Determinants of Premenopausal Bone Mineral Density: The Interplay of Genetic and Lifestyle Factors

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ABSTRACT

Bone mineral density (BMD) is a reflection of both genetic and lifestyle factors. The interplay of genetic (vitamin D receptor [VDR] gene polymorphisms) and lifestyle factors on BMD at the lumbar spine and proximal femur was examined in 470 healthy premenopausal women, aged 44–50 years, using a Hologic QDR 2000 densitometer. The objective of this study was to examine the genetic and lifestyle determinants of premenopausal BMD. Each participant was genotyped for BsmI polymorphism at the VDR gene locus. The presence of a restriction site within VDR, specified as bb (180, 40.2%) (n, %) was associated with reduced spinal BMD, whereas absence of this site in BB (97, 20.6%) conferred greater spinal BMD, as did the genotype Bb (184, 39.1%). Associations between smoking, alcohol use, oral contraceptives, education level, multivitamins, number of children, degree of obesity, body weight, physical activity, dietary calcium intake, and VDR genotype to BMDs were examined. VDR genotype, body weight, degree of obesity, physical activity, and dietary calcium intake were all significant determinants of BMD. The association of VDR genotype with BMD at the femoral neck appeared to be modified by calcium intake (BB and Bb: 0.797 ± 0.11 g/cm² vs. 0.844 ± 0.11 g/cm², interaction term, p = 0.06) for low (<1036 mg/day) and high (≥1036 mg/day; upper quartile) calcium intakes, respectively. A similar trend was demonstrated for physical activity. These findings suggest that prophylactic interventions aimed at achieving and maintaining optimal BMD, such as greater calcium intake or physical activity, may be important in maximizing one’s genetic potential for BMD. (J Bone Miner Res 1996;11:1557–1565)

INTRODUCTION

Genetic factors, either alone or through interactions with lifestyle factors known to affect bone mineral density (BMD), may contribute to the pathogenesis of osteoporosis. There is little disagreement that BMD has a strong genetic component; this is well-supported from twin(1,2) and family studies.3-6 Part of the mechanism for this genetic effect was recently ascribed to a polymorphism in the vitamin D receptor (VDR) gene locus, accounting for as much as 75% of the variance in BMD.(7) Specifically, Morrison and colleagues demonstrated that subjects with the bb genotype had 15% higher BMD than BB (signifying the absence of a restriction site), after adjusting for age and environmental effects.(8) Similar, but less pronounced, effects have been confirmed by some(9-11) but not all investigators.(12-15) For example, the authors have previously demonstrated that in a population of healthy premenopausal women residing in the United States, the presence of a polymorphic restriction site, specified as bb, was associated with significantly reduced spinal BMD (about 3%) as compared with those with BB (bb, 1.038 ± 0.11 g/cm² vs. BB, 1.069 ± 0.12 g/cm²; p < 0.05).(14,15) Clearly, the significance of this allelic change on BMD remains controversial.(8-15)

The impact of lifestyle factors, such as dietary calcium...
intake or physical activity, on one’s genetically determined BMD is potentially great. Indeed, it is possible that there is a significant interaction between VDR genotype and lifestyle factors. The lack of consistency in the VDR genotype and BMD literature may reflect variability in lifestyle factors, e.g., nutrition and activity levels across each population, suggesting that VDR may exert its influence through apparently non-genetic factors. For example, within the Australian Dubbo Osteoporosis Study population, largely of English and Irish descent, a 9.7% higher femoral neck BMD was observed in the bb genotype, compared with BB, whereas in a recent report, based on healthy women residing in Indiana, there was no relationship between polymorphisms at the VDR gene locus and BMD at either the proximal femur or spine among female twins (mean age 40s). The fact that BMD is multifactorially determined by both genetic and environmental factors is well-established, although the relative contributions of each remains largely unresolved. Since heritability is thought to contribute 46–62% of the variance in BMD, a substantial proportion of BMD may be determined by lifestyle factors. This possibility raises several important research questions: Can one’s genetic predisposition to BMD be mediated by lifestyle factors? If so, what are the most significant factors contributing to this modulation, and if not, are prophylactic recommendations for optimizing BMD equally as effective across VDR genotypes? In view of these important research questions, the objectives of this study were to assess genetic and lifestyle determinants of BMD in healthy premenopausal women and examine whether differences in lifestyle factors can alter one’s genetic predisposition for having either low or high bone density.

MATERIALS AND METHODS

Subjects

The 535 women enrolled in this study entitled the Women’s Healthy Lifestyle Project (WHLP) were recruited from a random sample of registered voters from selected zip codes in Allegheny County, Pennsylvania, U.S.A. The protocol was approved by the Human Investigation Review Board at the University of Pittsburgh, and written informed consent was obtained from each subject. The criteria for entry into this study were good general health, aged 44–50 years, menses in the past 3 months, body mass index (BMI) 20–33.9 g/cm², alcohol intake <5 drinks/day, diastolic blood pressure <95 mm Hg (on three occasions), total cholesterol 160–260 mg/dl, low-density lipoprotein (LDL) cholesterol 80–160 mg/dl, and blood glucose <140 mg/dl. Women were excluded if they had a history of cancer within 5 years, psychiatric hospitalization within 1 year, drugs for hypertension, cholesterol lowering, hormone replacement therapy (including thyroid medications and estrogens), psychiatric disorders, hysterectomy, or bilateral oophorectomy.

Of the 535 participants, 521 received dual-energy X-ray absorptiometry (DXA) scans. Fourteen women refused DXA scans. Of the 521 participants, 479 were Caucasian, 36 were African-American, and 6 were considered as other. Because of the small number of non-Caucasians in this study and because of the significant differences in BMD between Caucasians and African-American women (p < 0.0001), only Caucasian women were included in this analysis. Of the 479 participants, 470 had complete VDR genotyping and DXA measures and were included in the final analyses.

Parent study design

This cross-sectional study was part of a clinical trial designed to determine whether an increase in LDL cholesterol around the time of menopause can be prevented by dietary intervention aimed at a reduction in saturated fat and cholesterol, weight loss, or prevention of weight gain. This study was based in our previous observation in the Healthy Women’s Study that LDL cholesterol increased during the menopausal transition, as did body weight. Women were randomly assigned to one of two groups: assessment only controls or assessment plus dietary intervention. Women assigned to the intervention group participated in dietary and behavioral intervention aimed at reducing saturated fat and cholesterol eaten, while in the assessment group, subjects were given general nutrition and low-fat guidelines.

Measurements

Weight was measured using a balance beam scale, and height was measured with a stadiometer (Perspective Enterprises, Kalamazoo, MI, U.S.A.). BMI was calculated as weight in kilograms divided by height in meters squared. The Paffenbarger activity questionnaire, a standardized measure of leisure-time physical activity, was administered in an interview format. The Paffenbarger questionnaire measured three components of activity reported for the past week, including blocks walked per day, flights of stairs climbed per day, and sport or recreational activity. The estimate of total kilocalories (kcals) expended per week was computed by a summary of calculated values for blocks walked, flights of stairs climbed, and sport or recreational activity. Dietary calcium intake was estimated from a food-frequency questionnaire that included the 14 foods that account for more than 95% of calcium intake in the American diet. Dietary calcium was expressed as milligrams (mg) of calcium per day.

Demographic data were collected on smoking, alcohol use, oral contraceptive use, multivitamins, education level, and parity from questionnaires and interview at the baseline examination. Smoking, oral contraceptive, and multivitamins were dichotomized into ever (current and past) and never users. For alcohol consumption, we compared never and occasional drinkers to regular drinkers, as defined as those women who reported consuming alcohol more than occasionally. Education was dichotomized as those with ≥ a college education to < a college education. For number of children, we compared those having ≤2 children to >2 children. Obesity was examined by comparing BMI ≥ 27.3 to <27.3 kg/m².
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BMD at the AP lumbar spine (L2–L4) and hip (femoral neck, Ward’s triangle, trochanter, and intertrochanter) were measured with Model QDR 2000 densitometer (Hologic Inc., Waltham, MA, U.S.A.). DXA measurements were performed at baseline if time permitted, or another appointment was made at the participant’s earliest convenience within the first 12 months of randomization into participation into the study. A standardized procedure for patient positioning and utilization of the QDR software was used. From in vitro phantoms scanned each month, the % coefficient of variations (CVs) were 0.6 and 1.3% for the lumbar spine and femoral neck, respectively (unpublished data). The scans were analyzed with Hologic software version 7.10.

DNA analysis

High molecular weight DNA was extracted from peripheral leukocytes by the salting-out procedure of Miller.(19) DNA was amplified by polymerase chain reaction (PCR) using VDR-R and VDR-L primers, similar to the method of Morrison et al.(8) Each PCR was performed using 60-Î£1 final reaction volumes containing 100–200 ng of DNA, 0.46 Î¼M of each primer, 185 Î¼M of deoxyribonucleotide triphosphate (dNTP) mixture, 50 Mm KCl, 10 MM Tris-HCl (pH = 9.0), 1.5 mM MgCl2, 0.1% Trition X-100, and 0.8 U of Taq DNA polymerase. After PCR amplification, 5 U of BsmI (New England Biolabs, Beverly, MA, U.S.A.) were added to the 16 Î£1 of amplified product for digestion (overnight at 65°C). Each digested sample was loaded onto a 2% agarose gel containing ethidium bromide and electrophoresed for 3 h at 90 V. Internal controls were run on each of these gels to ensure complete digestion of the BsmI enzyme. After electrophoresis, the DNA fragments were visualized by ultraviolet illumination, and fragment sizes were estimated by comparison to a 1 kb ladder run on the same gel. The presence of a polymorphic restriction site at the VDR gene locus was specified as bb, whereas absence of this site was BB.

Statistical analyses

Statistical analyses were performed using the SAS statistical package (SAS Institute, Cary, NC, U.S.A.).(20) Since spinal and femoral neck BMDs were similar for VDR genotypes BB and Bb (lumbar spine, 1.069 ± 0.12 and 1.067 ± 0.12; femoral neck, 0.808 ± 0.11 and 0.808 ± 0.11, respectively), we combined subjects having BB (20.6%) plus Bb (39.1%) (59.8% [high BMD]) and compared them to those having the bb genotype (40.2%, low BMD) for our analyses. Standard ANOVA or the nonparametric Kruskal–Wallis ANOVA was conducted to test for differences in clinical characteristics between VDR genotypes (BB + Bb vs. bb). The statistical significance of the univariate associations of the dichotomous lifestyle factors to VDR genotype was assessed by the Chi-square test of the equality of proportions. Since dietary calcium and physical activity levels were not normally distributed, we dichotomized these variables, based on the frequency distribution, with the top 25% representing “high” calcium intake and “high” physical activity. Although body weight was normally distributed, it was dichotomized in a similar manner to be consistent. The cutpoints for calcium intake, physical activity, and body weight were 1036 mg/day, 1789 kcals/week, and 160 lb, respectively. All p values <0.05 indicate statistical significance.

Univariate analyses

For the entire study population (n = 470), we analyzed potential associations of lifestyle factors, including body weight, physical activity, dietary calcium, BMI, education level, multivitamins, number of children, oral contraceptives, cigarette smoking, and alcohol use, with bone density with analysis of variance (ANOVA) and simple linear regression. The regression coefficients and standard errors generated from regression models were used to calculate the percent differences in BMD per unit (1 SD) of the independent variable.

Multivariate analyses

Variables that were associated with bone density in univariate analyses (p < 0.05) were examined by two-way ANOVAs, including the VDR genotype (BB + Bb vs. bb), each lifestyle factor and their interaction. Selected variables, significant at p < 0.05, were also entered into multivariate models for the femoral neck and lumbar spine. Regression coefficients and standard errors generated from multivariate models were used to calculate percent differences in BMD per unit (1 SD) of the independent variable. Post hoc comparisons of adjusted means were made.

RESULTS

Descriptive statistics of the subjects

The mean (±SD) for variables that describe the study population are presented in Table 1. The mean age of the subjects was 46.9 ± 1.9 years (n = 470). Women within each VDR genotype group were remarkably similar to each other. There were no significant differences in BMI, dietary calcium intake, or physical activity levels between groups. Women with genotype bb had a slightly lower mean body weight, but there were no differences in BMI. A high proportion of women reported at least a college education in both groups. There were also no significant differences in the prevalence of use of medications thought to influence BMD or in other lifestyle factors such as alcohol use or cigarette smoking.

Genetic and lifestyle factors: Univariate summary for study population

From univariate analyses, VDR genotype, body weight, BMI, physical activity, and dietary calcium intake were
strongly associated with BMD (Table 2). Women with genotype bb had a 2.8 and 2.7% lower BMD at the lumbar spine and femoral neck (p < 0.05), respectively, as compared with the combined genotypes BB and Bb. Although weight and BMI were highly correlated (r = 0.85), body weight was more strongly associated with BMD than BMI (R² = 7.9–8.5% and 1.1–3.4%, respectively), and therefore, weight was used exclusively in subsequent analyses. There were no significant associations between smoking, alcohol use, multivitamins, oral contraceptive use, education level, or parity and BMD at either site (Table 2).
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4. Dietary calcium (~1036 mg/day)
3. Physical activity (~1789 kcals/week)
1. VDR genotype (BB + Bb)

There was a 4.3 and 2.6% greater femoral neck and spinal BMD, respectively, for low and high activity levels. Differences in BMDs for low and high levels of calcium intake were 4.0 and 2.7%, respectively.

Genetic and lifestyle factors: Multivariate summary for study population

In multivariate analyses, VDR genotype, body weight, physical activity, and dietary calcium were all related to lumbar spine and femoral neck BMD, explaining 7.7 and 9.0% of the total variance in BMD, respectively (Table 3). Nevertheless, the magnitude of the effect on BMD was relatively small, ranging from 2.1–7.5%, with larger effects observed for femoral neck BMD as compared with spinal BMD. For example, comparing low versus high body weight, a 7.5% difference was observed at the femoral neck, while only a 5.5% difference at the lumbar spine was seen. There was a 4.3 and 2.6% greater femoral neck and spinal BMD, respectively, in those reporting higher levels of activity; similarly, differences in BMDs for low and high levels of calcium intake were 4.0 and 2.7%, respectively.

Genetic and lifestyle factors: Test for interaction

The association of VDR genotype with BMD at the femoral neck appeared to be modified by calcium intake (interaction term, p = 0.06). Within BB and Bb genotypes, higher calcium intake (≥ 1036 mg/day) was associated with a 5.9% greater BMD as compared with a lower calcium intake (< 1036 mg/day) (BB and Bb, 0.844 ± 0.11 vs. 0.797 ± 0.11 g/cm², respectively, p < 0.05; Fig. 1). However, for the bb genotype, higher calcium intake had a only minor effect (0.13%) on femoral BMD as compared with lower calcium intake (bb, 0.787 ± 0.08 vs. 0.786 ± 0.11 g/cm², respectively). A similar pattern was not observed at the lumbar spine (interaction term, p = 0.78; Fig. 2).

Although there were no significant interactions between VDR genotype and physical activity, VDR genotype seemed to modify the association between physical activity and BMD, at least at the femoral neck (interaction term, p = 0.24; Fig. 2). Within the bb genotype, higher physical activity (≥ 1789 kcals/week) was associated with significantly greater femoral neck BMD (about 6.2%) compared with low activity (< 1789 kcals/week) (bb, 0.822 ± 0.10 vs. 0.774 ± 0.10 g/cm², respectively, p < 0.05). A similar, but less pronounced pattern was demonstrated for genotypes BB and Bb (BB and Bb, 0.832 ± 0.11 vs. 0.803 ± 0.11 g/cm², for high and low activity levels, respectively).

There was no significant interaction between VDR genotype and body weight at the femoral neck (interaction term, p = 0.69) or lumbar spine (interaction term, p = 0.59; Fig. 3), and VDR genotype did not appear to modify the association between body weight and BMD. Irrespective of VDR genotype, higher body weight was consistently associated with greater BMD, particularly at the weight-bearing femoral neck (bb, 0.836 ± 0.10 g/cm² and 0.773 ± 0.10 g/cm²; BB and Bb, 0.846 ± 0.11 g/cm² and 0.793 ± 0.11 g/cm²; for high [≥ 160 lb] and low [< 160 lb] body weights, respectively).

DISCUSSION

In this study of healthy premenopausal women, VDR genotype and lifestyle factors including body weight, physical activity, and dietary calcium intake appeared to be significant determinants of bone density at the femoral neck and lumbar spine. Comparing those having VDR genotypes BB and Bb versus bb, there was approximately a 3.0% greater BMD observed at both sites; whereas differences in BMD, comparing low and high levels of lifestyle factors ranged from 2.0 to over 7.0%. It is noteworthy that the author's original report relating VDR and BMD in this population was incorrect; indeed, it appears that the bb genotype rather than the BB genotype is associated with a 3.0% lower spinal and trochanteric BMD.(14,15)

The association of VDR genotype and BMD at the femoral neck appeared to be modified by calcium intake. The mechanism by which VDR genotype is modulated by calcium intake remains largely unresolved; however, the fact that dietary calcium is important implicates the intestine as a likely site for action of the VDR variants. Accordingly, certain genotypes may be less likely to maintain calcium homeostasis via 1,25-dihydroxyvitamin D-stimulated active calcium transport. Most recently, Dawson-Hughes et al.(21) reported similar mean Ca⁴⁵ absorption values in postmenopausal women (BB [n = 26] vs. bb [n = 34]) on a high calcium diet (1500 mg of calcium/day, p = NS), albeit on a low calcium diet (<300 mg of calcium/day) significant differences in calcium absorption were demonstrated (BB, 20.57 ± 1.10% [SEM]; bb, 23.66 ± 0.95%; p = 0.044). This, in part, may reflect an altered status at the 1,25-dihydroxyvitamin D receptor, resulting in poor utilization of available calcium in BB and Bb genotypes as compared with their bb counterpart. Evidence for a genetic and environmental interaction with VDR receptor-gene polymorphisms and di-
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**FIG. 1.** Mean femoral neck (a) and lumbar spine (b) BMDs by dietary calcium intake and VDR genotype. Dark-shaded bars represent “low” levels of calcium intake (<1036 mg/day) and light-shaded bars represent “high” levels of calcium intake (≥1036 mg/day). Interaction between VDR genotype and calcium intake, p = 0.06. (a) *Significant differences in BMD between VDR genotype Bb and BB, high calcium intake versus all other categories, p < 0.01. (b) §Significant differences in BMD between VDR genotype bb, low calcium intake vs. Bb and BB, low calcium intake and Bb and BB, high calcium intake, p < 0.05.

**FIG. 2.** Mean femoral neck (a) and lumbar spine (b) BMDs by physical activity and VDR genotype. Dark-shaded bars represent “low” levels of physical activity (<1789 kcals/week) and light-shaded bars represent “high” levels of physical activity (≥1789 kcals/week). (a) *Significant differences in BMD between VDR genotype bb, low physical activity and all other categories, p < 0.01. (b) §Significant differences in BMD between VDR genotype bb, low physical activity versus Bb and BB, low physical activity and Bb and BB, high physical activity, p < 0.05.

Although our studies are not directly comparable because of differences in the populations studied, i.e., pre- versus postmenopausal women, study design, cross-sectional versus longitudinal, or perhaps that fact that a clinical trial with a 500-mg calcium supplement is compared with a self-reported calcium intake from a food-frequency questionnaire, the demonstration that the BB genotype responds more favorably to calcium supplementation in attenuating postmenopausal bone loss is similar to our cross-sectional findings, which show a more favorable response to higher calcium intake in the BB and Bb genotypes versus bb.

Although the interaction term for VDR genotype and physical activity was not significant in determining BMD, the pattern observed in BMD, particularly at the femoral neck, is noteworthy. The observation that the bb genotype responded to greater levels of physical activity with a 6.2% higher BMD as compared with only a 2.5% higher BMD in
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Evidence from both cross-sectional and longitudinal studies support the generally held belief that higher body weight confers greater BMD. Both mechanical and hormonal hypotheses have been postulated to explain the effect of body weight on BMD. The observation that individuals with bb genotype had reduced BMD at both sites as compared with BB and Bb, irrespective of body weight (low, high), may reflect an inability to overcome one’s genetic predisposition for reduced skeletal mass simply with greater body weight. Most recently, Barger-Lux et al. suggested that VDR gene polymorphism may exert its effects through an influence on body size. Indeed, the association between VDR alleles and body size was similar to that observed for BMD with individuals with bb weighing less than BB (62.4 ± 7.6 kg vs. 71.9 ± 8.8 kg, respectively) 

The lack of significant associations of other lifestyle factors with BMD in this population is not well understood. Since most evidence suggests that peak BMD is attained by age 35 years, the absence of additional associations may reflect the fact that this population is too far beyond their peak BMD. Moreover, habitual exposure to these factors is thought to have an even greater effect on BMD than current exposure, although cumulative histories might not be reliably ascertained from current levels. In this study, the importance of current calcium intake and physical activity levels on premenopausal BMD was demonstrated; this observation might reflect the fact that a high-calcium food consumption pattern or perhaps sound exercise habits established in childhood might persist throughout adulthood. Identification of additional nongenetic determinants of BMD, which are open to intervention, is instrumental in developing the most targeted areas for the prevention of osteoporosis.

This study provides at least some evidence to support the hypothesis that one’s genetic predisposition for BMD may be modified by environmental factors. In an earlier study, Smith et al. observed a genetic–environmental interaction for radial BMD, demonstrating that within-pair variance for BMD was four times as great for juvenile dizygotic (DZ) twins as for monozygotic (MZ) twins (0.0013). However, among adult twins, the difference was only about twice as great for DZ twins (0.0137) as for MZ twins (0.0069). Because of the large variation among DZ twins, a strong genetic determination was indicated, although the increased intrapair difference with age suggested the importance of genetic and environmental interactions in determining BMD. Of further interest is the observation that the variance within MZ twins was significantly greater in postmenopausal than in premenopausal twins; this evidence supports a stronger genetic influence during the development of peak bone mass and a more dominant environmental role at menopause. These conclusions have been confirmed by others.

This study has several limitations. First, the distribution of genotypes at the VDR gene locus in this cohort does not...

FIG. 3. Mean femoral neck (a) and lumbar spine (b) BMD by body weight and VDR genotype. Dark-shaded bars represent “low” body weight (<160 lb) and light-shaded bars represent “high” body weight (≥160 lb). (a) Significant differences in BMD between VDR genotype bb, low body weight versus bb, high body weight and Bb and BB, high body weight, p < 0.01. (b) Significant differences in BMD between VDR genotype Bb and BB, low body weight versus Bb and BB, high body weight and bb, high body weight, p < 0.01. (b) Significant differences in BMD between VDR genotype Bb and BB, low body weight versus Bb and BB, high body weight and Bb and BB, high body weight, p < 0.05. Significant differences between Bb and BB, low body weight versus Bb and BB, high body weight, p < 0.01.
conform to the expectations of Hardy–Weinberg equilibrium based on allele frequencies. The fact that within this population the bb genotype was associated with significantly reduced BMD—a finding that is in contrast with Morrison et al. (8) and others (9–11)—further exemplifies the need for continued research efforts in population-based cohorts to reach a consensus about the relationship between VDR and BMD as well as the inclusion of additional restriction sites such as Apal and Taq1 in subsequent research. Because of self-selection of participants and the exclusion of participants with chronic health problems, this sample consisted of healthy subjects of higher socioeconomic status. Therefore, extrapolation of these findings to the general population must be done with caution. Also, current levels of weight, dietary calcium, and activity were reported, rather than assessment of longer-term status. Recognizing the beneficial effect of calcium intake and physical activity as being more related to habitual rather than to current patterns, even stronger associations with BMD might have been demonstrated.

From this study of healthy premenopausal women, it appears that prophylactic interventions, aimed at achieving and maintaining BMD, can modulate BMD, preferentially by VDR genotype. If VDR is acting through apparently nongenetic factors, it is conceivable that positive modifications in lifestyle factors may help to maximize one’s genetic predisposition for BMD. Even modest gains in BMD could have a significant effect on preventing osteoporosis and reducing osteoporosis-related fractures. Clearly, a prospective trial is necessary to demonstrate the extent to which BMD responds to appropriate lifestyle changes across VDR genotypes.

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