Genetic Variants in *CYP2R1*, *CYP24A1* and *VDR* Modify the Efficacy of Vitamin D_3 Supplementation for Increasing Serum 25-Hydroxyvitamin D Levels in a Randomized Controlled Trial

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Context: Adequate serum 25-hydroxyvitamin D concentrations [25(OH)D] are required for optimal bone health, and low levels are associated with chronic diseases.

Objective: We investigated whether 41 candidate single nucleotide polymorphisms (SNPs) in vitamin D and calcium pathway genes (*GC*, *DHCR7*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *VDR*, and *CASR*) are associated with [25(OH)D] or modify the increase in [25(OH)D] from vitamin D_3 supplementation.

Design and setting: Baseline and year one [25(OH)D] measurements from a randomized controlled trial conducted at 11 clinical centers in the United States.

Participants: 1,787 healthy non-Hispanic white participants aged 45–75 years.

Interventions: Vitamin D_3 (1000 IU/day), calcium carbonate (1200 mg/day elemental), both or placebo.

Main outcome measures: Genotype main effects and interactions with vitamin D_3 treatment estimated using multiple linear regression.

Results: The mean baseline serum [25(OH)D] was 25.4 $\pm$ 8.7 ng/ml. Associations with baseline levels were discovered for SNPs in *CYP24A1* (rs2209314, rs2762939) and confirmed for SNPs in *GC* and *CYP2R1*. After one year, [25(OH)D] increased on average by 6.1 $\pm$ 8.9 ng/ml on vitamin D_3 treatment and decreased by 1.1 $\pm$ 8.4 ng/ml on placebo. The increase in [25(OH)D] due to vitamin D_3 supplementation was modified by genotypes at rs10766197 near *CYP2R1*, rs6013897 near *CYP24A1*, and rs7968585 near *VDR*.

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Abbreviations: 25-hydroxyvitamin D, 25(OH)D; 25-hydroxyvitamin D concentration, [25(OH)D]; 1,25-dihydroxyvitamin D, $1,25(OH)_2D$; single nucleotide polymorphism, SNP; genome-wide association studies, GWAS; coefficient of variation, CV; GC, group specific component; CYP2R1, cytochrome P450 2R-1; CYP24A1, cytochrome P450 24A-1; CYP27B1, cytochrome P450 27B-1; DHCR-7, 7-dehydrocholesterol reductace; VDR, vitamin D receptor; CASR, calcium-sensing receptor; NADSYN1, glutamine-dependent nicotinamide adenine dinucleotide synthase; MAF, minor allele frequency; LD, linkage disequilibrium; CI, confidence interval.

Conclusions: The increase in [25(OH)D] attributable to vitamin D_3 supplementation may vary according to common genetic differences in vitamin D 25-hydroxylase (*CYP2R1*), 24-hydroxylase (*CYP24A1*), and the vitamin D receptor (*VDR*) genes. These findings have implications for achieving optimal vitamin D status and potentially for vitamin D-related health outcomes.

Vitamin D is a prohormone whose active metabolite, 1,25-dihydroxyvitamin D, $1,25(OH)_2D$, regulates calcium homeostasis and plays an important role in bone growth and remodeling (1). The main circulating metabolite is 25-hydroxyvitamin D, 25(OH)D, a biomarker of vitamin D status. Vitamin D metabolism is highly regulated, and variation in expression or activity of key proteins may modify its level or effects. Key metabolic enzymes include: 25-hydroxylase (*CYP2R1*), which converts vitamin D to 25(OH)D; 1-hydroxylase (*CYP27B1*), which activates 25(OH)D to $1,25(OH)_2D$; 24-hydroxylase (*CYP24A1*), which inactivates 25(OH)D and $1,25(OH)_2D$; and 7-dehydrocholesterol reductase (*DHCR7*), which shunts vitamin D precursors toward cholesterol biosynthesis. Other key components include: vitamin D-binding protein (*GC*), which transports circulating metabolites, and the vitamin D receptor (*VDR*), which binds 1,25(OH)D to activate gene transcription and regulates vitamin D metabolism. Finally, the calcium sensing receptor (*CASR*), which regulates parathyroid hormone levels, is another critical part of this endocrine system controlling calcium levels and vitamin D metabolism.

Given the established risk of vitamin D deficiency for bone health and potential risks for major nonskeletal diseases (1), it is important to understand the role genetic factors play in modulating vitamin D levels. In previous research, common SNPs in vitamin D pathway genes have been associated with circulating [25(OH)D] in candidate gene studies (see (2, 3)) and genome-wide association studies (GWAS) (4, 5). A potentially important clinical question is whether these or other SNPs previously associated with other health outcomes modify the efficacy of vitamin D supplementation to increase serum [25(OH)D]. We investigated this question among participants in a randomized controlled trial (RCT) of oral vitamin D_3 supplementation.

Materials and Methods

Study Design and Population

We analyzed associations between SNPs and serum [25(OH)D] among participants in the Vitamin D/Calcium Polyp Prevention Study, a randomized trial of vitamin D_3 (1000 IU/d) and/or calcium carbonate (1200 mg/d elemental calcium) supplementation for prevention of colorectal adenomas conducted at 11 clinical centers in the US from 2004–2013. Institutional review boards at each site approved the study and participants provided informed consent. Participants were 45–75 years old and in good general health. Exclusion criteria included serum 25(OH)D $<$ 12 ng/ml. At enrollment, participants provided information on demographic and lifestyle factors, physical activity (International Physical Activity Questionnaire, August 2002 short version, https://www.ipaq.ki.se) and usual dietary intake (Block Brief 2000 Food Frequency Questionnaire, https://www.nutritionquest.com). Height and weight were measured or documented by self-report. Participants agreed to avoid personal vitamin D or calcium supplements and multivitamins without these were provided. After a blinded placebo run-in period, participants who took $\geq$ 80% of their study pills were randomized in blocks using computer-generated random numbers stratified by study center, sex, and colonoscopy interval (3 or 5 years) in a modified 2 $\times$ 2 factorial design with equal probability to vitamin D, calcium, both, or placebo ("4-arm study"). Women who wanted calcium supplementation were randomized to vitamin D only (to calcium alone or to calcium plus vitamin D, "2-arm study"). After randomization, participants were interviewed semiannually regarding adherence, supplement use, and dietary calcium and vitamin D intake.

Measurement of 25(OH)D Concentrations

Serum [25(OH)D] was measured at enrollment and one year after randomization at the UCLA Center for Human Nutrition using a radioimmunoassay (RIA) kit [Immunodiagnostic Systems, intra-assay coefficient of variation (CV) 5.3%-6.1% (26.5–151 nmol/L), interassay CV 8.2–7.3% (19.6–136 nmol/L), http://us.idsplc.com]. Samples from the international Vitamin D External Quality Assessment Scheme program were analyzed with every kit for quality control (QC) purposes. Repeated assay of blinded replicates from a pooled sample (average 25(OH)D 21.6 ng/ml) indicated an intra-assay CV of 4.1%.

Single Nucleotide Polymorphism (SNP) Selection and Genotyping

We selected for genotyping 41 candidate SNPs in or near seven vitamin D or calcium pathway genes (*GC*, *DHCR7*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *VDR*, and *CASR*) that were previously associated with [25(OH)D] or other health outcomes (Supplemental Table 1). Genomic DNA was isolated from buffy coat by BioServe Biotechnologies using DNAQuiK (https://www.bioserve.com). Genotyping by KBioscience used KASP technology (https://www.lgcgenomics.com), or by Genome Quebec Innovation Center used Sequenom iPLEX Gold (https://www.sequenom.com) or predesigned TaqMan assays (rs228570 and rs10766197, https://www.invitrogen.com). Samples that could not be called on $>$ 4 of 41 SNPs were dropped; sample success rate was 96.1%. SNP call rates (samples successfully genotyped) ranged from 96.7% to 99.8% (median 99%). Concordance rates among blinded replicates were 100%. All

SNPs were in Hardy-Weinberg Equilibrium in non-Hispanic whites ($P > .05$).

Statistical Analyses

Only self-reported non-Hispanic whites were included in analyses to avoid spurious associations caused by population stratification. [25(OH)D] was log transformed. Multiple linear regression was used to analyze associations of SNP genotypes with baseline [25(OH)D]. Covariates included age, sex and season of baseline blood draw (coded as a nonordered categorical variable: 1-Winter:Dec.-Feb.; 2-Spring:Mar.-May; 3-Summer: June-Aug.; and 4-Fall:Sept.-Nov.). Genotype was modeled additively. Association P values were from Wald tests. Gene global P values were from likelihood ratio (LR) tests (joint contribution of all SNPs included in the model simultaneously).

Similarly, multiple linear regression was used to analyze whether SNP genotypes modified the effect of vitamin D_3 supplementation on year one [25(OH)D]. Covariates included age, sex, season of year one blood draw, baseline 25(OH)D, randomized vitamin D_3 treatment group (placebo or vitamin D_3), and SNP genotype. Statistical adjustment for baseline [25(OH)D] removes the effect of regression to the mean (6). Genotype estimates and 95% confidence intervals were for the association of the [SNP*vitamin D_3 treatment] interaction term on year one [25(OH)D]. Interaction P values were obtained from Wald tests and global P values from LR tests. To assess the impact of optimal adherence, some analyses included only participants who took ≥ 80% of their study pills with no gaps > 7 days, and no personal vitamin D supplementation.

Due to prior evidence for associations of these candidate SNPs with circulating [25(OH)D] or other health outcomes, we did not adjust for multiple comparisons. Analysis of study treatment was intent-to-treat, except as indicated. Statistical tests were two-sided and considered significant at $P < .05$. Analyses used SAS (version 9.3) or Stata (version 12).

Results

Of 1,861 non-Hispanic white participants randomized, 1,787 (96.1%) were included in baseline analyses and 1,755 (94.3%) in year one analyses (Supplemental Figure 1). Baseline characteristics were similar between participants randomized to vitamin D_3 treatment or placebo (Table 1). The mean baseline [25(OH)D] was 25.4 ng/ml. After one year of treatment, [25(OH)D] increased on average 6.1 ± 8.9 ng/ml (mean ± SD) among participants randomized to vitamin D_3 and decreased 1.1 ± 8.4 ng/ml among those randomized to placebo (likely due to cessation of personal supplement use upon enrollment in the trial). The increase in year one [25(OH)D] due to vitamin D supplementation was not modified by randomization to calcium supplementation ($P_{interaction} = 0.94$).

Associations with baseline [25(OH)D] for 33 SNPs are shown in Table 2 (results not shown for eight SNPs in high linkage disequilibrium, $r^2 > 0.95$). Associations were sta-

Table 1. Baseline Characteristics of Study Participants by Vitamin D_3 Treatment

Characteristics	Placebo[a] (*n* = 890)	Vitamin D_3[b] (*n* = 897)	P Value[c]
Male, %	571 (64%)	575 (64%)	0.98
Age at enrollment, years	58.0 ± 6.9	58.2 ± 6.8	0.66
Body Mass Index (BMI), kg/m^2	29.0 ± 5.2	28.8 ± 5.1	0.55
Current smoker, %	70 (8%)	90 (10%)	0.11
Currently drink alcohol, %	602 (68%)	630 (71%)	0.17
Physical activity, MET-minutes/week	3052 ± 2865	3065 ± 2950	0.93
Dietary vitamin D intake, IU/day	141 ± 98	133 ± 99	0.12
Dietary calcium intake, mg/day	692 ± 318	661 ± 300	0.04
Multivitamin use, %	504 (57%)	513 (57%)	0.85
Vitamin D supplement use, %	108 (12%)	133 (15%)	0.10
Calcium supplement use, %	169 (19%)	182 (20%)	0.50
Baseline serum 25(OH)D, ng/ml	25.4 ± 8.7	25.4 ± 8.3	1.00

Includes only non-Hispanic white participants with genotype data available.

Data are number of participants (percentage) or mean ± SD

[a]Includes participants randomized to placebo (*n* = 336) or to calcium alone (*n* = 329) in the "4-arm study" and to calcium alone (*n* = 225) in the "2-arm study". Counts of participants with missing data: 1 BMI, 3 alcohol, 13 activity, 48 dietary intake, 2 multivitamin, and 3 vitamin D supplement.

[b]Includes participants randomized to vitamin D alone (*n* = 339) or to vitamin D plus calcium (*n* = 337) in the "4-arm study" and to vitamin D plus calcium (*n* = 221) in the "2-arm study". Counts of participants with missing data: 8 alcohol, 12 activity, 61 dietary intake, and 1 vitamin D supplement.

[c]2-sample t-tests were used for continuous variables and Pearson $\chi 2$ tests for categorical variables.

Table 2. Associations of SNP Genotypes with Baseline 25(OH)D Level and Modification of the Increase in 25(OH)D Level Due to Randomized Vitamin D_3 Supplementation (1,000 IU/day) for One Year

	Baseline 25(OH)D			Year One Increase in 25(OH)D					
				All Participants			Optimally Adherent		
SNP	**N**	**Estimated % difference (95% CI)[a]**	**P Value[b]**	**N**	**Estimated % difference (95% CI)[c]**	**P Value[d]**	**N**	**Estimated % difference (95% CI)[c]**	**P Value[d]**
GC:			**<0.0001**				0.23		0.39
rs12512631	1759	**4.69 (2.50, 6.92)**	**<0.0001**	1728	0.47 (-3.04, 4.10)	0.80	1437	−0.51 (-4.20, 3.32)	0.79
rs4588[f]	1737	**-8.87 (-10.90, −6.78)**	**<0.0001**	1708	2.18 (-1.64, 6.15)	0.27	1424	3.15 (-1.02, 7.49)	0.14
rs7041	1748	**-6.69 (-8.57, −4.76)**	**<0.0001**	1717	2.42 (-1.01. 5.97)	0.17	1427	3.25 (-0.46, 7.10)	0.09
rs222020	1755	**3.21 (0.24, 6.26)**	**0.03**	1726	−3.06 (-7.66, 1.77)	0.21	1434	−1.46 (-6.48, 3.83)	0.58
rs16847015	1771	1.94 (-3.00, 7.13)	0.45	1740	−0.68 (-8.61, 7.94)	0.87	1448	0.75 (-8.03, 10.37)	0.87
rs1155563	1742	**-8.44 (-10.48, −6.35)**	**<0.0001**	1713	1.79 (-2.01, 5.75)	0.36	1426	2.33 (-1.77, 6.60)	0.27
rs2298849	1771	1.95 (-0.70, 4.67)	0.15	1740	−3.59 (-7.73, 0.74)	0.10	1448	−2.98 (-7.40, 1.65)	0.20
DHCR7:			0.16			**0.05**			0.11
rs12785878	1763	−2.25 (-4.51, 0.06)	0.06	1732	1.54 (-2.32, 5.56)	0.44	1442	3.04 (-1.14, 7.40)	0.16
rs3829251	1754	−2.04 (-4.75, 0.75)	0.15	1723	−2.27 (-6.72, 2.39)	0.33	1433	0.01 (-4.91, 5.18)	1.00
CYP2R1:			**0.0001**			**0.03**			0.05
rs12794714	1768	**-4.74 (-6.68, −2.75)**	**<0.0001**	1737	−2.93 (-6.23, 0.49)	0.09	1446	**-3.69 (-7.18, −0.07)**	**0.05**
rs10741657[f]	1764	**4.91 (2.76, 7.10)**	**<0.0001**	1733	−0.80 (-4.19, 2.72)	0.65	1442	0.07 (-3.58, 3.85)	0.97
rs1562902	1768	**3.30 (1.21, 5.42)**	**0.002**	1739	1.61 (-1.79, 5.13)	0.36	1448	0.95 (-2.65, 4.68)	0.61
rs10766197	1755	**-3.83 (-5.79, −1.83)**	**0.0002**	1724	**-4.12 (-7.40, −0.76)**	**0.02**	1437	**-4.21 (-7.66, −0.63)**	**0.02**
CYP27A1			0.93			0.23			0.19
rs703842[f]	1763	0.55 (-1.63, 2.78)	0.62	1732	0.25 (-3.36, 4.00)	0.89	1441	1.32 (-2.55, 5.34)	0.51
CYP24A1:			0.15			0.61		0.69	
rs6013897	1766	−0.66 (-3.14, 1.88)	0.61	1735	**-4.24 (-8.22, −0.09)**	**0.04**	1444	**-4.86 (-9.10, −0.42)**	**0.03**
rs2209314	1745	**2.67 (0.19, 5.21)**	**0.03**	1714	−3.88 (-7.70, 0.11)	0.06	1425	−3.62 (-7.66, 0.61)	0.09
rs2762939	1757	**2.75 (0.32, 5.23)**	**0.03**	1727	−3.40 (-7.15, 0.49)	0.09	1437	−3.51 (-7.46, 0.62)	0.09
rs4809958	1751	−1.02 (-3.75, 1.78)	0.47	1720	2.60 (-2.08, 7.49)	0.28	1434	2.71 (-2.22, 7.89)	0.28
rs2244719	1756	−1.96 (-3.99, 0.12)	0.06	1725	1.72 (-1.78, 5.34)	0.34	1433	1.20 (-2.50, 5.05)	0.53
rs2296241	1735	−1.24 (-3.30, 0.85)	0.24	1706	1.84 (-1.69, 5.49)	0.31	1420	1.78 (-1.98, 5.68)	0.36
rs17219315	1768	−5.41 (-11.33, 0.91)	0.09	1737	6.00 (-4.90, 18.15)	0.29	1447	5.33 (-6.05, 18.09)	0.37
VDR:			0.76			0.23			0.10
rs7968585	1784	−1.50 (-3.49, 0.53)	0.15	1752	**3.44 (-0.02, 7.01)**	**0.05**	1458	3.15 (-0.54, 6.98)	0.09
rs11574143	1758	−0.67 (-4.28, 3.09)	0.72	1728	−2.43 (-8.32, 3.84)	0.44	1441	−1.05 (-7.50, 5.85)	0.76
rs731236[f]	1768	0.88 (-1.23, 3.04)	0.41	1736	−0.52 (-3.97, 3.06)	0.77	1443	0.06 (-3.66, 3.91)	0.98
rs7975232	1769	−1.53 (-3.52, 0.50)	0.14	1737	2.87 (-0.57, 6.43)	0.10	1449	2.05 (-1.61, 5.84)	0.28
rs2239179	1752	0.57 (-1.54, 2.72)	0.60	1721	−1.04 (-4.49, 2.53)	0.56	1433	−0.50 (-4.23, 3.38)	0.80
rs2228570	1777	1.33 (-0.80, 3.50)	0.22	1745	−1.33 (-4.76, 2.23)	0.46	1453	−1.73 (-5.39, 2.08)	0.37
rs10783219	1769	0.36 (-1.78, 2.56)	0.74	1739	−2.50 (-5.95, 1.09)	0.17	1447	**-3.75 (-7.41, 0.06)**	**0.05**
rs7139166[f]	1772	0.02 (-2.02, 2.10)	0.99	1740	1.61 (-1.82, 5.17)	0.36	1447	**3.99 (0.20, 7.92)**	**0.04**
rs11568820	1730	−0.89 (-3.37, 1.66)	0.49	1700	1.49 (-2.69, 5.85)	0.49	1417	−0.28 (-4.69, 4.33)	0.90
CASR			0.27			0.41			0.15
rs1801725[f]	1761	−1.94 (-4.85, 1.05)	0.20	1731	−1.24 (-6.08, 3.84)	0.62	1444	−3.01 (-8.21, 2.48)	0.27
rs1042636	1739	0.59 (-3.34, 4.69)	0.77	1711	−3.41 (-9.61, 3.22)	0.30	1423	1.14 (-5.83, 8.61)	0.76
rs1801726	1766	1.46 (-3.64, 6.83)	0.58	1735	5.77 (-2.87, 15.18)	0.20	1444	8.69 (-0.77, 19.05)	0.07

Abbreviations: SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval.

Bold numbers indicate $P \leq 0.05$.

[a] Estimated % difference in baseline 25(OH)D level per variant allele compared to wild type under an additive genetic model using linear regression of log-transformed 25(OH)D, adjusting for age, sex, and season of baseline blood draw.

[b] Wald test P values for single SNPs. Likelihood ratio test P values for joint contribution of all SNPs in a gene (first row).

[c] Estimated % difference in year one 25(OH)D level due to vitamin D_3 supplementation per variant allele compared to wild type under an additive genetic model using linear regression of log-transformed year one 25(OH)D, adjusting for log-transformed baseline 25(OH)D, age, sex, season of year one blood draw, SNP genotype and vitamin D_3 treatment assignment. Estimate is for the interaction between genotype and vitamin D_3 treatment (genotype*vitamin D_3 treatment) and indicates how genotype modifies the effect of vitamin D_3 treatment on year one 25(OH)D level.

[d] Wald test P values for the interaction term (genotype*vitamin D_3 treatment). Likelihood ratio test P values for the joint contribution of all interaction terms for all SNPs in the gene (first row).

[e] Optimally adherent participants took ≥80% of their study pills, with no gaps in pill taking ≥7 days, and no personal vitamin D supplementation.

[f] SNPs in high linkage disequilibrium (r^2>95%) with these SNPs are not shown (see Supplemental Table 1).

tistically significant for SNPs in three genes: *GC* (rs12512631, rs4588, rs7041, rs222020, rs1155563; not shown: rs2282679, rs3755967), *CYP2R1* (rs12794714, rs10741657, rs1562902, rs10766197; not shown: rs2060793), and *CYP24A1* (rs2209314, rs2762939) with per allele effect sizes ranging from –9 to +5% differences in [25(OH)D].

Next, we investigated whether these SNPs modified the efficacy of vitamin D_3 treatment to increase year one [25(OH)D] (Table 2). Three SNPs had statistically significant interactions: rs10766197 near *CYP2R1*, rs6013897 near *CYP24A1*, and rs7968585 near *VDR*, with per allele effect sizes ranging from –4% to +3% differences in [25(OH)]. When these analyses were restricted to optimally adherent participants (N = 1460, 83%), three additional SNPs had statistically significant interactions (rs12794714 in *CYP2R1*; rs10783219 and rs7139166 in *VDR*) and for rs7968585 in *VDR* the magnitude of the interaction decreased slightly and was no longer statistically significant.

Finally, analyses used to investigate the joint effects of linked SNPs implicated haplotypes in *GC, CYP2R1*, *CYP24A1*, and *VDR* with differences in baseline [25(OH)D], and haplotypes in *CYP2R1* and *VDR* with differences in the effect of vitamin D_3 treatment on [25(OH)D] (Supplemental Table 2).

Discussion

We confirmed known associations between baseline [25(OH)D] and twelve SNPs in *GC* and *CYP2R1*, and identified new associations with two SNPs in *CYP24A1* (rs2209314 and rs2762939). These two SNPs have smaller effect sizes, which may explain why they weren't identified in GWAS, and have previously been associated with decreased breast cancer risk (7) or reduced coronary artery calcification (8).

In novel analyses, we observed three SNPs that significantly modified the efficacy of 1,000 IU/d vitamin D_3 supplementation for increasing [25(OH)D]: rs10766197 near *CYP2R1*, rs6013897 near *CYP24A1,* and rs7968585 near *VDR*. Two of these have previously been associated with clinical outcomes: rs6013897 with lower risk of aggressive prostate cancer (9) and rs7968585 with risks of major clinical outcomes in association with low [25(OH)D] (10). Surprisingly, many SNPs (in *GC* and *CYP2R1*) associated with baseline [25(OH)D] did not substantially modify the response to supplementation whereas two SNPs (in *CYP24A1* and *VDR*) not associated with baseline levels did modify response. These results suggests different mechanisms regulate 25(OH)D derived from diet vs. cutaneous synthesis or adipose stores.

We know of only two small studies that previously examined associations with response to supplementation. In a pooled analysis of three trials (n = 285), Didriksen et al reported that rs10741657 (*CYP2R1*) was associated with a larger increase in 25(OH)D response to vitamin D 20,000 IU twice/wk + 800 IU/daily (11). The reason for the discrepancy with our results is unknown, but may relate to the 6-fold higher dose of vitamin D. Also, two studies reported opposite effects of two *GC* SNPs: the rs4588 variant was associated with a larger proportional 25(OH)D increase from 4,000 IU/d vitamin D (n = 98) (12), whereas the rs2282679 variant (high LD with rs4588) was associated with a smaller increase in the Didriksen study (11). Regardless, the physiological relevance of changes in [25(OH)D] associated with *GC* variants is unknown because the amount of free 25(OH)D may not change substantially.

A main strength of our study is the randomized design, which maximizes internal validity by providing a consistent route and dose of vitamin D_3 supplementation and minimizes differences between treatment groups, which could confound results. Participant adherence to pill taking and avoidance of personal supplementation was good. Few potential participants (3.4%) were excluded due to low baseline 25(OH)D (<12 ng/ml). Measurement of 25(OH)D was centralized with good reproducibility. We used rigorous statistical methodology to examine interactions between genotype and vitamin D_3 supplementation removing effects of regression to the mean and taking into account small decreases in [25(OH)D] among placebo participants. Finally, we focused on candidate SNPs previously associated with [25(OH)D] or other health outcomes.

The limitations of our study suggest avenues for future research. Analyses were restricted to non-Hispanic whites; other populations should be studied. We assessed only one oral dose of vitamin D_3; the dosing regimen providing the optimal risk/benefit profile or [25(OH)D] for various health outcomes is unknown. To our knowledge the function of these SNPs (except missense mutations in *GC* and *CASR*) has not been investigated. A more comprehensive analysis of genetic variation in these key genes and their

effects is warranted. Finally, the clinical relevance of the variants identified here should be explored further for diseases that are associated with vitamin D status.

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