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Relation of Bone Mineral Density to *Vitamin D Receptor* Gene Polymorphism and Lifestyle Factors in Japanese Female Workers Aged 22–44 Years: A Cross-Sectional Study

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Summary Bone mineral density (BMD) reflects both genetic and lifestyle factors. The aim of this cross-sectional study was to investigate the influence of *vitamin D receptor* (*VDR*) gene polymorphism and lifestyle factors on BMD in premenopausal female workers. The subjects were 162 premenopausal female employees aged 22–44 y who worked at a large-scale integrated manufacturing facility in Japan. BMD was measured at the nondominant radius by dual energy X-ray absorptiometry. Lifestyle information was obtained by a questionnaire at the same time and genomic DNA was isolated from peripheral leukocytes. BMD was positively correlated with age, weight, and body mass index (BMI). The genotype frequencies of *VDR* gene polymorphism detected by *Taq*I analysis were 77.2, 22.8, and 0.0% for *TT*, *Tt*, and *tt*, respectively. Analysis of covariance with adjustment for age and BMI showed that the mean BMD was significantly lower in subjects with the *Tt* genotype than in those with the *TT* genotype. Subjects who drank alcohol also showed a significantly lower BMD if they had the *Tt* genotype than if they had the *TT* genotype. According to multiple linear regression analysis, the independent determinants of BMD were age, BMI, and *VDR* gene polymorphism. Our data show that BMD is negatively correlated with the *Tt* genotype of the *VDR* gene, but positively correlated with age and BMI. These findings suggest that analysis of *VDR* gene polymorphism may be useful for identifying individuals who are susceptible to osteoporosis so that early preventive measures can be provided.

Key Words bone mineral density, gene polymorphism, lifestyle factors, vitamin D receptor

In Japan, prevention of osteoporosis is a high-priority issue because osteoporotic fracture is one of the primary causes of elderly persons becoming bedridden (*1*). Measurement of bone mineral density (BMD) is the best tool available to assess osteoporotic fracture risk, since low BMD is an important risk factor for osteoporotic fracture (*2*). The peak BMD of Japanese women is reached around 35–40 y of age, and it falls rapidly during the first 10 y after menopause and then decreases gradually (*3*). Because a decrease of BMD is inevitable after menopause, it is necessary to identify and manage any premenopausal decrease of BMD. BMD can be influenced by various lifestyle factors, such as smoking, alcohol intake, exercise, and nutrition (*4*). Among the genes that may be associated with the development of osteoporosis, polymorphism of the *vitamin D receptor* (*VDR*) gene has been most widely studied (*5*), and a meta-analysis (*6*) suggested that BMD is influenced by *VDR* gene polymorphism. BMD is affected by both genetic and lifestyle factors (*5*), but most studies have separately investigated the effects of *VDR* gene polymorphism or lifestyle factors on BMD. Better understanding of this combined effect may allow us to identify a high-risk group for osteoporosis and lead to more effective osteoporosis prevention and to improvement of public health. A few previous studies (*7–10*) have examined the relation of BMD to *VDR* gene polymorphism and lifestyle factors in premenopausal women, but the subjects were Caucasian women (*7*, *8*), were beyond the cut-off age for young adults (20–44 y) (*8*, *9*) set by the Japan Osteoporosis Society (*4*), or were in their early 20 s (*7*). In other studies, the results obtained were inconsistent (*9*, *10*). Therefore, the aim of the present cross-sectional study was to identify *VDR* gene polymorphism and lifestyle factors associated with BMD in Japanese female workers aged 22–44 y.

METHODS

Subjects. The subjects were 162 premenopausal female employees working at a large-scale integrated manufacturing facility in Japan. The menstrual history was recorded at an individual interview and premenopausal status was defined on the basis of regular menstruation. The criteria for entry into this study were no prior diagnosis of osteoporosis, no systemic diseases, and no medications known to influence bone or calcium metabolism. The age of the women ranged from 22 to 44 y (mean: 30.7±5.3 y). Height, weight, BMD, urinary deoxypyridinoline (DPD), and serum bone alka-

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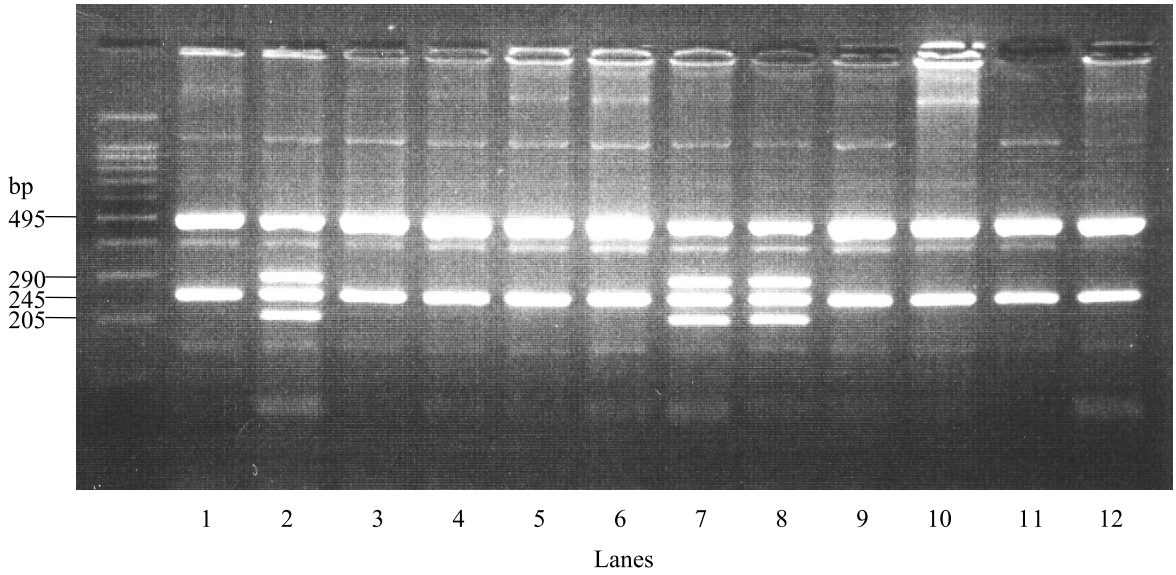

Fig. 1. PCR product patterns obtained for two possible genotypes after *Taq*I digestion of the 740 bp amplified region of the *vitamin D receptor* gene. Lanes 1, 3–6, 9–12: *TT* genotype, Lanes 2, 7, 8: *Tt* genotype.

line phosphatase (BAP) were measured during a comprehensive health check. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). Lifestyle assessment was performed at the same time as the above measurements were obtained. We followed the ethical guideline for human genome/genetic research endorsed by the Japanese government. The study protocol was approved by our university ethics committee, and all subjects provided written informed consent.

Bone metabolic markers. Serum BAP and urinary DPD concentrations were analyzed by enzyme immunoassay. The urinary creatinine (Cr) concentration was measured by a colorimetric assay. DPD excretion was expressed as a ratio of the urinary Cr concentration. DPD has been validated as a useful marker of bone resorption, while BAP is a marker of bone formation (*11*).

Measurement of bone mineral density (BMD). BMD was measured at the distal 1/3 site of the radius on the non-dominant side using dual energy X-ray absorptiometry (Osteometer DTX200) according to the manufacturer's protocol (precision error $<1.0\%$ CV in vivo). Quality control was carried out in accordance with the manufacturer's guidance.

Lifestyle assessment. The lifestyle of the subjects was investigated by using a self-reporting questionnaire, and the following information was obtained: past history of exercise (no or yes), current exercise (no or yes), daily milk intake (no or yes), number of meals per day (3 or 2), history of dieting (no or yes), smoking status (non-smoker or smoker), and alcohol intake (non-drinker or drinker). A past history of exercise was determined by reviewing exercise habits until the age of 20 y, while current exercise was defined as the performance of exercise at least once per week.

Genotyping. Genomic DNA was extracted from peripheral blood leukocytes by using a DNA Extractor WB Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). *Taq*I polymorphism of the *VDR* gene was determined by the polymerase chain reaction (PCR) method of Riggs et al. (*12*). A 740 base pair (bp) fragment was generated by PCR with primers located on intron 8 and exon 9. The primer sequences were 5′ cag agc atg gac agg gag caa 3′ (forward) and 5′ gca act cct cat ggc tga ggt ctc 3′ (reverse). PCR was performed for 35 cycles using *Taq* polymerase (Perkin Elmer Co., Ltd, New Jersey, USA) under the following conditions: denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. Then 10 μL of the PCR products were subjected to digestion with *Taq*I (Takara Shuzo Co., Ltd., Kyoto, Japan) at 65°C for 3 h and were separated on 3% Nusieve agarose gel (FMC Bioproducts, Rockland, ME, USA) (*13*). The presence of a C>T substitution at position 3 on codon 352 in exon 9, which codes for isoleucine, is associated with loss of the *Taq*I restriction site. The resulting alleles are designated as *T* (*Taq*I site absent; 2 fragments of 495 and 245 bp) or *t* (*Taq*I site present; 3 fragments of 290, 245 and 205 bp). The subjects were therefore classified as *TT* or *Tt* (Fig. 1) (*13*). There were no subjects with the *tt* genotype.

Statistical analysis. Statistical analysis was performed using SPSS 15.0 software. Results are presented as the mean±standard deviation (SD) and categorical variables are expressed as frequencies. The chi-square test was used to verify the Hardy-Weinberg equilibrium of genotype frequencies. Spearman's correlation analysis was used to examine the influence of continuous variables on BMD. Analysis of covariance (ANCOVA) with adjustment for age and BMI was performed to assess the influence of *VDR* gene polymorphism or lifestyle variables on BMD, because BMD was positively correlated with age and BMI by Spearman's correlation analysis. We then confirmed that these interactions were not significant. The Mann-Whitney U test was used to compare differences of lifestyle factors in rela-

tion to *VDR* gene polymorphism. Multiple linear regression analysis was performed to detect variables with an independent influence on BMD. To examine multi-collinearity of the regression model, we determined the variance inflation factor. (A variance inflation factor exceeding 10 was defined as indicating that collinearity is problematic.) Statistical significance was accepted at the $p<0.05$ level.

RESULTS

The characteristics of the subjects are summarized in Table 1. The mean age of the subjects was 30.7±5.3 y, and the mean BMD was 0.463±0.043 g/cm^2 (Table 1).

Correlations were analyzed to investigate factors with an influence on BMD. As a result, BMD was positively correlated with age ($r=0.22$, $p<0.001$), weight ($r=0.45$, $p<0.001$), and BMI ($r=0.48$, $p<0.001$). When high levels of DPD and BAP are detected, metastatic bone tumor, metabolic bone disease, or abnormal calcium metabolism can be suspected, but these parameters there were not significantly correlated with BMD. In addition, no significant correlation was found between height and BMD (Table 2).

The characteristics of the subjects stratified according to *VDR* gene polymorphism are shown in Table 3. *VDR* gene polymorphism showed a distribution that followed the Hardy-Weinberg equilibrium ($p=0.10$). The allele frequency of *VDR* gene polymorphism (*T* allele frequency of 0.77 and *t* allele frequency of 0.23) was similar to that reported previously in a Japanese population (0.80 and 0.20, respectively) (*10*). There were no subjects with the *tt* genotype. ANCOVA showed that the mean BMD adjusted for age and BMI was significantly lower in subjects with the *Tt* genotype than in those with the *TT* genotype ($F=4.046$, $p=0.046$), and there was no significant interaction among these variables ($F=1.489$, $p=0.229$). No significant differences of *VDR* gene polymorphism were seen in relation to age, height, weight, BMI, DPD, or BAP (Table 3).

Table 4 displays the combined influence of lifestyle factors and *VDR* gene polymorphism on BMD. No characteristic was significantly different between the *TT* and *Tt* genotypes according to the Mann-Whitney U test ($p>0.05$). After adjustment for age and BMI by ANCOVA, however, subjects who drank alcohol showed a significantly lower BMD if they had the *Tt* genotype than if they had the *TT* genotype ($F=8.487$, $p=0.005$), and there was no significant interaction among these variables ($F=2.506$, $p=0.092$). Among drinkers, there was no significant difference of the drinking frequency in relation to *VDR* gene polymorphism (sometimes: 79.5% ($n=35$) in *TT* vs. 81.8% ($n=9$) in *Tt*; every day: 20.5% ($n=9$) in *TT* vs. 18.2% ($n=2$) in *Tt*, $p=0.867$, Mann-Whitney U test). There was no significant association between the BMD and alcohol intake for any *VDR* genotype. There was also no significant association between the BMD and other lifestyle factors (past history of exercise, current exercise, daily milk intake, number of meals daily, history of dieting, and smoking status) (Table 4).

Table 5 shows the results obtained by multiple regression analysis that assessed factors with an influence on BMD, including age, BMI, *VDR* gene polymorphism, and lifestyle. Age, BMI, *VDR* gene polymorphism (*TT* vs. *Tt*), a past history of exercise (no vs. yes), current exercise (no vs. yes), daily milk intake (no vs. yes), number of meals daily (2 meals vs. 3 meals), a history of dieting (no vs. yes), smoking status (non-smoker vs. smoker), and alcohol intake (non-drinker vs. drinker) were selected from among subjects scoring less than 10.0 for the variance inflation factor, i.e., height and weight were excluded. The parameters showing an

Table 1. Characteristics of the subjects among 162 women.

Age (y)	30.7±5.3
Height (cm)	157.8±5.5
Weight (kg)	53.2±9.7
BMI (kg/m^2)	21.4±3.8
BMD (g/m^2)	0.463±0.043
DPD (nmol/mmol·Cr)	5.5±1.6
BAP (U/L)	18.4±4.7

Values are the mean±SD.
BMI, body mass index; BMD, bone mineral density; DPD, deoxypyridinoline; BAP, bone alkaline phosphatase.

Table 2. Correlation between various parameters and bone mineral density among 162 women.

	r	*p*
Age (y)	0.22	<0.001
Height (cm)	−0.05	NS
Weight (kg)	0.45	<0.001
BMI (kg/m^2)	0.48	<0.001
DPD (nmol/mmol·Cr)	0.11	NS
BAP (U/L)	−0.05	NS

Pearson correlation coefficient (*r*).
BMI, body mass index; DPD, deoxypyridinoline; BAP, bone alkaline phosphatase.

Table 3. Characteristics of the subjects stratified according to *vitamin D receptor* gene polymorphism.

	TT ($n=125$)	*Tt* ($n=37$)
Age (y)	30.5±5.1	31.5±5.8
Height (cm)	157.4±5.3	159.3±5.9
Weight (kg)	52.9±10.2	54.2±7.9
BMI (kg/m^2)	21.4±4.0	21.4±3.0
BMD (g/m^2)	0.466±0.043	0.453±0.043*
DPD (nmol/mmol·Cr)	5.5±1.7	5.3±1.5
BAP (U/L)	18.2±4.8	19.3±4.3

Data are the mean±SD.
BMI, body mass index; BMD, bone mineral density; DPD, deoxypyridinoline; BAP, bone alkaline phosphatase.
BMD was adjusted for age and BMI by ANCOVA (analysis of covariance).
*$p<0.05$ compared to *TT* subjects.

Table 4. Mean bone mineral density according to lifestyle factors and *vitamin D receptor* gene polymorphism.

Characteristic	All (n=162)		*TT* (n=125)		*Tt* (n=37)	
	Case (%)	BMD (mean±SD)	Case (%)	BMD (mean±SD)	Case (%)	BMD (mean±SD)
Past history of exercise						
No	59 (36.6)	0.465±0.049	49 (39.2)	0.468±0.050	10 (27.8)	0.449±0.045
Yes	102 (63.4)	0.462±0.040	76 (60.8)	0.464±0.039	26 (72.2)	0.454±0.043
Current exercise						
No	124 (80.0)	0.464±0.045	96 (80.0)	0.467±0.045	28 (80.0)	0.452±0.042
Yes	31 (20.0)	0.460±0.036	24 (20.0)	0.461±0.031	7 (20.0)	0.459±0.052
Daily milk intake						
No	117 (72.2)	0.464±0.045	93 (74.4)	0.466±0.045	24 (64.9)	0.455±0.047
Yes	45 (27.8)	0.459±0.038	32 (25.6)	0.464±0.039	13 (35.1)	0.449±0.034
Number of meals daily						
3 meals/d	117 (72.2)	0.463±0.045	94 (75.2)	0.466±0.045	23 (62.2)	0.452±0.042
2 meals/d	45 (27.8)	0.462±0.040	31 (24.8)	0.465±0.038	14 (37.8)	0.455±0.045
History of dieting						
No	54 (33.3)	0.459±0.042	40 (32.0)	0.457±0.041	14 (37.8)	0.466±0.046
Yes	108 (66.7)	0.465±0.044	85 (68.0)	0.470±0.044	23 (62.2)	0.455±0.040
Smoking status						
Non-smoker	124 (76.5)	0.460±0.044	97 (77.6)	0.464±0.044	27 (73.0)	0.447±0.043
Smoker	38 (23.5)	0.471±0.039	28 (22.4)	0.471±0.040	10 (27.0)	0.470±0.039
Alcohol intake						
Non-drinker	98 (64.1)	0.463±0.045	74 (62.7)	0.464±0.044	24 (68.6)	0.461±0.047
Drinker	55 (35.9)	0.462±0.041	44 (37.3)	0.469±0.042	11 (31.4)	0.438±0.030*

Data are the mean±SD.
BMD, bone mineral density.
BMD was adjusted for age and BMI by ANCOVA (analysis of covariance).
* $p<0.05$ compared to *TT* subjects.
No characteristic was significantly different between the *TT* and *Tt* genotypes by the Mann-Whitney *U* test ($p>0.05$).

Table 5. Multiple linear regression analysis on variables associated with bone mineral density among 162 women.

	Coefficient of variation	Standard error	p*
Age (year)	0.001	0.001	<0.05
BMI (kg/m^2)	0.006	0.001	<0.001
VDR gene polymorphism[a]	−0.016	0.007	<0.05
Past history of exercise[b]	0.003	0.007	0.676
Current exercise[c]	−0.003	0.008	0.667
Daily milk intake[d]	0.000	0.007	0.969
Number of meals daily[e]	−0.008	0.007	0.277
History of dieting[f]	−0.005	0.007	0.523
Smoking status[g]	0.013	0.008	0.101
Alcohol intake[h]	−0.006	0.007	0.348

BMI, body mass index; *VDR*, *vitamin D receptor*.
* $p<0.05$ by multiple linear regression analysis, adjusted $r^2=0.25$.
[a] *VDR* gene polymorphism was rated as 0 (*TT* genotype) or 1 (*Tt* genotype).
[b] Past history of exercise was rated as 0 (No) or 1 (Yes).
[c] Current exercise was rated as 0 (No) or 1 (Yes).
[d] Daily milk intake was rated as 0 (No) or 1 (Yes).
[e] Number of meals daily was rated as 0 (2 meals/d) or 1 (3 meals/d).
[f] History of dieting was rated as 0 (No) or 1 (Yes).
[g] Smoking status was rated as 0 (Non-smoker) or 1 (Smoker).
[h] Alcohol intake was rated as 0 (Non-drinker) or 1 (Drinker).

independent association with BMD were age (coefficient=0.001, $p<0.05$), BMI (coefficient=0.006, $p<0.001$), and *VDR* gene polymorphism (*TT* vs. *Tt*; coefficient=−0.016, $p<0.05$). However, none of the lifestyle factors (past history of exercise, current exercise, daily milk intake, number of meals daily, history of dieting, smoking status, or alcohol intake) were independent determinants of BMD. The variance inflation factors in this regression model were all less than 1.20 and the adjusted R^2 value was 25% (Table 5).

DISCUSSION

In the present study, we found that premenopausal Japanese female workers with the *Tt* genotype had a significantly lower BMD than those with the *TT* genotype. In addition, we found that independent determinants of the BMD among premenopausal female workers were age, BMI, and *VDR* gene polymorphism according to multiple linear regression analysis. The significant association between *VDR* gene polymorphism and BMD detected in this study agrees with the results of previous studies performed in Japanese women (*10*), Irish men and women (*7*), and Canadian women (*8*), which have indicated that allelic variation of *VDR* gene polymorphism contributes to the regulation of BMD. Only one previous study examined the association between polymorphism at the *Taq*I site of the *VDR* gene and BMD in young Japanese women (*10*), and we also showed that *VDR Taq*I polymorphism was an independent determi-

nant of BMD (BMD was lower in premenopausal female workers with *VDR* allele "*t*" than in those with "*T*"). The VDR is a nuclear transcription factor that belongs to the steroid-receptor gene family and mediates most of the actions of vitamin D (*14*). Vitamin D plays a central role in calcium homeostasis by regulating calcium absorption, bone resorption, bone cell differentiation, and parathyroid hormone secretion (*5*). The VDR activates osteocalcin gene expression through the vitamin D response element located in the promoter region of the gene (*15*), and the *VDR* gene regulates bone turnover as the receptor for vitamin D (*16*). The exact mechanism by which *VDR* gene polymorphism affects BMD is not fully understood, but such polymorphism is likely to alter the well-known actions of vitamin D on calcium homeostasis (*17*). A meta-analysis of 75 studies on *VDR* polymorphism and BMD concluded that *VDR* gene polymorphism had a significant influence on BMD (i.e., BMD was lower in persons with *VDR* allele "*t*" than "*T*" at the *Taq*I site), and that this genotype effect was more pronounced in premenopausal than postmenopausal women, suggesting that *VDR* gene polymorphism is correlated with bone accretion rather than bone loss (*6*). Riggs et al. reported that age modulated the influence of *VDR* gene polymorphism on BMD, with the effect of *VDR* polymorphism being greatest among younger (premenopausal) women and showing a decline with age until there was no discernible influence by the age of 70 y (*12*). It can therefore be suggested that *VDR* gene polymorphism is probably a useful genetic marker for predicting BMD in premenopausal Japanese women.

In the present study, age was an independent determinant of BMD. Soda et al. reported that the peak BMD of Japanese women is achieved around 35–40 y, and that BMD increases with age until the 30 s (*3*). The mean age of our subjects was 30.7±5.3 y, and their BMD was positively correlated with age (Spearman's r value=0.22, $p<0.001$). A longitudinal study (follow-up for 10 y) on age- and menopause-related changes of BMD in 172 Japanese women aged 31–69 y showed that there was no significant decrease of BMD among women before menopause, but there was a significant decrease of BMD (−1.59%/y) in the early postmenopausal period compared with the premenopausal and late postmenopausal periods (*18*). The importance of primary prevention of osteoporosis by maximization of the peak BMD during the premenopausal years was highlighted in a recent review article (*19*). Because a decrease of BMD is inevitable after menopause, an essential part of the preventive strategy for minimizing the future risk of significant osteoporosis involves maximizing the peak BMD during the premenopausal period.

In the present study, BMD was positively correlated with weight, and BMI was one of the most important determinants of BMD. A previous study of Japanese people (35 men and 81 women) aged 14–66 y revealed that BMD was significantly correlated with body weight and with the percentage of body fat (*20*). In addition, Zhang et al. performed a prospective 10-y trial of 153 postmenopausal Japanese women and found that the annual rate of decline in BMD was significantly smaller for obese women that for slim women, suggesting that fat tissue may be effective for preventing bone loss (*18*). Though no previous studies have exclusively targeted premenopausal Japanese women, a randomized clinical trial examined the BMD of 236 healthy premenopausal white women aged 44 to 55 y recruited from the community to participate in a lifestyle intervention program targeting weight loss (dietary modification and exercise). After 18 mo, the intervention group (n=115) had lost 3.2±4.7 kg vs. a weight gain of 0.42±3.6 kg for the controls (n=121), and the annual rate of BMD loss was significantly higher in the intervention group than the controls (0.81±1.3% vs. 0.42±1.1%; $p<0.001$) (*21*). As the potential mechanism underlying the possible relation of BMI or weight to BMD, a higher body weight would be associated with more mechanical strain on the skeleton than a lower weight and could lead to a compensatory increase of BMD (*22*). In addition, Reid has hypothesized that the fat-bone relationship involves the secretion of hormones that influence bone by the pancreatic beta cells (insulin, amylin, and preptin), as well as the secretion of other hormones influencing bone such as estrogens and leptin by adipocytes (*23*). Many studies (*20–22*) have shown that greater weight or a larger BMI is associated with a higher BMD. However, a high BMI cannot be recommended in relation to general health because a high BMI is associated with an increased risk of coronary heart disease in Japanese subjects (*24*).

In the present study, no lifestyle factor was an independent determinant of the BMD. After adjustment for age and BMI by ANCOVA, however, it was found that subjects who drank alcohol showed a significantly lower BMD if they had the *Tt* genotype than if they had the *TT* genotype. These results raise the possibility that the influence of alcohol on BMD is dependent on *VDR* gene polymorphism. A relationship between alcohol intake and BMD has been reported, but no previous studies have investigated this relationship and the influence of *VDR* gene polymorphism in premenopausal Japanese women. To our knowledge, this is the first report of an association between BMD and alcohol intake dependent on *VDR* gene polymorphism in premenopausal Japanese women. Alcohol causes several metabolic changes that can be unfavorable for the bones (*25*). Although the indirect mechanisms have not been studied extensively, chronic alcohol abuse could potentially affect BMD through nutritional deficiencies, alterations of calcium and vitamin D metabolism (*26*). In subjects with the *Tt* genotype, there seems to be a more adverse impact on the bone because deficiency of vitamin D and calcium alters the effectiveness of calcium absorption (*27*, *28*). On the other hand, there were no such adverse effects on the bone in subjects with the *TT* genotype. This difference might be mediated through *VDR* gene polymorphism. A dose-dependent effect of alcohol on bone metabolism has also been reported (*29*), but the precise influence of moderate alcohol con-

sumption on bone metabolism is still unknown. A recent meta-analysis indicated that men and women who consume more than 2 drinks per day have a higher risk of hip fracture (*30*). Little is known about the effects of alcohol on BMD, but excessive alcohol intake appears to have a modest adverse effect on the preservation of bone mass in premenopausal women, mainly by suppressing bone formation (*25*). Clearly, a large-scale prospective study will be needed to better define the relation between alcohol intake and BMD, as well as the influence of *VDR* gene polymorphism.

Our study had several limitations. First, there was no questionnaire to assess environmental factors in detail or for complete assessment of factors related to osteoporosis. However, the questionnaire was designed to cover the well-known risk factors for fracture (*4*). We should develop a reliable and valid questionnaire to assess environmental factors contributing to genetic susceptibility to osteoporosis. We did not assess the actual alcohol consumption of the drinkers. Further detailed studies about the association between alcohol consumption and BMD are required.

In conclusion, among premenopausal female workers aged 22 to 44 y, BMD was negatively correlated with the *Tt* genotype of the *VDR* gene, but was positively correlated with age and BMI. These findings suggest that genetic diagnosis of *VDR* gene polymorphism may be useful for identifying individuals who are susceptible to osteoporosis, thus allowing early preventive measures to be provided. Such studies could lead to more effective prevention of osteoporosis and improvement of public health. A prospective study will be necessary to determine the extent to which BMD responds to appropriate lifestyle changes in relation to *VDR* gene polymorphism.

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