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Genetic Ancestry, Skin Reflectance and Pigmentation Genotypes in Association with Serum Vitamin D Metabolite Balance

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Abstract

Background—Lower serum vitamin D (25(OH)D) among individuals with African ancestry is attributed primarily to skin pigmentation. However, the influence of genetic polymorphisms controlling for skin melanin content has not been investigated. Therefore, we investigated differences in non-summer serum vitamin D metabolites according to self-reported race, genetic ancestry, skin reflectance and key pigmentation genes (*SLC45A2* and *SLC24A5*).

Materials and Methods—Healthy individuals reporting at least half African American or half European American heritage were frequency matched to one another on age (+/− 2 years) and sex. 176 autosomal ancestry informative markers were used to estimate genetic ancestry. Melanin index was measured by reflectance spectrometry. Serum vitamin D metabolites (25(OH)D₃, 25(OH)D₂ and 24,25(OH)₂D₃) were determined by high performance liquid chromatography (HPLC) tandem mass spectrometry. Percent 24,25(OH)₂D₃ was calculated as a percent of the parent metabolite (25(OH)D₃). Stepwise and backward selection regression models were used to identify leading covariates.

Results—Fifty African Americans and 50 European Americans participated in the study. Compared with *SLC24A5* 111^{Thr} homozygotes, individuals with the *SLC24A5* 111^{Thr/Ala} and 111^{Ala/Ala} genotypes had respectively lower levels of 25(OH)D₃ (23.0 and 23.8 nmol/L lower, p-dominant=0.007), and percent 24,25(OH)₂D₃ (4.1 and 5.2 percent lower, p-dominant=0.003), controlling for tanning bed use, vitamin D/fish oil supplement intake, race/ethnicity, and genetic

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ancestry. Results were similar with melanin index adjustment, and were not confounded by glucocorticoid, oral contraceptive, or statin use.

Conclusions—The *SLC24A5* 111^{Ala} allele was associated with lower serum vitamin 25(OH)D₃ and lower percent 24,25(OH)₂D₃, independently from melanin index and West African genetic ancestry.

Keywords

African Continental Ancestry Group; European Continental Ancestry Group; *SLC24A5*; 25-hydroxyvitamin D; 24,25-Dihydroxyvitamin D₃

Introduction

Vitamin D metabolites play an important role in calcium homeostasis, phosphate reabsorption, and bone health.[1, 2] It is well-recognized that individuals with African Ancestry have substantially lower (~2-fold) serum 25(OH)D levels compared with other racial/ethnic groups.[3] These differences are attributed primarily to skin pigmentation.[4, 5] However, serum 25(OH)D levels have been associated with genetic differences, diet, sun exposure, and hormone status.[3] Paradoxically, there is a substantially lower risk of hip fracture among older African Americans, despite lower serum 25(OH)D levels.[6] Among individuals with European ancestry alone, a moderately high heritability of serum 25(OH)D levels (~50 to 70% for 25(OH)D) has been observed [7, 8], and trials among healthy individuals report up to 5-fold differences in serum vitamin D responses to dosage with vitamin D or UV light.[9-13] The importance of understanding differences in vitamin D metabolism is underscored by adverse health outcomes associated with the vitamin D supplementation in high-dose intervention trials.[13, 14]

The 25(OH)D metabolite is the principal hydroxylated metabolite circulating in the nanomolar range, and is considered a reasonable functional biomarker of vitamin D status. While subject to seasonal variation, it has a prolonged half-life of two to four weeks, within-individual consistency over time, and minimal diurnal variation.[15-17] 25(OH)D is comprised of both 25(OH)D₂ and 25(OH)D₃ which share similar metabolic pathways. 24,25(OH)₂D₃ is the major circulating dihydroxy metabolite of 25(OH)D₃ and is not frequently studied in relation to health outcomes.

The most biologically active vitamin D metabolite is 1,25(OH)₂D₃ (calcitriol, Figure 1), which circulates in the picomolar range, and may be the most relevant to long-term health outcomes. Nonetheless, 1,25(OH)₂D₃ is not frequently used as a biomarker in epidemiologic studies since it has a short *in vivo* half life of approximately 6 to 10 hours in serum, and is known to vary in concentration according to time of day, estrogen treatment, ovulatory cycle status, dietary phosphorous intakes, kidney function, and diuretic use.[18-20]

A clear definition of an adequate level of serum 25(OH)D has been hampered by inconsistent results from both observational studies and randomized clinical trials carried out for a number of different disease outcomes.[3] Part of this inconsistency may be due to genetic differences in the populations studied and the fact that genetic ancestry was not accounted for in the assessment of risk.

We therefore conducted a study of healthy individuals in order to examine the association between individual serum vitamin D metabolites, self-reported race, genetic ancestry, skin reflectance and polymorphisms in two genes known to participate in melanogenesis (*SLC45A2* and *SLC24A5*). The polymorphisms in these two genes were chosen because

they are the two most significant polymorphisms associated with skin reflectance, as measured through either p-value or R^2 value.[21, 22]

Materials and Methods

Study Participants and Human Subjects Approval

This work complies with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects, and was approved by the Penn State Institutional Review Board (IRB# 22524). Healthy volunteers from Pennsylvania State University (University Park, Pennsylvania) were recruited by inter-office communication, student list-servs, newspaper and word of mouth at the Penn State University Park Campus. Eligible participants were required to self-identify with at least 50% European or at least 50% African or African American ancestry. Individuals with a fever (100°F or higher) at the time of visit, severe chronic disease (i.e., end stage renal disease, chronic obstructive pulmonary disease, liver failure, HIV/AIDS), unable to provide a blood sample, or currently undergoing chemotherapy or radiation treatment for cancer, were excluded from participation. At recruitment, participants were frequency matched on age ($\pm$ 2 years), sex and self-reported race/ethnicity.

During one visit to the Penn State General Clinical Research Center (GCRC), participants were administered informed consent, donated a 50 ml blood sample, answered a questionnaire, and were measured for skin reflectance (melanin index), height, weight and blood pressure (systolic and diastolic). In order to minimize the variation in serum vitamin D level, blood collection occurred during non-Summer months (i.e., no blood collection from May 15th to August 31st). The questionnaire included information on age, sex, race/ethnicity, as well as sources of vitamin D. Sources of vitamin D included current tanning bed use (yes/no), number of hours spent outdoors in occupational and leisure activities; vitamin D-containing foods; supplement intake over the past year that included fish oil use (yes/no), and the form, quantity and frequency of vitamin D supplement use. The frequency of vitamin D-containing foods intake was recorded as never, a few times per year, once per month, 2-3 times per month, once per week, 2 times per week, 3-4 times per week, 5-6 times per week, or every day.

Participants reported all current medication use, including medications that may influence vitamin D metabolism, such as cholesterol-lowering drugs (“statins”), oral contraceptives, glucocorticoids, and non-prescription steroids.[23] Current glucocorticoid use was determined by review of the trade and generic drug names, as previously described,[24] and individuals indicating use of “inhaler” for asthma were classified as a current glucocorticoid user.

Skin Spectroscopy

Skin reflectance was measured by the DermaSpectrometer (CyberDerm, Media, PA, USA) which estimates melanin content of the skin based on reflectance of green and red light emitting diodes (LEDs). Two separate measurements were taken on the upper inner side (medial aspect) of each arm, avoiding moles and freckles. The measurements were averaged for the melanin index for each individual. Melanin index measurements using the DermaSpectrometer have a published physiologic range of 30 (very light skin) to 96 (very dark skin), and are highly reproducible with sufficient sensitivity to detect small induced changes in skin pigmentation in human volunteers, which is comparable to other instruments that use a broader light spectrum.[25]

Blood Collection and DNA Extraction

Serum was collected from whole blood drawn into a red top Vacutainer tube (BD Biosciences, USA) and allowed to clot for 30-60 minutes, followed by centrifugation, and the immediate transfer of the serum into 2 ml cryovials for storage at -80°C . Lymphocytes from blood collected in EDTA tubes were separated by differential centrifugation in Ficoll-Paque PLUS within 30 minutes of blood collection at the Penn State GCRC laboratory, aliquotted and placed into liquid nitrogen. DNA was extracted from the lymphocytes using a QIAamp DNA Mini Kit (QIAGEN, USA) and stored at -80°C .

Serum Calcium Assay

Total serum calcium was measured in duplicate with a colorimetric assay (BioVision, Mountain View, CA) at the Penn State Core Endocrine Lab. The average value between the two runs was used in the analysis. This assay spans the physiological range of calcium concentration 8.6-10.0 mg/dL (2.15-2.50 mM), CV=3.5%.

Vitamin D Metabolite Assays

Because of the recognized method differences in measuring vitamin D metabolites, levels of the major circulating vitamin D metabolite (25(OH)D) were measured both by radioimmunoassay (Immunodiagnosics, Inc., UK) and LC-MS/MS at the Penn State Core Endocrine Lab and the Penn State Mass Spectrometry Core Lab, respectively. The RIA assay has an inter-assay precision (coefficient of variation) of 8%, a within-run precision of 5%, and a reportable range of 7.0-120.0 ng/mL.

LC-MS/MS was used to measure serum levels of 25(OH)D₂, 25(OH)D₃ and 24,25(OH)₂D₃. Briefly, samples were prepared by solid phase extraction as follows: 400 μl of serum was spiked with 40 μl of deuterated standards (250 ng/ml of d₆-25(OH)D₃, and 100 ng/ml of d₆-25(OH)D₂, from Chemaphor, Inc., Ottawa, Canada). Protein precipitation was conducted by adding four volumes of 50/50 acetonitrile/methanol followed by centrifugation to pellet the insoluble matter. The supernatant was diluted with water to reduce the organic percentage below 25% prior to solid phase extraction using a Waters Oasis HLB Extraction Column (3cc 60mg) (Waters, Milford, MA, USA). Mass spectra were acquired using a hybrid triple quadrupole linear ion trap (4000 Q TRAP® system, Applied Biosystems) with Nitrogen as the collision gas. Multiple reaction monitoring (MRM) functions were applied as previously published.[26] Quantification was performed using Analyst software 1.5.1, and the standard curve was built with multi-point external calibrations with 1/x weighting.

In 10 repeated samples of pooled plasma samples from healthy individuals assayed on separate days, the CV% were 2.7, 12.2 and 6.3 for 25(OH)D₃, 25(OH)D₂ and 24,25(OH)₂D₃, respectively. The limit of quantitation (LOQ) for all 3 metabolites was 10 pg/ml with a standard curve range of 1 to 100 ng/ml for 25(OH)D₂ and 25(OH)D₃ and 0.1 to 10 ng/ml for 24,25(OH)₂D₃. Standards were prepared and run with each batch of samples.

Genotyping

Two separate panels of Ancestry Informative Markers (AIMs) across the genome were used to estimate genetic ancestry. The first panel of 176 AIMs was designed by DNAPrint Genomics using maximum likelihood methods for the determination of West African, European, East Asian and Native American Indian ancestry (DNAPrint, Inc., Sarasota, FL), as described in detail elsewhere.[27] This panel excludes the two skin pigmentation SNPs of interest in our study (i.e., SNP IDs rs1426654 and rs16891982). The second panel of 112 AIMS was designed by Dr. Jill Barnholtz-Sloan (Case Western) and was designed to robustly distinguish between West African and European as described in detail elsewhere [28] and individual ancestry proportions were estimated using maximum likelihood methods

(as reviewed in [29]). Genotyping for ancestry was conducted at the Penn State Genome Sciences Core Facility using an Illumina BeadXpress assay and by DNAPrint Genomics, Inc. using TaqMan genotyping.

Genotyping for solute carrier family 24, member 5 (*SLC24A5* (aka NCKX5, SNP ID rs1426654)) and solute carrier family 45, member 2 (*SLC45A2* (aka MATP, rs16891982)) polymorphisms was conducted by both TaqMan and Illumina BeadXpress. In the event genotyping between TaqMan and Illumina disagreed, the more conservative genotyping call was used (i.e., analyzed as heterozygote rather than homozygote). All genotyping was conducted by individuals blind to the race/ethnicity and vitamin D status of participants in the study. Duplicate genotyping on a 5% sample of participants was carried out to verify genotyping accuracy. Output of genotyping cluster algorithms were visually inspected and verified.

Statistical Methods

Because the amount of 24,25(OH)₂D₃ is likely a function of the available substrate (25(OH)D₃), [30] we calculated the percent 24,25(OH)₂D₃ as a proportion of total 25(OH)D₃ serum levels as a proxy for the catabolic rate of the active form of vitamin D (1,25(OH)₂D₃). [26] The statistical significance of the bi-variate difference in the distribution of continuous and categorical variables by self-reported race/ethnicity was tested by a two-sided t-test and Chi-Square test, respectively. In the event of categorical data with cell sizes less than 5, the two-sided Fisher's Exact test was used. Each frequency category of the dietary intake of vitamin D-containing foods were assigned corresponding values of 1 to 9 (1=never, 2=a few times per year, ... 9=every day). The difference in the frequency of dietary intake and the percent 24,25(OH)₂D₃ between African American and European American participants was tested using the non-parametric Wilcoxon Rank-Sum test. The Chi-square test for deviation from Hardy-Weinberg equilibrium was used to calculate the observed versus expected distribution of genotypes. The concordance between serum 25(OH)D values measured by RIA and LC-MS/MS and the concordance between percent West African Ancestry measured by DNAPrint Genomics and Barnholtz-Sloan were determined using the Concordance Correlation Coefficient. [31] The percent West African Ancestry estimated by DNAPrint Genomics were used in bi-variate analyses due to a lower frequency of missing observations. Agreement between *SLC24A5* and *SLC45A2* genotyping methods (Illumina and TaqMan) was calculated as the percent of exact genotype agreement out of all genotyping calls made.

Spearman's rank and Pearson's correlation coefficients were both calculated to investigate correlations between vitamin D metabolite levels and melanin index, genetic ancestry, self-reported race, oral contraceptive use, physical activity, glucocorticoids use, and genotype with genotype by Spearman's rank correlation coefficient. Stepwise and backward-selection variable models were used to identify genetic and environmental variables that were significantly associated with serum levels of 25(OH)D₃ and percent 24,25(OH)₂D₃ in both non-transformed and log-transformed outcome variable models. Variables included in selection models included melanin index (categorical and continuous forms), percent West African Ancestry (categorical and continuous forms), fish oil/vitamin D supplementation (yes/no), tanning bed use (yes/no), glucocorticoid use (yes/no), oral contraceptive use (yes/no), and skin pigmentation genotype. In presentation of the final model, tanning bed users were excluded and all models were adjusted for self-reported race/ethnicity and variables identified by both forward and backward selection methods. LC-MS/MS values for serum vitamin D metabolites and genetic ancestry estimates provided by DNAPrint Genomics were used in all population statistics.

Results

Methods Concordance

Values for the two analytical methods for serum vitamin D were well correlated (25(OH)D by RIA with LC-MS/MS (25(OH)D₃ + 0.75 * 25(OH)D₂: Concordance Correlation Coefficient (CCC)= 0.86 (95% Confidence Interval (CI): 0.81 to 0.91); as were the two methods of determination of the percent West African Ancestry: CCC=0.97 (95% CI: 0.96 TO 0.98), data not shown. The agreement between Illumina and TaqMan genotyping calls was 97.9% and 99.0% for *SLC24A5* and *SLC45A2*, respectively (data not shown).

Study Population Characteristics

Fifty African Americans and 50 European Americans participated in the study. There were no statistically significant differences between African American and European American participants with respect to age, sex, fish liver oil intake, vitamin D supplement use, calcium supplement use, glucocorticoid use, hours per week spent outdoors, current tobacco use, systolic blood pressure, diastolic blood pressure, nor the mean number of days past summer solstice when the blood was drawn (Table 1). No participants reported the location of their birth in a country on the African continent, or use of cholesterol lowering drugs. Body mass index was higher among African American participants, although African American participants also reported a higher prevalence of physical activity a few times/month and a higher prevalence of physical activity every day. Serum levels of 25(OH)D₃ and 24,25(OH)₂D₃ were significantly higher among European American participants compared with African American participants, and these differences persisted among men and women (Table 1). The percent 24,25(OH)₂D₃ was significantly lower only among women (9.5 v. 13.1, p<0.001). A higher frequency of African Americans had the *SLC45A2* 374^{Phe/Phe} genotype and the *SLC24A5* 111^{Ala/Thr} and 111^{Ala/Ala} genotypes, compared with European Americans. Within each racial/ethnic group the distribution of genotypes were within Hardy-Weinberg expectations (p > 0.05).

There were no significant differences between African Americans and European Americans in the intake frequency of vitamin D-containing foods, including: milk, milk/milk substitutes used on cereal, vitamin D-fortified orange juice, salmon, tuna (including tuna salads and tuna casseroles), sardines, oysters, other shellfish like shrimp/scallops/crabs, fish (not fried), ice cream, liver, and eggs (data not shown). European participants reported a higher frequency of intake of yogurt and frozen yogurt (p=0.029), while African American participants reported a higher frequency of intake of instant breakfast milkshakes (p=0.017), sardines (p=0.046), and fried fish/fish sandwiches (p<0.001, data not shown).

Serum Levels by Melanin Index, Genetic Ancestry and Self-Reported Race

The skin tones colorimetrically shown in Table 2 represent the tone at the median value within each quartile. Within the same skin melanin index quartile, differences in 25(OH)D₃ levels between African Americans and European Americans persisted, (23.4 nmol/L, Melanin Index Quartile 2, p=0.009), Table 2. Among African Americans, melanin index was significantly negatively correlated with serum levels of 25(OH)D₃ (Spearman's correlation = -0.3, p = 0.05), although this was not the case among European Americans (Spearman's correlation <+0.1, p > 0.05). There was not a statistically significant correlation between serum 24,25(OH)₂D₃, or the percent 24,25(OH)₂D₃ with either melanin index or genetic ancestry within each racial/ethnic group. Correlations between serum 25(OH)D₃ levels and percent East Asian or percent Native American Ancestry were not statistically significant (r=0.10, p=0.302 and r=0.03, p=0.801, respectively, data not shown.)

Serum Levels by Genotype, Melanin Index, Genetic Ancestry and Race/Ethnicity

As shown in Table 3, both *SLC45A2* and *SLC24A5* genotypes were significantly correlated with melanin index (both $r=0.8$), percent West African genetic ancestry (both $r=0.8$), and self-reported race/ethnicity ($r=0.6$ and 0.9), respectively. Within each melanin index quartile, there was no statistically significant correlation between serum 25(OH)D₃ levels and *SLC45A2* genotype, Table 3. Among individuals with the *SLC45A2* 374^{Leu/Leu} genotype, European Americans still had a significantly higher serum 25(OH)D₃ level compared with their African American counterparts (70.8 v. 41.6 nmol/L, $p=0.01$).

In contrast, as shown at the bottom of Table 3, the *SLC24A5* 111^{Ala} allele was significantly correlated with serum 25(OH)D₃ level within the three lightest skin melanin index quartiles (Q1-Q3: Spearman correlation coefficient range 0.4 to 0.5, all p -values ≤ 0.05), and within the two lowest tertiles of West African Genetic Ancestry (Spearman correlation coefficients range -0.4 to 0.6, all p -values ≤ 0.05). Among European Americans alone, individuals with the *SLC24A5* 111^{Thr/Thr} genotype had a mean serum 25(OH)D₃ level of 69.3 nmol/L, compared with the *SLC24A5* 111^{Thr/Ala} genotype (33.8 nmol/L, $p=0.004$). Upon closer review of the European American *SLC24A5* 111^{Thr/Ala} heterozygotes, two were classified with 0% West African Ancestry, and one with 49% West African Ancestry. Exclusion of the subject with 49% West African Ancestry resulted in a mean serum 25(OH)D₃ level of 38.8 nmol/L and an overall correlation of 0.3 ($P=0.04$) between 25(OH)D₃ and *SLC24A5* genotype among European Americans. Within each *SLC24A5* genotype, there was no statistically significant association between with serum 25(OH)D₃ levels and genetic ancestry or racial/ethnic group. Melanin index was significantly correlated with serum 25(OH)D₃, but only among *SLC24A5* heterozygotes. *SLC24A5* was not correlated with use of glucocorticoids, oral contraceptives, tanning beds or physical activity level (all $p > 0.05$, data not shown), within each racial/ethnic group. Figure 2 displays the regression line and R^2 value between serum 25(OH)D₃ levels according to skin melanin index for individuals homozygous for the *SLC24A5* 111^{Thr} allele (Figure 2a, $R^2=0.0003$) and homozygous or heterozygous for the 111^{Ala} allele (Figure 2b, $R^2=0.1081$) over the scatter plot (R^2 overall=0.268, data not shown).

In both backward and stepwise linear regression models, vitamin D/fish oil supplementation and *SLC24A5* genotype were retained in variable selection for serum 25(OH)D₃; *SLC24A5* genotype was retained in both variable selection methods for percent 24,25(OH)₂D₃. In the final multiple linear regression models (excluding tanning bed users) of serum 25(OH)D₃ levels and the percent 24,25(OH)₂D₃, *SLC24A5* genotype remained statistically significant after adjusting for vitamin D/fish oil supplement use, race/ethnicity, and West African Ancestry (Table 4). Thus, relative to the 111^{Thr/Thr} genotype, the 111^{Thr/Ala} genotype was associated with a 23.0 nmol/L decrease in serum 25(OH)D₃ and a 4.1 percentage point decrease in the percent 24,25(OH)₂D₃. Similarly, the 111^{Ala/Ala} genotype was associated with a 23.8 nmol/L decrease in serum 25(OH)D₃, and a 5.2 percentage point decrease in the percent 24,25(OH)₂D₃, relative to the 111^{Thr/Thr} genotype. Results for the *SLC24A5* genotype were similar when adjusted for melanin index or West African genetic ancestry (Table 4).

Discussion

Summary

This study is consistent with other studies reporting significantly lower serum 25(OH)D and 24,25(OH)₂D₃ levels among African Americans, compared with European Americans,[9, 32-34] and the inverse association between serum 25(OH)D and West African genetic ancestry.[35] There are two novel observations in this report. First, we identified a lower

percent of 24,25(OH)₂D₃ among African American women compared with European American women. Second, the *SLC24A5* Ala¹¹¹ allele was negatively associated with non-summer serum levels of 25(OH)D₃, 24,25(OH)₂D₃, and percent 24,25(OH)₂D₃, independent of skin melanin index or West African Genetic Ancestry.

Strengths and Limitations

The strengths of this study include an age- and sex-matched study design in healthy individuals without pre-existing conditions known to be associated with levels of 25(OH)D₃; the use of non-invasive skin spectroscopy to estimate skin melanin content which avoids the need for other subjective skin typing methods; [36] the use of genetic ancestry as one way of controlling for the effects of genetic background; and the use of a LC-MS/MS vitamin D metabolite assay which allowed for the assessment of multiple vitamin D metabolites and the exclusion of vitamin D₂, found exclusively in dietary sources and therefore not relevant to the study of skin pigmentation and serum vitamin D levels. The measurement of genetic ancestry was highly concordant with other methods in practice. [29] We could find no other published studies of genetic association with percent 24,25(OH)₂D₃.

The chief limitations of this study are the small sample size and the limited ability to generalize these results to pregnant women, populations with clinical disease, or older age individuals. There are other genetic loci known to be associated with skin pigmentation and/or serum vitamin D levels that were not included in this study, although the *SLC24A5* 111Ala/Thr and *SLC45A2* 374Phe/Leu polymorphisms we studied have been associated with the lowest p-value and highest R² value prediction of skin melanin index among individuals of European ancestry. [21] In addition, because some participants were drawn from a course on race/ethnicity where participants had previously been told their genetic ancestry results (i.e., Native American Indian, East Asian, West African and European American ancestry), the proportion of participants self-identifying with multiple racial/ethnic groups in this study population is likely higher than in other populations without previous knowledge of their genetic ancestry. Potential differences in the skin concentration of 7-dehydrocholesterol (7-DHC, the vitamin D precursor), the rates of glucuronidation of vitamin D metabolites, or the concentration of other inactive vitamin D conjugates were not assessed by this study. [37, 38] There are other biologically active vitamin D₃ metabolites, some of which have been identified to vary by race/ethnicity, but are not yet well characterized in different population groups. [39, 40] Finally, this study did not investigate differences in other vitamin D hydroxylated metabolites produced in the skin, nor other potential differences in fat storage of vitamin D that may be due to racial/ethnic differences in lipid storage.

Racial/ethnic Differences

The 24,25(OH)₂D₃ metabolite, the principal circulating dihydroxyvitamin D metabolite, is dependent on the activity of the 24-hydroxylase enzyme (encoded by the *CYP24A1* gene), which catabolizes both the active form of vitamin D (1,25(OH)₂D₃ to 1,24,25(OH)₃D₃) and the major circulating form of vitamin D (25(OH)D₃ to 24,25(OH)₂D₃). [41] The 24,25(OH)₂D₃ metabolite (also known as “secalciferol”) has an in vivo half-life of 15-40 days (several times longer than 1,25(OH)₂D₃), circulates at a concentration ~100-fold higher than calcitriol, can inhibit tumor development, induce calcium uptake in the duodenum (albeit at an effectiveness about 20 to 100-times lower than 1,25(OH)₂D₃ in animal models), and plays a role in bone physiology including the prevention of apoptosis in chondrocytes in the growth plate, bone mineralization (both Ca²⁺ and Pi), and bone fracture repair. [42-52] However, in clinical trials 24,25(OH)₂D₃ has not consistently increased bone mass or suppressed parathyroid hormone (PTH), and does not appear to influence serum concentrations of 1,25(OH)₂D₃ and 25(OH)D₃. [50, 53] Although *CYP24A1* is the gene

most highly-inducible by $1,25(\text{OH})_2\text{D}_3$, [54] there is little information regarding racial/ethnic differences in CYP24A1 expression and its influence on vitamin D metabolite levels.

In vivo, treatment with $1,25(\text{OH})_2\text{D}_3$ is associated with increased metabolic clearance of $25(\text{OH})\text{D}_3$, decreased serum $25(\text{OH})\text{D}_3$ levels, a higher $25(\text{OH})\text{D}_3$ to $24,25(\text{OH})_2\text{D}_3$ conversion rate, and (somewhat paradoxically) a decrease in $24,25(\text{OH})_2\text{D}_3$ serum levels in humans and animals. [55-57] If this model is applicable to racial/ethnic differences in vitamin D metabolism, then differences in the relative levels of $25(\text{OH})\text{D}_3$ and $24,25(\text{OH})_2\text{D}_3$, which we and others have observed, might be consistent with a higher average production rate of $1,25(\text{OH})_2\text{D}_3$ among African Americans. In general, there is not conclusive evidence regarding racial/ethnic differences in calcitriol, in part due to inaccuracy with earlier assays [58], and also because of the fact that studies do not account for diurnal variation [59].

Dietary calcium intake and estrogen concentrations may also have contributed to our observations. For example, a low calcium diet may decrease serum $25(\text{OH})\text{D}$ by upregulating *CYP27B1* production of $1,25(\text{OH})_2\text{D}_3$. While randomized animal studies and observational human studies report that higher intakes of calcium are associated with higher $25(\text{OH})\text{D}$ levels, cross-sectional data can not be used to predict individual $25(\text{OH})\text{D}$ levels. [60-62] Estrogen is also known to increase the production of $1,25(\text{OH})_2\text{D}_3$ and to suppress *CYP24A1* activity, thereby lowering the production of $24,25(\text{OH})_2\text{D}_3$. [63] Large population-based studies have identified higher levels of estrone, estradiol, and bio-available estradiol among African American women (both pre- and post-menopausal) compared with European American women. [64, 65] We did not measure estrogen in our study, and thus cannot rule out the possibility that higher estrogen levels may have contributed to the lower serum $24,25(\text{OH})_2\text{D}_3$ and lower percent $24,25(\text{OH})_2\text{D}_3$ among African American women in our study. Taken together, the present evidence suggests a significant difference in the balance of vitamin D₃ metabolites among healthy African Americans and Europeans—particularly among women.

Skin Reflectance, Genetic Ancestry and SLC24A5

The *SLC24A5* and *SLC45A2* polymorphisms (rs1426654 and rs16891982, respectively) have been associated with skin pigmentation in several human and *in vitro* studies, [22, 66-68] and are identified as the top 2 most significant polymorphisms associated with skin reflectance, as measured through either p-value or R^2 value. [21, 22] *SLC24A5* has been shown to influence pigmentation *in vivo* [69], and the SNP we investigated has two allelic forms occurring at amino acid 111, resulting in either Alanine (non-polar, aliphatic R group, non-essential) or Threonine (polar, uncharged R group, essential in mammals) residue. Both polymorphisms were similarly correlated to skin melanin index, but only the *SLC24A5* polymorphism was significantly associated with serum $25(\text{OH})\text{D}_3$ and percent $24,25(\text{OH})_2\text{D}_3$.

Among African American and European American participants, we observed a significantly lower serum $25(\text{OH})\text{D}_3$ (~22 to 24 nmol/L lower) and a lower percent $24,25(\text{OH})_2\text{D}_3$ (~3 to 5 percent lower) among individuals with either one or two copies of the ancestral *SLC24A5* 111^{Ala} allele. The magnitude of this association in relation to $25(\text{OH})\text{D}_3$ was greater than what we observed for tanning bed use and for vitamin D/fish oil supplement intake in our study; it is also greater than genotypic differences reported for genes related to vitamin D metabolism and cholesterol synthesis in other studies. [70] Previous genome wide association studies for serum $25(\text{OH})\text{D}$ have not reported an association with *SLC24A5*, although the variant of interest may have been thrown out due to the use of the HWE statistic which is used as an indicator for genotyping error. [71] The associations we

observed are not likely due to genotyping error as we observed a high agreement between two different genotyping assays (Illumina and TaqMan).

Disaggregating the role of this particular genetic variant from that of skin pigmentation and/or genetic background is complicated by the fact that this variant is simultaneously one of the highest ranked Ancestry Informative Markers (AIM) used to describe West African Ancestry [72, 73] and also is associated with the most significant proportion of variability in skin reflectance relative to all other known skin pigmentation polymorphic variants.[21] Nonetheless, our study provides some evidence that the *SLC24A5* 111^{Ala} allele (or some other closely linked causative allele) may have an effect on vitamin D status that is independent of both ancestry and skin reflectance.

First, although both the *SLC24A5* and *SLC45A2* genotypes we investigated were highly correlated with skin melanin index, only *SLC24A5* remained significantly associated with serum vitamin D metabolite levels, after adjustment for race/ethnicity, genetic ancestry, or melanin index. Second, within a reasonably small range of West African ancestry (0.0 to 2.0 percent), we observed a statistically significant correlation between the *SLC24A5* genotype and serum 25(OH)D₃ levels. Third, within each melanin index quartile group that included either only Europeans or both African Americans and Europeans, the correlation between *SLC24A5* and serum 25(OH)D₃ levels remained statistically significant (r range 0.4 to 0.5). The range of the melanin index in Quartile 1 of our study (24.2 to 30.6 Melanin Index Units) is the skin pigmentation change observed between approximately two applications of sun bronzing lotion to fair skin.[25] Finally, we observed a significant dominant p-value associated with the 111^{Ala} allele. Lamason and colleagues proposed a dominant/co-dominant effect of the 111^{Thr} allele on skin pigmentation.[69] If serum 25(OH)D₃ was tightly linked to pigmentation, then one would expect to observe a similar dominant or co-dominant effect of the 111^{Thr} allele on serum 25(OH)D₃ levels. Contrary to our initial hypothesis, our results suggest that there may be a dominant effect of the 111^{Ala} allele in relation to serum 25(OH)D₃.

The understanding of skin photoconversion of vitamin D related to skin pigmentation is quite limited. This is due to the few studies which have a small number of participants with dark pigmentation (i.e., 10 or fewer participants with Fitzpatrick skin types V and/or VI). [11, 30, 39, 74-77] Two previous epidemiologic studies have reported no association with serum 25(OH)D and skin pigmentation.[76, 78] Available evidence, along with the results of our study, suggests that non-summer serum 25(OH)D levels are not tightly linked to skin pigmentation.

The *SLC24A5* gene (also known as the potassium-dependent sodium/calcium exchanger 5, NCKX5), located in a genomic region of decreased heterozygosity among Europeans, also produces an integral intracellular membrane protein possibly located on the trans-Golgi and melanosomal membranes and is thought to regulate calcium content, pH, and osmolarity of the melanosome.[69, 79] Although other genes within the SLC gene family are expressed in melanocytes, *SLC24A5* is the predominant sodium-calcium exchanger transcript in human melanocytes and is the only one in the family found in the intra-cellular compartment.[80] The 111^{Thr/Ala} polymorphism is thought to occur in the trans-membrane domain.[81] Over-expression of the 111^{Thr} allele (found predominantly in Europeans with light skin) experimentally reduces the exchange rate of Ca²⁺ which is hypothesized to subsequently influence the Na⁺ gradient and influence downstream function of melanosomal and plasma membrane Na⁺/H⁺ exchangers and ultimately changes the pH within the melanosome.[69, 80] Conversely, calcitriol has not been found to upregulate *SLC24A5* expression, although this has not been investigated in melanocytes or keratinocytes.[82] It has been hypothesized that the more basic pH found in melanosomes from dark-pigmented individuals allows for

the maturation of tyrosinase, the rate-limiting catalyst of (eu-) melanogenesis,[69, 80, 83, 84] while acidity of the cytosol and dendritic processes of melanocytes is increased in skin with greater pigmentation.[85] Based on investigations of the chemical properties of vitamin D analogs, it is plausible that pH influences the isomerization, storage, transport or metabolism of vitamin D₃ in the dermis and epidermis *in vivo*. [86] It should also be noted that there are other pigmentation-related genes that have also been observed to influence the pH of the melanosome.[87] Thus, if there is some connection between skin pigmentation, pH, and vitamin D stability, it is possible that these events could be linked to polymorphic variants in other skin pigmentation genes.

Notably, both West Africans and East Asians have a high prevalence of the *SLC24A5* 111^{Ala} variant.[66, 69] Studies of healthy East Asians (in Hong Kong and Beijing), among age groups comparable to our study population, report non-summer serum 25(OH)D (32.0 to 37.7 nmol/L) levels that are comparable to the European and African American participants in our study that were *SLC24A5* 111^{Thr/Ala} heterozygotes (33.8 nmol/L and 38.8 nmol/L, respectively).[88, 89] We did not observe statistically significant associations between serum 25(OH)D₃ and either East Asian or Native American genetic ancestry, which diminishes the possibility that some other ancestral background effect explains the results we observed for *SLC24A5*, although our study was not designed explicitly to examine the association with East Asian ancestry.

Vitamin D and its metabolites themselves have effects upon the epidermis. Vitamin D analogs (calcipotriol and tacalcitol) are used as topical therapeutic agents for vitiligo.[90] Skin phenotyping studies consistently report that skin with darker pigmentation has a greater barrier function, more differentiated stratum corneum, increased epidermal lipid content, and increased lamellar body production.[85, 91, 92] Calcitriol is known to induce keratinocyte differentiation[93], and keratinocytes are one of the few cells in the body capable of producing calcitriol, 24,25(OH)₂D₃ and 25(OH)D₃. [94, 95] This paracrine function may be important in the ‘calcium switch’ defined as higher intra-cellular calcium concentrations in the upper, more-differentiated epidermis, that promotes the cornified envelope formation. [94] It remains to be determined whether the *SLC24A5* polymorphic variant or another variant may contribute to a skin calcium concentration gradient and/or whether vitamin D, its photoproducts or other active metabolites may influence phenotypic differences.

Conclusions

Compared with European American women, this study observed a lower serum percent 24,25(OH)₂D₃ among African American women, when evaluated as a proportion of the parent metabolite 25(OH)D₃. The *SLC24A5* 111^{Ala} allele was associated with lower serum vitamin 25(OH)D₃ and lower percent 24,25(OH)₂D₃, independently from skin melanin index and West African genetic ancestry. Mechanistic data are needed to investigate whether a true influence upon vitamin D metabolism exists.

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Figure 1. Selected Vitamin D₃ Metabolites

Following skin exposure to UVB light, 7-dehydrocholesterol is converted to pre-vitamin D₃, which undergoes a thermal isomerization to form vitamin D₃, undergoing activation and catabolism via three main cytochrome P450 enzymes. Vitamin D₃ is hydroxylated at C-25 by CYP2R1 [96] to generate the major circulating metabolite, 25(OH)D₃. A second hydroxylation step at C-1a occurs in the kidney by CYP27B1 [97], and produces the active form of vitamin D (calcitriol, or 1,25(OH)₂D₃). CYP24A1 [98] then catabolizes both 25(OH)D₃ and 1,25(OH)₂D₃ via hydroxylation at C-24 and further hydroxylation steps eventually leading to calcitroic acid or 1,25(OH)₂D₃-26,23 lactone. Major circulating metabolites are shown [99].

Figure 2.

Scatter plot of serum 25(OH)D₃ levels according to skin melanin index among individuals Homozygous for the *SLC24A5* Thr111 allele (Figure 2a) and homozygous or heterozygous for the Ala111 allele (Figure 2b). Regression lines and R^2 values appear in the figures. The regression line and R^2 value for melanin index in relation to serum 25(OH)D₃ for all subjects in the study, regardless of genotype: $y = -1.15x + 98.58$, $R^2 = 0.268$.

Table 1
Study Sample Characteristics by Self-Reported Race/Ethnicity

	African American ± std (N)	European American ± std (N)	p-value
% Female	54.0 (27)	54.0 (27)	1.000
Mean Age	24.9 ± 7.5	24.6 ± 6.3	0.807
Self-Reported Racial/Ethnic Groups (%)			
African American/African	100.0 (50)	34.0 (17)	<0.001
American Indian/Alaska Native	16.0 (8)	40.0 (20)	0.097
Asian	8.0 (4)	26.0 (13)	0.050
European	28.0 (14)	100.0 (50)	<0.001
Native Hawaiian/Pacific Islander	0.0 (0)	0.0 (0)	---
Vitamin D and Calcium Sources			
Vitamin D/Fish Oil Supplements (%)	8.0 (4)	14.0 (7)	0.525
Calcium Supplements (%)	14.0 (7)	20.0 (10)	0.396
Time Spent Outdoors (hrs/week)	10.2 ± 7.5	13.2 ± 9.7	0.090
Outdoor Job (%)	26.0 (13)	52.0 (26)	0.006
Tanning Bed Use (%)	0.0 (0)	18.0 (9)	0.003
Average Hours Per Week of Tanning Bed Use	---	1.0 ± 1.9	---
Oral Contraceptive Use (% of Women)	4.0 (2)	22.0 (11)	0.015
Corticosteroid Use	10.0 (5)	2.0 (1)	0.204
Statin Use	0.0 (0)	0.0 (0)	---
Body Mass Index (kg/m ²), Mean	27.7 ± 8.6	24.9 ± 4.1	0.045
Physical Activity (%)			
Less than a few times/month	28.0 (14)	70.0 (35)	<0.001
A few times/month	52.0 (26)	20.0 (10)	<0.001
Everyday	14.0 (7)	10.0 (5)	0.546
Current Smoker (%)	10.0 (5)	10.0 (5)	1.000
Systolic Blood Pressure (mmHg)	115.3 ± 10.9	113.1 ± 11.2	0.312
Diastolic Blood Pressure (mmHg)	70.0 ± 9.1	71.7 ± 8.2	0.353
Melanin Index Units, Mean	49.5 ± 9.7	31.5 ± 3.9	<0.001
West African Genetic Ancestry (%)	77.8 ± 17.2	3.3 ± 7.4	<0.001
Blood Draw Time Past Summer Solstice, Mean (days)	82.0 ± 51.3	92.8 ± 14.3	0.157
25-Hydroxyvitamin D ₃ (nmol/L), Mean	35.7 ± 16.0	67.7 ± 25.5	<0.001
Men	37.7 ± 16.9	58.0 ± 18.9	<0.001
Women	34.1 ± 15.3	75.9 ± 27.7	<0.001
25-Hydroxyvitamin D ₂ (nmol/L), Mean	2.5 ± 4.8	5.1 ± 7.3	0.037
Men	3.9 ± 6.7	4.2 ± 6.2	0.871
Women	1.3 ± 1.7	5.9 ± 8.2	0.008
24,25-Dihydroxyvitamin D ₃ (nmol/L), mean	4.2 ± 2.6	9.8 ± 4.9	<0.001
Men	4.9 ± 2.9	7.6 ± 3.4	0.007
Women	3.7 ± 2.2	11.6 ± 5.3	<0.001

	African American ± std (N)	European American ± std (N)	p-value
Percent [‡] 24,25 Dihydroxyvitamin D ₃	10.2 ± 2.6	12.2 ± 2.7	<0.001
Men	11.0 ± 2.6	11.3 ± 2.2	0.757
Women	9.3 ± 2.4	13.1 ± 2.8	<0.001
Serum Total Calcium (mg/dL)	9.9 ± 0.5	9.7 ± 0.4	0.028
Men	10.2 ± 0.5	9.9 ± 0.4	0.014
Women	9.7 ± 0.4	9.6 ± 0.4	0.435
<i>SLC45A2</i> coding SNP (dbSNP ID rs16891982)			
GG (374 ^{Leu/Leu})	10.0 (5)	70.0 (35)	
GC (374 ^{Leu/Phe})	26.0 (13)	28.0 (14)	
CC (374 ^{Phe/Phe})	62.0 (31)	0.0 (0)	
Hardy-Weinberg p-value	0.067	0.250	<0.001
<i>SLC24A5</i> coding SNP (dbSNP ID rs1426654)			
AA (111 ^{Thr/Thr})	4.0 (2)	90.0 (45)	
AG (111 ^{Thr/Ala})	38.0 (16)	6.0 (3)	
GG (111 ^{Ala/Ala})	62.0 (31)	0.0 (0)	
Hardy-Weinberg p-value	0.971	0.823	<0.001

--- No observations or statistic cannot be calculated.

[‡]Wilcoxon Rank Sums Test p-value for differences in the percent of 24,25(OH)₂D₃.

Table 2

Mean Non-Summer Serum Levels of 25(OH)D₃ (nmol/L), 24,25(OH)₂D₃ (nmol/L) and the Percent 24,25(OH)₂D₃ by Melanin Index, Genetic Ancestry, and Self-Reported Race/Ethnicity

	African American +/- std (N)	European American +/- std (N)	Difference (nmol/L or percent)	p-value of difference	Correlation with Serum Level [Overall correlation]
25(OH)D₃					
Melanin Index Quartile					
24.4 to 30.6	---	65.9 +/-17.0 (24)	---	---	---
30.7 to 37.0	46.1 +/-11.2 (6)	69.5 +/-31.5 (21)	-23.4	0.009	+0.4**
37.1 to 48.4	40.4 +/-12.2 (21)	68.2 +/-36.0 (5)	-27.8	0.160	+0.4
48.5 to 65.9	28.8 +/-17.4 (23)	---	---	---	---
Serum Level Correlation	-0.3**	<+0.1			[-0.6 [†]]
Genetic Ancestry, West African (%)					
0.0 to 2.0	---	69.6 +/-28.3 (32)	---	---	---
3.0 to 71.0	41.5 +/-12.2 (17)	64.2 +/-19.8 (18)	-22.7	0.003	+0.6 [†]
71.0 to 100.0	32.8 +/-17.0 (33)	---	---	---	---
Serum Level Correlation	-0.3	-0.1			[-0.6 [†]]
24,25(OH)₂D₃					
Melanin Index Quartile					
24.4 to 30.6	---	9.5 +/-4.3 (24)	---	---	---
30.7 to 37.0	5.5 +/-2.3 (6)	10.2 +/-5.6 (21)	-4.7	0.057	+0.5**
37.1 to 48.4	4.9 +/-2.4 (21)	9.5 +/-6.0 (5)	-4.6	0.165	+0.3
48.5 to 65.9	3.3 +/-2.6 (23)	---	---	---	---
Serum Level Correlation	-0.2	-0.1			[-0.5 [†]]
Genetic Ancestry, West African (%)					
0.0 to 2.0	---	9.6 +/-4.9 (32)	---	---	---
3.0 to 71.0	5.3 +/-2.5 (17)	10.1 +/-5.1 (18)	-4.8	0.001	+0.5 [†]
71.0 to 100.0	3.7 +/-2.5 (33)	---	---	---	---
Serum Level Correlation	-0.3	<-0.1			[-0.6 [†]]
Percent 24,25(OH)₂D₃a					
Melanin Index Quartile					
24.4 to 30.6	---	12.2 +/-2.5 (24)	---	---	---
30.7 to 37.0	10.3 +/-2.3 (6)	12.5 +/-2.6 (21)	-2.2	0.075	+0.4
37.1 to 48.4	10.5 +/-2.6 (21)	11.5 +/-4.3 (5)	-1.0	0.488	+0.1
48.5 to 65.9	9.7 +/-2.8 (23)	---	---	---	---
Serum Level Correlation	+0.1	+0.1			[-0.3 [†]]
Genetic Ancestry, West African (%)					

	African American +/- std (N)	European American +/- std (N)	Difference (nmol/L or percent)	p-value of difference	Correlation with Serum Level [Overall correlation]
0.0 to 2.0	---	11.9 +/-2.4 (32)	---	---	---
3.0 to 71.0	10.9 +/-2.8 (17)	12.9 +/-3.1 (18)	-1.0	0.054	+0.3
71.0 to 100.0	9.7 +/-2.5 (33)	---	---	---	---
Serum Level Correlation	-0.1	+0.2			[-0.3 [‡]]

--- No observations or the statistic cannot be calculated.

* Spearman's Correlation Coefficient calculated on continuous values. Cells with fewer than two subjects suppressed. Difference expressed as African American minus European American value. Spearman's Rank Correlation coefficient statistically significant:

**
p 0.05;

[‡]p 0.01;

[†]p 0.001.

◇ Wilcoxon Rank SumsTest p-value for differences in the ratio of vitamin D metabolites.

Table 3

Mean Serum 25(OH)D₃ (nmol/L) Levels by Melanin Index, Genetic Ancestry, and Skin Pigmentation Genotypes (*SLC45A2*, *SLC24A5*)

<i>SLC45A2</i> Genotype: (dbSNP ID rs16891982)	CC (374 ^{Phe/Phe}) +/-std (N)	GC (374 ^{Leu/Phe}) +/-std (N)	GG (374 ^{Leu/Leu}) +/-std (N)	<i>SLC45A2</i> Correlation with 25(OH)D ₃	Overall Correlation with <i>SLC45A2</i>
Melanin Index (Quartile)					0.8 [†]
24.4 to 30.6	---	57.4 +/-15.6 (6)	67.5 +/-16.5 (17)	0.2	
30.7 to 37.0	---	53.6 +/-33.5 (8)	69.6 +/-28.3 (18)	0.4	
37.1 to 48.4	42.0 +/-12.4 (8)	43.8 +/-17.4 (13)	57.0 +/-38.0 (5)	0.1	
48.5 to 65.9	29.2 +/-17.7 (22)	---	---	---	
Correlation with 25(OH)D ₃	-0.2	-0.3	-0.2		
Genetic Ancestry (Tertile), West African (%)					0.8 [†]
0.0 to 2.0	---	58.7 +/-36.2 (6)	71.4 +/-26.6 (25)	0.2	
3.0 to 71.0	39.8 +/-15.8 (8)	50.4 +/-19.2 (14)	64.4 +/-18.0 (13)	0.5 [‡]	
71.0 to 100.0	31.0 +/-17.6 (23)	40.8 +/-14.6 (7)	32.0 +/-20.7 (2)	0.2	
Correlation with 25(OH)D ₃	-0.1	-0.4 ^{**}	-0.3		
Self-Reported Race/Ethnicity					0.6 [†]
African American	33.3 +/-17.4 (31)	40.6 +/-11.6 (13)	41.6 +/-15.1 (5)	0.3	
European American	---	58.2 +/-27.7 (14)	70.8 +/-24.1 (35)	0.2	
Correlation with 25(OH)D ₃	---	+0.4 +/-	+0.5 [‡]		
<i>SLC24A5</i> Genotype (Thr111Ala): (dbSNP ID rs1426654)	GG (111 ^{Ala/Ala})	AG (111 ^{Thr/Ala})	AA (111 ^{Thr/Thr})	<i>SLC24A5</i> Correlation with 25(OH)D ₃	Correlation with <i>SLC24A5</i>
Melanin Index Quartile					0.8 [†]
24.4 to 30.6	---	38.3 +/-6.9 (2)	67.4 +/-14.9 (21)	0.5 ^{**}	
30.7 to 37.0	42.3 +/-16.1 (2)	46.3 +/-12.3 (3)	68.6 +/-31.7 (21)	0.4 ^{**}	
37.1 to 48.4	39.1 +/-13.1 (10)	39.3 +/-12.5 (11)	73.3 +/-29.5 (5)	0.4 ^{**}	
48.5 to 65.9	30.9 +/-18.5 (19)	18.9 +/-5.3 (3)	---	-0.2	
Correlation with 25(OH)D ₃	-0.2	-0.5 ^{**}	<0.1		
Genetic Ancestry (Tertile), West African (%)					0.8 [†]
0.0 to 2.0	---	38.3 +/-6.9 (2)	71.0 +/-28.7 (28)	0.4 ^{**}	
3.0 to 71.0	40.0 +/-17.4 (6)	38.7 +/-9.9 (10)	64.9 +/-17.3 (19)	0.6 [†]	
71.0 to 100.0	32.9 +/-16.8 (25)	34.4 +/-19.5 (7)	---	<0.1	
Correlation with 25(OH)D ₃	-0.2	-0.3	-0.2		
Race/Ethnicity					0.9 [†]
African American	34.3 +/-16.9 (31)	37.7 +/-14.3 (16)	51.4 +/-1.8 (2)	0.2	
European American	---	33.8 +/-9.2 (3)	69.3 +/-25.0 (45)	0.4 [‡]	

Correlation with 25(OH)D ₃	---	-0.1	+0.2
---------------------------------------	-----	------	------

*
p-interaction between genotype and melanin index; genotype and ancestry; or genotype and race/ethnicity calculated as a multiplicative interaction between the variables of interest in relation to ln(25-Hydroxyvitamin D).

--- No observations or the number of participants is less than 2. Spearman’s Rank Correlation coefficient statistically significant:

**
p 0.05;

*
p 0.01;

[†]
p 0.001.

Table 4Multiple Linear Regression Results for Serum 25(OH)D₃ (nmol/L) and Percent 24,25(OH)₂D₃

Variable	25(OH)D ₃ β-Coefficient*	p-value	Percent 24,25(OH) ₂ D ₃ β-Coefficient*	p-value
Melanin Index Quartile				
24.4 to 30.6	Reference		Reference	
30.7 to 37.0	-4.7	0.404	-0.3	0.704
37.1 to 48.4	-11.0	0.141	0.3	0.763
48.5 to 65.9	-19.8	0.024	0.1	0.941
p-trend		0.217		0.385
Genetic Ancestry (Tertile), West African (%)				
0.0 to 2.0	Reference		Reference	
3.0 to 71.0	5.4	0.338	1.6	0.047
71.0 to 100.0	0.1	0.987	0.9	0.374
p-trend		0.479		0.945
Vitamin D/Fish Oil Supplementation				
No	Reference		Reference	
Yes	11.6	0.012	1.7	0.011
<i>SLC24A5</i> Genotype (dbSNP ID rs1426654) [†]				
AA (111 ^{Thr/Thr})	Reference		Reference	
AG (111 ^{Thr/Ala})	-23.0	0.008	-4.1	<0.001
GG (111 ^{Ala/Ala})	-23.8	0.019	-5.2	<0.001
Cochran-Armitage p-trend		0.103		<0.001
p-dominant (Ala allele)		0.007		0.003
<i>SLC24A5</i> Genotype (dbSNP ID rs1426654) [‡]				
AA (111 ^{Thr/Thr})	Reference		Reference	
AG (111 ^{Thr/Ala})	-24.0	0.003	-4.0	<0.001
GG (111 ^{Ala/Ala})	-22.0	0.016	-5.5	<0.001
Cochran-Armitage p-trend		0.102		<0.001
p-dominant (Ala allele)		0.003		<0.001

* European American participants were frequency matched to African American participants on age (+/- 2 years) and sex. All models in Table 4 exclude individuals reporting tanning bed use and all beta coefficients are adjusted for vitamin D/fish oil supplementation, race/ethnicity and *SLC24A5* genotype (dbSNP ID rs1426654).

[†] Adjusted for vitamin D/fish oil supplementation, race/ethnicity and *SLC24A5* genotype plus West African genetic ancestry.

[‡] Adjusted for vitamin D/fish oil supplementation, race/ethnicity and *SLC24A5* genotype plus melanin index. *SLC24A5* genotype and vitamin D/fish oil supplementation identified as statistically significant in both stepwise and backward selection models for 25(OH)D₃ and ln(25(OH)D₃) that included the following co-variables: melanin index, West African genetic ancestry, vitamin D/fish oil supplementation, *SLC24A5* genotype and oral contraceptive use; *SLC24A5* genotype statistically significant in both stepwise and backward selection models for percent 24,25(OH)₂D₃ and ln(percent 24,25(OH)₂D₃) that included melanin index, genetic ancestry, vitamin D/fish oil supplementation, *SLC24A5* genotype, total serum calcium and oral contraceptive use.