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Vitamin D Receptor Gene *Fok*1 Polymorphism Predicts Calcium Absorption and Bone Mineral Density in Children*

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ABSTRACT

The vitamin D receptor (VDR) gene has been implicated as one of the major genetic components of osteoporosis. We evaluated the relationship between markers of mineral status and restriction fragment length polymorphisms of the VDR gene in 72 healthy children age 7–12 years. Using stable isotope techniques and dual-energy X-ray absorptiometry, we measured dietary calcium absorption, bone calcium deposition rates, and total body bone mineral density (BMD). The *Fok*1 polymorphism at the VDR translation initiation site was significantly associated with BMD ($p = 0.02$) and calcium absorption ($p = 0.04$). Children who were FF homozygotes had a mean calcium absorption that was 41.5% greater than those who were ff homozygotes and 17% greater absorption than Ff heterozygotes. BMD was 8.2% greater in the FF genotype than the ff genotype and 4.8% higher than the Ff genotype. These results suggest a substantial relationship between the VDR gene and bone metabolism at one or more levels, including dietary absorption of calcium and BMD in growing children. (J Bone Miner Res 1999;14: 740–746)

INTRODUCTION

PEAK BONE MASS in early adulthood is considered an important determinant of osteoporotic risk later in life. Acquisition of peak bone mass during childhood depends upon a variety of influences such as calcium intake, exercise, body mass, and genetic factors.[1–3] Attempts to define the genetic factors influencing bone density have largely focused on polymorphisms within the vitamin D receptor (VDR) gene, using adult, often elderly, subjects.[4] Several restriction fragment length polymorphisms have been reported for the VDR gene, including sites cleaved by *Bsm*1, *Apa*1, *Taq*1, and *Fok*1.[5–8] Previous reports have analyzed the anonymous *Bsm*1, *Apa*1, and *Taq*1 sites found within intron 8 and exon 9 of the VDR gene, either individually or by direct haplotyping. These studies have led to conflicting results, with some of these studies reporting no association of the polymorphic sites with bone mineral density (BMD),[9–13] and others finding decreased BMD associated with either the BB genotype[3,14–16] or the bb genotype.[17,18]

The *Fok*1 polymorphism, a C→T transition found within the VDR translation initiation site, creates an upstream initiation codon, resulting in VDR molecules elongated by

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three amino acids (f allele) compared with those initiating translation from the downstream site (F allele).[8,19,20] The *Fok*1 polymorphism corresponds to a single strand conformation polymorphism (M and m alleles) described by Arai et al.,[19] with the M and f alleles being equivalent. Significant associations of this polymorphism with low BMD have been described in some studies of adults,[19–21] but not in others.[22,23]

Little is known concerning the genetic factors influencing bone density in children. An association of the F (m) allele with increased BMD in children, who are actively gaining bone mass, has not been previously reported. Ferrari et al.[22] found no association of *Fok*1 genotype with BMD in a study of prepubertal Swiss girls, although their results did suggest a better bone mass response to calcium supplementation by girls with the FF genotype. They also reported cross-genotyping between the *Fok*1 and *Bsm*1 sites, which revealed lower BMD for bb/ff subjects than for those with the bb/FF genotype. When this same cohort was analyzed with respect to the *Bsm*1 polymorphism, the BB homozygotes were found to have lower BMD than the Bb or bb genotypes.[24] Other studies of the VDR genotype in relation to bone mass during childhood have been limited to the *Bsm*1 polymorphism. In a study of prepubertal American girls of Mexican descent, Sainz et al.[25] found evidence for association of the *Bsm*1 polymorphism, "bb" genotype, with increased femoral (2%) and vertebral (8%) density. Others, however, failed to find any association between VDR polymorphism and BMD or calcium accretion rates in children and young adults.[26]

To date, the effect of VDR genotype on other calcium-related phenotypes, such as absorption and bone calcium deposition, has not been addressed in children. A few absorption studies have been done in adults[16,27–29] with mixed results.

Our study addresses the question of whether VDR genotype, as determined by the *Fok*1 polymorphism, is associated with increased calcium absorption and BMD in a group of 72 healthy Caucasian, Mexican-American, and African-American children. We hypothesized that children homozygous for the F allele would have increased calcium absorption and higher BMD than ff homozygotes. Using dual tracer stable isotope techniques, we measured calcium absorption and the rate of calcium deposition into bone in children. In addition, we measured total body BMD by dual-energy X-ray absorptiometry.

MATERIALS AND METHODS

Subjects

We studied 72 children (38 Caucasian, 18 African-American, 16 Mexican-American) between 7.5 and 12 years of age living in the greater Houston area. Of these subjects, 8 were boys (Caucasian, Tanner stage 1). Data from 25 of these subjects (17 girls and 8 boys) for calcium absorption and kinetics have been reported previously.[30] We have previously demonstrated that there is no difference in calcium absorption between boys and girls in the age range reported here.[31] Furthermore, we performed statistical analyses for differences in calcium absorption, bone calcium turnover, and BMD among the various genotypes using data from all subjects, including the 8 boys, or using data from the 64 girls. No ff homozygotes were found in the African-American population studied. African-American children were excluded from some statistical analyses because of this unequal distribution among the three genotype groups. Fifty-seven prepubertal children (Tanner stage 1) age 7.5–11.9 years, 12 Tanner stage 2 children age 7.9–12.0 years, and 3 Tanner stage 3 children age 8.4–10.2 years were genotyped. Tanner stages for both breast and pubic hair were assigned by a pediatric endocrinologist as determined by physical examination. All subjects were between the 5th and 95th percentiles for weight for age and height for age. Subjects were adapted to diets containing ~1200 mg of calcium per day for at least 2 weeks prior to their initial study and maintained this intake level throughout the study period. Dietary compliance and actual intakes were determined from 24-h food records completed by the subjects and their parents for 3 days prior to admission to the Metabolic Research Unit.

This protocol was approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from all subjects and their parents or guardians.

Analysis of VDR gene polymorphisms

Genomic DNA was isolated from 3 ml of whole blood collected in EDTA-coated tubes using the Wizard™ Genomic DNA Purification Kit (Promega, Madison, WI, U.S.A.). Primers VDR2a: 5′-AGCTGGCCCTGGCACTGACTCTG CTCT-3′ and VDR2b: 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′, were used to amplify exon 2 of the VDR gene, which contains the *Fok*1 polymorphic restriction site, and have been previously described.[20,32] Briefly, the Expand™ High Fidelity PCR System and PCR Nucleotide Mix (0.2 mM each dNTP, final concentration) (Boehringer Mannheim, Indianapolis, IN, U.S.A.) were used with 1–2 μl of genomic DNA (without prior determination of concentration) in the presence of 2 mM $MgCl_2$ and 10% dimethylsulfoxide in 50-ml reactions. Expand™ Polymerase (2 U) was added to the reaction following an initial denaturation period of 3 minutes at 95°C. Thermocycling conditions were 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. A final elongation period of 5 minutes at 72°C was added after 30 cycles. PCR products were digested with *Fok*1 (Boehringer Mannheim) for 1.5–2 h at 37°C, and restriction fragments visualized by ethidium bromide staining following elecrophoresis through 2% agarose gels. Determination of VDR genotype as FF, Ff, or ff was made based on the *Fok*1 cleavage pattern, with capital letters denoting the absence, and small letters the presence, of the restriction site.

Bone mineral measurements

Bone mass was measured at each visit by dual-energy X-ray absorptiometry using a QDR 2000 W instrument

Table 1. Baseline Characteristics of Study Subjects

Genotype	*FF*	*Ff*	*ff*	p *value**
Number of subjects	30	32	10	
Age	9.3 (1.1)	9.1 (1.1)	8.6 (0.7)	0.27
Ethnicity				0.04
Caucasian	15	17	6	
Mexican-American	3	9	4	
African-American	12	6	0	
Tanner stage				0.12
1	21	6	10	
2 and 3	9	6	0	
BMI (kg/m^2)	17.9 (4.0)	16.7 (2.8)	17.1 (3.43)	0.35
Serum ALP (IU/l)	227.7 (68.8)	224.2 (40.3)	235.1 (48.8)	0.86
PTH (pg/ml)	29.3 (29.9)	31.7 (25.3)	29.8 (12.45)	0.96
25(OH)D (ng/ml)	33.4 (12.24)	36.9 (12.8)	27.24 (9.9)	0.09
Calcium intake (mg/day)	1150 (310)	1090 (290)	1080 (140)	0.57

Data are shown as mean ± SD.

*p values were determined by analysis of variance, except for ethnicity and Tanner stage, which were analyzed by χ^2 tests. Normal ranges: ALP activity, 60–415 IU/l; PTH, 18–80 pg/ml; 25(OH)D, 10–50 ng/ml.[42]

(Hologic Inc., Waltham, MA, U.S.A.) operated in total body mode. Bone mineral content and BMD were measured. The accuracy and precision (1–2%) of these measurements in children have been verified previously.[33,34]

Calcium kinetic measurements

On the day prior to the study, the calcium stable isotope tracer to be administered orally (^{46}Ca, 0.4 μg/kg) was mixed with ~6 oz of milk (containing 200 mg of Ca) and allowed to equilibrate overnight in a refrigerator. Subjects were admitted to the inpatient General Clinical Research Center at Texas Children's Hospital or the Metabolic Research Unit of the Children's Nutrition Research Center and a heparin lock catheter placed in the dorsum of their hand.

Blood was drawn (fasting) for measurement of routine chemistries, serum 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), and bone-specific alkaline phosphatase (ALP) activity. Subsequently, the subjects were given breakfast including the milk in which the isotopic tracer was equilibrated. Immediately following the breakfast meal, ^{42}Ca (0.08 mg/kg) was given by slow infusion over 5 minutes. Eight timed serum samples were drawn for calcium-stable isotope analyses over an 8-h interval after completion of the calcium isotope infusion.

Following breakfast, a complete 24-h urine sample was obtained in 8-h aliquots. At the conclusion of the 24-h period, the subjects were discharged and instructed to collect three "spot" urine samples daily (minimum two) for an additional 4 days at home.

Calcium isotope ratios were measured on all samples using a magnetic sector thermal ionization mass spectrometer (MAT 261; Finnigan, Bremen, Germany). We have shown that the isotope ratios obtained from these methods are highly accurate and precise (0.1–0.2% relative SD for accuracy and precision).[35,36]

We have described in detail our methods for determining calcium absorption and kinetic parameters.[35–38] Calcium absorption was determined by the relative urinary recovery of the oral versus the intravenous isotope during the 24-h after isotope administration.

Statistical analysis

Analysis of covariance (ANCOVA) was used to compare genotypes with respect to calcium metabolism and BMD (Minitab Inc., State College, PA, U.S.A.). Covariates included age, ethnicity, pubertal stage, and body mass index (BMI). We performed each of the analyses with and without the data from the eight male children. Because there was no difference in the results, the boys were included in the analyses presented in this report. We also performed separate analyses for prepubertal children (corrected for age, BMI, and ethnicity) and for Caucasian children (corrected for age, BMI, and pubertal status) to demonstrate that differences in calcium absorption and BMD among the genotype groups were not specific for ethnicity or pubertal status. Pair-wise comparisons among genotypes were made using Tukey's multiple comparison procedure.

RESULTS

Characteristics of the study population

Baseline characteristics for the 72 subjects genotyped are shown in Table 1. The overall prevalence of genotypes in this study was 41.6% FF, 44.5% Ff, and 13.9% ff. Genotype prevalence for Caucasians and Mexican-Americans was 33.3% FF, 48.2% Ff, and 18.5% ff. Only the FF (66.6%) and Ff (33.4%) genotypes were identified for the African-American children studied. Age, BMI, serum ALP, PTH, and 25(OH)D were not different between genotype groups, although 25(OH)D tended to be lower for the ff homozygotes ($p = 0.09$). We found no difference in p values or group mean values for calcium absorption, bone calcium

TABLE 2. ETHNIC AND PUBERTAL DIFFERENCES IN CALCIUM KINETICS AND BONE DENSITY

Ethnicity	*Caucasian*	*Mexican-American*	*African-American*	p *value*
Ca absorption (mg/day)	358 (21)	309 (34)	370 (33)	0.39
Ca Vo^{+}* (g/day)	1.68 (0.08)	1.51 (0.12)	1.74 (0.11)	0.30
BMD (g/cm^2)	0.82 (0.02)	0.79 (0.02)	0.85 (0.02)	0.08
Pubertal		*Prepubertal*	*Pubertal*	p *value*
Ca absorption (mg/day)		331 (16)	457 (38)	<0.01
Ca Vo^{+}* (g/day)		0.59 (0.06)	1.92 (0.12)	0.01
BMD (g/cm^2)		0.80 (0.01)	0.88 (0.02)	<0.01

Data are shown as mean ± SD. p values were determined by analysis of variance.
* Vo^{+} = bone calcium deposition rate as determined by stable isotope measurement.

turnover or BMD among the various genotypes whether the eight boys were included in the analyses or not.

Because the ff genotype group was not well matched to the FF and Ff groups for ethnicity and pubertal status, the influence of these variables on calcium absorption, bone calcium deposition, bone turnover, BMD, and average daily change in total body calcium was determined using ANCOVA. Table 2 shows the differences among Caucasians, African-Americans, and Mexican-Americans, and between prepubertal (Tanner stage 1) and pubertal (Tanner stage 2) children for these various parameters. Ethnic groups did not differ significantly for any of the calcium kinetic or bone density measurements (Table 2). Pubertal status, however, was associated with differences in calcium absorption, bone calcium deposition rate, and BMD (Table 2).

Calcium kinetic measurements

Analyses of genotype differences in calcium absorption and kinetics are shown in Table 3. Corrections for age, ethnicity, BMI, and pubertal status were used in the statistical model. Data are shown as adjusted means ± SD. Children with the FF genotype had 41.5% greater calcium absorption than ff homozygotes ($p = 0.04$), and 17% greater calcium absorption than the Ff heterozygotes ($p = 0.19$). Differences in absorption between the FF and Ff genotypes and between the Ff and ff groups were not significant. Mean values for age and BMI did not differ significantly between groups.

Although variations in ethnicity and pubertal status were controlled, the lack of African-Americans and Tanner stage 2–3 children in the ff group could potentially complicate interpretation of the results. To verify that the differences in calcium absorption observed between the genotype groups were not ethnicity- or puberty-specific, we performed an analysis using only the data from prepubertal (Tanner stage 1) Caucasians and Mexican-Americans (Table 3). In this analysis, calcium absorption was 52.9% greater for children with the FF genotype as compared with the ff genotype ($p = 0.02$), and 29.6% greater for the FF group as compared with the Ff heterozygotes ($p = 0.08$). Inclusion of prepubertal African-American children also gave a significant difference ($p = 0.01$; Table 3) for VDR genotypes for calcium absorption (as expected), as ethnicity was not found to influence absorption (Table 2). Similar results were seen for analysis of Caucasian children (prepubertal and pubertal combined, $p < 0.01$).

No differences among genotype groups were seen for deposition of bone calcium (Ca Vo^{+}; Table 3) when all subjects were analyzed together or when the analysis was limited to all prepubertal children or prepubertal Caucasians and Mexican-Americans.

Small differences that did not reach statistical significance ($p = 0.20$) in 24-h urinary calcium excretion were seen in the whole group based on *Fok*1 genotype. Mean (SD) urinary calcium excretion was 82 (62) mg/day in the FF group, 71 (38) mg/day in the Ff group, and 50 (34) mg/day in the ff group.

Bone densitometry

Results of total body BMD measurements are shown in Table 4. A significant relationship between BMD and VDR genotype ($p = 0.02$, adjusted for age, BMI, ethnicity, and pubertal status) was seen when all subjects were included in the analysis. FF homozygotes had 8.2% greater BMD than ff homozygotes ($p = 0.03$), and 4.8% greater BMD than Ff heterogygotes ($p = 0.08$). The significance of this association was maintained ($p = 0.03$) when all Caucasians (Tanner stages 1 and 2) were analyzed, but not when the analysis was limited to prepubertal children, regardless of ethnicity ($p = 0.12$).

Bsm*1 polymorphism*

We additionally evaluated the relationship between the *Bsm*1 polymorphism and the outcome values in this study, calcium absorption, bone calcium deposition, and total body BMD. We did not find any significant effect of the *Bsm*1 polymorphism on any of these values either in the whole population or the subgroup analysis. For total body BMD (whole group, $n = 72$), values were: BB ($n = 11$) 0.807 (0.02); Bb ($n = 34$) 0.822 (0.01); and bb ($n = 27$) 0.818 (0.01); $p = 0.86$ for group differences.

DISCUSSION

In this study, we identified a significant association of VDR genotype, based on the *Fok*1 polymorphism, with calcium absorption and BMD in children ages 7.5–12 years.

TABLE 3. VDR GENOTYPE AND CALCIUM KINETIC MEASUREMENTS

Genotype	*FF*	*Ff*	*ff*	p *value**
All subjects (Caucasian, Mexican-American, African-American; $n = 72$)				
Ca absorption (mg/day)	391 (23)	334 (22)	276 (41)	0.04‡
Ca $Vo^{+\dagger}$ (g/day)	1.63 (0.08)	1.69 (0.07)	1.63 (0.13)	0.80
All Caucasians ($n = 38$)				
Ca absorption (mg/day)	438 (31)	319 (29)	249 (55)	<0.01§
Ca $Vo^{+\dagger}$ (g/day)	1.61 (0.11)	1.71 (0.10)	1.69 (0.18)	0.87
All prepubertal (Caucasian, Mexican-American, African-American; $n = 57$)				
Ca absorption (mg/day)	387 (25)	312 (22)	256 (37)	0.04‖
Ca $Vo^{+\dagger}$ (g/day)	1.57 (0.09)	1.62 (0.08)	1.55 (0.12)	0.86
Prepubertal (Caucasian, Mexican Americans; $n = 48$)				
Ca absorption (mg/day)	393 (30)	303 (25)	257 (39)	0.02¶
Ca $Vo^{+\dagger}$ (g/day)	1.59 (0.10)	1.61 (0.09)	1.54 (0.12)	0.90

Data are shown as mean ± SD. Measurements of calcium absorption and bone calcium deposition rate (Ca $Vo^{+})^{\dagger}$ were made using dual-tracer stable isotopes. ANCOVA was done to determine differences in these measurements among the FF, Ff, and ff genotype groups.

* *p* value for FF vs. ff.

‡ Adjusted for age, BMI, ethnicity, and pubertal status.

§ Adjusted for age, BMI, and pubertal status.

‖ Adjusted for age, BMI, and ethnicity.

¶ Adjusted for age and BMI.

TABLE 4. VDR GENOTYPE AND BMD MEASUREMENTS

Genotype	*FF*	*Ff*	*ff*	p *value**
All subjects (Caucasian, Mexican-American, African-American; $n = 72$)				
BMD (g/cm^2)	0.846 (0.01)	0.807 (0.01)	0.782 (0.02)	0.02†
All Caucasians ($n = 38$)				
BMD (g/cm^2)	0.843 (0.02)	0.799 (0.01)	0.778 (0.02)	0.03‡
All prepubertal (Caucasian, Mexican-American, African-American; $n = 57$)				
BMD (g/cm^2)	0.819 (0.02)	0.799 (0.01)	0.767 (0.02)	0.12§
Prepubertal (Caucasians, Mexican-Americans; $n = 48$)				
BMD (g/cm^2)	0.814 (0.02)	0.795 (0.01)	0.767 (0.02)	0.19‖

Data are shown as mean ± SD. BMD was measured by DXA. Differences in BMD among the genotype groups were determined by ANCOVA.

* *p* value for FF vs. ff.

† Adjusted for age, BMI, ethnicity, and pubertal status.

‡ Adjusted for age, BMI, and pubertal status.

§ Adjusted for age, BMI, and ethnicity.

‖ Adjusted for age and BMI.

These are the first data demonstrating a relationship between VDR genotype and both calcium metabolism and BMD in children.

We found a significant relationship between the FF genotype and longitudinal changes in calcium absorption. Children with the FF genotype absorbed on average 115 mg more calcium per day than those with the ff genotype. We found that pubertal status, but not ethnicity, was associated with calcium absorption in our study population. The association of VDR genotype with calcium absorption was significant both when prepubertal children were analyzed separately and when all subjects were analyzed together with pubertal status included in the statistical model as a covariate.

Furthermore, we found the FF genotype to be associated with higher BMD, although only when all subjects or all Caucasian subjects were analyzed together. The decreased sample size resulting from separate analyses of pubertal and ethnic groups could explain the loss of significant association of VDR and BMD within these subpopulations. These results are consistent with previous studies reporting an association between the *Fok*1 polymorphism and BMD in Japanese,[19] Mexican-American,[20] and American Caucasian women.[21] Studies evaluating both the physical and hormonal determinants of puberty would be necessary to clarify the interaction of puberty and genetic factors in calcium absorption.

We did not find a significant difference in the bone calcium deposition rate for the 6- to 12-month period for any of the genotype groups. The kinetically determined bone

calcium deposition rate is not closely correlated with fractional calcium absorption in growing children.[39] Furthermore, the bone calcium deposition rate is closely related to early changes in pubertal development which may have obscured identifying a relationship in our study population which included such children.[30] In adults, an association was found between the ff genotype and a marker of skeletal resorption, N-telopeptide, but not other turnover markers.[23] Therefore, failure to find a relationship between the VDR *Fok*1 polymorphism and the bone calcium deposition rate is not unexpected, because vitamin D regulation of calcium metabolism is more likely related to calcium absorption. Longer term longitudinal studies of children may provide additional insights into the mechanisms by which VDR alleles affect calcium kinetics.

The prevalence of VDR alleles, as determined by the *Fok*1 polymorphism, has been determined for African-American women in one previous study.[21] We found 66.6% of the African-Americans in our study to be homozygous for the F allele and 33.4% to be Ff heterozygotes. This genotype distribution is similar to that reported by Harris et al.[21] (65% FF, 31% Ff, 4% ff). The small number of African-American children enrolled in our study (n = 18) may explain the absence of ff homozygotes and precludes extrapolation of allele prevalence observed here to the population as a whole. It is interesting to speculate, however, that higher frequencies of the more favorable FF genotype (double that found for Caucasians and Mexican-Americans in our study) might partially explain the increased BMD seen in African-Americans, if similar allele frequencies are seen with larger study populations.

Although a body of evidence generated over the past 20 years suggests that BMD and the development of osteoporosis are, at least in part, genetically determined,[40] conclusive evidence for the identification of the genetic loci involved, and the mechanisms by which BMD is modulated by genetic elements, has been lacking. Of particular controversy is the effect of the VDR gene on BMD.[41] In the present study, we evaluated these relationships in growing children, in whom the contribution of polymorphic VDR alleles is less likely to be masked by an accumulation of variable and potentially unidentifiable environmental events. By examining calcium kinetics and bone density during this dynamic period of bone development, genetic factors ultimately influencing BMD may be more evident than in older populations where bone loss is the significant factor affecting BMD.

In their study of Mexican-American girls, Sainz et al.[25] also found an association of VDR polymorphism with BMD. However, in that study the *Bsm*1 polymorphism was analyzed and measurements of calcium absorption were not performed. In addition, Ferrari et al.[22] reported a trend toward association of *Fok*1 genotype with BMD at high calcium intakes in prepubertal girls, a finding consistent with our results. They also cross-genotyped subjects for the *Fok*1 and *Bsm*1 polymorphisms and found no evidence of linkage disequilibrium between these loci. One previous study investigated the association of the VDR genotype with calcium absorption in postmenopausal women.[16] The authors reported an association of the *Bsm*1 VDR polymorphism (BB) with decreased calcium absorption, but only at calcium intakes below 300 mg/day. We did not identify a relationship with the *Bsm*1 polymorphism in this study, however.

Our finding that the VDR *Fok*1 polymorphism influences absorption of calcium at least partially explains the mechanism by which VDR genotype contributes to differences in BMD. VDR molecules encoded by the f allele initiate translation from an upstream ATG (at the site of the *Fok*1 polymorphism) and are three amino acids longer than the F allele product. Arai et al. demonstrated that the protein encoded by the F allele produced 1.7-fold greater transactivation of transcription from a promoter containing a vitamin D-responsive element than did the f allele product.[19] Consistent with these results showing functional differences between the F and f alleles in vitro, our study demonstrates the association of the F allele with increased calcium absorption and BMD in children. The nature of the relationship between the expression of the VDR genotype and calcium metabolism in children requires further studies, especially longitudinal ones that include consideration of the effects of gender, ethnicity, and pubertal development.

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REFERENCES

1. Kelly PJ, Eisman JA, Sambrook PN 1990 Interaction of genetic and environmental influences on peak bone density. Osteoporos Int **1:**56–60.
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S 1987 Genetic determinants of bone mass in adults. J Clin Invest **80:**706–710.
3. Kelly PJ, Morrison NA, Sambrook PN, Nguyen TV, Eisman JA 1995 Genetic influences on bone turnover, bone density and fracture. Eur J Endocrinol **133:**265–271.
4. Cooper GS, Umbach DM 1996 Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res **11:**1841–1849.
5. Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA 1991 Genetic factors in bone turnover. J Clin Endocrinol Metab **72:**808–813.
6. Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW 1988 Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA **85:**3294–3298.
7. Saijo T, Ito M, Takeda E, Huq AH, Naito E, Yokota I, Sone T, Pike JW, Kuroda Y 1991 A unique mutation in the vitamin D receptor gene in three Japanese with vitamin D–dependent rickets type II: Utility of single-strand conformation polymorphism analysis for heterozygous carrier detection. Am J Hum Genet **49:**668–673.
8. Gross C, Eccleshall TR, Feldman D 1996 Vitamin D receptor gene alleles and osteoporosis. In: Bilezikian JP, Raisz LG, Ro-

dan GA (eds.) Principles of Bone Biology, 1st Ed. Academic Press, San Diego, CA, U.S.A., pp. 917–933.
9. Vandevyver C, Wylin T, Cassiman J-J, Raus J, Geusens P 1997 Influence of the vitamin D receptor gene alleles on bone mineral density in postmenopausal and osteoporotic women. J Bone Miner Res **12:**241–247.
10. Garnero P, Borel O, Sornay-Rendu E, Arlot ME, Delmas PD 1996 Vitamin D receptor gene polymorphisms are not related to bone turnover, rate of bone loss, and bone mass in postmenopausal women: The OFELY study. J Bone Miner Res **11:**827–834.
11. McClure L, Eccleshall TR, Gross C, Villa ML, Lin N, Ramaswamy V, Kohlmeier L, Kelsey JL, Marcus R, Feldman D 1997 Vitamin D receptor polymorphisms, bone mineral density, and bone metabolism in postmenopausal Mexican-American women. J Bone Miner Res **12:**234–240.
12. Spotila LD, Caminis J, Johnston R, Shimoya KS, O'Connor MP, Prockop DJ, Tenenhouse A, Tenenhouse HS 1996 Vitamin D receptor genotype is not associated with bone mineral density in three ethnic/regional groups. Calcif Tissue Int **59:**235–237.
13. Willing M, Sowers M, Aron D, Clark MK, Burns T, Bunten C, Crutchfield M, D'Agostino D, Jannausch M 1998 Bone mineral density and its change in white women: Estrogen and vitamin D receptor genotypes and their interaction. J Bone Miner Res **13:**695–705.
14. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen V, Sambrook PN, Eisman JA 1994 Prediction of bone density from vitamin D receptor alleles. Nature **367:**284–287.
15. Riggs BL, Nguyen TV, Melton LJ, Morrison NA, O'Fallon WM, Kelly PJ, Egan KS, Sambrook PN, Muhs JM, Eisman JA 1995 The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J Bone Miner Res **10:**991–996.
16. Dawson-Hughes B, Harris SS, Finneran S 1995 Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. J Clin Endocrinol Metab **80:**3657–3661.
17. Uitterlinden AG, Pols HA, Burger H, Huang Q, Van Daele PLA, Van Duijn CM, Hofman A, Birkenhager JC, Van Leeuwen JPTM 1996 A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. J Bone Miner Res **11:**1241–1248.
18. Houston LA, Grant SFA, Reid DM, Ralston SH 1996 Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fractures: Studies in a UK population. Bone **18:**249–252.
19. Arai H, Miyamoto K-I, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E 1997 A vitamin D receptor gene polymorphism in the translation initiation codon: Effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res **12:**915–921.
20. Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D 1996 The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res **11:**1850–1855.
21. Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D 1997 The vitamin D receptor start codon polymorphism (*Fok*1*)* and bone mineral density in premenopausal American black and white women. J Bone Miner Res **12:**1043–1048.
22. Ferrari S, Rizzoli R, Manen D, Slosman D, Bonjour J-P 1998 Vitamin D receptor gene start codon polymorphisms (*Fok*1) and bone mineral density: Interaction with age, dietary calcium, and 3′-end region polymorphisms. J Bone Miner Res **13:**925–930.
23. Eccleshall TR, Garnero P, Gross C, Delmas PD, Feldman D 1998 Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premenopausal French women: The OFELY study. J Bone Miner Res **13:**31–35.
24. Ferrari S, Rizzoli R, Slosman D, Bonjour J-P 1998 Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? J Bone Miner Res **13:**363–370.
25. Sainz J, VanTornout JM, Loro L, Sayre J, Roe TF, Gilsanz V 1997 Vitamin D-receptor gene polymorphisms and bone mineral density in prepubertal American girls of Mexican descent. N Engl J Med **337:**77–82.
26. Gunnes M, Berg JP, Halse J, Lehmann EJ 1997 Lack of relationship between vitamin D receptor genotype and forearm bone gain in healthy children, adolescents, and young adults. J Clin Endocrinol Metab **82:**851–855.
27. Kinyamu HK, Gallagher JA, Knezetic JA, DeLuca HF, Prahl JM, Lanspa SJ 1997 Effect of vitamin D receptor genotypes on calcium absorption, duodenal vitamin D receptor concentration, and serum 1,25-dihydroxyvitamin D levels in normal women. Calcif Tissue Int **60:**491–495.
28. Zmuda JM, Cauley JA, Danielson ME, Wolf RL, Ferrel RE 1997 Vitamin D-receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. J Bone Miner Res **12:**1446–1452.
29. Kinyamu HK, Gallagher JA, Prahl JM, DeLuca HF, Petranick KM, Lanspa SJ 1997 Association between intestinal vitamin D receptor, calcium absorption, and serum 1,25-dihydroxyvitamin D in normal young and elderly women. J Bone Miner Res **12:**922–928.
30. Abrams SA, O'Brien KO, Stuff JE 1996 Changes in calcium kinetics associated with menarche. J Clin Endocrinol Metab **81:**2017–2020.
31. Abrams SA, Grusak MA, Stuff J, O'Brien KO 1997 Calcium balance in 9–14-y-old children. Am J Clin Nutr **66:**1172–1177.
32. Hughes MR, Malloy PJ, Kieback DK, Kesterson RA, Pike JW, Feldman D, O'Malley BW 1988 Point mutations in the vitamin D receptor gene associated with hypocalcemia in rickets. Science **242:**1702–1705.
33. Ellis KJ, Shypailo RJ, Pratt JA, Pond WG 1993 Accuracy of DXA-based body composition measurements for pediatric studies. Basic Life Sci **60:**153–156.
34. Ellis KJ, Shypailo RJ, Pratt JA, Pond WG 1994 Accuracy of dual energy x-ray absorptiometry for body composition measurements in children. Am J Clin Nutr **60:**660–665.
35. Abrams SA 1993 Pubertal changes in calcium kinetics in girls assessed using ^{42}Ca. Pediatr Res **34:**455–459.
36. Abrams SA, Stuff JE 1994 Calcium metabolism in girls: Current dietary patterns lead to low rates of calcium absorption and retention during puberty. Am J Clin Nutr **60:**739–745.
37. Abrams SA, Sidbury JB, Muenzer J, Esteban NV, Vieira NE, Yergey AL 1991 Stable isotopic measurements of endogenous fecal calcium excretion in children. J Pediatr Gastroenterol Nutr **12:**469–473.
38. Abrams SA, Yergey AL, Heaney RP 1994 Relationship between balance and dual tracer isotopic measurements of calcium absorption and excretion. J Clin Endocrinol Metab **79:**965–969.
39. Bronner F, Abrams SA 1998 Development and regulation of calcium metabolism in healthy girls. J Nutr **128:**1474–1480.
40. Kelly PJ, Nguyen TV, Hopper J, Pocock NA, Sambrook PN, Eisman JA 1993 Changes in axial bone density with age. J Bone Miner Res **8:**11–17.
41. Civitelli R, Ziambararas K 1998 Does vitamin D receptor gene polymorphism affect bone mineral density and calcium absorption? Curr Opin Gastroenterol **14:**164–172.

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