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Review Article

Vitamin D, Genetic Polymorphism and Bone Health in African Americans

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Abstract

Vitamin D deficiency has been associated with many skeletal and extra skeletal diseases such as bone disease, several types of cancers, cardiovascular disease, diabetes and autoimmune disorders. Many of these chronic diseases are disproportionately distributed in our population, with African Americans being more affected than other races/ethnicities. Differences in 25-hydroxyvitamin D concentration among racial/ethnic groups have been suspected to be one of the sources of these health disparities. Darker skin pigmentation has been identified as interfering with UVB radiation of the sun resulting in lower vitamin D synthesis in the skin. In addition, presence of genetic polymorphism in vitamin D-binding proteins and vitamin D receptors that have different affinities for vitamin D may explain differences seen in vitamin D levels between ethnicities. Circulating levels of vitamin D are also predicted by season of blood draw, smoking, sex, age and body mass index which is independent of ethnicity. Regardless of vitamin D levels, African Americans have higher bone mineral density and lower fracture risks than other ethnicities/races. In this review, several clinical trials have been analyzed in order to explain vitamin D deficiency, genetic polymorphism and bone health in African Americans compared to other races.

Keywords: Vitamin D; Genetic polymorphism; Vitamin D binding protein; Bone mineral density; African Americans

Abbreviations

BMD: Bone Mineral Density; VDR: Vitamin D Receptor; SNP: Single Nucleotide Polymorphism; SCP: Start Codon Polymorphism; BMI: Body Mass Index; BMC: Bone Mineral Composition; PCR: Polymerase Chain Reaction; IRAS: Insulin Resistance Atherosclerosis Study; DBP: Vitamin D Binding Protein; (25[OH] D): 25-hydroxyvitamin D; ($1,25[OH]_2D$): 1,25-dihydroxyvitamin D; SCCS: Southern Community Cohort Study; SD: Standard Deviation; DEXA: Dual Energy X-ray Absorptiometry; HANDLS: Healthy Aging in Neighborhoods of Diversity across the Life Span; PTH: Parathyroid Hormone; GWAS: Genome Wide Association Studies; WAA: West African Ancestry

Introduction

Vitamin D refers to a group of fat soluble structures that are responsible for enhancing the absorption of calcium, magnesium, iron, phosphate and zinc from the intestines. The two important compounds in humans are Vitamin D2 also referred to as ergocalciferol and vitamin D3 also known as cholecalciferol. Both compounds can be obtained from diet and supplements. However, diet and supplements are not considered a major source of vitamin D. Synthesis of vitamin D in the skin which is dependent on exposure to the UVB radiation of the sun is the major source of this vitamin. Regardless of the source, vitamin D is biologically inactive when it enters the body and requires enzymatic hydroxylation in the liver and kidney to become active [1,2]. The first hydroxylation will yield 25-hydroxyvitamin D which is the major circulating metabolite and is a marker of the vitamin D status in the body. The final hydroxylation will yield 1,25-dihydroxyvitamin D, which is the more biologically active metabolite [3].

Other than playing an important role in absorption of calcium, vitamin D regulates over 900 genes that are responsible in various physiologic functions in the body. Vitamin D deficiency has been recognized as a major public health issue that has been linked to many skeletal related diseases such as osteomalacia and osteoporosis and extra skeletal disorders such as cancer, diabetes and cardiovascular disease [4,5].

African Americans have higher bone mineral density and are therefore at lower risk for fractures than Caucasians [6]. However, they have been identified as having a higher deficiency in Vitamin D levels than Caucasians even when they live in sunlight intense southern and southwestern states or who are taking higher dietary vitamin D intake than the recommended daily allowance or greater [7]. While, higher amount of melanin in skin which corresponds to darker skin pigmentation can effect vitamin D synthesis, circulating levels of 25-hydroxyvitamin D are predicted by season of blood draw, smoking, sex, age and body mass index that are independent of race/ethnicity [7,8].

Elevated levels of parathyroid hormone, is considered a sensitive marker of vitamin D deficiency which is more common in African Americans than Caucasians. African Americans have lower levels of total 25-hydroxyvitamin D and vitamin D binding protein than their Caucasian counterparts. Genetic polymorphism in the vitamin D-binding protein gene produces variant proteins that have different affinity for vitamin D. The prevalence of this polymorphism differs between racial groups [9]. In this review several clinical trials have been analyzed in order to better explain the connection between

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vitamin D deficiency, genetic polymorphism and the bone health in African Americans.

Methods

A Pub Med search (1966-May 2016) was conducted using the following MeSH terms: African Americans; Vitamin D; Vitamin D Binding protein; and Polymorphism, genetic. A free-text search was conducted using the same search terms. An International Pharmaceutical Abstracts (IPA) search was conducted using the terms vitamin D, vitamin D receptor, polymorphism and African American (1970-May 2016). A Cochrane Central database search was conducted with the following terms: African Americans, Vitamin D, Vitamin D-Binding Protein and Polymorphism, Genetic. A total of 46 articles were identified. Articles were included if the patients were African American, were clinical studies and included information related to genetic polymorphism associated with vitamin D or vitamin D binding protein. A bibliographic search was conducted as well. A total of 12 articles were identified for further analysis. Study quality was assessed by National Institutes of Health Quality Assessment of Controlled Intervention Studies was ranked by good, fair or poor [10,11].

Clinical trial analysis

A cross-sectional study was conducted by Harris, et al. to compare *Fok*I genotype distribution to race, bone mineral density (BMD) and another vitamin D receptor (VDR) polymorphism, *Bsm*I. The FokI single nucleotide polymorphism (SNP) is defined by the presence or absence of a second start codon for translation of the vitamin D receptor (VDR) and genotypes are denoted as follows: homozygous subjects with both start codons are ff, homozygous subjects with only the second start codons are FF and heterozygous subjects are Ff. Inclusion in this study required that subjects be black or white American women, premenopausal, between 20 and 40 years old and in good general health. BMD of the total body, femoral neck and lumbar spine (L2-L4) was assessed. There were 154 women included in this analysis: 72 black women and 82 white women. The FF genotype was most common among black women (n=47; 65%), followed by Ff (n=22; 31%) and ff (n=3; 4%). However, the Ff genotype was most common for white women (n=37; 45%), followed by FF (n=30; 37%) and ff (n=15; 18%). The mean age was 30.5±5.9 years for the FF genotype group, 28.9±5.5 years for Ff and 30.7±5.6 years for ff (P=0.231). Total body BMD was significantly lower for white patients compared to black patients (1.230±0.076 g/cm^2 compared to 1.161±0.075, difference = 0.068, 95% CI; 0.042-0.095). *Bsm*I genotypes were found to be similarly distributed between the 2 original groups. Women with the BB genotype had lower BMD of the femoral neck and total body than those with the bb genotype. In the final sample population of this study, there was no statistical significance between the mean age, height, weight and calcium intake across the different genotypes. The one exception was an increased weight in the ff group of white women. Average dietary calcium intake was 701±365 mg/day, 700±306 mg/day and 802±327 mg/day, respectively (P=0.510). A much higher percentage of white women than black women were homozygous ff (18% compared to 4%), while more black patients had the FF genotype (65% vs 50%) (P< 0.001). Black patients had higher femoral neck BMD scores across the various genotypes (p=0.001). When both black and white women were pooled and adjustments were made for race, weight and age, a statistical difference was detected in the BMD of the femoral neck. Patients with FF genotype had higher BMD at the femoral neck (1.08±0.01) compared to Ff (1.03±0.02) and ff (1.00±0.03; P=0.015). Those who were homozygous ff had 2.9% lower BMD scores than heterozygous Ff and 7.4% less than homozygous FF. The same patterns were evident for the lumbar spine and total body, but no statistical significance was detected. No differences in plasma $1,25(OH)_2D$, serum parathyroid hormone (PTH) and serum osteocalcin were observed between the genotypes. When genotype, age and weight were adjusted for, the difference in BMD at the femoral neck between black and white women decreased considerably (0.069±0.023 to 0.045±0.024; P=0.015). Relationships between start codon polymorphism (SCP) and *Bsm*I were analyzed for the entire sample as well black and white women separately. SCP and *Bsm*I were not found to be independent for the group as a whole or for white women considered separately. African American patients have higher BMD levels at the femoral neck region. The author's concluded that SCP polymorphism appears to have an effect on peak bone density, specifically at the femoral neck [12].

A cross-sectional cohort analysis by Zmuda, et al. examined the effect that polymorphisms in the VDR gene have on bone mass and rate of bone loss in African American women over 2 years. The cohort of African American women (n=101) were at least 65 years old, able to ambulate independently and had no history of bilateral hip replacement. One hundred and sixty-five women completed the initial evaluation and 113 returned for the second examination. Associations between the *Bsm*I, *Apa*I and *Taq*I VDR gene polymorphisms, bone mass and the rate of bone loss were evaluated. Genotypes are identified as follows: lowercase letters denote the presence of the restriction enzyme site; conversely, absence of the restriction enzyme site is denoted by an uppercase letter. The frequency distribution of the various genes was reported as follows: B=0.36, b=0.64; A=0.67, a=0.33; T=0.58, t=0.42. As observed in previous studies involving Caucasian and Japanese women, a significant association between b and T alleles was found (n=76 and n=86, respectively; P< 0.001). Further analyses were adjusted for age and body weight due to differences noted between genotypes. No association was found between BMD or broadband ultrasound attenuation (BUA) for any site or any polymorphism. For the oldest group of women (≥ 70 years old), there was a significant correlation between genotype and bone mass. Patients with the tt genotype had five times more bone loss than those with the (*Taq*I) Tt genotype at the hip (P=0.04). Heterozygous women experienced intermediate bone loss; less than tt, but more than TT. At the *Bsm*I site, women who were heterozygous (*Bsm*I) Bb experienced the most bone loss (P=0.004). The change in BMD per year was not found to be significantly different for any of the genotypes. No statistical significance was found when calculations were further adjusted for baseline BMD, baseline body weight or weight change. A 14% difference was noted in calcium absorption between the BB and bb genotypes; BB subjects had less absorption than those who were bb (P=0.08). No associations of this kind were found with the *Apa*I polymorphism. In this study, polymorphisms at the VDR gene (e.g., BsmI, ApaI and TaqI) were not associated with BMD or bone turnover in this population of African American women [13].

A cross-sectional cohort study to analyze how this polymorphism

Table 1: Summary of the clinical trials.

Ref.	Design	Quality	Objective	Poly-morphism	Demographics	Endpoint Results	Conclusion
[12]	Cross-sectional study	Poor	Compare *Fok*I genotype distribution to race, BMD, and another VDR polymorphism, *Bsm*I. *Fok*I genotypes	FokI, BsmI genotypes for the VDR gene	Patients: 154 women included in this analysis; 72 black and 82 white women. The FF genotype was most common among black women (n=47; 65%), followed by Ff (n=22; 31%), and ff (n=3; 4%). However, the Ff genotype was most common for white women (n=37; 45%), followed by FF (n=30; 37%), and ff (n=15; 18%). The mean age was 30.5 ± 5.9 years for the FF genotype group, 28.9 ± 5.5 years for Ff, and 30.7 ± 5.6 years for ff (P = 0.231).	Black and white women were pooled and adjustments were made for race, weight, and age, a statistical difference was detected in the BMD of the femoral neck (*P* = 0.001). Patients with FF genotype has higher BMD at the femoral neck (1.08±0.01) compared to FF (1.03±0.02), and ff (1.00± 0.03) (*P* = 0.015). Those who were homozygous ff had 2.9% less BMD than heterozygous Ff and 7.4% less than homozygous FF.	SCP genotype may influence BMD and helps to explain why African Americans, who typically have lower vitamin D levels than white patients, consistently have higher BMD.
[13]	Cross sectional cohort analysis	Good	Examine the associations between the BsmI, ApaI, and TaqI VDR gene polymorphisms and bone mass and the rate of bone loss in a sample of community-dwelling African-American women aged 65 years and older	BsmI polymorphisms	Patients: n=113 Lower case letters indicate no polymorphisms. The frequency distribution of the various genes was reported as follows: B = 0.36, b = 0.64; A = 0.67, a = 0.33; T = 0.58, t = 0.42	Patients with the tt genotype had five times more bone loss than those with the Tt genotype at the hip (P = 0.04). At the BsmI site, women who were heterozygous Bb experienced the most bone loss (P = 0.004). The change in BMD per year was not found to be significantly different for any of the genotypes. No statistical significance was found when calculations were further adjusted for baseline BMD, baseline body weight, or weight change.	Polymorphisms at the VDR gene (e.g., BsmI, ApaI, and Taq I) are not associated with BMD or bone turnover in this population of African American women.
[14]	Cohort of African American women	Good	To examine further the associations between the *VDR* start codon polymorphism and several markers of osteoporotic risk in community-dwelling older African-American women	Patients (n=104) were genotyped for FokI SNP. Genotypes were defined as FF, Ff, and ff; homozygous: FF denotes absence of the restriction site and homozygous ff represents presence of the restriction site.	The frequencies of the F allele and f allele were 78.4% and 21.6%; respectively.	FF was the most frequent genotype (n=65; 62.5%), followed by Ff (n=33; 31.7%), and ff (n=6; 5.8%). Low prevalence of the ff genotype is consistent with previous findings in African Americans. Total hip BMD was 0.87 g/cm^2 (SD = 0.13) for patients with the FF genotype, 0.86 g/cm^2 (SD = 0.17) for Ff patients, and 0.83 g/cm^2 (SD = 0.08) for the ff group (*P* = 0.69).	No evidence was identified to verify that *Fok*I genotype had any effect on bone turnover or osteoporosis risk. Although African Americans were more likely to have variations in the VDR, this did not appear to have any observable clinical effect on BMD.
[15]	Cross-sectional cohort analysis	Poor	Determine if there is any correlation between variants in the VDR gene and BMD or BMC.	Investigators compared the *Bsm*I gene variation patterns between African American and white patients.	Recruitment for this sub study was closed after 50 mother-child pairs were identified. From this group, genotype and bone mass data were collected on a total of 43 mothers and 41 children. Of the mothers, 19 were African American and 24 were white; all were premenopausal. Sixteen of the children were African American and 25 were white; all were prepubertal. The mean maternal age was 38.2 years old with no significant difference between groups.	Heterozygous samples were labeled as “Bb” and produced both an intact product as well as restriction fragments. Outcomes of this study indicated that there was a significant difference in VDR genotype distribution according to ethnicity (p=0.01 mothers and p=0.02 children). No African Americans included in this study (either mothers or children) were BB homozygous; however, they were nearly equally split between the Bb and bb genotypes, either heterozygous or homozygous for the polymorphism. Among the adults, 10 African Americans had the Bb genotype (53%) and 9 had the bb genotype (47%). There were 6 white adults with the BB genotype (25%), 7 with Bb (29%), and 11 with bb (46%). Eight African American children were Bb (50%), and the other 8 were bb. White children were 24% BB (n=6), 56% Bb (n=14), and 20% bb (n=5). A correlation was shown between bone mass and genotype for white mothers; however, this association was not found for white children, African American adults, or African American children (values reported were not separated by ethnicity).	There may be a difference in the genetic polymorphism variations between African Americans and white Americans. Additionally, it appears that African American patients seem to have a much lower prevalence of the genotype that result in lower BMC and BMD.
[3]	Cross-sectional cohort analysis	Good	Examine differences in vitamin D levels and genotypes between African and Hispanic Americans	SNPs associated with DBP, VDR, and CYP27B1 were evaluated between Hispanics and African Americans.	A total of 504 San Antonio Hispanics, 513 San Luis Valley Hispanics, and 513 Los Angeles African Americans were included in this study. Females comprised 59%, 56%, and 58% of participants and median age for each group was 38.0, 39.0, and 40.0 years respectively. BMI was significantly different between treatment center groups (P< 0.001): median 29.2, 26.4, and 29.8, respectively.	Linkage disequilibrium for two SNPs in the vitamin D binding protein (DPB) were calculated (r^2 = 0.31, 0.26, and 0.03 in San Antonio Hispanics, San Luis Valley Hispanics, and Los Angeles African Americans, respectively [*P*< 0.001 for all]). After adjusting for gender and age, these 2 SNPs were found to be significantly associated with 25-hydroxyvitamin D, as well as one SNP in the VDR. Further, one SNP in the DPB was associated with 1,25-dihydroxyvitamin D. Variation in the first DBP SNP (rs4588) was associated with an increase in serum 25-hydoxyvitamin D for all groups, whereas variation in the second (rs7041) was associated with a decrease in 25-hydroxyvitamin D for all groups. Variation in the VDR SNP (rs10783219) was associated with decreased serum 25-hydroxyvitamin D levels in San Antonio Hispanics, but was not significant for the other groups.	This finding is in agreement with other studies which have also found rs4588 in the DBP to be significantly associated with vitamin D level.

[16]	Cross-sectional cohort analysis	Poor	Investigate whether and to what extent genetic polymorphisms in GC, CYP27B1, CYP24A1, CYP2R1, and VDR determine 25(OH)D levels in African Americans and Caucasians; and whether polymorphisms in these genes mediate the association between African ancestry and 25(OH)D levels among African Americans that we previously reported.	SNPs in the group-specific complement gene (GC; associated with the vitamin D binding protein), CYP27B1, CYP24A, CYP2R1, and vitamin D receptor (VDR) were analyzed	A total of 792 patients were randomized, 22 to each group. Groups were determined by sex, self-reported race (African American or Non-Hispanic white), smoking status (current, former, never), and body mass index (BMI; 18 – 24.99, 25 – 29.99, 30 – 45). Both groups were 50.7% male, and the mean ages were 51.9 (SD = 9.0) and 54.1 years (SD = 9.5) for African Americans and Caucasians, respectively. Mean percentage African ancestry for the African American group was 0.929 (range: 0.505 – 0.999) and 0.009 (range: 0.001 – 0.171) for the Caucasian group. Mean percentage European ancestry was 0.071 (range: 0.001-.0495) and 0.991 (range: 0.829-0.999, respectively).	Three different SNPs were found to be significantly associated with 25-hydroxyvitamin D levels in African Americans. Two were located in the gene coding region for vitamin D binding protein (DBP), and one was located in CYP27B1 (rs10877012; P = 0.02). The two contained in GC were rs2298849 and rs2282679 (P = 0.005 and P = 0.03, respectively); they were not in linkage disequilibrium (r^2 = 0.01 in African Americans and 0.08 in Caucasians). Together, variation in these SNPs was linked to a difference of 2.1 to 3.6 ng/mL in serum 25-hydroxyvitamin D levels. Overall, genotype accounted for 28.5% of the variation in vitamin D levels when added to a linear regression model including dietary vitamin D intake, UVR score, age, BMI, smoking, sex, and percentage of African American ancestry.	Combined with information from other studies, SNPs account for less than 5% of variation in 25-hydroxyvitamin D levels, but genetic associations within the vitamin D pathway warrant further investigation.
[17]	RCT All patients were supplemented with calcium (Tums tablets) in order to achieve a daily intake of 1,000 mg beginning 1 month before randomization. Women in the treatment group received 1,000 IU of vitamin D3 daily, while the control group received a matching placebo; both were to be taken in the morning.	Good	Determine the effect of genetic polymorphisms in the vitamin D receptor (VDR) on vitamin D supplementation and bone density.	Five known SNPs were genotyped, including *Apa*I, *Taq*I, *Bsm*I, *Fok*I, and *Cdx*-2.	Patients: 127; and 103 were included in the analysis The mean ages of the 2 groups were 62.3±8.5 years and 61.2±7.6 years for the intervention and control groups, respectively. Years since onset of menopause were 17.8±23.1 years and 19.3±21.3 years. Calculated BMI for each group was 31.2±6.3 and 31.6±5.7.	SNPs *Apa*I, *Taq*I, and *Fok*I were found to be in Hardy-Weinberg equilibrium (estimates alleles in a sample), whereas *Bsm*I and *Cdx*-2 were not. However, the only SNP that displayed an association with vitamin D supplementation was *Fok*I. Patients were classified as FF, Ff, or ff, with the following distribution: 60 % FF (n = 47), 38% Ff (n = 29), and 2% ff (n = 2). The FF group increased in serum 25(OH)D from a mean of 12±4 to 20±10 ng/mL and from 15±8 to 26±8 ng/mL in the Ff/ff group. Each group experienced approximately a 35% increase in serum 25-hydroxyvitamin D levels. No significant changes were seen in the placebo group. There was a significant difference between the FF and Ff/ff groups in maintenance of BMD at the femoral hip (P = 0.009). Women in the FF group who received placebo lost approximately 1.4% BMD compared to the group supplemented with vitamin D who had -0.17% bone loss at this site (P = 0.02, n = 27). When comparing femoral neck BMD change between FF and Ff groups, the results were also significant (P = 0.009).	Vitamin D supplementation had a significant and sustainable effect on serum vitamin D levels. This did not appear to have a significant effect on BMD, except in a subgroup of women who had the FF genotype. In these women, BMD at the femoral neck was maintained at a higher rate than those with either the Ff or ff genotype.
[9]	Cross-sectional cohort analysis	Good	Determine whether vitamin D–binding protein genotypes and concentrations of circulating vitamin D–binding protein differ between black Americans and white Americans.	Two common SNPs, rs4588 and rs7041 were evaluated.	In total, 1181 black patients and 904 white patients were included in the final results of this study with an average age of about 48 years old (range 30-64 years) and average BMI of 29.6.	a variant allele at either location was associated with lower levels of total 25-hydroxyvitamin D. Black patients were more likely than white patients to have a variation at rs7041, while white patients were more likely to have the reference allele at this location (P< 0.001 for both associations). At rs4588, however, white patients were more likely to have the variant than black patients (P < 0.001). Overall, this means that a variation at either location was associated with a decrease in total vitamin D levels. Gc1F homozygous was predictive of the lowest levels of vitamin D-binding protein.	In conclusion, low levels of total 25-hydroxyvitamin D levels may not correlate to true vitamin D deficiency when levels of DBP are low in black Americans.
[7]	Cross-sectional cohort study	Good	Investigate if 39 SNPs in eight vitamin D pathway genes were associated with serum 25(OH)D con- centrations in AAs and EAs.	A total of 39 SNPs in 8 vitamin D pathway genes were evaluated to determine the association between the genes and serum 25-hydroxyvitamin D concentrations between AAs and EAs. Genetic analysis was conducted on 30 SNPs in 8 different vitamin D-associated genes: GC, DHCR7/NADSYN1, VDR, CYP2R1, CYP27A1, CYP27B1, CYP3A4, and CYP24A1.	In total, 652 African American men and 405 European American men were selected for inclusion. The average ages of the participants were 59.0 years (SD = 10.0) and 60.9 (SD = 8.4) years for African American and European Americans, respectively (P = 0.001). Mean vitamin D levels differed significantly: 47.2 nmol/L African American vs. 64.9 nmol/L European American (P< 0.001).	A strong correlation between SNP and 25-hydroxyvitamin D levels was found in 2 of the CYP2R1 SNPs (rs12794714 and rs10741657; P = 0.01). Logistic regression analysis of vitamin D deficiency reinforced this association and found that rs12794714 had the strongest association with vitamin D deficiency (P = 0.003, OR = 0.72, 95% CI; 1.20-2.47). SNP in GC (rs115563) had significant—though weak—correlations to serum 25-hydroxyvitamin D levels (P = 0.048). In HCR7/NADSYN1, rs12800438 was identified as significantly associated with vitamin D deficiency in African Americans (P = 0.04, OR = 0.76, 95% CI; 0.58-0.99), but was not found to be associated with 25-hydroxyvitamin D in regression analysis.	Genetic variation accounted for a small amount of variation in 25-hydroxyvitamin D; however, when combined with environmental and biological factors it was able to account for more of the 25-hydroxyvitamin D variation in European Americans as compared to African Americans (28.2 % vs 19.1%, respectively; R^2 = 0.241 after adjustment for age, total vitamin D intake, season of blood draw, BMI, and UVR exposure).

[18]	Cohort analysis based on Health, Aging, and Body Composition (Health ABC) study (n=3075) or the Multi-Ethnic Study of Atherosclerosis (MESA; n=1198). Only included African Americans from Health ABC study (N=1281)	Good	Investigate whether certain environmental and genetic factors are predictors of circulating 25(OH) D in 989 elderly African Americans participating in the Health, Aging, and Body Composition (Health ABC) Study	SNPs in or near cytochrome P450, family 2 subfamily R, polypeptide 1 (CYP2R1), group-specific component (vitamin D binding protein) (GC) and 7-dehydrocholesterol-reductase (DHCR7/ NAD synthetase 1 (NADSYN1).	Serum 25 (OH), D, (mg/L): 20.7 ±9 Mean age: 74.5±2.9	Ten SNPs were chosen for analysis based on data from prior genome-wide association studies (GWAS). Only 8 of these 10 were documented in the Health ABC study. None showed a statistically significant association with serum 25-hydroxyvitamin D (P> 0.05 for all comparisons). SNP rs7041, associated with the vitamin D binding protein, was used for further analysis because it had the P value nearest to significance (P = 0.08). Participants with the rs7041 GG or GT alleles who took multivitamins were found have higher increases in serum 25-hydroxyvitamin D than those with the TT alleles (29.0 vs 25.3 mcg/L; P = 0.002). When adjustment was made for age, study site, gender, season of blood draw, and principle components, serum 25-hydroxyvitamin D was still higher in patients with GG or GT alleles who took multivitamins (P = 0.04). This effect was only significant for patients who took multivitamins (no multivitamin use: P = 0.24).	Following genetic analysis, one SNP, rs7041, was also associated with higher levels of serum vitamin D in participants who also supplemented with a multivitamin. Many SNPs analyzed (with no significance found) were originally determined to be significant in a Caucasian population

affects risk factors associated with osteoporosis, biochemical markers of bone health and calcium absorption was also done by Zmuda, et al. The study population was drawn from a cohort of 156 African American women who were at least 65 years old, community-dwelling, able to ambulate independently and who had no history of a bilateral hip replacement. Of these women, 104 were genotyped for the *Fok*I SCP. Genotypes were defined as FF, Ff and ff; homozygous FF denotes absence of the restriction site and homozygous ff represents presence of the restriction site. The frequencies of the F allele and f allele were 78.4% and 21.6%, respectively. FF was the most frequent genotype (n=65; 62.5%), followed by Ff (n=33; 31.7%) and ff (n=6; 5.8%). Low prevalence of the ff genotype is consistent with previous findings in African Americans. No significant differences in genotypes were observed for age, weight, height, body mass index (BMI), dietary calcium intake, calcium supplementation, alcohol use, exercise (walking), smoking status, receipt of thiazide diuretics oral estrogen replacement therapy, history of fracture since age 50 or poor health status. There was no significant difference found between genotypes for hip or calcaneal BMD, calcaneal ultrasound or pelvic radiograph. Total hip BMD was 0.87 g/cm^2 (SD=0.13) for patients with the FF genotype, 0.86 g/cm^2 (SD=0.17) for Ff patients and 0.83 g/cm^2 (SD=0.08) for the ff group (P=0.69). Calcaneal BMD for all groups was 0.46 g/cm^2 (SD=0.10; P=0.99). Hip axis length was 128.6 mm (SD=9.1), 125.6 mm (SD=9.3) and 127.0 (SD=3.5; P=0.35). The other markers of bone health and turn over (serum osteocalcin, cross-linked NTx) as well as fractional absorption of calcium were also found to be similar across the genotype groups. After adjustment for factors such as age, weight, dietary calcium intake, health status, estrogen replacements therapy, thiazide diuretic use, smoking history and walking for exercise, no statistical difference was identified between the genotypes. Analyses were conducted with and without the rare f allele with similar and non-significant differences. Results of this study reveal that the ff allele is rare in the African-American community compared to others and the significance of differences in this allele has yet to be elucidated. However, it appears that the VDR start codon polymorphism is not associated with several markers of osteoporotic risk [14].

Although multiple studies have been conducted linking polymorphisms in the vitamin D receptor (VDR) gene to differences in bone mass, few have compared these differences in minorities. Nelson, et al. performed a cross-sectional cohort analysis in order to determine if there is any correlation between variants in the VDR gene and BMD or bone mineral composition (BMC). They compared the *Bsm*I gene variation patterns between African American and white patients. Subjects were recruited from a cohort of children in the City of Southfield Public School District near Detroit, Michigan. To be eligible for this cohort children had to be in the third grade, be able to lie still for at least 10 minutes and their parents were required to fill out the appropriate surveys and letter of consent. Children with congenital or chronic health problems were included in the cohort, but their data was excluded from analysis. When patients in this original cohort came to their routine study visit they were asked if they would participate in a special sub-study, requiring blood draws from both the child and the biological mother. Fathers were identified as being of the same race as the mother. Recruitment for this sub-study was closed after 50 mother-child pairs were identified. The average age of the children included was 9 years old. Genotype and bone mass data were collected on a total of 43 mothers and 41 children. Of the mothers, 19 were African American and 24 were white; all were premenopausal. Sixteen children were African American and 25 were white; all were prepubertal. The mean maternal age was 38.2 years old with no significant difference between groups; mean height, weight and BMI were similar between groups. Mothers were scanned only once, but children were scanned at the beginning of the study and again at age 11. Resulting PCR products were classified as BB, Bb or bb. Homozygotes without the *Bsm*I site variation were identified as BB; these samples displayed a single, intact PCR product. Homozygotes in which both genes contained the *Bsm*I site variation (labeled bb) resulted in 2 differently sized restriction fragments. Heterozygous samples were labeled as Bb and produced both an intact product as well as restriction fragments. Outcomes of this study indicated that there was a significant difference in VDR genotype distribution according to ethnicity (P=0.01 mothers and P=0.02 children). No African Americans included in this study (either mothers or children) were BB homozygous; rather, they were nearly equally distributed between the Bb and bb genotypes, either heterozygous or homozygous for the polymorphism. Among the adults, 10 African Americans had the Bb genotype (53%) and 9 had the bb genotype (47%). There were 6 white adults with the BB genotype (25%), 7 with Bb (29%) and 11 with bb (46%). Eight African American children were Bb (50%) and the other 8 were bb. White children were 24% BB (n=6), 56% Bb (n=14) and 20% bb (n=5). Using the Hardy-Weinberg model and the mothers' genotypes, the genotypes of the children were predicted. No statistical difference was found between the actual and predicted genotypes of the children (P >1.0). A correlation was shown between

bone mass and genotype for white mothers; however, this association was not found for white children, African American adults or African American children (values reported were not separated by ethnicity). Thus, further calculations were performed with combined groups of white and African Americans patients together. VDR genotype was found to be predictive of both BMC and BMD for the combined adult groups (*P*< 0.003 and *P*< 0.001 respectively). BMC for adults was 1863±491 for BB patients, 2472±477 for Bb patients and 2479±319 g for bb patients. BMD for these patients was 0.970±.014, 1.1±0.11 and 1.15±0.07 g/cm^2 respectively. Children had mean BMC as follows: 1003±468, 973±284 and 926±233 g respectively. BMD was reported as 0.80±0.10, 0.79±0.06 and 0.79±0.05 g/cm^2. BB genotype was associated with the lowest BMC and BMD, while Bb and bb were found to have the highest BMC and BMD during post hoc analysis ((*P*≤ 0.01; specific data not shown). Bone mass and genotype were not found to be correlated in children at age 9 or 11. Increase in bone mass was highest for the bb group (44%) followed by Bb (43%) and lowest in the bb group (12.7%). After conducting a regression analysis, investigators concluded that maternal bone mass alone should not be used to predict children's bone mass (r^2=0.08). The authors concluded that ethnic differences in VDR genotype frequencies may help explain ethnic differences in bone mass [15].

The differences in vitamin D levels and genotypes between African and Hispanic Americans were evaluated in a cross-sectional analysis performed by Engelman, et al. Data was collected from the Insulin Resistance Atherosclerosis Study (IRAS) Family Study cohort. The IRAS study was designed to evaluate genetic influence on insulin resistance and adiposity. The initial IRAS cohort (prior to the Family Study) was made up of self-identified Hispanic, non-Hispanic white, African American men and women aged 40-69. Recruitment for the Family Study was done in 3 different locations: Los Angeles, California (African Americans), San Luis Valley, Colorado (Hispanics) and San Antonio, Texas (Hispanics). Those recruited to the Family Study were required to have a large family who were willing to participate- at least 12-13 family members. At least 9 of these family members were required to undergo full genotyping and phenotyping, but the parents of the proband (reference family member) along with spouses were simply genotyped. Both 25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D (1,25[OH]$_2$D) levels were measured. Three databases (Ensembl, dbSNP and HapMap) were searched for SNPs associated with vitamin D binding protein (DBP), VDR and CYP27B1. Additionally, participants from the San Luis Valley center filled out a questionnaire on sun exposure and multivitamin use. Covariates for the environmental factors were adjusted for based on gender, age sun exposure in the month before blood draw, BMI, physical activity quartile and 25(OH)D level (for the 1,25[OH]$_2$D analysis). In total, 504 San Antonio Hispanics, 513 San Luis Valley Hispanics and 513 Los Angeles African Americans were included; the number of families recruited was 58, 30 and 42, respectively. Females comprised 59%, 56% and 58% of participants and median age for each group was 38.0, 39.0 and 40.0 years, respectively. BMI was also significantly different between treatment center groups (*P*< 0.001): median 29.2, 26.4 and 29.8, respectively. Both vitamin D levels differed by group (*P*< 0.001 for both). Median levels of 25(OH)D were 14.0, 18.0 and 9.0 ng/mL, respectively, while median levels of 1,25(OH)$_2$D were 46.0, 42.0 and 42.0 ng/mL. Both of these levels were significantly associated for all centers (r=0.43, 0.33 and 0.38 for African Americans, Hispanics from San Antonio and Hispanics from San Luis Valley, respectively. Higher concentration of 1,25(OH)$_2$D blood levels was only associated with younger age (San Antonio Hispanics) and lower BMI (Los Angeles African Americans) following adjustment for 25(OH)D. Heritability across all groups was significant for both 25(OH)D and 1,25(OH)$_2$D levels, although it was only able to explain part of the variability. After accounting for environmental factors, residual heritability estimates of coefficients of effects were as follows: 25(OH)D was 0.227±0.105 (*P*=0.005), 0.413±0.100 (*P*< 0.001) and 0.283±0.097 (*P*< 0.001) for San Antonio Hispanics, San Luis Hispanics and Los Angeles African Americans, respectively; 1,25(OH)$_2$D was 0.196±0.089 (*P*=0.004), 0.162±0.083 (*P*=0.006) and 0.484±0.099 (*P*< 0.001), respectively. Lastly, genetic associations were determined. Linkage disequilibrium for two SNPs in the DBP were calculated (r^2=0.31, 0.26 and 0.03 in San Antonio Hispanics, San Luis Valley Hispanics and Los Angeles African Americans, respectively [*P*< 0.001 for all]). After adjusting for gender and age, these 2 SNPs were found to be significantly associated with 25(OH)D, as well as one SNP in the VDR. Further, one SNP in the DBP was associated with 1,25(OH)$_2$D. Variation in the first DBP SNP (rs4588) was associated with an increase in serum 25(OH)D for all groups, whereas variation in the second (rs7041) was associated with a decrease in 25(OH)D for all groups. Variation in the VDR SNP (rs10783219) was associated with decreased serum 25(OH)D levels in San Antonio Hispanics, but was not significant for the other groups. It is possible that the variation in SNP variation between groups could be contributing to the racial differences in vitamin D status. The authors concluded that SNPs in the vitamin D binding receptor are associated with levels of 25(OH)D and 1,25(OH)$_2$D in Hispanic and African Americans [3].

The effects of various SNPs on serum 25(OH)D levels in both African Americans and Caucasians was evaluated in a cross-sectional cohort analysis (Signorello). SNPs in the group-specific complement gene (GC; associated with the vitamin D binding protein), CYP27B1, CYP24A, CYP2R1 and VDR were analyzed. Patient data from the Southern Community Cohort Study (SCCS) were used for analysis. Patients were recruited from 12 states in the southeastern U.S. (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Virginia and West Virginia) and randomized to one of 36 groups using a 2x2x3x3 factorial design. A total of 792 patients were randomized, 22 to each group. Groups were determined by sex, self-reported race (African American or Non-Hispanic white), smoking status (current, former, never) and BMI (18-24.99, 25-29.99, 30-45). Both groups were 50.7% male and the mean ages were 51.9 (SD=9.0) and 54.1 years (SD=9.5) for African Americans and Caucasians, respectively. Mean percentage African ancestry for the African American group was 0.929 (range: 0.505-0.999) and 0.009 (range: 0.001-0.171) for the Caucasian group. Mean percentage European ancestry was 0.071 (range: 0.001-0.495) and 0.991 (range: 0.829-0.999, respectively). Results indicated that mean serum 25(OH)D levels were significantly lower for African Americans (17.5 ng/mL, 95% CI; 16.6-18.4 ng/mL) than Caucasians (27.2 ng/mL, 95% CI; 26.1-28.3). Mean dietary intake of vitamin D was 218 IU (SD=213) for African Americans and 269 IU (SD=236) for Caucasians. Three different SNPs were found to be significantly associated with 25(OH)D levels in African Americans. Two were

located in the gene coding region for DBP and one was located in CYP27B1 (rs10877012; *P*=0.02). The two contained in GC were rs2298849 and rs2282679 (*P*=0.005 and *P*=0.03, respectively); they were not in linkage disequilibrium (r^2=0.01 in African Americans and 0.08 in Caucasians). Together, variation in these SNPs was linked to a difference of 2.1-3.6 ng/mL in serum 25(OH)D levels. Genetic risk scores were calculated for these three SNPs in order to determine if there was an association between the serum 25(OH)D levels and the number of high risk alleles for each patient. Genetic risk score proved a good predictor of vitamin D levels for African Americans. Higher scores (indicating a higher number of variant alleles) were associated with an average vitamin D level of 7.1 ng/mL lower than those with a score of 1. This trend continued for all scores (P_{trend}< 0.001). Scores of 2, 3, 4 and 5 were correlated to increasingly larger decreases in serum vitamin D level: OR=1.35 (*P*=0.56), 2.67 (*P*=0.05), 2.54 (*P*=0.06) and 6.01 (*P*=0.01), respectively. Genetic risk score was not significantly related to 25(OH)D levels in Caucasians (data not provided). Further analysis was done by dividing African Americans into tertiles based upon extent of African ancestry. These tertiles were then compared to the high risk genotypes. African Americans in the highest tertile of African ancestry were *less* likely to have the high risk alleles for rs2282679 (*P*=0.003). Overall, genotype accounted for 28.5% of the variation in vitamin D levels when added to a linear regression model including dietary vitamin D intake, UVR score, age, BMI, smoking, sex and percentage of African American ancestry. Alone, genotype only accounted for 2.6% of the variation in this study. Combined with information from other studies, SNPs account for less than 5% of variation in 25(OH)D levels, but genetic associations within the vitamin D pathway warrant further investigation. The investigators found that genetic variation does play a role in determining 25(0H)D levels in African Americans [16].

African American women are at a higher risk of vitamin D deficiency than white women; however, African American women are also at a lower risk of fracture. To further explore this discrepancy, investigators sought to determine the effect of genetic polymorphisms in the VDR on vitamin D supplementation and bone density. This randomized, placebo-controlled clinical trial was conducted by Nieves, et al. in order to determine the effect of vitamin D supplementation (1000 IU/day) on bone turnover in older African American women. Patients were required to be at least 45 years old, be in menopause for at least one year (natural spontaneous menopause or surgical ovariectomy), verify that at least 3 of their 4 grandparents were African American and have a serum 25(OH)D level of <20 ng/mL. A total of 348 patients were originally screened, 154 underwent the first blood test and 127 were randomized to study groups. Only 103 participants were included in the final analysis, representing the women who completed the follow-up bone density screenings. Five known SNPs were genotyped, including *Apa*I, *Taq*I, *Bsm*I, *Fok*I and Cdx-2. At months 6, 12, 18 and 24, BMD of the hip and lumbar spine were measured. Primary outcomes were BMD in the total hip and femoral neck. All patients were supplemented with calcium (Tums^R tablets) in order to achieve a daily intake of 1,000 mg beginning 1 month before randomization. Women in the treatment group received 1,000 IU of vitamin D_3 daily, while the control group received a matching placebo; both were to be taken in the morning. Mean ages of the 2 groups were 62.3±8.5 years and 61.2±7.6 years for the intervention and control groups, respectively. Years since onset of menopause were 17.8±23.1 years and 19.3±21.3 years. Calculated BMI for each group was 31.2±6.3 and 31.6±5.7. The only baseline demographic that was significantly different was BMD at the spine: 1.154±0.16 g/cm^2 vs. 1.212±0.15 g/cm^2 for the intervention and control groups (*P*=0.05). Although statistically significant, this discrepancy was not treated as clinically significant. As predicted by prior research, BMD was above average, while 25(OH)D levels (11.6±5.0 ng/mL and 11.6±5.7 ng/mL) were below the normal range for both groups. At baseline, about 20% of participants had PTH levels >65 pg/mL and 50% had serum 25(OH)D levels <10 ng/mL. No significant differences in biochemical markers were found and on average all of the other levels fell within normal ranges. After 2 years, approximately 80% of the participants had completed the study with adherence rates of 95% and 93% for the intervention and control groups, respectively. Levels of 25(OH)D doubled in the group receiving vitamin D_3 supplementation (*P*< 0.001) after three months. PTH values were decreased by 10% (*P*< 0.02). The effects on measured 25(OH)D were persistent after 2 years, but PTH levels had increased leading to insignificance at the 24-month follow-up. A decrease in BMD of both the total hip and lumbar spine was noted for both groups. This decline was slightly lower in the vitamin D treatment group, but not enough to be statistically significant (*P*=0.10 for femoral neck; unreported for other sites). Baseline characteristics for those who underwent genotyping did not differ significantly from the group as a whole. Adjustment for age and BMI had no impact on significance, nor did per-protocol analysis. SNPs *Apa*I, *Taq*I and *Fok*I were found to be in Hardy-Weinberg equilibrium (used to measure the distribution of alleles in a sample), whereas *Bsm*I and *Cdx*-2 were not. However, the only SNP that displayed an association with vitamin D supplementation was *Fok*I. Patients were classified as FF, Ff or ff, with the following distribution: 60% FF (n=47), 38% Ff (n=29) and 2% ff (n=2). Ff and ff groups were combined for analysis. Baseline characteristics between the FF and Ff/ff groups were similar with no significant differences reported (*P* >0.4 for all comparisons). Twenty-four women in the vitamin D supplementation group were classified as FF, with 13 Ff. Similarly, the placebo group had 23 in the FF group and 18 in the Ff group. Similar rates of increase in serum 25(OH)D level were seen between both groups receiving vitamin D supplementation (*P*< 0.02). The FF group experienced an increase in serum 25(OH)D from a mean of 12±4 to 20±10 ng/mL and from 15±8 to 26±8 ng/mL in the Ff/ff group. Each group experienced approximately a 35% increase in serum 25(OH)D levels. No significant changes were seen in the placebo group. There was a significant difference between the FF and Ff/ff groups in maintenance of BMD at the femoral hip (P=0.009). Women in the FF group who received placebo lost approximately 1.4% BMD compared to the group supplemented with vitamin D who had -0.17% bone loss at this site (*P*=0.02, n=27). When comparing femoral neck BMD change between FF and Ff groups, the results were also significant (P=0.009). Similar trends were found at the spine and elsewhere in the hip. The authors concluded that vitamin D supplementation does not appear to influence bone loss in black women; however, black patients with the *FF* polymorphisms of the VDR gene group may experience less bone loss [17].

Since low levels of total 25(OH)D are more common in the

African American population compared to white Americans, Powe, et al. conducted a study to determine DBP genotypes and concentrations of circulating DBP differ between black Americans and white Americans via a cross-sectional cohort analysis. The authors utilized patients in the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) cohort in Baltimore, MD, to assess differences between black and white patients. To be included in the HANDLS cohort, patients had to be age 30-64, able to give informed consent, provide a photo ID and be able to complete at least 5 measures outlined in the study protocol (i.e. household survey, DEXA scan, cognitive testing, medical history, physical exam, dietary recall, etc.). Study investigators genotyped the participants and analyzed them for 2 common SNPs, rs4588 and rs7041. This data was compiled along with race, total 25(OH)D level, DBP level, PTH level and calcium level of patients and correlations were evaluated. In total, 1181 black patients and 904 white patients were included in the final results of this study with an average age of about 48 years old (range 30-64 years) and average BMI of 29.6. Concentrations of bioavailable 25(OH)D (vitamin D unbound by DBP) were assessed. Patients were divided further to evaluate correlations between 25(OH)D status and several markers (e.g., PTH level, calcium level and BMD). Participants included in the two groups were comparable in regards to age, sex, BMI and menopausal status. Medications which could have potentially affected the measured levels, such as hormone replacement therapy, antiepileptic medications or glucocorticoids, were only reported by very low percentages of patients. However, there were statistically significant differences in several areas; black patients were more likely than white patients to be smokers, have microalbuminuria or have a household income below 125% of the federal poverty line. In addition, black patients were less likely to receive a diagnosis of osteoporosis or receive medications for osteoporosis. Unadjusted total levels of 25(OH)D were significantly lower among black patients compared with white patients (15.6±0.2 ng/mL and 25.8±0.4 ng/mL, respectively, P< 0.001). This difference remained significant after adjustment for multiple variables. Both unadjusted and adjusted levels of vitamin D-binding protein were significantly different between groups, with black patients averaging 168±3 µg/mL and white patients averaging 337±5 µg/mL (P< 0.001). Adjusted femoral neck BMD was higher in black patients, who averaged 1.05±0.01 g/cm^2 vs 0.84±.0.1 g/cm^2 (P< 0.001), which confirmed previous findings. Adjusted PTH levels were slightly higher for black patients than for white patients: 39±1 pg/mL vs 34±1 pg/mL (P< 0.001). Further, investigators analyzed the differences in genetic polymorphisms between the black and white patients. They focused on 2 different locations: rs7041 and rs4588. A variant allele at either location was associated with lower levels of total 25(OH)D. Black patients were more likely than white patients to have a variation at rs7041, while white patients were more likely to have the reference allele at this location (P< 0.001 for both associations). At rs4588, however, white patients were more likely to have the variant than black patients (P< 0.001). Overall, this means that a variation at either location was associated with a decrease in total vitamin D levels. Black patients were found to have mean total 25(OH)D levels that were significantly lower than whites (15.6±0.2 ng/mL vs. 25.8±0.4 ng/mL, P< 0.001), which may be a result of the variation at rs7041. Additional analysis was conducted on homozygous patients. Approximately 93% of black patients were Gc1F homozygous (i.e., they had the variant allele at rs7041, but the reference allele at rs4588). This genotype was predictive of the lowest levels of DBP. On the other hand, 76% of white patients were classified as Gc1S homozygous (reference allele at both locations) which was associated with the highest levels of DBP. For these homozygous patients there was no correlation between BMD and bioavailable or total 25(OH)D for black patients; however, for white patients there was a trend of increased BMD in patients with higher total or bioavailable 25(OH)D levels. In conclusion, low levels of total 25(OH)D levels may not correlate to true vitamin D deficiency when levels of vitamin D-binding proteins are low in black Americans. Even though white patients had higher levels of total 25(OH)D than black patients, the levels of bioavailable 25(OH) D were very similar between groups. This has been attributed to the lower levels of DBP observed in black patients which leads to a relatively higher bioavailability. Thus, the threshold value for vitamin D deficiency in black patients may be lower than white patients when total vitamin D concentrations are measured [9].

In a cross-sectional cohort analysis, Batai, et al. sought to identify relationships between SNPs in the vitamin D pathway and serum vitamin D levels in both African Americans and European Americans. Pertinent genome wide association studies (GWAS) have revealed variants located in the GC sequence. A total of 39 SNPs in 8 vitamin D pathway genes were evaluated to determine the association between the genes and serum 25(OH)D concentrations between African Americans and European Americans. Subjects were recruited from Washington, D.C. and Chicago, IL for inclusion in the study. Genetic analysis was conducted on 30 SNPs in 8 different vitamin D-associated genes: GC, DHCR7/NADSYN1, VDR, CYP2R1, CYP27A1, CYP27B1, CYP3A4 and CYP24A1. Four genes were removed from analysis due to the presence of more than 2 alleles, deviation from Hardy-Weinberg equilibrium or lack of a polymorphism (monomorphic). These genes were rs2228570 (*FokI*), rs1989969, rs11568820 and rs116071925. Therefore, a total of 35 genes were analyzed. Baseline adjustments were as follows: African American data were adjusted for age, West African ancestry (WAA) and study site, whereas European American data were adjusted for age and environmental factors. In total, 652 African American men and 405 European American men were selected for inclusion. All European American men were selected from Chicago; however, 226 of the African American men were recruited from Washington D.C. None of the participants were related and race was self-identified. Patients were excluded from the study if they had liver disease or chronic kidney disease. The average ages of the participants were 59.0 years (SD=10.0) and 60.9 (SD=8.4) years for African American and European Americans, respectively (P=0.001). Mean vitamin D levels differed significantly: 47.2 nmol/L African American vs. 64.9 nmol/L European American (P< 0.001). More African Americans were recruited during high UVR exposure months (June to November; P< 0.001); however, this did not correlate to higher vitamin D levels. Only 36.4% of African Americans had 400 or more IU per day of vitamin D as compared to 49.5% of European Americans (P=0.001). Prior to genetic analysis, statistical analysis was performed to recognize other factors affecting serum vitamin D concentrations. Study site, total vitamin D intake and season of blood draw were all shown to be significantly associated with 25(OH)D levels in African Americans (P< 0.001; specific data not shown). For European Americans, serum vitamin D was associated with age, total vitamin D intake, season of blood draw and BMI (P< 0.001). UV

exposure was close to being significantly associated with vitamin D concentration, but the p value did not reach significance (P=0.06). Overall, there was a significant difference in the vitamin D status between the two races. Prevalence of vitamin D deficiency and insufficiency was much higher in African Americans than European Americans (85.8% vs 67.7%, respectively). Before analyzing the SNP correlations, environmental and biological factors affecting 25(OH)D levels were determined. Study site, total vitamin D intake and season of blood draw were significant indicators of vitamin D level in African Americans (P< 0.001). No correlation was found for skin pigmentation or WAA. European American 25(OH)D levels were significantly associated with age (P=0.006), season of blood draw (P< 0.001), total vitamin D intake (P< 0.001) and BMI (P< 0.001). These significant interactions were adjusted for in the genetic analyses. Following adjustments a total of 6 SNPs were found to be significantly associated with 25(OH)D levels in African Americans: five SNPs defined by GWAS (1 in GC, 4 in CYP2R1) as well as one non-GWAS-identified SNP in GC. Of these, a strong correlation between SNP and 25(OH) D levels was found in 2 of the CYP2R1 SNPs (rs12794714 and rs10741657; P=0.01). There was only a weak association between these SNPs (r^2=0.07) and regression models showed independent association (P=0.04 for both). Logistic regression analysis of vitamin D deficiency reinforced this association and found that rs12794714 had the strongest association with vitamin D deficiency (P=0.003 or=0.72, 95% CI; 1.20-2.47). Two additional SNPs in GC were identified as correlating to serum 25(OH)D levels (rs115563 and rs115316390). The former, which was previously reported in GWAS, had significant-though weak-correlations to serum 25(OH)D levels (P=0.048). The latter was not reported in GWAS and though it had stronger associations with serum 25(OH)D levels and was initially significant, it was not significant after adjustment for multiple testing. The two SNPs were not in linkage disequilibrium (r^2< 0.001) and were found to be independently associated through regression models (P=0.03 for both). In DHCR7/NADSYN1, rs12800438 was identified as significantly associated with vitamin D deficiency in African Americans (P=0.04 or=0.76, 95% CI; 0.58-0.99), but was not found to be associated with 25(OH)D in regression analysis. SNP correlations in European Americans were quite different. More SNPs were identified with strong associations, but for different SNPs than seen in the African American group. Nine GWAS-identified SNPs were associated with serum 25(OH)D levels. The strongest correlation was with rs1993116, a CYP2R1 SNP (P=0.0006). Logistic regression analysis verified this finding (P=0.0008 or=0.51, 95% CI; 0.35-0.76). The second strongest association with 25(OH)D concentrations was in a GC SNP, rs7041 (P=0.0007). After adjustment for these 2 SNPs in the regression analysis, other SNPs in CYP2R1 and GC were no longer significant. One CYP24A1 SNP, rs73913757, showed an originally significant association with vitamin D levels (P=0.04), but significance was lost after controlling for multiple testing. After adjusting for age, WAA, study site, total vitamin D intake and season of blood draw, the two most significant SNPs were combined in a linear regression analysis in order to determine additive effects. This combination represented 19.1% of the 25(OH)D level variations (adjusted R^2=0.191) in African Americans. Genetic Risk Scores, ranging from 1 to 4, were calculated by counting the number of A alleles for the top 2 SNPs (rs12794714 and rs115316390). Proportion of patient with vitamin D deficiency was found to be directly related to risk score: as the score increased, so did the proportion of vitamin D-deficient patients. For example, 76% of African Americans with a risk score of 4 were vitamin D deficient. A small group of African American patients with only 1 risk allele were an exception to this trend, as 28.6% were vitamin D deficient. When slightly different adjustments were made (age, WAA, study site, season and vitamin D intake), a linear regression model showed that Genetic Risk Score and serum 25(OH)D were significantly correlated (β=-0.044; P=0.005). Due to a difference in the regression coefficients (β) between the 2 SNPs, a weighted Genetic Risk Score was also calculated; significance was still found (β=-0.947; P=0.001). Genetic variation accounted for a small amount of variation in 25(OH)D; however, when combined with environmental and biological factors it was able to account for more of the 25(OH)D variation in European Americans as compared to African Americans (28.2% vs 19.1%, respectively; R^2=0.241 after adjustment for age, total vitamin D intake, season of blood draw, BMI and UVR exposure). Genetic risk score for European Americans was also calculated and trends resembled those for African Americans. Serum vitamin D levels were significantly correlated (β=0.043; P<0.001) in a linear regression model adjusted for age, BMI, season, vitamin D intake and UV exposure. In conclusion, this study was able to confirm many findings of the GWAS, which associate vitamin D levels with several SNPs. In particular, strong correlations with SNPs in CYP2R1 and GC suggest that these may be the most important genes in this pathway for determining variation. SNPs within GC may reduce the circulating levels of 25(OH)D [7].

Both elderly and African American patients are at higher risk of vitamin D deficiency; yet, very few analyses of this kind have been conducted. In this cross-sectional cohort analysis, Hansen, et al. investigated the genetic and environmental determinants of vitamin D status in elderly African American women. Patients included in this analysis were from two previously studied cohorts: Health, Aging and Body Composition (Health ABC) study (n=3075) or the Multi-Ethnic Study of Atherosclerosis (MESA; n=1198). Patients in the Health ABC cohort that were studied in the present trial were African American, between the ages of 70-79, Medicare-eligible and lived near Memphis, Tennessee or Pittsburgh, Pennsylvania (n=1281). To be included, they had to be capable of walking 0.25 miles, climbing 10 stairs and conducting activities of daily living independently. Patients in the MESA cohort were between the ages of 45-85 and were without diagnosis of clinical cardiovascular disease at baseline. Mean age of the African Americans in the Health ABC study was 74.5±2.9 years. Mean serum 25(OH)D level was 20.7±9.0 mcg/L. Females comprised 57.3% of the population and 55.2% of participants were recruited from Pittsburgh. Vitamin D deficient patients were defined as having a serum 25(OH)D<20 mcg/L and comprised 55% of the participants. Similarly, patients in the MESA cohort had a mean serum 25(OH)D level of 19.0 mcg/L, with 60% of participants classified as deficient. Per the Health ABC study, male gender, residence in Memphis and summer season of blood draw were all positively correlated to higher vitamin D levels. An inverse relationship with BMI was observed and was most significant for obese patients. Multivitamin use was the single highest predictor of vitamin D status in this population and accounted for 8% of variation. Supplementation with either vitamin D or calcium was associated with a significant increase in serum 25(OH) D status (adjusted P=0.0002 for both). Consumption of cereal or

dairy products also produced clinically significant increases in serum vitamin D levels (adjusted *P*=0.004 and *P*=0.0008, respectively). Ten SNPs were chosen for analysis based on data from prior GWAS. Only 8 of these 10 were documented in the Health ABC study. None showed a statistically significant association with serum 25(OH)D (*P*>0.05 for all comparisons). SNP rs7041, associated with DBP, was used for further analysis because it had the *P* value nearest to significance (*P*=0.08). Participants with the rs7041 GG or GT alleles who took multivitamins were found have higher increases in serum 25(OH)D than those with the TT alleles (29.0 vs 25.3 mcg/L; *P*=0.002). When adjustment was made for age, study site, gender, season of blood draw and principle components, serum 25(OH)D was still higher in patients with GG or GT alleles who took multivitamins (*P*=0.04). This effect was only significant for patients who took multivitamins (*P*=0.24 if no multivitamin use). Genetic analysis was conducted on 205 additional SNPs that were reported by the GWAS because the original 10 were primarily identified in Caucasian patients; due to genetic differences between Caucasians and African Americans, different SNPs may be important in the African American vitamin D pathway. However, none of these proved significant. SNP rs4588 was also analyzed, but even after adjustments for age, gender, study site, season of draw and principle components, no discernible association was found (*P*=0.76). The results of the two studies were combined for meta-analysis. In combination, genetic variation accounted for 23% of serum 25(OH)D variance. Following genetic analysis, one SNP, rs7041, was also associated with higher levels of serum vitamin D in participants who also supplemented with a multivitamin. Many SNPs analyzed (with no significance found) were originally determined to be significant in a Caucasian population, further verifying prior data suggesting that African Americans genotypes in the vitamin D pathway differ significantly from those of Caucasians. Several modifiable factors can explain variability in serum 25(OH)D levels. Additional studies need to be conducted to determine the effect of genetics on circulating levels of 25(OH)D [18].

Discussion

Several factors can determine circulating levels of 25(OH)D. In the current analysis, the effect of genetic polymorphisms of the vitamin D receptor binding protein was assessed to determine if there is an association between polymorphisms and vitamin D levels and/or overall bone health. A number of genetic polymorphisms have been identified in white Americans that seem to effect the vitamin D pathway; however, few have evaluated this effect in African American patients [3,12-15].

The majority of trials assessed were of good quality; however, there is conflicting information related to the effect of genetic polymorphism on bone health. Several investigators have found an association between genotype and vitamin D levels [3,7,10,15-17]. If genetic polymorphisms have a significant effect on vitamin D levels, the effect appears to be small (~5%) [7,16] or results in a small increase in circulating vitamin D levels. Changes in BMD may also be small in patients who have genetic polymorphisms at the vitamin D receptor and the changes are inconsistent among locations in the body [10,14,17]. Several of the studies only assessed changes in BMD and not circulating levels of vitamin D. Other investigators did not record adherence to vitamin D and/or calcium levels [8,10,16-18]. Additionally, vitamin D levels were drawn at variable seasons, which may affect serum concentrations of vitamin D. There were inconsistent measures of BMD at different regions [10,14,16]. At this time, it is uncertain as to the etiology of differences with the potential for genetic polymorphisms and resultant BMD levels on different sites in the body.

The level of evidence associated with vitamin D polymorphism and African American patients is variable. Only one clinical trial was identified [17]; the remainder of the studies was conducted in previous studies and may not have had the power to sufficiently detect a difference between the groups. Further, the majority of SNPs analyzed were first identified in Caucasian patients; but, it is possible that different SNPs cause more significant differences in the African American population. Additional studies need to be conducted to assess vitamin D polymorphisms in the African American population to determine the relationship between free vitamin D levels and BMD levels.

No information was located related to the genetic polymorphism of the vitamin D receptor in men. Only one study assessed premenopausal women [9] and the remainder of the studies evaluated the effects in postmenopausal women. There are so many alleles associated with genetic polymorphisms of the vitamin D receptor that it is difficult to determine which allele has the most profound effect on the receptor.

In addition, none of the investigators evaluated genetic polymorphism of the vitamin D receptor and its effect on fracture risk. Additional studies need to be performed in order to evaluate this effect on fracture risk.

Conclusion

There appears to be a genetic polymorphism to vitamin D binding protein. There is conflicting information as to how this may affect individual patients and whether or not additional vitamin D/vitamin D binding protein lab markers should be assessed in African American populations. Since the majority of studies that were conducted were observational studies, additional clinical trials need to be performed to determine the clinical impact of vitamin D/vitamin D binding protein on BMD levels.

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