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Polymorphisms in Vitamin D related genes and Risk of Uterine Leiomyomata

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Abstract

Objective—To investigate UL incidence in relation to polymorphisms in genes involved in vitamin D metabolism and skin pigmentation. Rates of uterine leiomyomata (UL) are 2–3 times higher in African Americans than European Americans. Recent studies suggest that vitamin D deficiency is associated with an increased risk of UL.

Design—Nested case-control study.

Setting—Black Women's Health Study, a prospective cohort study of African American women.

Patient(s)—2,232 premenopausal women diagnosed with UL confirmed by ultrasound or surgery during 1997–2011 (cases) and 2,432 premenopausal women never diagnosed with UL through 2011 (controls).

Intervention—None.

Main outcome measure—Self-reported UL. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between each polymorphism and UL, controlling for age, geographic region, and ancestry.

Results—Three of twelve polymorphisms were associated with UL at the nominal significance level: rs4944957 and rs12800438 near *DHCR7*, and rs6058017 in *ASIP*. After correction for multiple hypothesis testing, two SNPs remained significantly associated with UL (rs12800438 and rs6058017). Compared with the AA genotype for rs12800438 (correlated with higher serum 25(OH)D levels), ORs were 1.09 (95% CI: 0.92, 1.29) and 1.23 (95% CI: 1.03, 1.47) for the GA and GG genotypes, respectively. Compared with the AA genotype for rs6058017 (correlated with higher serum 25(OH)D levels), ORs were 1.01 (95% CI: 0.83, 1.22) and 1.18 (95% CI: 0.97, 1.44) for the GA and GG genotypes, respectively.

Conclusions—Our data support the hypothesis that vitamin D deficiency is involved in UL etiology.

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Keywords

prospective studies; African Americans; vitamin D; genetics; uterine neoplasms

INTRODUCTION

Uterine leiomyomata (UL), or fibroids, are benign neoplasms of the myometrium and are clinically-recognized in 25–30% of reproductive-aged women (1–3). Studies have documented a 2–3-fold higher incidence of UL in African Americans than European Americans (4, 5), and African Americans tend to have younger ages at diagnosis and greater symptom severity (6). None of the identified established risk factors explain this racial disparity (7).

Vitamin D deficiency is more common among African Americans than other ethnic populations (8, 9). In 2008, Baird hypothesized that vitamin D deficiency increases UL risk, and that black-white differences in serum levels of 25-hydroxyvitamin D3 [25(OH)D] contribute to the racial disparity (8). Since then, one case-control (10) and two cross-sectional (9, 11) studies have found significantly lower serum vitamin D3 levels among UL cases than controls.

Determinants of circulating 25(OH)D—the inactive circulating form of vitamin D and an established marker of vitamin D status—include sun exposure, dietary intake (e.g., oily fish, cod liver oil, fortified milk), and vitamin supplements. Most vitamin D is produced in the skin following sufficient sunlight exposure. Heritability may also play a role: several genes are involved in vitamin D3 metabolism, and genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with 25(OH)D concentrations (12, 13). In 2010, a GWAS in 30,000 individuals of European descent identified SNPs at four loci that were associated with 25(OH)D levels: *GC* (rs2282679), *DHCR7* (rs12785878), *CYP2R1* (rs10741657), and *CYP24A1* (rs6013897) (12). A second GWAS examining 25(OH)D levels of 4,501 individuals of European ancestry confirmed the previous study's findings for *GC*, *DHCR7*, and *CYP2R1* (13). These variants were located within or near genes involved in vitamin D transport (*GC*), cholesterol synthesis (*DHCR7*), and hydroxylation (*CYP2R1* and *CYP24A1*). Thus, GWAS studies suggest that SNPs in enzymes related to activation or degradation of vitamin D and its metabolites predict serum 25(OH)D levels (14, 15).

We hypothesized that vitamin D-related genetic variants would be associated with UL. In a large cohort of African American women, we examined UL risk in relation to twelve polymorphisms in eight genes: two in *GC* (12, 16, 17), two in *VDR* (18), two in *CYP2R1* (12, 19), two near *DHCR7* (12, 19), and one each in *SLC24A5* (20, 21), *OCA2* (22), *ASIP* (23), and *CYP24A1* (18). The selected SNPs have been associated with vitamin D levels in previously-described GWAS (12, 13), or with vitamin D levels (14–19, 24, 25) and skin pigmentation (14, 20–23, 26) in other studies.

MATERIALS AND METHODS

Study population

The Black Women's Health Study (BWHS) is an ongoing prospective cohort study of 59,000 women who self-identify as "black" (27). The study began in 1995 when women 21–69 years of age from across the United States completed a 14-page postal health questionnaire. Follow-up questionnaires have been completed by participants every two years and cohort retention has exceeded 80% through 2011. During 2004–2007, we obtained saliva samples as a source of DNA from 26,814 participants using the mouthwash-swish method (28). Participants who provided DNA were slightly older than those who did not (49.7 vs. 47.7 years), but were similar with respect to education (≤12 years: 18% vs. 19%), region (Northeast: 27 vs. 28%; South: 31 vs. 30%; Midwest: 24 vs. 23%; West: 18 vs. 19%), body mass index (BMI: 28.1 vs. 27.8 kg/m^2), and family history of UL (38.5 vs. 36.5%). The present analysis includes 4,664 premenopausal women aged 23–50 years in 1997. The study protocol was approved by the Institutional Review Board of Boston University Medical Center.

Assessment of uterine leiomyomata

Ultrasound is the clinical standard used to confirm UL diagnoses (3) and it has high sensitivity (99%) and specificity (91%) relative to histologic evidence (29, 30). Every two years, beginning in 1999, women reported whether they had been diagnosed with "uterine fibroids," the calendar year of first diagnosis, and whether their diagnosis was confirmed by ultrasound or surgery. Analyses were restricted to premenopausal women because new UL diagnoses are rare after menopause (3).

The case group (N=2,232) consisted of all premenopausal women with incident UL diagnosed during 1997–2011 who provided DNA. Controls (N=2,432) were a random sample of similarly-aged healthy women who provided DNA and had never been diagnosed with UL through 2011. We assessed the accuracy of self-report in a random sample of 248 incident cases and confirmed the diagnosis for 96% (122/127) by medical record (31). There were no systematic differences in characteristics according to the release of medical records (31).

Assessment of covariates

Baseline and biennial follow-up questionnaires collected data on reproductive, contraceptive, and medical history, height, current weight, smoking, alcohol, physical activity, geographic region, and various indicators of socioeconomic status. Recency of pelvic ultrasound was reported in 2007 ("never, <5, 5–9, ≥10 years ago"), as well as 2009 and 2011 ("previous two years"). Family history of UL ("Has your mother or any of your sisters ever been diagnosed with uterine fibroids?") was ascertained in 2009.

In 1995 and 1997, participants reported their use of "calcium with vitamin D" supplements in the past year; in 1997, use of "vitamin D alone" was additionally ascertained under an open-ended question about other vitamins taken ≥3 times a week. Diet was assessed in 1995 using a modified version of the NCI-Block food frequency questionnaire (FFQ) (32, 33). In

1995, a 68-item FFQ was used to collect data on the consumption of specified foods and beverages during the previous year (33). Nutrient values for vitamin D were calculated using DIETCALC software (version 1.41). In a validation study conducted among 400 BWHS participants in 1996–1998 (33), energy-adjusted and deattenuated Pearson correlations comparing nutrient estimates from the FFQ with averages from the combined recall/record data ranging from 0.5 to 0.8 (33).

Genotyping and quality control

DNA isolation and amplification—DNA was isolated from mouthwash swish samples at the Boston University Molecular Core Genetics Laboratory using the QIAAMP DNA Mini Kit (Qiagen, Valencia, CA). Whole genome amplification was performed with Qiagen RePLI-g Kits using the method of multiple displacement amplification. Amplified samples underwent purification and PicoGreen quantification at the Broad Institute Center for Genotyping and Analysis (Cambridge, MA) before being plated for genotyping.

Selection of SNPs and DNA genotyping—Genotyping was performed at the Broad Institute using a Sequenom iPLEX assay (Sequenom, San Francisco, CA). Sixteen SNPs were selected for analysis because only a portion of a Sequenom panel was available for genotyping. We prioritized SNPs that had been associated with serum vitamin D levels in previously-described GWAS (12, 13), or with vitamin D levels (14–19, 24, 25) or skin pigmentation (14, 20–23, 25, 26) in other studies, especially SNPs associated with vitamin D concentrations in African Americans (25). For example, among darker pigmented African Americans, rs12800438 in *DHCR1* had the strongest association (P=0.004) with risk of vitamin D deficiency (<20 ng/ml) (25). Skin pigmentation genes (e.g., *ASIP*) were also considered because skin pigmentation is a strong predictor of vitamin D synthesis from sun exposure (20, 22, 23, 26). Sequenom multiplex limitations precluded us from being able to fit all prioritized SNPs into the same design. For example, a variant located in *CYP27B1* (major gene in vitamin D pathway) was prioritized for inclusion but got eliminated based on its inability to fit in the same design as other GWAS-identified SNPs (12, 13).

One percent of samples were blinded duplicates included to assess reproducibility of genotypes. An average reproducibility of 96% was obtained. Four SNPs with a call rate of less than 90% were excluded. We also excluded 125 subjects with call rates <80% and 5 duplicate sample pairs with discordance rates >5%. After these exclusions, 12 SNPs, 2,232 UL cases and 2,432 controls remained for analysis. All SNPs analyzed were in Hardy-Weinberg equilibrium (P>0.01 in controls).

We genotyped the top 30 ancestral informative markers (AIMs) from a list of 1,536 validated SNPs to estimate percent European ancestry and adjust for population stratification due to European admixture. These 30 AIMs had allele frequency differences between Africans and Europeans ≥0.75 (34). We used a Bayesian approach as implemented in the ADMIXMAP software (35, 36) to estimate individual admixture proportions. In our cohort, the correlation between percent European admixture determined by the reduced panel of 30 AIMs as compared with the full panel of 1,536 AIMs was significant (r=0.87, P=0.0001), confirming the validity of the reduced panel (37).

Data analysis

We used PLINK software version 1.06 (38) to compute summary statistics for the genetic data. We used a 1-df chi-square test that does not assume any particular genetic model, correcting for multiple testing through 10,000 permutations. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between each SNP and UL, with adjustment for age in 1997 (<30, 30–34, 35–39, 40–44, ≥45 years), percent European ancestry (continuous variable), and region of residence (Northeast, South, Midwest, and West) (39). We considered the variant associated with low vitamin D levels or dark skin pigmentation to be the risk allele. We constructed models that additionally controlled for UL determinants, including age at menarche (years), parity (births), age at first birth (years), years since last birth (<5, 5–9, 10–14, 15, ≥20), age at first oral contraceptive use (years), BMI (<20, 20–24, 25–29, 30–34, ≥35 kg/m^2), smoking (current, past, never), current alcohol consumption (<1, 1–6, ≥7 drinks/week), education (≤12, 13–15, 16, ≥17 years), marital status (married/partnered, divorced/separated/widowed, single), occupation (white collar, non-white collar, unemployed, missing), and household income (≤ $25,000, $25,001–50,000, $50,001–100,000, >$100,000). Because multivariable models gave slightly stronger results than models adjusted for age, region, and ancestry, and it is unlikely that lifestyle factors are confounders because they are “downstream” from exposure, we presented the more parsimonious models. We stratified the data by age and surgery because early diagnosis may reflect a genetic predisposition to disease, and surgically-confirmed cases often have more symptomatology (40). In secondary analyses, we restricted controls to those with a recent pelvic ultrasound (“5 years ago” in 2007, or within the “previous two years” in 2009 or 2011).

We tested whether the general genotypic model (i.e., model that includes separate categories for each of the three genotypes) fit the data better than the recessive model (i.e., model that assumes risk is observed only for homozygotes of the risk allele, combining the protective allele homozygotes and heterozygotes into a single reference category). We conducted a likelihood ratio test comparing the recessive model (null) to the genotypic model (alternative) with 1 degree of freedom.

RESULTS

Characteristics of cases and controls are shown in Table 1. Mean age at the start of follow-up (1997) was 34.3 years for UL cases and 34.5 years for controls. Mean age of UL cases at diagnosis was 38.4 years. As expected, cases had reduced parity, lower percent European ancestry, earlier ages at first birth, and greater years since last birth than controls (7).

In three of the twelve SNPs examined (Supplemental Table 1), the risk alleles were more frequent in cases compared with controls, and were associated with UL at the nominal significance level of $p<0.05$ (Table 2): rs4944957 (49.2% in cases vs. 46.8% in controls), rs12800438 (61.4% vs. 58.3%) near *DHCR7*, and rs6058017 (66.2% vs. 62.9%) in *ASIP*. Positive associations also were found between UL and other SNPs (i.e., rs10741657 in *CYP2R1;* rs1426654 in *SLC24A5*), but these associations were not statistically significant, possibly because the risk allele was rare. After correction for multiple testing, two SNPs remained significantly associated with UL (rs12800438, *P-permutated*=0.028; rs6058017,

P-permutated=0.013). Relative to the AA genotype for rs12800438 (low-risk genotype), ORs were 1.09 (95% CI: 0.92, 1.29) for the GA genotype and 1.23 (95% CI: 1.03, 1.47) for the GG genotype. Relative to the AA genotype for rs6058017 (low-risk genotype), ORs were 1.01 (95% CI: 0.83, 1.22) for the GA genotype and 1.18 (95% CI: 0.97, 1.44) for the GG genotype.

The genotypic model did not provide a significantly better fit of the data than the simpler recessive model for rs12800438 (*P*=0.37) and rs6058017 (*P*=0.99). Because rs12800438 and rs6058017 are part of the same metabolic pathway, we created a summary score variable representing the number of "high-risk" homozygous GG genotypes for each individual (0, 1, or 2). For this new variable, the adjusted ORs were 1.12 (95% CI: 0.98, 1.28) and 1.42 (95% CI: 1.18, 1.71) for 1 and 2 homozygous GG genotypes, respectively, relative to none (Table 3).

There was little evidence that the associations of rs12800438 or rs6058017 with UL risk differed across strata of age (data not shown). Similarly, results did not vary by method of confirmation (ultrasound vs. surgery), BMI, or family history of UL (data not shown). When the control group was restricted to those with a recent pelvic ultrasound, thereby excluding subclinical UL cases, results were stronger for rs6058017 (GG genotype vs. others: OR=1.36, 95% CI: 1.15, 1.61) but not for rs12800438 (GG genotypes vs. others: OR=1.05, 95% CI: 0.89, 1.23).

We assessed the role of dietary vitamin D intake and vitamin D supplementation on UL risk, and their modifying effects on the genotype-UL associations (Table 4). ORs for 2.5–4, 5–7.4, ≥7.5 mcg/day vs. <2.5 mcg/day of dietary vitamin D in 1995 were 0.88 (95% CI: 0.78, 1.00), 0.71 (95% CI: 0.54, 0.92), and 0.52 (95% CI: 0.30, 0.91), consistent with published reports from our cohort showing an inverse association between dairy (major contributor to dietary vitamin D) and UL (41, 42). The OR for vitamin D supplementation in 1995 or 1997 was 0.97 (95% CI: 0.79, 1.18). Among women using vitamin D supplements or consuming ≥5 mcg/day dietary vitamin D, the association was attenuated for two homozygous GG genotypes versus no homozygous GG genotypes (OR=1.05, 95% CI: 0.64, 1.71). Among non-users of supplements consuming <5 mcg/day dietary vitamin D, the OR for having two homozygous GG genotypes vs. none was 1.53 (95% CI: 1.24, 1.88). Although these results suggest possible gene-environment interaction, differences in the effect of genotype by vitamin D supplements/diet were not statistically significant (*P-interaction*=0.235).

DISCUSSION

This is the first study to assess polymorphisms in vitamin D-related genes in relation to UL incidence. UL risk was positively associated with the GG genotype of SNPs rs12800438 near *DHCR7* and rs6058017 in *ASIP*. A second SNP near *DHCR7*, rs4944957, was nominally associated with UL risk but not after correcting for multiple testing. The high correlation ($r^2 = 0.62$) between the two *DHCR7* SNPs suggests that they may be tagging the same causal variant. Results did not vary appreciably by age, BMI, or family history of UL, and results for *ASIP* were stronger when the control group was restricted to those with a recent pelvic ultrasound (among whom UL misclassification is reduced). The direction of

the associations was consistent with the hypothesis that vitamin D deficiency increases UL risk.

Agouti signaling protein (ASP), encoded by the *ASIP* gene, was first shown to inhibit eumelanogensis in human melanocytes in 1997 (26). The *ASIP* gene is located on chromosome 20q and consists of three exons (26). The rs6058017 polymorphism is associated with light and dark pigmentation across the globe (43). The G-allele confers darker skin in a recessive manner in African Americans (23), consistent with the recessive risk model observed in our study. In fact, some researchers argue that SNPs in *ASIP* (and other skin pigmentation genes) should be considered genetic proxies for lifetime sun exposure (44).

The rs12800438 is located in the third intron of the *NADSYN1* (NAD synthetase 1) gene. However, the causal variant tagged by this SNP is most likely affecting vitamin D metabolism through the nearby *DHCR7* gene (~4.7 kb away from *NADSYN1*). *DHCR7* is located on chromosome 11 (11q13.4) and encodes 7-dehydrocholesterol reductase, an enzyme involved in the conversion of 7-dehydrocholesterol (precursor of cholecalciferol) into cholesterol in human skin (45). A recent genome-wide methylation study among African Americans found differences of methylation in 79 CpG sites comparing extremes of the 25(OH)D plasma distribution (≤25 vs. >75 nmol/L), including 2 CpG sites in the 5' upstream region of *DHCR7* (46). This suggests that genetic variants near *DHCR7* affect vitamin D3 levels through gene expression regulation. Evidence of positive natural selection at the *DHCR7* gene in European and Northeast Asian populations was recently found, possibly due to selective advantage of having low activity of the 7-dehydrocholesterol reductase and greater availability of 7-dehydrocholesterol in skin (19). These observations indicate the presence of high-frequency variants associated with high vitamin D3 levels in Northern latitude populations, and a lower frequency of those variants in African ancestry populations. Although rs12800438 was examined in only one (12) of the two GWAS among European Americans (12, 13), a study investigating 19 of the GWAS-identified SNPs found that, among darker pigmented African Americans, rs12800438 had the strongest association with vitamin D deficiency (<20 ng/ml) (OR=0.40; 95% CI: 0.22, 0.75; P=0.004) (25).

Vitamin D supplementation or dietary intake of vitamin D (≥5 mcg/day) appeared to negate the harmful effect of having two of the high-risk genotypes. Both *ASIP* and *DHCR7* affect vitamin D synthesis in the skin: *ASIP* determines skin pigmentation and the ability of skin to synthesize vitamin D when exposed to sunlight; and 7-dehydrocholesterol reductase (i.e. enzyme coded by *DHCR7*) converts 7-dehydrocholesterol into cholesterol and therefore regulates availability of the substrate for UV light action. If the high-risk *ASIP* and *DHCR7* genotypes are related to deficient vitamin D synthesis in the skin, then we would expect vitamin D supplementation or dietary intake (not dependent on skin) to reduce the risk conferred by rs12800438 or rs6058017.

Our data are consistent with three epidemiologic studies published in 2013—two cross-sectional studies (9, 11) and one case-control study (10)—all showing significantly lower serum vitamin D3 levels among UL cases than controls. Vitamin D3 may protect against UL risk by reducing cell proliferation, increasing apoptosis and differentiation, and regulating

angiogenesis and extracellular matrix synthesis (8, 9, 47, 48), important processes in UL pathogenesis (7). Specifically, tissue culture studies show that UL treated with calcitriol, the hormonally active form of vitamin D3 (1,25(OH)2D), exhibit reduced cell proliferation and diminished extracellular matrix production (48–51). Studies in the Eker rat show that treatment with calcitriol inhibits UL growth *in vivo* (52).

Limitations of our investigation include reduced statistical power to detect significant associations when allele frequencies were low, and the possibility that we may have missed other important SNPs involved in the vitamin D pathway. The vitamin D related genes we examined included 4 of the 5 genes in the major pathway of production, metabolism, and activity, but not *CYP27B1*. Furthermore, we analyzed only a subset of the GWAS-identified SNPs (12, 13). However, we incorporated variants of other critical loci (*GC, DHCR7*, and *CYP2R1*) associated with vitamin D levels in GWAS (12, 13) and studies of African Americans (25). Genes are an imperfect measure of actual vitamin D status. Nevertheless, some SNPs in the vitamin D pathway have shown strong correlations with serum vitamin D concentrations (12, 13, 25) and skin pigmentation (23, 44). Our analyses of gene-environment interactions were underpowered because large sample sizes are needed to be able to detect differences in genotype by environmental factors; however, there was a suggestion in the data that dietary and supplemental vitamin D modified the genotype effect in the expected direction. Because vitamin D supplementation was ascertained along with calcium supplementation in the earlier years of the study (1995 and 1997), at a time when supplementation was less common, underascertainment of actual vitamin D supplementation throughout the follow-up period was likely. Finally, we lacked prospective data on an important environmental predictor of vitamin D status: sun exposure.

Strengths of our study include adjustment for age, geographic region, and ancestry, potential confounders of the vitamin D-UL association. Observed minor allele frequencies were consistent with 1000 Genomes Project data for populations of African ancestry (53). Cohort retention was high, reducing potential for differential loss to follow-up. Because we did not screen all women for UL, we may have misclassified true cases (5). However, our validation study of UL indicated high accuracy in reporting (>96%) and results were similar when we restricted the control group to women with a recent pelvic ultrasound. Although misclassification of UL was likely because small tumors may not have been recorded or reported to the participant, and the 5-year ultrasound interval was fairly broad, any misclassification would be non-differential (unrelated to genes under study) and lead to attenuation of effects. The large sample size conferred high statistical power to detect relatively small differences in risk. Because 87% of cases had UL-related symptoms or a palpable tumor on pelvic exam (31), our results likely apply to symptomatic UL, which represents the disease burden in reproductive-aged women.

In summary, two SNPs involved in the vitamin D pathway were associated with UL risk: rs12800438 near *DHCR7* and rs6058017 in *ASIP*. Our data provide support for the hypothesis that vitamin D deficiency is involved in UL etiology, but we did not have serum measurements of vitamin D to test the hypothesis directly. It remains to be shown whether vitamin D3 deficiency explains a large fraction of the black-white disparity in UL incidence.

Prospective studies involving direct measurement of vitamin D3 levels before UL diagnosis are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Buttram VC, Reiter RC. Uterine leiomyomata: etiology, symptomatology, and management. Fertil Steril. 1981; 36:433–445. [PubMed: 7026295]
2. Coronado GD, Marshall LM, Schwartz SM. Complications in pregnancy, labor, and delivery with uterine leiomyomas: a population-based study. Obstet Gynecol. 2000; 95:764–769. [PubMed: 10775744]
3. Stewart EA. Uterine fibroids. Lancet. 2001; 357:293–298. [PubMed: 11214143]
4. Marshall LM, Spiegelman D, Barbieri RL, Goldman MB, Manson JE, Colditz GA, et al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol. 1997; 90:967–973. [PubMed: 9397113]
5. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol. 2003; 188:100–107. [PubMed: 12548202]
6. Kjerulff KH, Guzinski GM, Langenberg PW, Stolley PD, Moye NE, Kazandjian VA. Hysterectomy and race. Obstet Gynecol. 1993; 82:757–764. [PubMed: 8414322]
7. Wise, LA.; Laughlin-Tommaso, SK. Uterine Leiomyomata. In: Goldman, MB.; Troisi, R.; Rexrode, KM., editors. Women And Health. San Diego, CA: Academic Press; 2013. p. 285-306.
8. Baird DD. Hypothesis: vitamin D protects against uterine fibroid development [Abstract]. Ann Epidemiol. 2008; 18:710.
9. Baird DD, Hill MC, Schectman JM, Hollis BW. Vitamin D and the Risk of Uterine Fibroids. Epidemiology. 2013; 24:447–453. [PubMed: 23493030]
10. Paffoni A, Somigliana E, Vigano P, Benaglia L, Cardellicchio L, Pagliardini L, et al. Vitamin D status in women with uterine leiomyomas. J Clin Endocrinol Metab. 2013; 98:E1374–E1378. [PubMed: 23824422]
11. Sabry M, Halder SK, Allah AS, Roshdy E, Rajaratnam V, Al-Hendy A. Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: a cross-sectional observational study. Int J Womens Health. 2013; 5:93–100. [PubMed: 23467803]
12. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010; 376:180–188. [PubMed: 20541252]
13. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet. 2010; 19:2739–2745. [PubMed: 20418485]

14. Lucas RM, Ponsonby AL, Dear K, Valery PC, Taylor B, van der Mei I, et al. Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. J Steroid Biochem Mol Biol. 2013; 136:300–308. [PubMed: 23395985]
15. Didriksen A, Grimnes G, Hutchinson MS, Kjaergaard M, Svartberg J, Joakimsen RM, et al. The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, BMI, and baseline levels. Eur J Endocrinol. 2013; 169:559–567. [PubMed: 23935129]
16. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, et al. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. J Clin Endocrinol Metab. 2008; 93:3381–3388. [PubMed: 18593774]
17. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013; 369:1991–2000. [PubMed: 24256378]
18. Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, Zhao H, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. Breast Cancer Res. 2012; 14:R58. [PubMed: 22480149]
19. Kuan V, Martineau AR, Griffiths CJ, Hypponen E, Walton R. DHCR7 mutations linked to higher vitamin D status allowed early human migration to northern latitudes. BMC Evol Biol. 2013; 13:144. [PubMed: 23837623]
20. Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, Aros MC, et al. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science. 2005; 310:1782–1786. [PubMed: 16357253]
21. Basu Mallick C, Iliescu FM, Mols M, Hill S, Tamang R, Chaubey G, et al. The Light Skin Allele of SLC24A5 in South Asians and Europeans Shares Identity by Descent. PLoS Genet. 2013; 9:e1003912. [PubMed: 24244186]
22. Shriver MD, Parra EJ, Dios S, Bonilla C, Norton H, Jovel C, et al. Skin pigmentation, biogeographical ancestry and admixture mapping. Hum Genet. 2003; 112:387–399. [PubMed: 12579416]
23. Bonilla C, Boxill LA, Donald SA, Williams T, Sylvester N, Parra EJ, et al. The 8818G allele of the agouti signaling protein (ASIP) gene is ancestral and is associated with darker skin color in African Americans. Hum Genet. 2005; 116:402–406. [PubMed: 15726415]
24. Signorello LB, Shi J, Cai Q, Zheng W, Williams SM, Long J, et al. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. PLoS One. 2011; 6:e28623. [PubMed: 22205958]
25. Kittles, RA.; Batai, K.; Shah, S.; Ruden, M.; Newsome, J.; Agate, S., et al. Genetic and environmental predictors of serum vitamin D3 levels in African Americans [Abstract]. The 62nd American Society of Human Genetics (ASHG) Annual Meeting in San Francisco, CA; 2012 Nov. p. 6-10.
26. Scherer D, Kumar R. Genetics of pigmentation in skin cancer--a review. Mutation Research. 2010; 705:141–153. [PubMed: 20601102]
27. Rosenberg L, Adams-Campbell LL, Palmer JR. The Black Women's Health Study: a follow-up study for causes and preventions of illness. J Am Med Womens Assoc. 1995; 50:56–58. [PubMed: 7722208]
28. Cozier YC, Palmer JR, Rosenberg L. Comparison of methods for collection of DNA samples by mail in the Black Women's Health Study. Ann Epidemiol. 2004; 14:117–122. [PubMed: 15018884]
29. Dueholm M, Lundorf E, Hansen ES, Ledertoug S, Olesen F. Accuracy of magnetic resonance imaging and transvaginal ultrasonography in the diagnosis, mapping, and measurement of uterine myomas. Am J Obstet Gynecol. 2002; 186:409–415. [PubMed: 11904599]
30. Loutradis D, Antsaklis A, Creatsas G, Hatzakis A, Kanakas N, Gougoulakis A, et al. The validity of gynecological ultrasonography. Gynecol Obstet Invest. 1990; 29:47–50. [PubMed: 2190879]
31. Wise LA, Palmer JR, Stewart EA, Rosenberg L. Age-specific incidence rates for self-reported uterine leiomyomata in the Black Women's Health Study. Obstet Gynecol. 2005; 105:563–568. [PubMed: 15738025]

32. Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. Epidemiology. 1990; 1:58–64. [PubMed: 2081241]
33. Kumanyika SK, Mauger D, Mitchell DC, Phillips B, Smiciklas-Wright H, Palmer JR. Relative validity of food frequency questionnaire nutrient estimates in the Black Women's Health Study. Ann Epidemiol. 2003; 13:111–118. [PubMed: 12559670]
34. Smith MW, Patterson N, Lautenberger JA, Truelove AL, McDonald GJ, Waliszewska A, et al. A high-density admixture map for disease gene discovery in African Americans. Am J Hum Genet. 2004; 74:1001–1013. [PubMed: 15088270]
35. Hoggart CJ, Shriver MD, Kittles RA, Clayton DG, McKeigue PM. Design and analysis of admixture mapping studies. Am J Hum Genet. 2004; 74:965–978. [PubMed: 15088268]
36. Shlush LI, Bercovici S, Wasser WG, Yudkovsky G, Templeton A, Geiger D, et al. Admixture mapping of end stage kidney disease genetic susceptibility using estimated mutual information ancestry informative markers. BMC Med Genomics. 2010; 3:47. [PubMed: 20955568]
37. Ruiz-Narvaez EA, Rosenberg L, Wise LA, Reich D, Palmer JR. Validation of a Small Set of Ancestral Informative Markers for Control of Population Admixture in African Americans. Am J Epidemiol. 2011; 173:587–592. [PubMed: 21262910]
38. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]
39. SAS. SAS/STAT® 9.2 User's Guide. Cary, NC. Cary, NC: SAS Institute; 2008. SAS Institute Inc. 2008.
40. Schwartz SM, Marshall LM, Baird DD. Epidemiologic contributions to understanding the etiology of uterine leiomyomata. Environ Health Perspect. 2000; 108:821–827. [PubMed: 11035989]
41. Wise LA, Palmer JR, Ruiz-Narvaez E, Reich DE, Rosenberg L. Is the observed association between dairy intake and fibroids in african americans explained by genetic ancestry? Am J Epidemiol. 2013; 178:1114–1119. [PubMed: 23825169]
42. Wise LA, Radin RG, Palmer JR, Kumanyika SK, Rosenberg L. A prospective study of dairy intake and risk of uterine leiomyomata. Am J Epidemiol. 2010; 171:221–232. [PubMed: 19955473]
43. Norton HL, Kittles RA, Parra E, McKeigue P, Mao X, Cheng K, et al. Genetic evidence for the convergent evolution of light skin in Europeans and East Asians. Mol Biol Evol. 2007; 24:710–722. [PubMed: 17182896]
44. Bonilla C, Gilbert R, Kemp JP, Timpson NJ, Evans DM, Donovan JL, et al. Using genetic proxies for lifecourse sun exposure to assess the causal relationship of sun exposure with circulating vitamin d and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2013; 22:597–606. [PubMed: 23441100]
45. Waterham HR, Wanders RJ. Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith-Lemli-Opitz syndrome. Biochim Biophys Acta. 2000; 1529:340–356. [PubMed: 11111101]
46. Zhu H, Wang X, Shi H, Su S, Harshfield GA, Gutin B, et al. A genome-wide methylation study of severe vitamin D deficiency in African American adolescents. J Pediatr. 2013; 162:1004–1009. e1. [PubMed: 23219444]
47. Hossein-Nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc. 2013; 88:720–755. [PubMed: 23790560]
48. Halder SK, Osteen KG, Al-Hendy A. 1,25-Dihydroxyvitamin D3 Reduces Extracellular Matrix-Associated Protein Expression in Human Uterine Fibroid Cells. Biol Reprod. 2013
49. Blauer M, Rovio PH, Ylikomi T, Heinonen PK. Vitamin D inhibits myometrial and leiomyoma cell proliferation in vitro. Fertil Steril. 2009; 91:1919–1925. [PubMed: 18423458]
50. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, Al-Hendy A. Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertil Steril. 2011; 95:247–253. [PubMed: 20736132]
51. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 Reduces TGF-{beta}3-Induced Fibrosis-Related Gene Expression in Human Uterine Leiomyoma Cells. J Clin Endocrinol Metab. 2011; 96:E754–E762. [PubMed: 21289245]

52. Halder SK, Sharan C, Al-Hendy A. 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod. 2012; 86:116. [PubMed: 22302692]

53. Clarke L, Zheng-Bradley X, Smith R, Kulesha E, Xiao C, Toneva I, et al. The 1000 Genomes Project: data management and community access. Nat Methods. 2012; 9:459–462. [PubMed: 22543379]

Table 1

Baseline characteristics of cases and controls. Black Women's Health Study, USA, 1997–2011.

Characteristics	Controls (N=2,432)	Cases (N=2,232)
Age in 1997 (y), mean	34.5 ± 7.0	34.3 ± 6.2
Age at diagnosis (cases) or end of follow-up (controls) (y), mean	45.5 ± 6.0	38.4 ± 6.3
Percent European ancestry, mean	21.2 ± 11.1	19.7 ± 10.3
Age at menarche (y), mean	12.4 ± 1.6	12.2 ± 1.6
Parous in 1997, %[a]	59.6	53.7
Age at first birth in 1997 (y), mean[a]	23.9 ± 6.5	22.9 ± 5.0
Time since last birth in 1997 (y), mean[a]	8.6 ± 8.2	9.8 ± 6.8
Education in 1995 (y), mean	14.9 ± 1.8	14.9 ± 1.7
BMI in 1997 (kg/m^2), mean	27.6 ± 6.8	27.6 ± 6.7
Current alcohol intake (drinks/wk), mean	1.3 ± 3.0	1.4 ± 3.1
Smoking history, %		
Current	9.8	10.8
Former	18.1	15.3
Geographic region of residence, %		
Northeast	18.3	21.9
South	38.9	37.9
Midwest	23.9	23.2
West	18.9	17.0
Recent Papanicolau smear, %	95.2	94.9

Abbreviations: UL, uterine leiomyomata; BMI, body mass index.

Means (± SD) and percentages are standardized to age distribution of sample in 1997.

[a]Restricted to parous women only.

Table 2

Association between Vitamin D SNPs and UL. Genotypic model.

Gene	SNP[a]	Risk allele frequency (%)		*P-value for allele frequency difference*		Cases/controls	Unadjusted model	Adjusted model[b]
		Cases	Controls	Nominal[b]	Permutated[b]		OR (95% CI)	OR (95% CI)
GC	rs7041							
	TT	18.2	19.0	*0.350*	*0.993*	1,369/1,283	1.00	1.00
	TC					596/589	0.95 (0.83, 1.09)	1.01 (0.88, 1.16)
	CC					72/75	0.90 (0.65, 1.25)	0.98 (0.70, 1.37)
GC	rs2298849							
	AA	37.0	36.9	*0.974*	*0.999*	887/950	1.00	1.00
	AG					1,026/1,131	0.97 (0.86, 1.10)	0.96 (0.85, 1.09)
	GG					308/322	1.02 (0.86, 1.23)	1.01 (0.84, 1.21)
CYP2R1	rs10766196							
	AA	15.9	16.0	*0.889*	*0.999*	1,558/1,685	1.00	1.00
	AG					590/642	0.99 (0.87, 1.13)	1.05 (0.92, 1.20)
	GG					56/62	0.98 (0.68, 1.41)	1.04 (0.72, 1.51)
CYP2R1	rs10741657							
	GG	28.5	26.7	*0.059*	*0.503*	1,155/1,259	1.00	1.00
	GA					855/977	0.95 (0.85, 1.08)	0.96 (0.85, 1.09)
	AA					203/149	1.49 (1.19, 1.86)	1.48 (1.18, 1.85)
DHCR7	rs4944957							
	GG	49.2	46.8	*0.022*	*0.225*	588/669	1.00	1.00
	GA					1,069/1,201	1.01 (0.88, 1.16)	1.01 (0.88, 1.16)
	AA					554/518	1.22 (1.03, 1.43)	1.19 (1.01, 1.40)
DHCR7	rs12800438							
	AA	61.4	58.3	*0.002*	*0.028*	327/402	1.00	1.00
	GA					1,053/1,187	1.09 (0.92, 1.29)	1.09 (0.92, 1.29)
	GG					829/796	1.28 (1.07, 1.53)	1.23 (1.03, 1.47)
VDR	rs739837							
	AA	42.3	42.1	*0.837*	*0.999*	726/756	1.00	1.00

Gene	SNP[a]	Risk allele frequency (%)		*P-value for allele frequency difference*		Cases/controls	Unadjusted model	Adjusted model[b]
		Cases	Controls	Nominal[b]	Permutated[b]		OR (95% CI)	OR (95% CI)
	AG					997/1,135	0.92 (0.80, 1.04)	0.92 (0.81, 1.05)
	GG					399/394	1.05 (0.89, 1.25)	1.05 (0.88, 1.25)
VDR	rs886441							
	AA	39.5	39.3	*0.825*	*0.999*	735/713	1.00	1.00
	AG					988/935	1.02 (0.89, 1.17)	0.99 (0.86, 1.14)
	GG					309/296	1.01 (0.84, 1.22)	0.97 (0.80, 1.18)
OCA2	rs1800404							
	CC	22.3	23.2	*0.332*	*0.991*	1,300/1,382	1.00	1.00
	TC					785/811	1.03 (0.91, 1.16)	1.10 (0.97, 1.25)
	TT					94/134	0.75 (0.57, 0.98)	0.88 (0.66, 1.17)
SLC24A5	rs1426654							
	GG	22.2	22.6	*0.668*	*0.999*	1,344/1,429	1.00	1.00
	GA					757/846	0.95 (0.84, 1.08)	1.04 (0.91, 1.18)
	AA					114/118	1.03 (0.79, 1.34)	1.31 (0.99, 1.74)
ASIP	rs6058017							
	AA	66.2	62.9	*0.001*	*0.013*	253/309	1.00	1.00
	GA					929/1,104	1.03 (0.85, 1.24)	1.01 (0.83, 1.22)
	GG					941/908	1.27 (1.05, 1.53)	1.18 (0.97, 1.44)
CYP24A1	rs2762941							
	GG	49.9	49.7	*0.846*	*0.999*	562/626	1.00	1.00
	GA					1,076/1,140	1.05 (0.91, 1.21)	1.03 (0.90, 1.19)
	AA					558/612	1.02 (0.86, 1.19)	0.98 (0.83, 1.16)

[a] Order of genotype categories is as follows: top row = homozygous for the reference allele, middle row =heterozygous, bottom row = homozygous for the risk allele.

[b] Adjusted for age in 1997, geographic region, and percent European ancestry.

Numbers of cases and controls do not sum to 2,432 and 2,232, respectively, due to missing genotype data.

Table 3

Association between Vitamin D SNPs and UL. Recessive model.

SNP	Cases/controls	Unadjusted model OR (95% CI)	Adjusted model OR (95% CI)[a]
rs12800438			
AA/GA	1,380/1,589	1.00	1.00
GG	829/796	1.20 (1.06, 1.35)	1.16 (1.02, 1.31)
rs6058017			
AA/GA	1,182/1,413	1.00	1.00
GG	941/908	1.24 (1.10, 1.40)	1.17 (1.04, 1.33)
Sum of homozygous GG genotypes			
None	744/933	1.00	1.00
One	995/1,049	1.19 (1.05, 1.35)	1.12 (0.98, 1.28)
Two	368/302	1.53 (1.28, 1.83)	1.42 (1.18, 1.71)

[a] Adjusted for age in 1997, geographic region, and percent European ancestry.

Numbers of cases and controls do not sum to 2,432 and 2,232, respectively, due to missing genotype data.

Table 4

Association between Vitamin D SNPs and UL by dietary/supplemental intake of vitamin D

	Dietary or supplemental intake of vitamin D			
	<5 mcg/d (<200 IU) and no supplements		≥5 mcg/d (≥200 IU) or supplements	
SNP	**Cases/ controls**	**Adjusted model[a] OR (95% CI)**	**Cases/ controls**	**Adjusted model[a] OR (95% CI)**
rs12800438				
AA/GA	1,183/1,323	1.00 (ref.)	197/266	1.00 (ref.)
GG	715/665	1.16 (1.01, 1.33)	114/131	1.15 (0.84, 1.57)
rs6058017				
AA/GA	999/1,177	1.00 (ref.)	183/236	1.00 (ref.)
GG	820/757	1.23 (1.07, 1.41)	121/151	0.99 (0.72, 1.35)
Sum of homozygous GG genotypes				
None	329/251	1.00 (ref.)	107/154	1.00 (ref.)
One	842/873	1.31 (1.00, 1.72)	153/176	1.19 (0.85, 1.65)
Two	637/779	1.53 (1.24, 1.88)	39/51	1.05 (0.64, 1.71)

[a] Adjusted for age in 1997, geographic region, and percent European ancestry.

Numbers of cases and controls do not sum to 2,432 and 2,232, respectively, due to missing genotype data.