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Vitamin D Intake and Season Modify the Effects of the *GC* and *CYP2R1* Genes on 25-Hydroxyvitamin D Concentrations[1–3]

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Abstract

Vitamin D deficiency {defined by the blood concentration of 25-hydroxyvitamin D [25(OH)D]} has been associated with many adverse health outcomes. Genetic and nongenetic factors account for variation in 25(OH)D, but the role of interactions between these factors is unknown. To assess this, we examined 1204 women of European descent from the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative Observational Study. Twenty-nine single nucleotide polymorphisms (SNPs) in 4 genes, *GC*, *CYP2R1*, *DHCR7*, and *CYP24A1*, from recent meta-analyses of 25(OH)D genome-wide association studies were genotyped. Associations between these SNPs and 25(OH)D were tested using generalized linear regression under an additive genetic model adjusted for age, blood draw month, and ancestry. Results were stratified by season of blood draw and, separately, vitamin D intake for the 6 SNPs showing a significant association with 25(OH)D at the $P < 0.01$ level. Two nonsynonymous SNPs in *GC* and 4 SNPs in *CYP2R1* were strongly associated with 25(OH)D in individuals whose blood was drawn in summer ($P \leq 0.002$) but not winter months and, independently, in individuals with vitamin D intakes ≥400 ($P \leq 0.004$) but not <400 IU/d (10 μg/d). This effect modification, if confirmed, has important implications for the design of genetic studies for all health outcomes and for public health recommendations and clinical practice guidelines regarding the achievement of adequate vitamin D status. J. Nutr. 143: 17–26, 2013.

Introduction

Over the past 10–15 y, vitamin D has been associated with many health outcomes, including skeletal health, cancer, immune responses, type 2 diabetes and metabolic syndrome, cardiovascular diseases and hypertension, neuropsychological functioning, and all-cause mortality (1–7). Moreover, the prevalence of blood concentrations of 25-hydroxyvitamin D [25(OH)D][17] that put individuals at risk for vitamin D inadequacy [≤50 nmol/L (≤20 μg/L)] by recent Institute of Medicine (IOM) standards (7) is quite high. National Health and Nutrition Examination Survey data from 2005 to 2006 (n = 4495) revealed that 31, 63, and 82% of non-Hispanic whites, Hispanics, and non-Hispanic blacks, respectively, were at risk for vitamin D inadequacy (8).

Vitamin D status, measured by 25(OH)D, is known to be influenced in part by sunlight exposure and vitamin D intake. Dietary and supplemental intake has been shown to account for 1–8% of the inter-individual variation in 25(OH)D (9–11). Estimates of season and sunlight exposure have been shown to explain 1–15% of the inter-individual variation (9–11), although the exact contribution is likely underestimated due to the challenges of measuring individual-level sunlight exposure. Accounting for

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[3] A list of Women's Health Initiative investigators is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[17] Abbreviations used: AIM, ancestry informative marker; CAREDS, Carotenoids in Age-Related Eye Disease Study; GWA, genome-wide association; IOM, Institute of Medicine; LD, linkage disequilibrium; 25(OH)D, 25-hydroxyvitamin D; PCA, principal components analysis; SNP, single nucleotide polymorphism; WHI-OS, Women's Health Initiative Observational Study.

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these external sources of vitamin D plus additional characteristics, such as age and adiposity, accounts for 21–32% of the variation in 25(OH)D (9–11).

The genetic contribution to 25(OH)D has been estimated to range from 23 to 77% (10,12–17). Recent evidence indicates that estimates of heritability may vary by season of blood draw (16,17), which affects the amount of vitamin D produced upon exposure to sunlight. Yet the effect of season on heritability estimates is inconsistent, with one study finding moderate heritability in the summer (48%) and no genetic contribution in winter (17) and another study finding no genetic contribution in summer but a moderate contribution in winter (70%) (16).

Two large meta-analyses of Caucasian cohorts with measured 25(OH)D and genome-wide association (GWA) data were recently published (18,19). In both meta-analyses, genetic variants in 3 genes were associated with 25(OH)D: *GC* (vitamin D binding protein), *DHCR7* (encodes the enzyme that produces cholecalciferol in the skin), and *CYP2R1* [encodes the enzyme that produces 25(OH)D in the liver]. An additional gene, *CYP24A1* [encodes the enzyme that degrades 25(OH)D], was also significant in the SUNLIGHT Consortium report (19), which included a much larger sample size (n = 33,868) than the other meta-analysis (n = 4501) (18). However, the role of interactions between these genes and nongenetic factors, such as season and vitamin D intake, has rarely been investigated. One study examined the contribution of a genetic variant in *GC*, stratified by season of blood draw, and found a stronger association in the fall compared with the winter, although the sample sizes for the subgroups were rather small ($n \approx 100$) (20).

We have genotyped single nucleotide polymorphisms (SNPs) in these 4 genes in participants from the Carotenoids in Age-Related Eye Disease Study (CAREDS), an ancillary study of the Women's Health Initiative Observational Study (WHI-OS), with the goals of testing for association between SNPs in these genes and 25(OH)D and determining if the SNP effects are modified by season of blood draw, a proxy for the available solar radiation in the environment, and/or vitamin D intake.

Participants and Methods

Participants. The WHI-OS, a prospective cohort study, assessed morbidity and mortality in 93,676 postmenopausal women recruited from 40 sites throughout the United States (21–23). The serum, DNA, and data (except self-reported sunlight exposure) for the current study were collected at the WHI-OS baseline visit (1993–1998). The baseline characteristics of the participants and reliability of baseline measures were previously reported (24). The CAREDS is an ancillary study of the WHI-OS that examined the association between dietary intake of carotenoids (lutein and zeaxanthin) and the prevalence of age-related eye disease, including macular degeneration (25). Participants with a baseline lutein+zeaxanthin intake >78th or <28th percentiles were eligible for the CAREDS (n = 3143) and were recruited between 2001 and 2004 from 3 of 40 WHI centers: the University of Iowa, Iowa City, IA (latitude: 42° N); the University of Wisconsin, Madison, WI (latitude: 43° N); and the Kaiser Center for Health Research, Portland, OR (latitude: 46° N). The demographic and other health-related data did not differ between the overall sample of women enrolled in the WHI-OS and the subsample enrolled in the CAREDS (25).

Among the 3143 women eligible for enrollment in the CAREDS, 96 (3%) died or were lost to follow-up during the recruitment phase, leaving 3047 women who were invited to participate, of whom 2005 (66%) agreed to enrollment. DNA was requested from 1787 (89%) participants who had data on age-related macular degeneration (the primary outcome of interest in the CAREDS). Of those, 1772 (99%) approved the use of their stored DNA and had an adequate volume of the DNA sample to be sent for genotyping; 1697 (96%) of these had sufficient DNA quantity for genotyping. Of these samples, 1663 (98%) passed the quality assurance/quality control checks, of which 1230 (74%) had 25(OH)D available at the baseline WHI-OS visit and 1204 (98%) were of European descent (based on self-report and ancestry informative genetic markers). The analyses were conducted with and without women with conditions that could affect vitamin D absorption in the gut (a history of ulcerative colitis, surgery to remove part of the intestine, or use of a special diet for malabsorption; n = 42), but the results did not substantially change (data not shown). Therefore, these women were included in the final analyses to preserve sample size. Thus, the final sample available for analysis was 1204 women (60% of the total CAREDS population).

Women who were included in this study (n = 1204) did not differ (P > 0.05) from the CAREDS women who were excluded (n = 801) with respect to 25(OH)D, vitamin D intake, season of blood draw, waist circumference, total cholesterol, self-reported time in sunlight, or allele frequencies for the significant SNPs in this report.

The Institutional Review Boards at each participating institution approved all protocols and consent forms. All women provided written informed consent.

Measurement of serum 25(OH)D. The measurement of serum 25(OH)D in the CAREDS samples was previously described (26). Serum 25(OH)D is reported in nmol/L, but values can be divided by 2.5 to convert them to μg/L.

SNP selection and genotyping. We selected the 2 nonsynonymous (coding) SNPs in the *GC* gene that result in a change in the vitamin D binding protein (GC) that affects its affinity for 25(OH)D (27). Variation at rs4588 results in a base pair change of ACG→AAG, leading to an amino acid change in codon 436 [previously known as 420 (20)] of Thr→Lys, a protein change from GC-1 to GC-2, and lower affinity for 25(OH)D. Similarly, variation at rs7041 results in a base pair change of GAT→GAG, leading to an amino acid change in codon 432 (previously known as 416) of Asp→Glu and a protein change to the GC-1s protein, which has an affinity for 25(OH)D that is higher than that for GC-2 but lower than the other form of GC-1 (GC-1f). Moreover, there is evidence of differences in glycosylation (28–30), metabolism of the GC protein (31), and concentration of the GC protein (28) between the GC-1 and GC-2 isoforms (coded for by variation in rs4588). Because these 2 SNPs are functional and directly influence 25(OH)D, no additional genotyping was done in the *GC* gene. For the *DHCR7* and *CYP2R1* genes, where functional SNPs have not yet been found, we selected a set of SNPs (tagSNPs) that "tag" SNPs in the region that are not genotyped. Tagging was completed using the HapMap Genome Browser (32). TagSNPs were chosen using the Utah residents with ancestry from northern and western Europe reference population and filtering for a minor allele frequency ≥0.05 and an r^2 ≥0.80. Nine tagSNPs in *DHCR7* were selected, including rs1790349 from the Ahn et al. (18) GWA meta-analysis and rs12419279, which is in linkage disequilibrium (LD) (r^2 = 1.0) with rs12785878 from the SUNLIGHT Consortium GWA meta-analysis (19). Eleven tagSNPs in *CYP2R1* were selected, including rs2060793 from the Ahn et al. (18) GWA meta-analysis, which is in LD (r^2 = 1.0) with rs10741657 from the SUNLIGHT Consortium GWA meta-analysis (19). Functional SNPs have also not been found for the *CYP24A1* gene. However, this gene required a very large number of tagSNPs and showed the weakest evidence for association with 25(OH)D, so only 6 SNPs from the literature and 1 nonsynonymous SNP were selected. In all, 29 SNPs were chosen. An additional 186 ancestry informative markers (AIMs), developed and validated by Price et al. (33), were also genotyped, 95 to discern the northwest to southeast cline in European ancestry and 91 to discern the southeastern European to Ashkenazi Jewish ancestry cline (http://genepath.med.harvard.edu/~reich/EUROSNP.htm).

DNA was extracted from the buffy coats of blood obtained at the WHI-OS baseline visit that were stored frozen at −80°C. Genotyping was done at Case Western Reserve University using an Illumina Custom GoldenGate Assay or via the KASPar Assay at LCG Genomics (formerly KBiosciences) if a SNP failed the Illumina design. The GoldenGate Assay genotypes were called using Illumina Genome Studio and the KASPar Assay genotypes were called via the KASP SNP Genotyping System.

Duplicate quality control samples from 42 individuals were placed randomly throughout each of the nineteen 96-well plates. The genotype concordance rate was 99.86%. Genotype quality assurance checks were performed using the PLINK software v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) (34). Of the 1772 samples sent for genotyping, 75 had insufficient DNA quantity, 14 failed genotyping, 7 had call rates <90%, 7 had heterozygosity >44.5% (an indicator of sample cross-contamination), and 3 pairs ($n = 6$) had concordance rates ≥99% (an indicator of a sample mix-up), resulting in 1663 samples that passed the quality assurance/control checks. Call rates for the SNPs in the 4 vitamin D genes ranged from 98.2 to 100% and there was no deviation from Hardy-Weinberg equilibrium at a Bonferroni-corrected global significance level $\alpha = 0.05$.

Proxy measures for sunlight exposure: season as a proxy for the available solar radiation. To divide the season of blood draw at the WHI-OS baseline visit into 2 sunlight exposure categories, season was categorized as summer/fall (June to November) or winter/spring (December to May) based on a cluster analysis using the average linkage clustering method in the SAS CLUSTER procedure (version 9.2; SAS Institute). Season of blood draw served as a proxy measure of the available solar radiation in the environment.

Proxy measures for sunlight exposure: physical activity as a proxy for individual-level sunlight exposure. The measurement of physical activity and its association with 25(OH)D in the CAREDS population was previously described (26). In that study, the observed association between physical activity and 25(OH)D was found to largely reflect the effect of sunlight exposure during outdoor physical activity. In the current study, the weekly duration of total recreational physical activity and yard work were summed and used as a proxy for individual-level sunlight exposure, because sunlight exposure information was not collected at the WHI-OS baseline visit (1993–1998).

Proxy measures for sunlight exposure: retrospective self-reported sunlight exposure. The measurement of self-reported sunlight exposure in the CAREDS population was previously described (26). Briefly, a sunlight exposure questionnaire was administered at the CAREDS baseline (2001–2004) to collect information about the time spent in direct sunlight at the time of the baseline enrollment in WHI-OS (1993–1998) when 25(OH)D was measured.

Vitamin D intake. The methods used to collect and calculate vitamin D intake from food were previously described (35). An interviewer-administered form was used to collect information on intake of nutrients from supplements (such as vitamin D) at the WHI-OS baseline (36,37). Total vitamin D intake was calculated by summing vitamin D intake from foods and supplements. Vitamin D intake is reported in IU but can be divided by 40 to convert to μg.

Body size measures. The methods used to measure body size were previously described (26). All body size measurements were performed at the WHI-OS baseline.

Other potential correlates. Self-reported age, education, smoking, alcohol intake, hormone therapy use, overall general health (5-point scale ranging from poor to excellent), systolic and diastolic blood pressure (averaged from 2 measurements; mm Hg), TG (mmol/L), and total cholesterol (mmol/L) were also obtained at the WHI-OS baseline (21).

Statistical analysis. The 25(OH)D values were not normally distributed; therefore, a square root transformation was used to better approximate a normal distribution. The unadjusted percent of variability in square root-transformed 25(OH)D explained by nongenetic characteristics was estimated using linear regression implemented in the SAS GLM procedure (version 9.2). These characteristics were then evaluated using forward step-wise linear regression in the SAS REG procedure with the entry P value < 0.10 and stay P value < 0.05 to build the best-fitting final model.

To minimize the risk of confounding in the genetic analyses due to population stratification, we used the genotypes from the panel of AIMs and principal components analysis (PCA), using the SmartPCA program in the EIGENSOFT package version 3.0 (38), to detect genetic outliers and estimate principal components (eigenvalues) that capture genotypic variation due to ancestry. The PCA was initially performed while including genotypes for the HapMap Utah residents with ancestry from northern and western Europe, Yoruba in Ibadan, Nigeria, Japanese in Tokyo, Japan, and Han Chinese in Beijing, China populations in order to remove women who self-reported being non-Hispanic Caucasian but whose AIMs were not consistent with European ancestry. A subsequent PCA included only women who were of European descent. The first principal component explained 1.4% of the genetic variance and was then used to correct for population stratification in the genetic analyses.

For the genetic analyses, 25(OH)D values were adjusted for month of blood draw using the nonparametric SAS LOESS procedure to minimize the seasonal effect on 25(OH)D and then square root transformed. The association between each of the vitamin D SNPs and 25(OH)D was tested using linear regression in the PLINK software v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) (34). An additive genetic model was tested while adjusting for age and the principal component for ancestral admixture. For SNPs showing a significant association with 25(OH)D at the $P < 0.01$ level, the analyses were then stratified by season of blood draw (winter or summer) and, separately, vitamin D intake (< or ≥400 IU/d). Unadjusted and untransformed mean 25(OH)D and sample sizes for each genotype were obtained using the SAS MEANS procedure. Gene-environment interaction testing was performed using the SAS GLM procedure. Haplotypes were probabilistically inferred by Haploview version 4.2 (http://www.broadinstitute.org/haploview/haploview) (39). Haplotype association analysis was performed in PLINK. The SAS REG procedure was used to conduct a conditional analysis to identify SNPs with independent effects. A genetic risk score was calculated as the sum of the number of risk alleles for SNPs that were independently associated with 25(OH)D at the $P < 0.01$ level.

Results

Not adjusting for other factors, vitamin D intake from foods and supplements accounted for the highest percent of variability in 25(OH)D (9.6%); mean 25(OH)D increased for each higher quartile of vitamin D intake (**Table 1**). Waist circumference accounted for 8.8% of the variability in 25(OH)D, with higher mean concentrations at each lower quartile of waist circumference. A similar relationship was seen with BMI, but it accounted for slightly less variability in 25(OH)D (7%). Season of blood draw accounted for 8.6% of the variability in 25(OH)D, with higher concentrations found in blood drawn from June through November. Additional variation in 25(OH)D was explained by total cholesterol; physical activity; TG; retrospective, self-reported hours in sunlight; overall general health; education; hormone use status; systolic blood pressure; alcohol intake; and Madison, WI study site.

An overall predictive model was developed using forward step-wise linear regression. In order of the percent of variability in 25(OH)D accounted for, the following variables were entered and remained in the final model: vitamin D intake (9.5%), waist circumference (7.6%), season of blood draw (7.1%), total cholesterol (1.5%), and hours in sunlight (1.0%) (**Table 2**). The fully adjusted model accounted for 26.7% of the variation in square root-transformed 25(OH)D.

Of the 29 genotyped SNPs, one SNP in the *GC* gene (rs4588) and 3 SNPs (rs10500804, rs11023380, and rs2060793) in the *CYP2R1* gene were significantly associated with 25(OH)D at a Bonferroni-corrected significance of α/n of SNPs tested = 0.05/29 = 0.0017 (**Table 3**). Two additional SNPs, rs7041 in *GC* and rs11023374 in *CYP2R1*, were associated with 25(OH)D at the $P < 0.01$ significance threshold. All 6 of these SNPs were therefore examined for potential modification of their effect by external sources of vitamin D. For all 6 SNPs and both external sources of

TABLE 1 Serum 25(OH)D in postmenopausal women of European descent in the CAREDS by WHI-OS baseline (1993–1998) characteristics[1]

Characteristic	*n*	25(OH)D (*nmol/L*)[2]	*P* value[3]	r^2 [3]
Demographic				
Age group, y			0.16	<0.01
50–59	384	59.3 ± 22.9		
60–69	564	56.1 ± 24.3		
70–79	256	56.8 ± 21.9		
Education, highest level completed			0.001	0.01
High school diploma/GED or lower	296	54.4 ± 23.6		
Vocational training/some college	452	56.4 ± 22.4		
College degree	116	57.2 ± 20.9		
Postgraduate coursework/degree	337	60.9 ± 24.9		
Environmental				
Study site (latitude)			0.006[4]	0.01[4]
Iowa City, IA (42° N)	435	56.4 ± 22.1		
Madison, WI (43° N)	402	60.0 ± 24.2		
Portland, OR (46° N)	367	55.2 ± 23.6		
Season of blood draw			<0.0001	0.09
Winter/spring (December to May)	549	50.1 ± 22.1		
Summer/fall (June to November)	655	63.3 ± 22.7		
Behavioral				
Vitamin D intake from foods and supplements,[5] IU/d range			<0.0001	0.10
Quartile 1, 4.1–220.1	301	47.0 ± 23.2		
Quartile 2, 220.3–488.0	301	56.1 ± 22.5		
Quartile 3, 488.3–669.5	301	60.0 ± 20.9		
Quartile 4, 669.8–2459.0	301	66.0 ± 22.8		
Physical activity, min/wk range			<0.0001	0.03
Quartile 1, 0.0–110.0	302	52.3 ± 24.0		
Quartile 2, 115.0–232.5	297	56.9 ± 22.4		
Quartile 3, 235.0–395.0	302	56.5 ± 21.1		
Quartile 4, 400.0–1920.0	303	63.4 ± 24.5		
Retrospective, self-reported time in sunlight,[6] h/wk			<0.0001	0.01
<1	445	54.2 ± 22.6		
1–3	618	58.6 ± 23.4		
>3	130	62.3 ± 24.7		
Smoking status			0.40	<0.01
Never smoked	698	57.8 ± 23.3		
Past smoker	467	56.4 ± 23.2		
Current smoker	39	58.3 ± 26.9		
Alcohol intake[7]			0.005	0.01
Never drank	119	54.6 ± 22.7		
Past drinker	202	57.6 ± 21.8		
<1 drink/mo	163	52.4 ± 21.9		
<1 drink/wk	258	55.9 ± 22.9		
1–7 drinks/wk	336	59.3 ± 23.9		
>7 drinks/wk	125	62.9 ± 26.3		
Hormone use status			0.005	0.01
Never used	395	54.7 ± 23.3		
Past user	166	57.5 ± 24.0		
Current user	643	58.8 ± 23.1		
Overall general health			0.0003	0.01
Excellent	210	61.9 ± 23.4		
Very good	542	57.8 ± 24.0		
Good	378	54.5 ± 21.7		
Fair	65	56.3 ± 23.2		
Poor	6	48.4 ± 41.9		
Physiological				
BMI, kg/m^2			<0.0001	0.07
Under-/normal weight, <25.00	425	65.1 ± 25.6		
Overweight, 25.00–29.99	447	55.8 ± 20.7		
Obese, ≥30.00	332	49.3 ± 20.5		

(Continued)

TABLE 1 *Continued*

Characteristic	*n*	25(OH)D (*nmol/L*)[2]	*P* value[3]	r^2 [3]
Waist circumference, cm range			<0.0001	0.09
Quartile 1, 57.0–74.4	297	65.4 ± 25.6		
Quartile 2, 74.5–81.6	294	61.5 ± 24.3		
Quartile 3, 82.0–92.0	318	54.0 ± 18.8		
Quartile 4, 92.5–142.0	295	48.4 ± 20.8		
Systolic blood pressure, mm Hg range			0.004	0.01
Quartile 1, 86.0–114.0	314	59.4 ± 23.0		
Quartile 2, 115.0–124.0	284	58.5 ± 23.4		
Quartile 3, 125.0–137.0	305	56.0 ± 24.1		
Quartile 4, 138.0–200.0	301	55.1 ± 22.8		
Diastolic blood pressure, mm Hg range			0.44	<0.01
Quartile 1, 46–68	320	57.6 ± 22.8		
Quartile 2, 69–73	284	57.4 ± 23.1		
Quartile 3, 74–79	271	56.6 ± 23.9		
Quartile 4, 80–107	329	57.5 ± 23.7		
Total cholesterol, mmol/L range			<0.0001	0.03
Quartile 1, 2.7–5.1	296	61.1 ± 24.2		
Quartile 2, 5.2–5.7	305	60.1 ± 24.2		
Quartile 3, 5.7–6.4	296	56.8 ± 22.8		
Quartile 4, 6.4–9.9	304	51.4 ± 21.0		
TG, mmol/L range			<0.0001	0.02
Quartile 1, 0.5–1.2	299	63.8 ± 25.3		
Quartile 2, 1.2–1.6	299	58.6 ± 23.5		
Quartile 3, 1.6–2.2	301	55.0 ± 21.9		
Quartile 4, 2.2–13.5	302	51.9 ± 20.9		

[1] CAREDS, Carotenoids in Age-Related Eye Disease Study; 25(OH)D, 25-hydroxyvitamin D; WHI-OS, Women's Health Initiative Observational Study.
[2] Untransformed and unadjusted mean ± SD 25(OH)D.
[3] *P* value and r^2 estimated from unadjusted linear regression where 25(OH)D values were square root transformed.
[4] *P* value and r^2 for dichotomous variable of Madison, WI, no/yes.
[5] Vitamin D intake can be divided by 40 to convert from IU to μg.
[6] Retrospective, self-reported time in sunlight at the time of the WHI-OS baseline visit was assessed at the CAREDS baseline visit.
[7] Alcohol intake was self-reported by the participant, not measured.

vitamin D (season and vitamin D intake), the effect of the SNP was approximately double in the high exposure group and the *P* value was much lower compared with the low-exposure group (**Table 4**). However, the statistical test for interaction was significant ($P < 0.05$) for only one gene-environment pair (rs7041-season; $P = 0.01$), where the β-coefficient for the high-exposure group was much more than twice that in the low-exposure group (−0.33 vs. −0.02, respectively).

Results from association analyses of haplotype blocks in the *GC* and *CYP2R1* genes were consistent with the results from the individual SNP analyses (data not shown). When both *GC* SNPs were included in the model, only rs4588 remained significant; rs7041 was not independently significant (data not shown). Similarly, when all 4 *CYP2R1* SNPs were included in the linear regression model, only rs2060793 remained significant; the other 3 SNPs were not independently significant. Therefore, further analyses focused on rs4588 in *GC* and rs2060793 in *CYP2R1*.

To determine the joint effect of these 2 SNPs, a genetic risk score was calculated as the sum of the number of A alleles for rs4588 and G alleles for rs2060793. It was not necessary to weight the number of risk alleles by the correlation coefficient, because the coefficients for rs4588 and rs2060793 were nearly identical in a multiple regression model including both SNPs. The range of the genetic risk score was 0–4. This score was

TABLE 2 Final multivariable model for nongenetic correlates of 25(OH)D in the CAREDS population, $n = 1204$[1]

Characteristic	β (SEE)	*P* value	Semi-partial R^2
Vitamin D intake from foods and supplements,[2] IU/d	0.001 (0.0001)	<0.0001	0.10
Waist circumference, cm	−0.03 (0.003)	<0.0001	0.08
Season of blood draw, summer/fall vs. winter/spring	0.83 (0.079)	<0.0001	0.07
Total cholesterol, mmol/L	−0.21 (0.043)	<0.0001	0.02
Retrospective, self-reported time in sunlight, h/wk	0.17 (0.044)	<0.0001	0.01
Fully adjusted model R^2			0.27

[1] Final multivariable model was developed using forward step-wise linear regression. All characteristics with significant *P* values in Table 1 were considered. β-Coefficients, *P* values, and semi-partial R^2 for each characteristic in the order in which it entered the final model are shown. CAREDS, Carotenoids in Age-Related Eye Disease Study; 25(OH)D, 25-hydroxyvitamin D.
[2] Vitamin D intake can be divided by 40 to convert from IU to μg.

TABLE 3 Characteristics of genotyped SNPs and their association with 25(OH)D in the CAREDS population, $n = 1204$[1]

Gene/SNP	Chromosome/position[2]	Alleles[3]	MAF	P_{HWT}	$\beta^4_{additive}$	P^4
GC	4					
rs4588	72618323	A/C	0.28	0.67	−0.25	<0.001
rs7041	72618334	T/G	0.43	0.51	−0.19	0.002
CYP2R1	11					
rs10832312	14887830	G/A	0.10	0.52	−0.11	0.28
rs11023371	14896271	A/G	0.07	0.19	0.13	0.27
rs11023374	14903636	G/A	0.29	0.78	−0.19	0.005
rs10500804	14910273	C/A	0.44	0.64	−0.19	0.001
rs7129781	14912417	G/A	0.07	0.10	−0.20	0.09
rs2060793	14915310	A/G	0.38	0.39	0.25	<0.001
rs16930609	14915908	C/A	0.10	0.62	−0.04	0.66
rs11819875	14917297	C/A	0.18	1.00	−0.07	0.39
rs10832313	14922363	G/A	0.07	0.19	−0.11	0.34
rs12418214	14926755	G/A	0.10	0.33	0.00	1.00
rs11023380	14930058	G/A	0.48	0.91	−0.20	0.001
DHCR7	11					
rs11233570	71126315	C/G	0.03	1.00	−0.12	0.49
rs1540130	71129192	G/C	0.22	0.93	0.03	0.66
rs1540129	71129523	C/G	0.23	0.68	−0.04	0.55
rs12419279	71139061	T/A	0.26	0.14	−0.04	0.56
rs1790349	71142350	G/A	0.15	0.37	0.05	0.53
rs1792272	71142518	G/A	0.05	0.18	−0.11	0.42
rs7122671	71144468	A/G	0.07	0.65	−0.09	0.45
rs1790334	71155153	A/G	0.05	0.73	−0.12	0.41
rs1790373	71166337	A/G	0.05	0.31	−0.09	0.53
CYP24A1	20					
rs6013897	52742479	A/T	0.20	0.66	−0.07	0.35
rs4809957	52771171	G/A	0.21	0.73	−0.06	0.39
rs1570669	52774427	G/A	0.34	0.06	−0.04	0.54
rs1570670	52774579	G/A	0.21	0.79	−0.06	0.44
rs2274130	52774601	G/A	0.21	0.93	−0.06	0.44
rs2296239	52775528	A/G	0.21	0.86	−0.05	0.47
rs35051736	52788189	A/G	0.005	1.00	0.61	0.17

[1] CAREDS, Carotenoids in Age-Related Eye Disease Study; MAF, minor allele frequency; 25(OH)D, 25-hydroxyvitamin D; P_{HWT}, *P* value from the Hardy-Weinberg equilibrium test; SNP, single nucleotide polymorphism.
[2] Chromosome and chromosomal position in base pairs from the P arm telomere for the GRCh37.p5 assembly of genome build 37.3 in the NCBI database.
[3] The allele listed first is the minor allele.
[4] Month of blood draw-adjusted, square root-transformed 25(OH)D under an additive genetic model, adjusting for age and ancestry.

associated with 25(OH)D ($P < 0.0001$) after adjustment for age and ancestry (data not shown). When the genetic risk score was added to the model shown in Table 2, it accounted for 2.4% of the variation in 25(OH)D. The fully adjusted model R^2 increased from 26.7 to 29.1%, indicating that the joint effect of the 2 SNPs accounted for a 9% relative increase in the model's ability to explain variation in 25(OH)D.

The mean 25(OH)D was highest in the groups with no copies of the rs4588 or rs2060793 risk alleles who also had high external sources of vitamin D (**Fig. 1**). Mean 25(OH)D was lowest in the groups with 3 risk alleles and low external sources of vitamin D or 4 risk alleles, regardless of external sources of vitamin D. There was a trend toward a modification of the effect of the genetic risk score by both external sources of vitamin D, such that there was a much weaker effect of the genetic risk score when external sources of vitamin D were low and a stronger effect when external sources were high. This is similar to the findings from the single SNP analyses and is consistent with a quantitative gene-environment interaction, in which the magnitude, but not the direction, of the effect of one factor varies across the levels of another (40). A test for the statistical interaction between genetic risk score and external source of vitamin D was significant for season of blood draw ($P = 0.04$) but not for vitamin D intake ($P = 0.26$).

Vitamin D intake, which can be easily modified with little risk of adverse effects below the upper level of intake of 4000 IU/d set by the IOM committee of experts (7), was examined further. Individuals were stratified by quartile of vitamin D intake. For the genetic risk score, individuals with either 3 or 4 risk alleles were combined due to small sample sizes in the 4-risk alleles subgroup after stratification into 4 vitamin D intake groups. The percentage of individuals with 25(OH)D that is adequate for good bone health [i.e., does not put the individual at risk for inadequacy; >50 nmol/L (>20 μg/L)] (7) was calculated. All of the women with no risk alleles who consumed at least 670 IU/d vitamin D (slightly more than the RDA of 600 IU/d for 1–70 y olds) had 25(OH)D >50 nmol/L; this fell to 84, 72, and 62% for individuals consuming at least 670 IU/d of vitamin D but who had 1, 2, or 3–4 risk alleles, respectively (**Fig. 2**). Only 30% of women with 3–4 risk alleles and in the lowest quartile of vitamin D intake had adequate 25(OH)D. Encouragingly, even among women with 3–4 risk alleles, the percentage with adequate 25(OH)D did rise with each increasing quartile of vitamin D intake.

Discussion

In this study of 1204 postmenopausal women of European descent, we replicated findings in the *GC* and *CYP2R1* genes that were previously reported in 2 large GWA meta-analyses of Caucasian cohorts (18,19) and demonstrated that levels of external sources of vitamin D modify the effects of the SNPs in these genes. The effects of 2 SNPs in the *GC* gene and 4 SNPs in the *CYP2R1* gene, as well as the genetic risk score formed by one independent SNP in each of these genes, were highly significant, with effects twice the magnitude in individuals with high external sources of vitamin D compared with individuals with low external vitamin D, where the associations were generally not significant. This is consistent with a previous study in which rs4588 in the *GC* gene was associated with 25(OH)D in Canadians of European descent in the fall ($n = 111$; $R^2_{A\ allele} = -0.256$; $P = 0.009$) but not in the winter ($n = 97$; $R^2_{A\ allele} = -0.066$; $P = 0.535$) (20), although, to our knowledge, the effect of modification by vitamin D intake has not been examined before. These findings have implications for discovery and replication genetic studies, where discounting important environmental factors can lead to false-negative findings and lack of replication.

SNPs in *DHCR7*, the other gene reported by both of the previous meta-analyses, were not significantly associated with 25(OH)D in our study. There are a few possible explanations for this lack of replication. First, although we genotyped either an identical SNP to the meta-analysis SNP or a SNP in perfect LD ($r^2 = 1.0$) with the meta-analysis SNP, none of these SNPs are known to have functional consequences; they are likely to be in LD with another, not yet identified, functional SNP. Therefore, the *DHCR7* SNPs in our study may not have been in strong enough LD with the truly functional SNP, reducing our power to detect the association. This reduction in power may have been exacerbated by our smaller sample size ($n = 1204$) relative to that of the meta-analyses [$n = 4501$ (18) and $n = 33{,}868$ (19)]. Alternately, our study population consisted of postmenopausal women between the ages of 50 and 79 y. There is evidence that production of cholecalciferol in the skin following sunlight exposure decreases with increasing age (41). This may be the result of linear

TABLE 4 Association between 6 significant SNPs and 25(OH)D stratified by high and low exposure to external sources of vitamin D in the CAREDS population[1]

	Stratified by winter/summer					Stratified by low/high vitamin D intake				
	Mean 25(OH)D[2]			$\beta^3_{additive}$	P^3	Mean 25(OH)D[2]			$\beta^3_{additive}$	P^3
	nmol/L					*nmol/L*				
GC										
rs4588	AA	AC	CC			AA	AC	CC		
Low exposure[4]	54.2 (n = 39)	46.8 (n = 232)	52.3 (n = 278)	−0.15	0.17	51.1 (n = 35)	48.0 (n = 205)	51.8 (n = 265)	−0.18	0.11
High exposure[4]	53.5 (n = 50)	62.0 (n = 258)	65.6 (n = 347)	−0.33	0.0002	55.6 (n = 54)	59.7 (n = 285)	65.5 (n = 360)	−0.31	<0.0001
Interaction P[5]					0.17					0.33
rs7041	TT	TG	GG			TT	TG	GG		
Low exposure[4]	51.2 (n = 94)	48.7 (n = 264)	51.2 (n = 172)	−0.02	0.81	47.6 (n = 86)	49.8 (n = 239)	51.9 (n = 162)	−0.18	0.07
High exposure[4]	57.4 (n = 114)	63.1 (n = 314)	67.8 (n = 197)	−0.33	<0.0001	59.6 (n = 122)	61.2 (n = 339)	66.4 (n = 207)	−0.23	0.003
Interaction P[5]					0.01					0.75
CYP2R1										
rs2060793	AA	AG	GG			AA	AG	GG		
Low exposure[4]	51.3 (n = 78)	52.3 (n = 245)	47.4 (n = 226)	0.18	0.07	51.3 (n = 84)	52.7 (n = 227)	46.9 (n = 194)	0.20	0.04
High exposure[4]	67.3 (n = 107)	65.6 (n = 311)	58.4 (n = 237)	0.31	0.0001	68.2 (n = 101)	64.6 (n = 329)	57.5 (n = 269)	0.32	<0.0001
Interaction P[5]					0.28					0.33
rs10500804	CC	AC	AA			CC	AC	AA		
Low exposure[4]	48.8 (n = 112)	49.1 (n = 268)	52.9 (n = 168)	−0.13	0.17	47.9 (n = 100)	50.8 (n = 254)	51.0 (n = 150)	−0.10	0.30
High exposure[4]	57.1 (n = 129)	63.9 (n = 317)	66.0 (n = 208)	−0.25	0.002	57.1 (n = 141)	62.0 (n = 331)	66.1 (n = 226)	−0.25	0.0004
Interaction P[5]					0.31					0.20
rs11023380	GG	AG	AA			GG	AG	AA		
Low exposure[4]	50.9 (n = 128)	48.0 (n = 270)	53.6 (n = 150)	−0.09	0.34	49.0 (n = 112)	49.9 (n = 259)	52.2 (n = 132)	−0.12	0.22
High exposure[4]	58.2 (n = 148)	63.4 (n = 327)	67.7 (n = 178)	−0.29	0.0002	58.8 (n = 164)	61.4 (n = 338)	67.4 (n = 196)	−0.26	0.0002
Interaction P[5]					0.09					0.25
rs11023374	GG	AG	AA			GG	AG	AA		
Low exposure[4]	50.3 (n = 49)	49.0 (n = 219)	51.0 (n = 281)	−0.06	0.55	50.1 (n = 45)	48.6 (n = 205)	51.5 (n = 255)	−0.13	0.23
High exposure[4]	59.4 (n = 52)	60.2 (n = 269)	66.4 (n = 333)	−0.29	0.001	58.9 (n = 56)	59.9 (n = 283)	64.9 (n = 359)	−0.23	0.004
Interaction P[5]					0.09					0.42

[1] CAREDS, Carotenoids in Age-Related Eye Disease Study; 25(OH)D, 25-hydroxyvitamin D.
[2] Unadjusted and untransformed genotypic means and sample sizes.
[3] β and P values are for the effect of the minor allele on month of blood draw adjusted (within each stratum), square root-transformed 25(OH)D under an additive genetic model, adjusting for age and ancestry.
[4] Low exposure is defined as winter season of blood draw for the stratified by winter/summer analyses and vitamin D intake <400 IU/d for the stratified by low-/high-diet analyses; high exposure is defined as summer season of blood draw for the stratified by winter/summer analyses and vitamin D intake ≥400 IU/d for the stratified by low-/high-diet analyses. Vitamin D intake can be divided by 40 to convert from IU to μg.
[5] Interaction P values are for a test of the gene-environment interaction term using generalized linear regression, where the gene variable was a count of the number of minor alleles for the SNP and the environmental variable was 0 for low exposure (winter blood draw or vitamin D intake <400 IU/d) and 1 for high exposure (summer blood draw or intake ≥400 IU/d).

decreases in the concentration of 7-dehydrocholesterol in the epidermis, the main site for cholecalciferol production, beginning in middle age (42). Therefore, the postmenopausal women in our study may have had lower epidermal concentrations of 7-dehydrocholesterol that would be the equivalent of low exposure (as in Table 4) for our entire sample. This could result in a lack of replication for the *DHCR7* SNPs, because this gene encodes the 7-dehydrocholesterol reductase enzyme that catalyzes the conversion of 7-dehydrocholesterol in the skin to pre- cholecalciferol, which is rapidly transformed to cholecalciferol. Therefore, this gene would be relevant only if adequate concentrations of the substrate, 7-dehydrocholesterol, were present in the epidermis.

This study has potentially important implications for public health recommendations and clinical practice guidelines. Individuals with more copies of the rs4588 and/or rs2060793 risk alleles may require higher vitamin D intake to achieve adequate 25(OH)D (Fig. 2). Based on the report from the IOM committee of experts, the RDA (the vitamin D intake sufficient to meet the needs of ~97.5% of the population) for adults age 70 y or younger is 600 IU/d and for adults >70 y it is 800 IU/d (7). However, in our study population, of the 288 women age 70 y or younger who reported taking at least 600 IU/d, only 66% of those with 3–4 risk alleles had achieved the 25(OH)D recommended by the IOM committee of experts for good bone health (≥50 nmol/L) (7) compared with 91% of those with 0–1 risk alleles (data not shown). Of the 102 women over age 70 y who reported taking at least 800 IU/d, only 50% of the those 3–4 risk alleles had achieved adequate 25(OH) compared with 77% of those with 0–1 risk alleles. This suggests that individuals with multiple genetic risk factors may need to consume higher amounts of vitamin D to achieve adequate 25(OH)D. If health outcomes beyond those related to calcium metabolism were confirmed to be causally associated with vitamin D by large ongoing clinical trials (43), the findings of this study would have even broader implications. Fortunately, our data suggest that, even in individuals with 3–4 risk alleles, 25(OH)D does increase with each increasing quartile of vitamin D intake (average increase of 16.7 nmol/L from the lowest to highest quartile of intake), but at a lower rate than in individuals with fewer risk alleles (average increase of 27.7 nmol/L from the lowest to highest quartile of intake) (Fig. 2), although the P value for the interaction was not significant.

One limitation of this study is that, despite the striking effect modification for all 6 SNPs and both external sources of

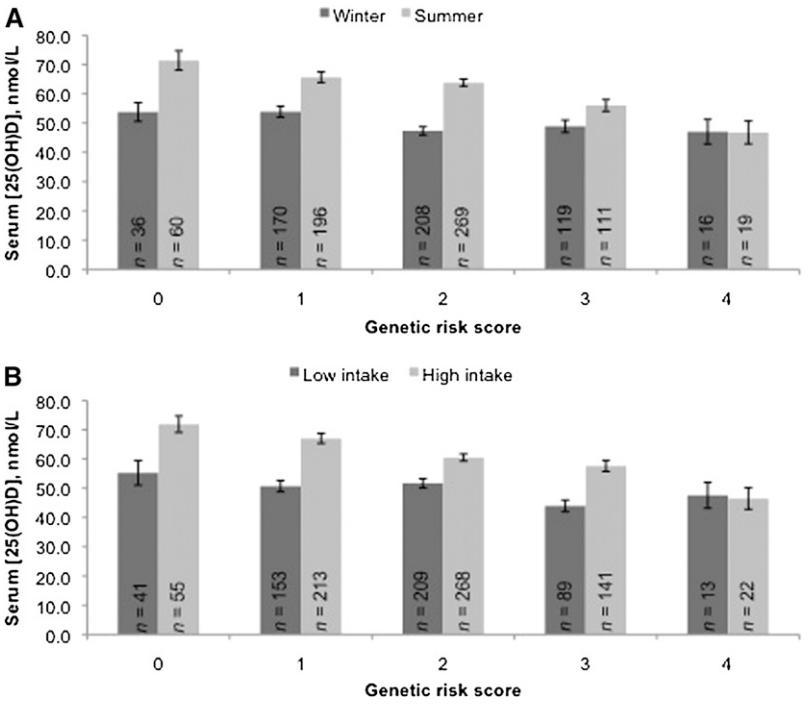

FIGURE 1 Mean serum 25(OH)D for each genetic risk score category stratified by high and low exposure to external sources of vitamin D. Plots of the mean unadjusted serum 25(OH)D (nmol/L, *y*-axis) for each genetic risk score category (*x*-axis) stratified by winter or summer season (*A*) and, separately, by low vitamin D intake (<400 IU/d) or high intake (≥400 IU/d) (*B*). Error bars indicate the SEM for each group. The genetic risk score was calculated as the sum of the number of A alleles for rs4588 and G alleles for rs2060793. Vitamin D intake can be divided by 40 to convert from IU to μg. 25(OH)D, 25-hydroxyvitamin D.

vitamin D, we had a low power to detect significant gene-environment interactions and were only able to do so with the rs7041-season and genetic risk score-season interaction terms. For example, the power to detect an interaction between season and rs4588 was 0.28 and with rs206793 it was 0.20. Similarly, the power to detect an interaction between vitamin D intake and rs4588 was 0.17 and with rs206793 it was 0.19. However, the consistency of the effect and *P* values among multiple SNPs in 2 different but relevant genes interacting with 2 different and uncorrelated external sources of vitamin D (*P* value = 0.53 for the concordance between winter/summer and low/high vitamin D intake) provides internal validation of our effect modification findings, suggesting that they are real findings and not artifacts of our subgroup analyses. Moreover, similar results were reported by Gozdzik et al. (20), providing additional evidence in support of a true gene-environment interaction.

An additional limitation is that our study population was drawn from the CAREDS, an ancillary study of WHI-OS women with baseline lutein+zeaxanthin intake above the 78th or below the 28th percentiles. Although demographic and other health-related data did not differ between the overall sample of women enrolled in the WHI-OS and the subsample enrolled in the CAREDS (25), vitamin D intake may be correlated with lutein +zeaxanthin intake, such that individuals with higher lutein +zeaxanthin may also have higher vitamin D intake. If this were the case, women selected for the CAREDS may have had higher and lower vitamin D intakes than the WHI-OS population, with fewer women in the middle of the distribution than would be expected from a random sample. This may have resulted in more women in the higher and lower distributions of 25(OH)D. Although a plot of vitamin D intake in the women in our study showed a slight bimodal distribution, this is to be expected, because intake is summed across diet and supplements, where the lower peak represents the distribution in individuals who do not take supplements and the higher peak is representative of individuals who do take supplements. In fact, a nearly identical bimodal distribution is seen in both the high lutein+zeaxanthin intake group and the low one. Moreover, a plot of the 25(OH)D in these women shows a purely unimodal distribution.

Additional research is needed to replicate these findings in populations that include both men and women and in populations that include ancestral groups beyond strictly European,

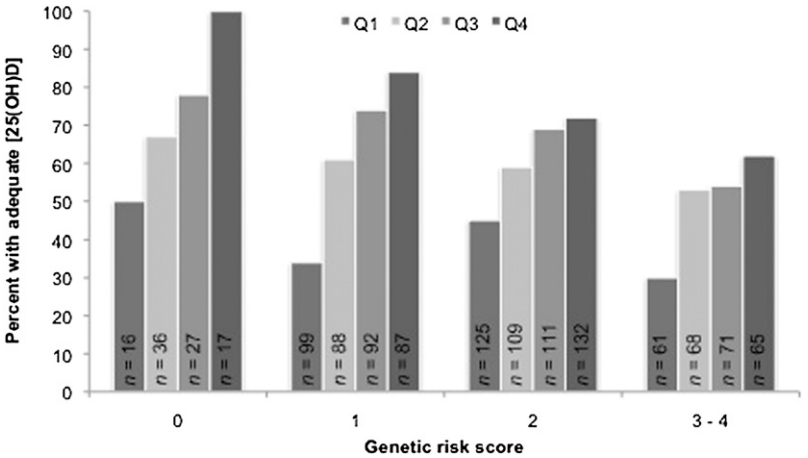

FIGURE 2 Percent of individuals with adequate 25(OH)D for each genetic risk score category stratified by quartile of vitamin D intake. Plot of the percentage of individuals with adequate serum 25(OH)D, defined as >50 nmol/L (>20 μg/L, *y*-axis) for each genetic risk score category (*x*-axis) stratified by quartile of vitamin D intake (Q1: 14–220 IU/d; Q2: 221–488 IU/d; Q3: 489–669 IU/d; Q4: 670–2459 IU/d). The genetic risk score was calculated as the sum of the number of A alleles for rs4588 and G alleles for rs2060793. Vitamin D intake can be divided by 40 to convert from IU to μg. 25(OH)D, 25-hydroxyvitamin D.

especially those with darker skin color where the prevalence of vitamin D deficiency is much higher (8). Moreover, despite including both genetic and nongenetic factors in our final predictive model, we were able to explain only 29.1% of the variation in 25(OH). This may be in part due to imperfect measurement of nongenetic factors, especially sun exposure. Future studies should aim to measure environmental factors more accurately.

In summary, we not only replicated 2 of the 3 genes from previous GWA meta-analyses of 25(OH)D, but we provided evidence that external sources of vitamin D modify these genetic effects. This has important implications for both the design of discovery and replication genetic studies for all health outcomes and for public health recommendations and clinical practice guidelines regarding achievement of adequate vitamin D status, demonstrating that a "one size fits all" approach may not work well for vitamin D.

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Literature Cited

1. Holick MF. Vitamin D deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. Nat Rev Endocrinol. 2011;7:73–5.
2. Bikle DD. Vitamin D regulation of immune function. Vitam Horm. 2011;86:1–21.
3. Davis CD, Milner JA. Vitamin D and colon cancer. Expert Rev Gastroenterol Hepatol. 2011;5:67–81.
4. Grant WB, Boucher BJ. Requirements for Vitamin D across the life span. Biol Res Nurs. 2011;13:120–33.
5. Scragg R. Vitamin D and public health: an overview of recent research on common diseases and mortality in adulthood. Public Health Nutr. 2011;14:1515–32.
6. Hewison M. Antibacterial effects of vitamin D. Nat Rev Endocrinol. 2011;7:337–45.
7. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.
8. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutr Res. 2011;31:48–54.
9. Millen AE, Wactawski-Wende J, Pettinger M, Melamed ML, Tylavsky FA, Liu S, Robbins J, LaCroix AZ, LeBoff MS, Jackson RD. Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative Calcium plus Vitamin D clinical trial. Am J Clin Nutr. 2010;91:1324–35.
10. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB Sr, Ordovas JM, O'Donnell CJ, Dawson-Hughes B, Vasan RS, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr. 2009;63:458–64.
11. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr. 2009;89:634–40.
12. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW, Norris JM. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin d levels in Hispanic and African Americans. J Clin Endocrinol Metab. 2008;93:3381–8.
13. Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, Spector TD. Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. J Bone Miner Res. 2001;16:371–8.
14. Orton SM, Morris AP, Herrera BM, Ramagopalan SV, Lincoln MR, Chao MJ, Vieth R, Sadovnick AD, Ebers GC. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. Am J Clin Nutr. 2008;88:441–7.
15. Arguelles LM, Langman CB, Ariza AJ, Ali FN, Dilley K, Price H, Liu X, Zhang S, Hong X, Wang B, et al. Heritability and environmental factors affecting vitamin D status in rural Chinese adolescent twins. J Clin Endocrinol Metab. 2009;94:3273–81.
16. Karohl C, Su S, Kumari M, Tangpricha V, Veledar E, Vaccarino V, Raggi P. Heritability and seasonal variability of vitamin D concentrations in male twins. Am J Clin Nutr. 2010;92:1393–8.
17. Snellman G, Melhus H, Gedeborg R, Olofsson S, Wolk A, Pedersen NL, Michaelsson K. Seasonal genetic influence on serum 25-hydroxyvitamin D levels: a twin study. PLoS ONE. 2009;4:e7747.
18. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet. 2010;19:2739–45.
19. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010;376:180–8.
20. Gozdzik A, Zhu J, Wong BY, Fu L, Cole DE, Parra EJ. Association of vitamin D binding protein (VDBP) polymorphisms and serum 25(OH)D concentrations in a sample of young Canadian adults of different ancestry. J Steroid Biochem Mol Biol. 2011;127:405–12.
21. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, Shumaker S, Wang CY, Stein E, Prentice RL. Implementation of the Women's Health Initiative study design. Ann Epidemiol. 2003;13:S5–17.
22. Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. The Women's Health Initiative recruitment methods and results. Ann Epidemiol. 2003;13:S18–77.
23. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Control Clin Trials. 1998;19:61–109.
24. Langer RD, White E, Lewis CE, Kotchen JM, Hendrix SL, Trevisan M. The Women's Health Initiative Observational Study: baseline characteristics of participants and reliability of baseline measures. Ann Epidemiol. 2003;13:S107–21.
25. Mares JA, LaRowe TL, Snodderly DM, Moeller SM, Gruber MJ, Klein ML, Wooten BR, Johnson EJ, Chappell RJ. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. Am J Clin Nutr. 2006;84:1107–22.
26. Kluczynski MA, Lamonte MJ, Mares JA, Wactawski-Wende J, Smith AW, Engelman CD, Andrews CA, Snetselaar LG, Sarto GE, Millen AE. Duration of physical activity and serum 25-hydroxyvitamin D status of postmenopausal women. Ann Epidemiol. 2011;21:440–9.
27. Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet. 1993;92:183–8.
28. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. Clin Chem. 2001;47:753–6.
29. Ravnsborg T, Olsen DT, Thysen AH, Christiansen M, Houen G, Hojrup P. The glycosylation and characterization of the candidate Gc macrophage activating factor. Biochim Biophys Acta. 2010;1804:909–17.
30. Borges CR, Jarvis JW, Oran PE, Nelson RW. Population studies of vitamin D binding protein microheterogeneity by mass spectrometry lead to characterization of its genotype-dependent O-glycosylation patterns. J Proteome Res. 2008;7:4143–53.
31. Kawakami M, Blum CB, Ramakrishnan R, Dell RB, Goodman DS. Turnover of the plasma binding protein for vitamin D and its metabolites in normal human subjects. J Clin Endocrinol Metab. 1981;53:1110–6.
32. HapMap Genome Browser Release 27 [cited April 21, 2011]. Available from: http://hapmap.ncbi.nlm.nih.gov/.
33. Price AL, Butler J, Patterson N, Capelli C, Pascali VL, Scarnicci F, Ruiz-Linares A, Groop L, Saetta AA, Korkolopoulou P, et al. Discerning the ancestry of European Americans in genetic association studies. PLoS Genet. 2008;4:e236.
34. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–75.

35. Millen AE, Pettinger M, Freudenheim JL, Langer RD, Rosenberg CA, Mossavar-Rahmani Y, Duffy CM, Lane DS, McTiernan A, Kuller LH, et al. Incident invasive breast cancer, geographic location of residence, and reported average time spent outside. Cancer Epidemiol Biomarkers Prev. 2009;18:495–507.
36. Patterson RE, Kristal AR, Levy L, McLerran D, White E. Validity of methods used to assess vitamin and mineral supplement use. Am J Epidemiol. 1998;148:643–9.
37. Patterson RE, Levy L, Tinker LF, Kristal AR. Evaluation of a simplified vitamin supplement inventory developed for the Women's Health Initiative. Public Health Nutr. 1999;2:273–6.
38. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904–9.
39. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263–5.
40. Wang X, Elston RC, Zhu X. The meaning of interaction. Hum Hered. 2010;70:269–77.
41. Lester E, Skinner RK, Wills MR. Seasonal variation in serum-25-hydroxyvitamin-D in the elderly in Britain. Lancet. 1977;1:979–80.
42. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest. 1985;76:1536–8.
43. Manson JE, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, Zaharris E, Macfadyen JG, Danielson E, Lin J, et al. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. Contemp Clin Trials. 2012;33:159–71.