

Association between Vitamin D receptor polymorphisms and the rate of bone loss in elderly women—importance of adjusting for dietary and lifestyle factors[☆]

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Abstract

The association between the restriction length polymorphisms of the Vitamin D receptor (VDR) gene and the bone mineral density (BMD) or the rate of bone loss is still under debate. In a longitudinal study of untreated postmenopausal elderly women, we evaluated the relationship between the VDR gene polymorphisms (BsmI, TaqI, ApaI, and FokI) and the rate of bone loss over a 3-year period. We also examined the effect of adjustments for dietary and lifestyle factors on these associations. Before adjustments, the rate of femoral neck bone loss was $-3.76 \pm 1.58\%$ in women with BB genotype and $0.45 \pm 0.65\%$ in women with bb genotype, which was not significantly different. Upon adjustment for dietary and lifestyle factors, statistically significant ($P = 0.03$) bone loss was observed at femoral neck in women with BB genotype ($-3.66 \pm 2.44\%$) compared to that of bb genotype ($2.39 \pm 1.32\%$). Similar results were observed with TaqI genotypes. The rates of bone loss at other skeletal sites were not different between VDR genotypes defined by BsmI and TaqI. VDR gene polymorphisms defined by ApaI and FokI were not related to the rate of bone loss.

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1. Introduction

Family and twin studies demonstrated that about 60–80% of the variance in the bone mineral density (BMD), a major contributing factor for fracture risk, is determined by genetics [1,2]. In 1994, Morrison et al. [3] proposed that 75% of the total genetic variance of BMD could be predicted by polymorphisms in the VDR gene locus. Following this initial report, the relationships between VDR gene polymorphisms and BMD, bone turnover and bone loss, have been evaluated in a plethora of studies. Studies carried out in a variety of populations have reported divergent conclusions with some investigators confirming the earlier view (reviewed in [4,5]), albeit, the effect being of lesser magnitude than reported by Morrison et al. [3], while others refuting it (reviewed in [4,5]). Two meta-analyses [6,7] revealed a modest effect of VDR genotypes on BMD.

Despite extensive research, no consensus has yet been reached regarding the association of VDR genotypes and BMD. One possible reason for the controversy that has been proposed is the differences in the confounding factors in different studies, which include ethnic background, age, calcium intake [2], caffeine intake [8], and other complex and genetic–environmental interactions. The importance of controlling for environmental and lifestyle factors to detect true genotypic effect has been reported by Deng et al. [9].

In the present study, we examined the relationship between the genotypes defined by polymorphisms for BsmI, TaqI, ApaI, and FokI in the VDR gene, and the rate of bone loss in postmenopausal elderly women, addressing the issue of adjustment for dietary and lifestyle factors in detecting the associations.

2. Materials and methods

The results discussed in this report were derived from the data of 96 postmenopausal elderly women assigned to the placebo group, and completed a 3-year randomized

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clinical trial (STOP IT: Sites Testing Osteoporosis Prevention/or Intervention) of the effect of hormone replacement with estrogen and progestin with or without calcitriol, on BMD. These women were a subset of 489 normal elderly Caucasian women aged 66–77 years initially enrolled into the study. All subjects signed an informed consent to participate in the study. The study was approved by Creighton University Institutional Review Board.

2.1. Dietary intake, smoking, and alcohol history

Dietary calcium, Vitamin D, protein, fiber, calorie, and caffeine intakes were calculated from 7-day food dairies collected at baseline, by a trained dietician with the use of the Food Processor II plus nutrition and diet analysis system (version 5.1; Esha Research, Salem, OR). Current smokers were considered as smokers while past and women who never smoked were classified as nonsmokers. Alcohol use was stratified into drinkers and nondrinkers.

2.2. Bone mineral density

Bone mineral density (g/cm^2) at the lumbar spine (L1–L4), total hip, three sites in the proximal femur (femoral neck, trochanter, and Ward's triangle), whole body and mid-radius was measured at baseline and at the end of the study using a dual-energy X-ray absorptiometry (Lunar Model DPX-L). Standardized protocols for uniform subject positioning, scan mode, and scan analysis, were used for determining the BMD. Duplicate measurements were made for hip and spine and the mean was used for the analysis. The percent change in BMD was calculated as the difference between baseline and follow-up BMD (36-month value), divided by baseline BMD, and multiplied by 100.

2.3. VDR genotyping using RFLP analysis

Genomic DNA was extracted from the patient's white blood cells by a standard, phenol/chloroform extraction procedure [10]. The DNA samples were genotyped for BsmI, TaqI, ApaI, and FokI RFLPs at the VDR gene locus as described earlier [10,11]. The RFLPs were coded as B-b, T-t, A-a, or F-f upper case lettering signifying the absence and the lower case lettering, the presence of the restriction site. The presence (t) or absence (T) of the TaqI polymorphic site is in linkage with absence (B) or presence (b), respectively, of the BsmI polymorphic site.

2.4. Statistical analysis

All analyses were done using the SPSS statistical package for Windows (version 10.0, SPSS Inc., Chicago). Baseline characteristics of the VDR genotypes defined by BsmI, TaqI, ApaI, and FokI, respectively were compared using a one-way analysis of variance (ANOVA). The categorical variables, smoking and alcohol use was compared between

genotypes using the Chi-square test. The adjusted measurements of percent change in BMD were compared between the VDR genotypes defined by BsmI, TaqI, ApaI, and FokI, respectively, using a univariate general linear model. The fixed effects in the model were baseline smoking, alcohol intake, and VDR genotypes (done separately for BsmI, TaqI, ApaI, and FokI). The common covariates in all models were the average total calcium intake, each respective baseline BMD and average caffeine intake. Other significantly correlated covariates identified from correlation analysis were included only if significant. Fisher's least significant difference (LSD) post hoc multiple comparison test was used to determine post hoc significance between the VDR genotypes. A P value less than 0.05 was considered significant and a P value less than 0.10 was considered border line significant.

3. Results

3.1. Study population characteristics (Table 1)

The distribution of VDR genotypes (BsmI, TaqI, ApaI, and FokI) in the present study population followed the Hardy–Weinberg equilibrium. No significant differences existed with regard to any of the study characteristics between the three genotypes defined by BsmI (BB, Bb, and bb), TaqI (TT, Tt, and tt), ApaI (AA, Aa, and aa), and FokI (FF, Ff, and ff), respectively (Table 1).

3.2. Bone mineral density

The unadjusted percent rate of bone loss at femoral neck was tending to be significantly ($P = 0.054$) higher in women with the BB ($-3.76 \pm 1.58\%$) compared to that of women with the bb ($0.45 \pm 0.65\%$) genotype, while the rate of bone loss was significantly ($P = 0.03$) different between tt (-3.56 ± 1.64) and TT ($0.35 \pm 0.64\%$) genotypes (Fig. 1). Upon adjustment for significantly correlated dietary and lifestyle factors, it was observed that women with the BB genotype ($-3.66 \pm 2.49\%$) lost significantly ($P = 0.027$) higher bone mass at femoral neck compared to that of women with the bb genotype ($2.39 \pm 1.32\%$). Similarly, on adjustment for significantly correlated dietary and lifestyle factors, women with the tt genotype (-4.34 ± 3.26) lost significantly ($P = 0.021$) higher bone mass at femoral neck compared to that of women with the TT genotype (2.31 ± 1.33). The difference in the rate of bone loss between the BB and bb, and tt and TT genotypes was much higher after adjustment for covariates. The unadjusted and adjusted percent BMD change at spine, total body, radius-mid, and total femur were not significantly different between the genotypes, BB-bb and TT-tt. No significant differences were noted between any of the genotypes of ApaI and FokI with regard to any of the skeletal sites measured.

Table 1
Characteristics of study population according to VDR-BsmI, TaqI, ApaI, and FokI polymorphisms

Genotype	N (%)	Age (year)	Height (cm)	Weight (kg)	Total calcium intake (mg per day)	Age at menopause (year)	Smokers ^a (%)	Alcohol drinkers ^a (%)
BB	11 (12.6)	68.3 ± 1.02	159.9 ± 1.65	69.1 ± 4.55	675 ± 101	46.5 ± 1.4	18.2	18.2
Bb	39 (44.8)	71.2 ± 0.61	159.8 ± 0.80	68.0 ± 1.90	748 ± 42	49.1 ± 1.0	12.8	38.5
bb	37 (42.5)	70.8 ± 0.56	161.7 ± 1.08	70.7 ± 2.55	855 ± 59	49.5 ± 1.0	10.8	32.4
TT	38 (43.7)	70.6 ± 0.56	161.6 ± 1.06	70.5 ± 2.49	856 ± 58	49.6 ± 0.9	10.5	31.6
Tt	38 (43.7)	71.1 ± 0.59	160.0 ± 0.82	67.4 ± 1.92	732 ± 44	48.7 ± 1.0	15.8	39.5
tt	11 (12.6)	69.2 ± 1.32	159.6 ± 1.65	71.7 ± 4.57	719 ± 97	47.0 ± 1.4	9.1	18.2
AA	26 (29.9)	70.5 ± 0.88	160.5 ± 1.11	71.9 ± 2.69	685 ± 57	48.8 ± 1.0	11.5	34.6
Aa	40 (46.0)	70.6 ± 0.49	160.0 ± 0.80	67.9 ± 2.28	831 ± 51	49.1 ± 1.0	15	32.5
aa	21 (24.1)	70.9 ± 0.79	162.0 ± 1.58	68.6 ± 2.82	821 ± 72	48.1 ± 1.3	9.5	33.3
FF	37 (41.6)	70.6 ± 0.65	160.2 ± 1.07	66.2 ± 2.30	781 ± 58	49.1 ± 1.1	16.2	37.8
Ff	38 (42.7)	70.6 ± 0.54	159.8 ± 0.95	70.6 ± 2.03	787 ± 52	49.4 ± 0.9	7.9	23.7
ff	14 (15.7)	71.5 ± 1.03	161.7 ± 1.60	70.5 ± 4.24	797 ± 70	47.7 ± 1.3	14.3	50

The values represented in the table are means ± S.E.M. Comparisons were done between genotypes using the one-way analysis of variance.

^a The distribution of smoking and alcohol use was tested within genotypes using the Chi-square statistic.

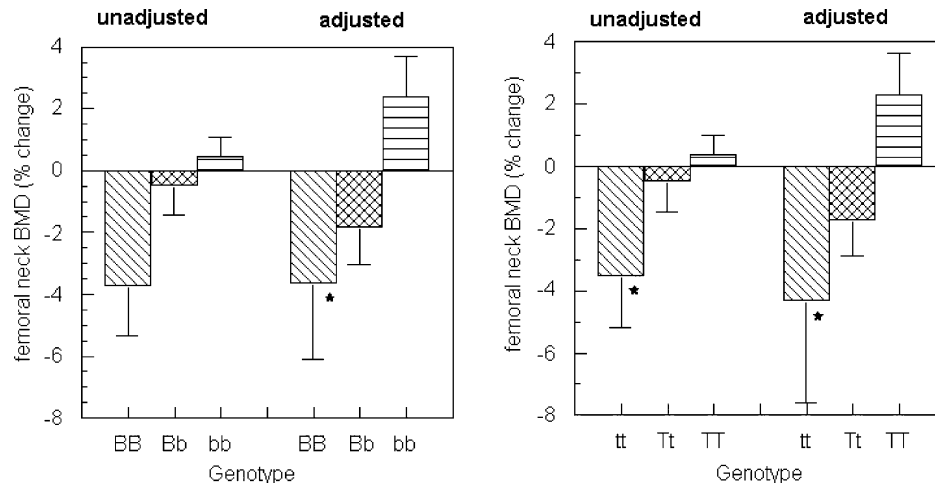


Fig. 1. Unadjusted and adjusted rates of bone loss at femoral neck in relation to VDR genotypes defined by BsmI and TaqI restriction enzymes. Unadjusted: values are means ± S.E.M. Comparison between the VDR genotypes was done by one-way analysis of variance. The LSD post hoc multiple comparison test was used to test for differences between genotypes. Adjusted: values are adjusted means ± S.E.M. Comparison between VDR genotypes was done using a GLM, adjusting for significant covariates. Multiple comparisons were done using the LSD post hoc test. * $P < 0.05$ compared with respective homozygous (BB vs. bb and TT vs. tt) genotypes.

4. Discussion

We report here an association between rate of bone loss and VDR genotypes defined by BsmI and TaqI restriction enzymes but not with ApaI and FokI genotypes in postmenopausal elderly women. Conflicting reports have been published with regard to the association of rate of bone loss with VDR genotypes (reviewed in [4,5]). In line with the positive observations reported, we found a significant association between the VDR genotypes defined by BsmI and TaqI, and the rate of bone loss at femoral neck, but not at the other skeletal sites measured. Adjustments for dietary and lifestyle factors increased the magnitude of difference in the rate of bone loss between the homozygous genetic variants (BB/tt versus bb/TT) of the VDR gene. Discrepancies

among studies in relation to the association of VDR genotypes and BMD or rate of bone loss are suggested to be caused by differences in methodology and recruitment designs, differences in dietary factors and lifestyle habits and due to heterogeneity of gene–environmental interactions. The influence of calcium intake as an environmental factor on the association of VDR genotypes and BMD has been reported [12–14]. Recently, Deng et al. [9,15] also emphasized the importance of adjusting for life style and environmental factors in studying the genotype-BMD associations and suggested that there is potential danger of missing the associations if these are not taken into account. Our results further strengthen this suggestion.

Inconsistent reports are published regarding the association of VDR genotypes defined by ApaI and BMD or the

rate of bone loss in postmenopausal women, the majority being negative [16,17]. On the other hand, VDR genotypes defined by FokI were reported to be related to BMD [18,19] and rate of bone loss [11]. In the current study, however, we did not find any significant relation between FokI, ApaI genotypes and rate of bone loss. Eccleshall et al. [20] and Ferrari et al. [21] also failed to detect a significant segregation of FokI with BMD at multiple skeletal sites.

In conclusion, our results from a longitudinal study in postmenopausal women support the contention that the rate of bone loss is associated with the VDR polymorphisms defined by restriction enzymes BsmI and TaqI. Caucasian women with the BB/tt genetic variant have a high probability of net bone loss compared to those with the bb/TT genotype. Our data also suggests that one should consider the variations in dietary and lifestyle factors in effectively detecting these associations.

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