Differences in BMD among two and three groups in each genotype were tested using Student's non-paired t test and analysis of variance (ANOVA), respectively. Further comparisons of the BMD adjusted for covariates were performed by analysis of covariance (ANCOVA). The covariates were 7 environmental factors: age (years), BMI (kg/m^2), current smoking status, current Ca intake, alcohol intake, menoxenia, and physical activities as reported previously[1,20]. To examine the effect of genetic and environmental factors on BMD at different sites, we used multiple regression analysis. Variables entered in the model were 8 genetic factors plus the 7 environmental factors listed above. Current smoking status and menoxenia were rated as yes (=1) or no (=0). Alcohol intake was categorized as times of drinking in one week: 7 times (=4), 5–6 times (=3), 3–4 times (=2), 1–2 times (=1), less than once (=0). Physical activity was categorized as the sum of the positive periods among junior high school, senior high school, and the present: 3 periods (=3), 2 periods (=2), 1 period (=1), no period (=0). All analyses were accomplished using Statview 5.0 (Abacus Concepts, Berkeley, CA, USA) and SPSS 6.1J (SPSS, Chicago, Illinois, USA).

Results

Characteristics

Table 1 shows the characteristics and lifestyles of all study subjects. The participants in this study had not used oral contraceptives and were not using them at the time of this study and were not currently pregnant, and they did not have a systemic disease or medication that would affect bone and mineral metabolism. The mean age of subjects was 32.0±7.1 years (range 16–44). Height, weight, and BMI in the subjects were similar to means for the genetic population of Japanese women (20–39 years) reported by the Ministry of Health and Welfare[21]. Although 47.9% of subjects drank alcohol, none was defined as a heavy drinker, that is, drinking a mean amount greater than 150 ml ethanol per day. Eight subjects (5.7%) drank 7 times per week, 3 (2.1%) drank 5–6 times, 11 (7.9%) drank 3–4 times, 45 (32.1%) drank 1–2 times, and 73 (47.9%) drank less than once a week. The percentage of current smokers among subjects was 12.9%. The percentage of subjects experiencing menoxenia was 40.7%. None of our subjects had primary amenorrhoea, defined as the absence of menstruation upon reaching the age of 18 years or older. One subject had secondary amenorrhoea (absence of menstruation during the previous 6 months). As she recovered regular menstruation in the follow-up study of the next year, we did not exclude this subject from this study. Fifty-two (37.1%) subjects had no delivery history, 55 (39.3%) and 33 (23.6%) had delivered 1–2 times and 3–4 times, respectively. There was no significant association between times of delivery and BMD at any site. With regard to activity level, 46.4% of the women were presently participating in a regular physical activity, and those who participated in junior high school and senior high school were 70.7% and 47.1%, respectively. Mean calorie intake was 7,546.6±1,629.8 J/day (1,805.4±389.9 kcal/day), and Ca intake was 15.7±7.2 mmol/day (628.9±290.3 mg/day). The participant's occupations were public servants (20.7%), housewives (17.1%), office workers (14.3%), public health nurses (10.7%), senior high school or college students (9.3%), nurses (8.6%), teachers (8.6%), and various others.

Table 1 Characteristics of subjects

Age (y)	32.0±7.1
Height (cm)	157.9±5.4
Body weight (kg)	52.5±7.4
BMI (kg/m^2)	21.0±2.6
% Menoxenia	40.7
% Current smokers	12.9
% Current drinkers	47.9
% Regular physical activity at present	46.4
Energy intake (J/day)[a]	7,546.6±1,629.8
Calcium intake (mmol/day)[b]	15.7±7.2

Values are means±SD.
[a] 1 kcal=4.18 kJ.
[b] 1 mg/24 h=0.02495 mmol/day.

Bone Mineral Density

Lumbar spine mineral density was 1.15±0.13 (mean±SD) g/cm², with a minimum of 0.72 g/cm² and a maximum of 1.50 g/cm². Radius mineral density was 0.48±0.06 g/cm², and whole-body mineral density was 1.19±0.09 g/cm².

Lumbar spine BMD was 1.12±0.07 (mean±SD) g/cm² in 16- to 19-years-old subjects, that of participants in their twenties was 1.13±0.11 g/cm², and that of participants in their thirties and forties (40–44 years old) was 1.16±0.15 g/cm² and 1.16±0.09 g/cm², respectively. There was no difference among age groups in the mean lumbar spine BMD, with similar trends seen in whole-body and radius BMD.

Genetic markers and BMD

Genotype frequencies and means of BMD for each RFLP are shown in Tables 2–5. There was no evidence of deviation from Hardy-Weinberg equilibrium for any of the eight polymorphisms in the chi-square test. The χ^2 values (p-value) were 3.26 (p=0.20) in VDR *Bsm*I polymorphism, 0.31 (p=0.86) in VDR *Fok*I polymorphism, 0.70 (p=0.71) in ER polymorphism, 0.02 (p=0.99) in CTR polymorphism, 0.36 (p=0.84) in PTH polymorphism, 0.13 (p=0.94)

in OC polymorphism, 0.06 (p=1.00) ApoE polymorphism, and 0.57 (p=0.75) in FABP2 polymorphism, respectively.

Table 2 shows the genotype frequencies and BMD for the VDR intron-8 (*Bsm*I site) and start codon (*Fok*I site). The prevalence of *Bsm*I VDR was similar to that in previous studies, whereas the prevalence of *Fok*I VDR was slightly different from that in a previous Japanese study[22]. In these polymorphisms, there was no association with BMD. After adjustment for covariates, there were no significant differences, either.

The genotype frequencies and BMD for the ER (*Pvu*II) and CTR (*Alu*I) are presented in Table 3. The prevalence of ER and also CTR was similar to the findings reported previously. Using crude BMD and adjusted BMD at any site, no significant association was found in ER or CTR polymorphisms.

Table 4 illustrates the genotype frequencies and BMD for the PTH (*Bst*BI) and OC (*Hin*dIII). These genotype frequencies did not differ from those of the Japanese observed previously. Regarding OC polymorphisms, there were genotype-related differences in BMD at all sites; however, the BMD tended to be higher in the HH genotype than in the Hh and hh genotype. Furthermore, comparing the adjusted BMD in HH/Hh with hh, the mean BMD

Table 2 VDR (*Bsm*I and *Fok*I) polymorphism and BMD

Characteristics	Genotype			p	p*	p**
	BB	Bb	bb			
Number (%)	6 (4.3)	23 (16.4)	111 (79.3)			
BMD (g/cm²)						
Whole-body	1.23±0.08	1.18±0.07	1.19±0.10	0.89	0.93	0.52
Lumbar spine	1.18±0.11	1.15±0.11	1.15±0.14	0.68	0.78	0.40
Radius	0.46±0.06	0.48±0.05	0.48±0.06	0.89	0.98	0.73
	FF	Ff	ff			
Number (%)	63 (45.0)	65 (46.4)	12 (8.6)			
BMD (g/cm²)						
Whole-body	1.18±0.11	1.19±0.08	1.21±0.07	0.51	0.65	0.65
Lumbar spine	1.16±0.15	1.14±0.11	1.17±0.10	0.66	0.84	0.61
Radius	0.47±0.06	0.49±0.05	0.47±0.05	0.84	0.81	0.95

Values are means±S.D.

p values are shown BB+Bb vs. bb and FF+Ff vs. ff.

* Adjusted for age (years), BMI (body mass index, kg/m²), current Ca intake, current smoking, alcohol intake, menoxenia, physical activity.

** Adjusted for the 7 environmental factors given above and the genotypes of the other 7 genes.

Table 3 ER (*Pvu*II) and CTR (*Alu*I) polymorphisms and BMD

Characteristics	Genotype			p	p*	p**
	PP	Pp	pp			
Number (%)	16 (11.4)	72 (51.4)	52 (37.1)			
BMD (g/cm²)						
Whole-body	1.20±0.08	1.18±0.09	1.19±0.11	0.58	0.55	0.59
Lumbar spine	1.19±0.12	1.14±0.12	1.15±0.14	0.21	0.21	0.26
Radius	0.49±0.07	0.48±0.05	0.48±0.05	0.51	0.70	0.86
	CC	CT/TT				
Number (%)	113 (80.7)	27 (19.3)				
BMD (g/cm²)						
Whole-body	1.19±0.10	1.18±0.08		0.52	0.79	0.53
Lumbar spine	1.16±0.13	1.12±0.12		0.17	0.27	0.15
Radius	0.48±0.06	0.47±0.05		0.33	0.59	0.49

Values are means±S.D.

p values are shown PP vs. Pp+pp and CC vs. CT+TT.

* Adjusted for age (years), BMI (body mass index, kg/m²), current Ca intake, current smoking, alcohol intake, menoxenia, physical activity.

** Adjusted for the 7 environmental factors given above and the genotypes of the other 7 genes.

Table 4 PTH (*Bst*BI) and OC (*Hin*dIII) polymorphisms and BMD

Characteristics	Genotype			p	p*	p**
	BB	Bb/bb				
Number (%)	114 (81.4)	26 (18.6)				
BMD (g/cm²)						
Whole-body	1.18±0.10	1.21±0.07		0.17	0.08	0.04
Lumbar spine	1.15±0.14	1.15±0.11		0.89	0.79	0.41
Radius	0.48±0.06	0.48±0.05		0.95	0.85	0.86
	HH	Hh	hh			
Number (%)	5 (3.6)	40 (28.6)	95 (67.9)			
BMD (g/cm²)						
Whole-body	1.24±0.07	1.20±0.10	1.18±0.09	0.32	0.04	0.02
Lumbar spine	1.18±0.15	1.18±0.15	1.14±0.12	0.06	0.02	0.01
Radius	0.51±0.04	0.48±0.07	0.47±0.05	0.25	0.17	0.13

Values are means±S.D.

p values are shown BB vs. Bb+bb and HH+Hh vs. hh.

* Adjusted for age (years), BMI (body mass index, kg/m²), current Ca intake, current smoking, alcohol intake, menoxenia, physical activity.

** Adjusted for the 7 environmental factors given above and the genotypes of the other 7 genes.

in HH/Hh was significantly higher than that of the hh genotype at whole-body BMD (mean±SD, 1.20±0.10 vs. 1.18±0.09 g/cm²; HH/Hh vs. hh, p=0.04) and at lumbar spine BMD (mean±SD, 1.18±0.14 vs. 1.14±0.12 g/cm²; HH/Hh vs. hh, p=0.02).

The relationships between ApoE and FABP2 RFLPs and BMD are illustrated in Table 5. The ApoE allele distribution in subjects was similar to that seen in subjects in a previous Japanese study. The BMD at the radius was modestly higher at E3/4+E4/4 than at E3/3 or E2/3; however, the differences were not significant. In addition, the unadjusted and adjusted BMD did not significantly differ among FABP2 polymorphism at any sites.

Determinants for BMD

We used multiple regression analysis to assess the association of genetic and environmental factors with BMD at each site (Table 6). The results showed the most important and independent contributions of BMI at all sites (whole-body and lumbar BMD p<0.0001, radius BMD p=0.0029). Next, OC polymorphism (p=0.0099), physical activity (p=0.0245), menoxenia (p=0.0384), and PTH polymorphism (p=0.0425) were significant related to

whole-body BMD, and OC polymorphism (p=0.0137) was significantly correlated with lumbar BMD. Physical activity (p=0.0421) was the only independent factor to affect radius BMD, except for BMI.

Discussion

In this study, we analyzed the VDR (*Bsm*I, *Fok*I), ER, CTR, PTH genotypes as polymorphisms of hormones or hormone receptors, and OC (bone Gla protein) as a metabolite of bone formation. In addition, ApoE and FABP2 were included as polymorphisms relating to lipid metabolism. Vitamin K1 promotes the generation of Gla (γ-carboxyglutamate residues in osteocalcin) in osteoblasts[23]. Although little is known about vitamin K metabolism, vitamin K is fat-soluble and is absorbed in the intestine. In one study, the FABP2 gene was expressed at epithelial cells in the small intestine, and on Ala54Thr substitution for this gene was reported to affect the lipid absorption rate[19]. ApoE plays an important role in the receptor-mediated clearance of lipoprotein particles from plasma, and a relationship between apoE polymor-

Table 5 ApoE (*Hha*I) and FABP2 (*Hha*I) polymorphisms and BMD

Characteristics	Genotype			p	p*	p**
	E2/3	E3/3	E3/4, E4/4			
Number (%)	10 (7.1)	109 (77.9)	21 (15.0)			
BMD (g/cm²)						
Whole-body	1.18±0.10	1.19±0.09	1.20±0.12	0.78	0.91	0.95
Lumbar spine	1.15±0.16	1.15±0.13	1.16±0.13	0.93	1.00	0.87
Radius	0.45±0.06	0.48±0.06	0.49±0.05	0.13	0.15	0.13
	Ala/Ala	Ala/Thr	Thr/Thr			
Number (%)	67 (47.9)	56 (40.0)	17 (12.1)			
BMD (g/cm²)						
Whole-body	1.20±0.10	1.18±0.10	1.19±0.08	0.30	0.79	0.24
Lumbar spine	1.17±0.13	1.13±0.14	1.15±0.11	0.08	0.12	0.07
Radius	0.48±0.05	0.48±0.06	0.46±0.06	0.30	0.62	0.48

Values are means±S.D.

p values are shown E2/3 vs. E3/3 vs. E3/4+E4/4 and Ala/Aa vs. Ala/Thr+Thr/Thr.

* Adjusted for age (years), BMI (body mass index, kg/m²), current Ca intake, current smoking, alcohol intake, menoxenia, physical activity.

** Adjusted for the 7 environmental factors given above and the genotypes of the other 7 genes.

Table 6 Multiple-regression analysis for factors of bone mineral density

Factors	Standardized regression coefficient	p	Standardized regression coefficient	p	Standardized regression coefficient	p
	Whole-body BMD (R=0.636)		Lumbar BMD (R=0.506)		Radius BMD (R=0.444)	
VDR (*Bsm*I)[a]	0.084	NS	0.081	NS	-0.008	NS
VDR (*Fok*I)[b]	0.021	NS	0.076	NS	-0.068	NS
ER[c]	0.004	NS	0.068	NS	0.028	NS
CTR[d]	-0.058	NS	-0.129	NS	-0.075	NS
PTH[e]	0.154	0.0425	0.086	NS	-0.038	NS
Osteocalcin[f]	0.191	0.0099	0.204	0.0137	0.143	0.0946
ApoE[g]	0.009	NS	-0.013	NS	0.169	0.0495
FABP2[h]	0.025	NS	0.104	NS	0.091	NS
Age	-0.120	NS	-0.066	NS	0.011	NS
BMI	0.559	<0.0001	0.396	<0.0001	0.255	0.0029
Ca intake	0.021	NS	-0.004	NS	0.026	NS
Smoking	-0.012	NS	0.062	NS	-0.050	NS
Alcohol intake[i]	0.126	NS	0.063	NS	0.097	NS
Menoxenia	-0.155	0.0384	-0.144	0.0859	-0.134	NS
Physical activity[j]	0.172	0.0245	0.131	NS	0.180	0.0421

Variables entered in the model were 8 genetic factors, age (years), BMI (body mass index, kg/m^2), current Ca intake, current smoking, alcohol intake, menoxenia, and physical activity.

[a] Vitamin D receptor (*Bsm*I) polymorphism was rated as 2 (BB genotype) or 1 (Bb genotype) or 0 (bb genotype).

[b] Vitamin D receptor (*Fok*I) polymorphism was rated as 2 (FF genotype) or 1 (Ff genotype) or 0 (ff genotype).

[c] Estrogen receptor polymorphism was rated as 2 (PP genotype) or 1 (Pp genotype) or 0 (pp genotype).

[d] Calcitonin receptor polymorphism was rated as 2 (TT genotype) or 1 (CT genotype) or 0 (CC genotype).

[e] Parathyroid hormone polymorphism was rated as 2 (TT genotype) or 1 (Tt genotype) or 0 (tt genotype).

[f] Osteocalcin polymorphism was rated as 2 (HH genotype) or 1 (Hh genotype) or 0 (hh genotype).

[g] Apolipoprotein E polymorphism was rated as 2 (E2/3) or 3 (E3/3) or 4 (E2/4 and E3/4).

[h] Fatty acid binding protein 2 polymorphism was rated as 2 (AA genotype) or 1 (AT genotype) or 0 (TT genotype).

[i] Alcohol intake was rated for the times of drinking in one week: 7 times (=4), 5–6 times (=3), 3–4 times (=2), 1–2 times (=1), less than once (=0).

[j] Physical activity was rated for the sum of activities in 3 periods: 3 periods (=3), 2 periods (=2), 1 period (=1), no period (=0).

phism and BMD was reported by Shiraki et al.[8]. For these reasons, we added the two polymorphisms to the candidate genes.

Multiple regression analysis showed BMI contributed significantly to BMD at all sites (Table 6). Furthermore, OC polymorphism, physical activity, menoxenia, and PTH polymorphism were independent factors in whole-body BMD. OC polymorphism and physical activity as well as BMI were observed as significant factors influencing BMD in lumbar and radius BMD, respectively. In addition, the present findings suggest that a genetic contribution of lumbar BMD was strong compared with that of the other sites[24].

We showed that physical activity was a significant factor in affecting whole-body and radius BMD. We categorized the physical activity as the sum of the positive periods among 3 periods (junior high school, senior high school, and the present) for the analysis in this study. We reported previously a study that focused on the relation between physical activity and BMD among some of the present subjects[25], and the results suggested that physical activity in adolescence and the continuation of physical activity in adulthood were both important determinants for BMD in young adults. After taking genetic factors into account in this study, we found that physical activity was an independent factor in peak bone mass, suggesting that the intervention in physical activity was useful to protect people from osteoporosis even in subjects with genetic susceptibility.

Menoxenia occurred in 40.7% of the present subjects, and the percentage was similar to that of Japanese females, 45.9% (25–34 years), reported by the Ministry of Health and Welfare[21]. Since menopause is well known as the factor most clearly related to BMD[1], only one previous study has examined the association between menoxenia and low bone mass in young women[20]. The

present study also showed that menoxenia influenced whole-body BMD in healthy premenopausal women. The loss of bone mass was correlated with estrogen insufficiency in peri- and postmenopausal periods, and it has been demonstrated that the decrease in BMD can be prevented by hormone replacement therapy. As oral contraceptives had not been approved by June, 1999 in Japan, none of our subjects had used them or were using them at the time of this study, so it was very interesting that the association of menoxenia with BMD was illustrated in our subjects without oral contraceptive use.

Whole-body BMD and lumbar spine BMD were associated with the OC polymorphism after adjustment for some covariates (Table 4). OC is one of the major noncollagenous proteins; it is synthesized exclusively by osteoblasts[26]. Although the functional effect of the OC polymorphism was not clear, this OC polymorphism is a C to T transition at position -198 nucleotides in the promoter region. Since Dohi et al. also reported the relation between OC polymorphism and BMD[7], it was likely that OC polymorphism is associated with peak bone mass in Japanese females.

The allele frequency of VDR (*Fok*I) polymorphism (F allele frequency 0.68, f allele frequency 0.32) was slightly different from that (F allele frequency 0.59, f allele frequency 0.41) reported by Arai et al.[21]; however, allele frequencies of other genes were similar to those previously reported. In addtion, Arai et al. demonstrated that BMD with the FF genotype was significantly greater than that with either the Ff or ff genotypes, we did not detect a significant association among genotypes. The *Fok*I polymorphism site is in a start codon (ATG) which changes to ACG, so alleles with ACG would give rise to VDR proteins that shorten in length by three amino acids. Two functional analyses of this *Fok*I site

were reported positively by Arai et al.[22] and negatively by Gross et al.[27], showing that this subject is controversial and further studies are needed.

Since VDR (*Bsm*I) was the first genetic variation shown to be associated with BMD by Morrison et al.[15], the association has continued to be controversial. Most positive results were reported for perimenopausal or postmenopausal women. Although there have been a few studies on premenopausal women, the subject has not yet been fully discussed. In the present study we showed that VDR (*Bsm*I) polymorphism was not an independent factor in BMD. On the other hand, we previously reported that VDR (*Bsm*I) was a strong genetic factor in BMD in postmenopausal Japanese women[28]. It is suggested that these different findings occurred because, first, there is a great difference in the nutritional condition between childhood and young adulthood in postmenopausal women faced with osteoporosis and the young females in the present study. The traditional high carbohydrate diet in Japan with low lipids and proteins has changed, and more recently the diet is similar to a Western diet. Genetic factors such as VDR (*Bsm*I) polymorphism may have bigger effects in malnutrition. A second reason for the different finding is that genetic factors may particularly affect BMD during and after menopause.

The findings of the present study suggest that even in developed countries where the population has a modern diet, peak bone mass in females is affected by environmental factors as well as genetic factors. The present findings imply that peak bone mass is multi-factorial, which means that appropriate intervention in lifestyle could improve BMD. Since the regression coefficients were not so high, there was the possibility of other factors, which were associated with the BMD. For the differences in the bone components, the regression coefficient at radius BMD may be found to be smaller than those of other sites. As this was a cross-sectional study, there were many limitations to analyzing the effects of genetic and environmental factors on BMD. We are currently engaged in a 5-year follow-up study to investigate the relation of genetic and environmental factors prospectively.

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