Determinants of Bone Mineral Density in Postmenopausal White Iowans

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Background. Osteoporosis is a major health problem for older individuals. For women, development of osteoporosis is a function of the accretion of "peak" bone mass in the third decade, age at menopause, and rate of bone loss with aging. Low bone mineral density (BMD) is a major risk factor for osteoporosis and fracture. The purpose of this study was to identify life style, nutritional, medical, and genetic predictors of low BMD in postmenopausal Iowa women.

Methods. One hundred thirty-four postmenopausal White women ranging in age from 57 to 81 years were included in this case-control study. Bone mineral density was measured at the femoral neck, using dual photon X-ray absorptiometry (Hologic 2000 QDR). Sixty-six women with BMD measurements below 0.68 g/cm² (the bottom quartile of the BMD distribution in the population from which participants were recruited), and 68 women with values at or above 0.83 g/cm² (the top quartile of the BMD distribution in the same population) were included. Information about environmental, nutritional, medical, and life style modifiers of BMD was obtained by written questionnaire and telephone interview. To assess familial factors that might influence BMD, we obtained a detailed family history for each participant. In addition, we tested the hypothesis that allelic variation at the Vitamin D receptor (VDR), and the type I collagen gene (COL1A1 and COL1A2) loci influence BMD.

Results. Weight, loss of height, age, and age at menopause were strong predictors of BMD in our population. After adjustment for these differences, we found no effect of genotype at the COL1A1, COL1A2, and VDR loci on BMD.

Conclusions. Bone mineral density is a complex trait that is influenced by several different modifiers; in the present study, weight was the best predictor of postmenopausal BMD. While several studies suggest that VDR genotype is an important determinant of BMD, we did not find this association in our population, nor did we identify an association between allelic variation at the type I collagen gene loci and BMD. Identification of genes that determine body mass index may provide additional insight into risk factors for low BMD, and osteoporotic fractures.

Bone mass is influenced by genetic and environmental factors, including nutritional and life-style characteristics. How these modifiers interact to determine the phenotype we measure as bone mineral density (BMD) is not well understood. Numerous family and twin studies have demonstrated that a strong genetic component is involved in the determination of BMD in both males and females (1). Comparison of intrapair differences in BMD between monozygotic and dizygotic twin pairs suggests that the heritability of BMD is between 70 and 80%, depending on the skeletal site examined. Although causative mutations have yet to be identified in large numbers of individuals with low BMD, studies of relatively rare inherited bone conditions, such as osteogenesis imperfecta and vitamin D resistant rickets, suggest that the type I collagen genes (COL1A1 and COL1A2), and the vitamin D receptor gene (VDR) are good candidate genes for more common conditions that involve the bone matrix (2–4). In addition, some population-based studies have shown a strong association between BMD and allelic variants at the VDR locus (5–7).

It has been shown that Whites are at risk for low BMD and osteoporosis-related fractures, compared with other racial groups (8). For this reason, we sought to identify predictors of postmenopausal BMD, and to examine potential gene-environment interactions relevant to BMD determination, using a case-control format with a population of White Iowans. We obtained detailed information about nutritional, reproductive, and life-style characteristics, medical conditions, medication use, and family history of osteoporosis for each study participant, and examined possible interaction effects with known risk factors for BMD. In addition, we tested the hypothesis that allelic variation at the VDR, COL1A1, and COL1A2 gene loci is associated with BMD. Weight, loss of height, age, and age at menopause were strong predictors of BMD. After adjusting for these factors, we found no association between any of the allelic variants and BMD in this population.

METHODS

Study participants. — One hundred thirty-four postmenopausal healthy White women, ages 57 to 81 years, were included in the study. These individuals were originally part of a larger group of women from Eastern Iowa who were recruited to participate in the Fracture Intervention Trial (FIT) at the University of Iowa (9). As part of the initial FIT recruitment process, BMD at the femoral neck was measured in a blinded manner, and information about bone-related problems was obtained. There were 1,197 women who were not included in the FIT study because ei-
ther they had normal BMD, or they had no history of spinal fracture, despite having low BMD. From the 1,197, we randomly selected 66 case women who had femoral neck BMD measurements of less than 0.68 g/cm² (the bottom quartile of the BMD distribution for a similar-age population), and 68 control women who had BMD measurements greater than 0.83 g/cm² (the top quartile of the BMD distribution for similar-age population) (10). We specifically chose women with BMD measurements at the extremes in order to maximize the likelihood of identifying genetic and environmental factors which influence BMD. No participant had metabolic bone disease, or osteogenesis imperfecta. All study participants considered themselves to be healthy.

Questionnaires. — Each study participant completed a written standardized baseline health questionnaire at the initial screening visit for the FIT trial. These data were collected in a blinded manner, and were obtained prior to BMD determination. The questionnaire asked about current and past medical history, including bone-related problems and symptomatology, medication use, estrogen replacement, calcium and vitamin D supplementation, alcohol and caffeine consumption, tobacco use, physical activity in the past 12 months, reproductive characteristics, breast-feeding practices, age of menopause, and family history of osteoporosis. Subsequently, one of the authors (ETS), blinded to all genetic analyses, contacted each study participant by telephone to verify information on the FIT questionnaire, and to obtain a pedigree on each participant.

Height and weight measurements were available on each study participant from their initial FIT screening visit. A second set of measurements was obtained at the clinic visit for this study, and a blood sample was obtained from each participant for the purpose of genotyping. Genotyping was performed in the laboratory of one of the authors (MCW), who was blinded to the questionnaires and the BMD measurements. All aspects of this study were approved by the Institutional Review Board at the University of Iowa.

Bone densitometry. — Bone mineral density at the femoral neck was measured by dual energy x-ray absorptiometry, using a Hologic 2000 QDR densitometer.

Genotyping. — Genomic DNA, isolated from lymphocytes, served as the template for amplification by the polymerase chain reaction [PCR (10)]. Oligonucleotide primers used for amplification of the domain of the VDR gene known to contain the polymorphic BsmI restriction site were from Morrison et al. (5). Primers for the TaqI and ApaI polymorphisms were derived from Spector et al. (7). Three polymorphic sites (MnlI, MspI and Rsal) were analyzed for COL1A1; two sites (PvuII and Rsal) were analyzed for COL1A2. Sequences for the PCR primers were from Baker et al. (11). Following the PCR, an aliquot of amplified material was cleaved with the appropriate restriction endonuclease, according to manufacturers’ specifications (New England Biolabs, Beverly, MA). The presence (+) or absence (−) of the enzyme recognition site was identified by ethidium bromide staining of fragments separated in 6% polyacrylamide. Genotypes were assigned as (+/+, +/− and −/−). By convention, the VDR (+/+) and (−/−) genotypes correspond to bb (aa and tt) and BB (AA, TT), respectively (5,12). These genotypes were determined blinded to BMD and questionnaire data.

Statistical analysis. — Descriptive statistics were calculated as means, standard deviations (SD), and ranges for continuous variables and proportions for categorical variables. Comparison of the means of continuous variables for women with lower vs upper quartile BMD was carried out using least squares analysis of covariance. Comparison of categorical variables and allele frequency distributions utilized chi-square analysis. Logistic regression using the Statistical Analysis Program was used to determine if any genotype was a risk factor for low BMD, after adjusting for other significant variables, including age. Significance was defined as a p-value less than .05. With the available sample size, we would be able to detect an odds ratio of 3 with a power of 80%, using that significance level.

RESULTS

To identify predictors of BMD, we studied 134 healthy postmenopausal Iowans who were selected on the basis of hip BMD, measured at the femoral neck. Since a major component of our study focused on the effect of genotype on BMD, we sought women whose BMD measurements fell in either the bottom or top quartile of the BMD distribution, in order to optimize the likelihood of detecting an association. The first group (case women) consisted of 66 participants with low hip BMD (mean .56 g/cm²), while the second (control) group comprised 68 individuals with high hip BMD (mean .91 g/cm²). The mean ages for case and control women were 70.2 years and 66.4 years, respectively (p < .001).

We compared weight and height in the case and control women. As a group, case women weighed less (p < .001) and were shorter than control women (p < .01) (Table 1).

Reproductive characteristics were assessed for each woman. As a group, case women had fewer pregnancies than control women (p < .05) and had their first pregnancy at an older age, compared to control women (p < .025) (Table 2). There was no difference in age at menarche between the two groups, but case women experienced menopause at a mean age of 47.3 years, compared to 49.5 years for control women (p < .05). Within the case group, there were nine women (13.6%) who had undergone a hysterectomy prior to age 40 years, compared to six (8.8%) control women. Twenty-one percent of case women had undergone unilateral oophorectomy, compared to 13% of control women.

Current and past medical problems were reviewed for each study participant. Obesity was identified as a current medical problem in nine control women, but in only one case woman (p < .01), corroborating our objective assessment of weight in the two groups. Control women also had a higher prevalence of diabetes (p < .01) and hypertension (p < .01), compared to case women. Arthritis was reported more often in the control group (p < .05), although it was not reflected in the use of analgesic or anti-inflammatory
drugs. Bone-related problems also differed between the two groups (Table 3): 56% of case women reported loss of height, compared to 25% of control women (p < .001), whereas 67% of case women had experienced a nonspinal fracture, compared to 41% of controls (p < .005).

Medication use was similar among the cases and controls except for minor differences in estrogen replacement (6% in cases and 0% in controls) and diazide use (3% in cases and 14% in controls). Four percent of controls used vitamin D supplements, compared to 5% of cases, while 26% of controls used calcium supplements, compared to 35% of cases. These differences were not statistically significant (data not shown). Alcohol and tobacco use were also assessed for all study participants. There was no significant exposure difference. Finally, family history of osteoporosis was determined in both case and control women. In our cohort, more control women had at least two relatives with osteoporosis, compared to case women (p < .05).

Vitamin D receptor, COL1A1, and COL1A2 genotypes were determined for each participant; genotype frequencies for each marker closely followed the distribution expected under Hardy-Weinberg equilibrium (Figures 1–3), and were similar to those reported in other White populations (12). We observed the previously described linkage disequilibrium for VDR markers (13,14) with the BsmI bb genotypes being associated with Apal aa and TaqI TT genotypes, while the BB genotype was more commonly found with the AA and tt genotypes (p < .001). Because differences existed between cases and controls in terms of age, weight, height, and years since menopause, logistic regression analysis was used to adjust for the effects of these factors prior to consideration of genotype effects. After adjustment, none of the genetic markers was related to low BMD status. From multivariate analysis, body size as measured by weight or body mass index was significantly related to case or control status. The genotypes, however, were not related to body size.

**TABLE 1. Characteristics of 66 Case and 68 Control Women**

|                        | Control | Cases | p-value  
|------------------------|---------|-------|----------
| Weight (kg)            | 85.2    | 63.5  | <.001    
|                        | (17.2)  | (11.5)|          
| Height (cm)            | 161.7   | 157.9 | <.01     
|                        | (9.5)   | (5.4) |          
| Age (years)            | 66.4    | 70.2  | <.001    
|                        | (5.4)   | (5.5) |          
| Hip BMD (g/cm²)        | 0.91    | 0.56  | <.001    
|                        | (0.08)  | (0.07)|          
| Hip BMD (g/cm²) adjusted for age, weight | 0.89    | 0.58  | <.001    

*Note: Values are expressed as means and standard deviations (in parentheses).*

**TABLE 2. Reproductive Characteristics of 66 Case and 68 Control Women**

|                        | Controls | Cases | p-value  
|------------------------|----------|-------|----------
| Age (years) of menarche| 12.6     | 13.0  | n.s.     
|                        | (1.4)    | (1.7) |          
| No. of pregnancies     | 3.5      | 2.8   | <.05     
|                        | (2.2)    | (1.9) |          
| Ever pregnant          | 63       | 59    | n.s.     
| Age (years) at first pregnancy | 23.7   | 25.5  | <.025    
|                        | (4.1)    | (4.6) |          
| Age (years) at menopause | 49.5   | 47.3  | <.05     
|                        | (5.3)    | (6.1) |          

*Notes: Values are expressed as means and standard deviations (in parentheses); n.s. = not significant.*

**TABLE 3. Bone-Related Problems, Behavioral Characteristics, and Family History of Osteoporosis in 66 Case and 68 Control Women**

|                        | Controls | Cases | p-value  
|------------------------|----------|-------|----------
| Back pain              | 7 (10)   | 16 (24)| <.05    
| Dowaguer hump          | 4 (6)    | 8 (12) | n.s.     
| Loss of height         | 17 (25)  | 37 (56)| <.001    
| History of nonspinal fracture | 28 (41) | 44 (67)| <.005    
| Current smoker         | 3 (4)    | 7 (11) | n.s.     
| Alcohol use (>1/week)  | 11 (16)  | 8 (12) | n.s.     
| Family history of osteoporosis | 22 (32) | 27 (41)| n.s.     
| Two or more relatives with osteoporosis | 9 (13) | 2 (3) | <.05    

*Notes: n = number with the characteristic; n.s. = not significant.*

**Discussion**

For this study, we identified weight, loss of height, age, and age at menopause as predictors of BMD. Our data corroborate earlier findings of a community-based study of postmenopausal Iowa women, in which radial BMD values were positively associated with body size and composition, including height, weight, percent body fat, and muscle mass (15). In addition, they are consistent with the results of a larger population-based epidemiological study which included more than 9,000 women from different parts of the United States (16). While the latter study also identified estrogen use as a predictor of BMD, we were unable to identify an effect of estrogen use on BMD because there was no difference in its use among our case and control women. These women were also similar in their use of dietary supplements and medications, smoking history, and alcohol consumption, suggesting that they represent a relatively homogeneous population with respect to nutritional and environmental exposures that might influence BMD. This made them an ideal cohort for studying the effect of genotype on BMD. It should be noted that the age of case and control women differed by an average of 3.8 years. Prior to considering the effect of genotype on BMD, we adjusted for age differences between the two groups, including age at menopause and age since menopause. Even given the estimated age-related bone loss, the controls would not have reached BMD measurements in the range observed in cases (17).
The type I collagen genes were chosen for analysis because type I collagen is the major protein component of the organic bone matrix; alterations in type I collagen structure or synthesis result in osteogenesis imperfecta (OI), an inherited brittle-bone disorder characterized by bone fractures, osteopenia, and an altered bone matrix. Several case reports suggest clinical overlap between OI and osteoporosis (2). In addition, an analysis of individuals with low BMD identified a COL1A1 mutation that led to a proline-to-alanine substitution in two unrelated individuals (3). Fol-
found no difference in the distribution of genotypes between cases and controls, after controlling for differences in age, weight, height, and reproductive characteristics. This does not eliminate the possibility of subtle alterations in one of the type I collagen genes in some of the case women, but suggests that there is not a common genetic background which predisposes to mutations that cause low BMD. Although we did not genotype study participants with the Spl marker, it would be difficult to explain why there would be an association between BMD and this COL1A1 marker, but not the other intragenic markers. Our data are in agreement with recent analyses that failed to demonstrate linkage of either COL1A1 or COL1A2 in seven families with familial osteopenia and low BMD (19).

We also analyzed the relationship between BMD and polymorphic sites in the VDR gene in our cases and controls because of previously published reports demonstrating a strong association between VDR genotype and BMD in Australian and British women (5,7). This relationship has become controversial, and much of the original Australian data has been retracted. As with many other studies (14,20,21), we did not identify an association between VDR genotype and BMD in postmenopausal White women from Iowa. The VDR allele frequencies in the Iowa population are similar to those reported in Australian (13) and American populations (12,14); genotype distributions exhibit linkage disequilibrium, with the VDR BsmI BB genotype being most often associated with the ApaI AA and TaqI tt genotypes, while the bb genotype is commonly found with aa and TT genotypes. Our study focused only on the relationship between VDR genotype and hip BMD. Although an association between VDR and BMD could be site specific, and therefore missed in the present study, inclusion of multiple skeletal sites did not influence the results of a large American study (14).

Recent studies suggest that the effect of VDR genotype on BMD may be age-dependent, with genotype influencing peak bone mass more dramatically than rate of bone loss (22,23). Ethnic differences among populations could also potentially impact the relationship. In support of this notion is the observation that VDR genotype modulated lumbar spine BMD in premenopausal women from Boston (22), but had no effect on this site in premenopausal women from Minnesota (23). Based on all of the available data about VDR, it seems likely that in some populations, VDR genotype is a significant factor in determining bone mass and subsequent risk for osteoporosis. The original estimate of its significance for most populations, however, appears to be unsubstantiated, even with the argument about sample size (24). Given our sample size, we calculated that an odds ratio of 3 or greater was detectable with a power of 80%.

We conclude that, for postmenopausal Iowans, VDR genotype is not a predictor of BMD and risk for osteoporosis.

It is likely that more than one gene influences bone mass, whether it be the accretion of peak bone mass, or the rate of loss with age. Although VDR, COL1A1, and COL1A2 do not predict BMD in our postmenopausal population, potential candidate genes might include those which encode other important bone matrix proteins, such as osteocalcin. In addition, as Kobayashi et al. (25) demonstrate, genetic variation at the estrogen receptor gene locus may also influence bone mass and BMD. Finally, since most epidemiological studies, including our own, identify weight or body mass index as one of the strongest predictors of BMD and subsequent risk for osteoporosis, it would seem appropriate to consider genes that determine this complex trait as well.

Figure 3. Distribution of COL1A2 genotypes in case (hatched bars) and control (darkened bars) women. The (+) signifies the presence of the PvuII and RsaI endonuclease restriction sites; the (−) signifies the absence of the restriction sites.
REFERENCES


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