Environmental, Personal, and Genetic Determinants of Response to Vitamin D Supplementation in Older Adults

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Context and Objective: Suboptimal vitamin D status can be corrected by vitamin D supplementation, but individual responses to supplementation vary. We aimed to examine genetic and non-genetic determinants of change in serum 25-hydroxyvitamin D (25(OH)D) after supplementation.

Design and Participants: We used data from a pilot randomized controlled trial in which 644 adults aged 60 to 84 years were randomly assigned to monthly doses of placebo, 30 000 IU, or 60 000 IU vitamin D3 for 12 months. Baseline characteristics were obtained from a self-administered questionnaire. Eighty-eight single-nucleotide polymorphisms (SNPs) in 41 candidate genes were genotyped using Sequenom MassArray technology. Serum 25(OH)D levels before and after the intervention were measured using the Diasorin Liaison platform immunoassay. We used linear regression models to examine associations between genetic and nongenetic factors and change in serum 25(OH)D levels.

Results: Supplement dose and baseline 25(OH)D level explained 24% of the variability in response to supplementation. Body mass index, self-reported health status, and ambient UV radiation made a small additional contribution. SNPs in CYP2R1, IRF4, MC1R, CYP27B1, VDR, TYRP1, MCM6, and HERC2 were associated with change in 25(OH)D level, although only CYP2R1 was significant after adjustment for multiple testing. Models including SNPs explained a similar proportion of variability in response to supplementation as models that included personal and environmental factors.

Conclusion: Stepwise regression analyses suggest that genetic variability may be associated with response to supplementation, perhaps suggesting that some people might need higher doses to reach optimal 25(OH)D levels or that there is variability in the physiologically normal level of 25(OH)D. (J Clin Endocrinol Metab 99: E1332–E1340, 2014)

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2014 by the Endocrine Society
Received November 14, 2013. Accepted March 18, 2014.
First Published Online April 2, 2014

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Abbreviations: BMI, body mass index; CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; GWAS, genome-wide association study; LD, linkage disequilibrium; MET, metabolic equivalent task; SNP, single-nucleotide polymorphisms; UVR, UV radiation.
Vitamin D is essential for maintaining bone health. There is also evidence to suggest that vitamin D is associated with some nonskeletal health outcomes, including type 2 diabetes mellitus (1–3), cardiovascular disease and its risk factors (4–7), and colorectal cancer (8). For some of these extraskeletal diseases, biologically plausible mechanisms have been proposed to explain the observed associations (9), but causality has not been established (10).

Serum concentrations of 25-hydroxyvitamin D (25(OH)D) are widely regarded to be the best measure of vitamin D status (11), but there is debate regarding the optimal level (9, 11). A recent report from the Institute of Medicine in the United States concluded that 50 nmol/L is sufficient to ensure adequate bone health in most people (10). Other studies have suggested that a level of serum 25(OH)D of at least 60 nmol/L is required to achieve a reduction in the risk of fractures (12) and falls (13).

The predominant source of vitamin D is cutaneous production after exposure to sunlight, with few foods naturally containing significant amounts of vitamin D. In Australia, the estimated average dietary intake of vitamin D is relatively low (48–104 IU/d) (14), and growing awareness of vitamin D deficiency has led to increasing demand for testing and supplementation. There is, however, considerable variability in individual responses to supplementation, suggesting that a one-size-fits-all approach to supplementation may not be appropriate or that there is physiological variation in normal serum 25(OH)D levels. Studies have found associations between an increase in serum 25(OH)D level after supplementation and supplement dose (15), body size (16–20), baseline serum 25(OH)D level (18, 19, 21–23), and the season in which supplementation is initiated (21). Although there is strong evidence that vitamin D status may be affected by genotype (24, 25), few studies have investigated genetic influences on response to supplementation (19, 26, 27). In this study, we aimed to examine environmental, personal, and genetic determinants of change in serum 25(OH)D levels after supplementation using data from a randomized controlled trial of vitamin D supplementation in older Australians.

Subjects and Methods

Study population and design

The methods and protocols of the study have been described previously (28). Briefly, the pilot D-Health trial was a population-based, randomized, placebo-controlled, double-blind chemoprevention trial of vitamin D3 in older adults. The Human Research Ethics Committee at the QIMR Berghofer Medical Research Institute approved the study, and all participants gave informed consent.

We recruited 644 people aged 60 to 84 years who were residents of 1 of the 4 eastern states of Australia, using the Australian Electoral Roll as the sampling frame. Participants were sampled in strata of 5-year age bands, sex, and state and location of residence (capital city and elsewhere). We excluded anybody who was taking more than 400 IU of vitamin D per day or who had a history of kidney stones, hyperparathyroidism, osteomalacia, osteoporosis, or sarcoidosis. Recruitment took place between October 2010 and March 2011, and the randomization rate was 10%. Participants were randomized to receive monthly doses of placebo or 30 000 or 60 000 IU vitamin D3 for 12 months. Participants, study investigators, and staff were unaware of the treatment allocation until analysis of the primary outcome (change in serum 25(OH)D level) was complete.

Questionnaires and blood collection

Participants were asked to complete questionnaires within 2 weeks of having blood drawn at study entry and exit, in which they reported their height, weight, skin color, burning and tanning ability, ancestry, smoking and alcohol consumption, and any history of cancer, diabetes, or cardiovascular disease. They reported the time they spent outdoors for each day of the previous week but were not asked about use of sun protection or the time of day they were outside. They were asked about weekly physical activity (walking, moderate and vigorous) and this was multiplied by typical energy expenditure requirement in metabolic equivalent tasks (METs), to calculate MET-hours per week (29). We asked participants to categorize their overall health on a 5-point scale ranging from poor to excellent. This was collapsed into 2 groups: good (excellent, very good, or good) and fair or poor.

Dietary intake of vitamin D in the last month was estimated by asking participants to recall their frequency of intake of 14 specific foods, including oily fish, margarine (which is routinely fortified in Australia), cheese, and milk fortified with vitamin D. We also asked about use of oral supplements containing vitamin D. We derived quantity of vitamin D intake (in international units) from foods using an Australian foods database (AUSNUT 2006) and food and supplement composition details provided by the manufacturers. Intakes of vitamin D from foods and supplements were combined to calculate total vitamin D intake.

25(OH)D assay

Nonfasting blood samples collected in serum separator tubes before and after the intervention were transported on ice by overnight courier to the Queensland University of Technology (Brisbane, Australia). Samples were centrifuged at 4°C for 15 minutes at 2000g, and the serum was stored at −80°C. Serum 25(OH)D concentration was measured in a single batch using a commercial chemiluminescent immunoassay (LIASON 25(OH)D Vitamin D TOTAL Assay; DiaSorin, Inc). Intra-assay and interassay variability was 3% to 6% and 6% to 9%, respectively. The laboratory undertaking the testing is certified by the international Vitamin D External Quality Assessment Scheme.

Assigning ambient UV radiation to location of residence and month of initiating the intervention

We estimated available ambient UV radiation (UVR) for each participant by matching the postcode of their residential address.
at baseline with UVR data available in a 1° latitude by 1.25° longitude grid from NASA’s Ozone Monitoring Instrument. Where there were multiple cells within a postcode, we averaged over the postcode using GIS. This database was used to assign the average month-specific erythemally weighted UVR irradiance (J/m²) to each postcode area (30).

Genotyping

Blood samples for genotyping were collected in ACD tubes and were transported by courier at room temperature to Queensland University of Technology, where buffy coats were separated. DNA was extracted from buffy coats using the QIAamp DNA Mini Blood Kit (QIAGEN). All DNA samples were blindly genotyped for 88 single-nucleotide polymorphisms (SNPs) located within or near 41 candidate genes. Genes were selected based on known or putative functions that are primarily involved in the vitamin D metabolism pathways or related to determinants of serum 25(OH)D level or vitamin D intake (skin pigmentation, body mass index [BMI], bone density, and lactase intolerance). SNP sequences were downloaded from the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and were cross-checked using Sequenom databases (https://mysequenom.com) before assay design. Multiplexed assays were designed for all SNPs using the Sequenom MassARRAY Assay Design software (version 3.1). SNPs were typed using iPLEX Gold chemistry and analyzed using a Sequenom MassARRAY Compact Mass Spectrometer (Sequenom Inc). All reactions were carried out using a modified half-volume reaction. The post-PCR products were spotted on a Sequenom SpectroChip 2, and the data were processed and analyzed using Sequenom MassARRAY TYPER version 4.0 software.

Statistical analysis

For all analyses, we excluded data from participants with more than 5 SNPs with missing data. For analyses of change in serum 25(OH)D level, we also excluded data from participants who were randomized to take the placebo or did not provide blood samples both at baseline and after intervention. Stepwise regression analyses were further restricted to participants with complete data for all candidate predictors.

Although not the primary aim of this manuscript, we also analyzed associations between genetic variants and baseline serum 25(OH)D level (associations with nongenetic factors have been reported previously) (31). To increase power, this analysis used data from all participants who provided a baseline blood sample.

For the presentation of descriptive statistics, baseline ambient UVR, physical activity, and time outdoors were categorized into approximate tertiles, whereas age, BMI, total vitamin D intake, and alcohol consumption were categorized into prespecified groups. In all other analyses, these variables were used in their continuous format.

We used a paired t test or McNemar’s test to assess whether the distributions of selected variables differed between the baseline and post-intervention questionnaires. We derived the change in serum 25(OH)D level as the difference between post-intervention and baseline levels.

Departure from Hardy-Weinberg equilibrium was tested using the HardyWeinberg package in R (32), with SNPs excluded from all further analyses if P < .05. Linear regression was used in preliminary analyses of association between change in serum 25(OH)D level and each SNP adjusted for supplement dose, both with and without adjustment for baseline serum 25(OH)D level. We used the pwr package in R (32) to perform post hoc power calculations for these preliminary analyses. We also used linear regression in our supplementary analyses of baseline serum 25(OH)D level.

We used forward stepwise variable selection to generate 3 multiple linear regression models to predict change in serum 25(OH)D level. Variables considered for inclusion in model 1 were supplement dose and baseline measures of serum 25(OH)D level, age, sex, BMI, ambient UVR, physical activity, time outdoors, vitamin D intake, and self-reported health status. Variables considered for inclusion in model 2 were supplement dose, baseline serum 25(OH)D level, and SNPs for which P < .05 in preliminary analyses, excluding those SNPs with a minor allele count greater than zero and less than 5. SNPs were treated as continuous terms based on the number of minor alleles. Variables selected in models 1 and 2 were forced into model 3, and stepwise regression was used to evaluate whether any variables not previously selected should be included. P values of .15 and .2 were used as criteria for variable inclusion and exclusion, respectively. Adjusted R² was used to compare models, with 95% confidence intervals (CIs) estimated using percentiles from 500 bootstrap samples.

To evaluate the possible effect of behavioral changes over the study period, we first repeated the analysis used to generate model 1, replacing physical activity and time outdoors at baseline with the change in these variables over time. Second, we added change in physical activity and time outdoors to models 1 and 3.

To test whether the effect of dose was modified by any other variables in a model, we included interaction terms for dose by each predictor. We also performed subgroup analyses, with models 1, 2, and 3 fitted to the data stratified by supplement dose.

Analyses were performed in SAS version 9.2 (SAS Institute, Inc). All P values are two-sided and, unless otherwise specified, we used a statistical significance level of P < .05. When evaluating stepwise regression models, a Bonferroni-corrected significance level was obtained by adjusting for the number of predictors in the final model. For other analyses, the Bonferroni-corrected significance level was adjusted for the number of regression models generated.

Results

Characteristics of participants

The total number of participants was 644, and 643 had a baseline 25(OH)D measurement. Of these, 32 had more than 5 SNPs with missing data, leaving 611 participants for the supplementary analysis of baseline serum 25(OH)D level. For analyses of change in serum 25(OH)D level, we further excluded 207 participants who were randomized to placebo and 19 participants who were randomized to active vitamin D supplementation but did not provide a post-intervention blood sample, leaving 385 participants (Figure 1). There were no statistically significant differences in age, sex, or baseline serum 25(OH)D level between those excluded from or retained in the anal-
644 participants recruited for Pilot D-Health trial

611 participants included in analysis of baseline serum 25(OH)D level

207 participants randomised to placebo

404 participants randomised to active vitamin D

385 participants included in analysis of change in serum 25(OH)D level

350 participants included in stepwise regression analyses

170 randomised to 30,000 IU vitamin D, per month

180 randomised to 60,000 IU vitamin D, per month

19 did not provide a post-intervention blood sample

35 had incomplete data for ≥ 1 candidate explanatory variable

Figure 1. Flow of participants through the study.

Genetic determinants of baseline circulating 25(OH)D

For brevity, Supplemental Table 2 shows results for associations between baseline serum 25(OH)D level and selected SNPs. An SNP was included if the P value and/or P trend was <.1, it appears in our stepwise regression model, or a genome-wide association study (GWAS) has reported an association with baseline 25(OH)D level. Fifteen SNPs in 7 genes were associated with baseline 25(OH)D level with P < .05. Only rs2282679 (CYP2R1) was significant at the Bonferroni-adjusted significance level (P trend = 2 × 10⁻⁴).

Change in serum 25(OH)D level

The mean serum 25(OH)D level increased from 42 nmol/L (SD 13) to 64 nmol/L (SD 17) in the 30 000 IU group and from 42 (SD 14) to 78 nmol/L (SD 20) in those assigned to 60 000 IU/mo. Table 1 presents pre- and post-intervention mean serum 25(OH)D level by categories of baseline demographic and lifestyle factors. Supplemental Table 1 displays results of tests of association between SNPs and change in serum 25(OH)D level. Although we found a number of SNPs associated with response to supplementation at P < .05, none was significant at the Bonferroni-corrected significance level. Assuming supplement dose explains 10% of variability in change in serum 25(OH)D levels, given our sample size (n = 385), we had a power between 0.54 and 0.84 to detect a significant SNP effect at P < .05, if the SNP explains an additional 1% to 2% of variability. Using a Bonferroni-corrected significance level, the power decreases to 0.09 to 0.31. For the model that also adjusts for baseline serum 25(OH)D level, we obtain marginally higher power, assuming that dose and baseline level explain 20% of the variability.

Regression models for response to supplementation

In model 1, including only personal and environmental variables, stepwise selection resulted in inclusion of supplement dose, baseline serum 25(OH)D level, BMI, self-reported health status, and ambient UVR in the best predictive model for change in 25(OH)D level (Table 2). Including changes in physical activity and time outdoors as potential predictors did not alter variable selection. Baseline serum 25(OH)D level, BMI, and ambient UVR were negatively correlated with change in serum 25(OH)D level. People receiving the higher dose and those with a fair or poor self-reported health status experienced a greater change than those receiving the lower dose and those with good self-reported health, respectively.

Fifteen SNPs for which P < .05 in preliminary analyses were included as candidate variables; 8 were selected in model 2. Characteristics of the selected SNPs are shown in Table 3. SNPs in or near genes related to vitamin D metabolism (rs10766197), sun sensitivity, and skin, eye, and hair pigmentation (rs12203592 and rs1805009) was significant at P = 4.8 × 10⁻³. Some SNPs selected in model 2 were in linkage disequilibrium (LD) with SNPs not selected. These were rs10766197 and rs12794714 (both CYP2R1) and rs182549 and rs4988235 (both MCM6).
Supplement dose and baseline serum 25(OH)D level were the first and second variables selected in both models 1 and 2. A regression model that included only these 2 variables had an adjusted $R^2$ of 0.24 (95% CI = 0.16–0.33). The model that incorporated dose, baseline 25(OH)D level, and SNPs (model 2) accounted for a slightly greater proportion of the variability in change in 25(OH)D level (adjusted $R^2 = 0.30$, 95% CI = 0.23–0.41) than the model that incorporated dose, baseline 25(OH)D level, and personal and environmental variables (adjusted $R^2 = 0.26$, 95% CI = 0.18–0.36) (model 1). Including both SNPs and lifestyle factors (model 3) marginally increased adjusted $R^2$ (0.32, 95% CI = 0.25–0.44) (Table 2). Using a Bonferroni-adjusted significance level, the only significant predictors in model 3 were dose, baseline serum 25(OH)D level, and BMI. Adding change in physical activity and change in time outdoors to models 1 and 3 did not substantially alter coefficients or $P$ values.

Only the interaction between dose and SNP rs1805099 (MC1R) had $P < .05$ ($P = .02$ in model 3). In subgroup analyses, rs1805099 was significant at the .05 level in models 2 and 3 in the 60 000 IU dose group but not in the 30 000 IU dose group. In the 60 000 IU dose group, people with 1 copy of the minor allele C had a greater change in serum 25(OH)D level than those with no minor alleles. No participants had the homozygous CC genotype for this SNP.
SNPs in or near genes regulating vitamin D metabolism, largely explained by supplement dose and baseline 25(OH)D levels after 12 months of monthly supplementation.

A Stepwise linear regression was used to select variables for models 1, 2, and 3 in the combined analysis of change in serum 25(OH)D level. Model variables included Supplement dose (per 30 000 IU), Baseline 25(OH)D (per 1 nmol/L), BMI (per 5 kg/m²), Self-reported health status, Ambient UVR (per 200 J/m²), rs10766197 (CYPIA1), rs12203592 (IFIT4), rs1805009 (MCIR), rs10877012 (CYPIA2), rs2228570 (VDR), rs1408799 (TYRP1), rs182549 (MCIA), rs1667394 (H(ERC2), Adjusted P value (% CI) ) 0.01 0.05

Discussion

For this group of older Australians, changes in serum 25(OH)D levels after 12 months of monthly supplementation with either 30 000 or 60 000 IU of vitamin D₃ were largely explained by supplement dose and baseline 25(OH)D levels. There was also evidence of a relationship between response to supplementation and BMI and with SNPs in or near genes regulating vitamin D metabolism, sun sensitivity, and skin, eye, and hair pigmentation.

Serum 25(OH)D level has been shown to increase by 1.5 to 2.5 nmol/L for every 100 IU/d of vitamin D intake (9), although the relationship may be nonlinear (10). The increases we observed were within this range, although the mean increase per 100 IU vitamin D per day in the 60 000 IU group was somewhat lower than that in the 30 000 IU group (1.8 vs 2.2 nmol/L).

We found that the response to supplementation decreased as baseline serum 25(OH)D level increased, consistent with previous reports (18, 19, 21–23). Some conclude that this is due to regression to the mean (23). Others have suggested that the process by which vitamin D₃ is converted to 25(OH)D is saturable (33), which may also explain the lower response per 100 IU intake per day in our higher dose group than in those randomized to the lower dose.

Table 2. Determinants of Change in Serum 25(OH)D Level: Multiple Linear Regressiona

<table>
<thead>
<tr>
<th>Variableb</th>
<th>Regression Coefficient (95% CI)</th>
<th>P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement dose (per 30 000 IU)</td>
<td>3.0 (0.5, 5.5)</td>
<td>.02</td>
</tr>
<tr>
<td>Baseline 25(OH)D (per 1 nmol/L)</td>
<td>-0.5 (-0.8, -0.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (per 5 kg/m²)</td>
<td>-3.0 (-5.2, -0.9)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Self-reported health status</td>
<td>7.5 (11.7, 13.2)</td>
<td>.01</td>
</tr>
<tr>
<td>Ambient UVR (per 200 J/m²)</td>
<td>-0.3 (-0.8, 0.1)</td>
<td>.11</td>
</tr>
<tr>
<td>rs10766197 (CYPIA1)</td>
<td>-3.7 (-6.2, -1.1)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>rs12203592 (IFIT4)</td>
<td>4.0 (0.9, 7.1)</td>
<td>.01</td>
</tr>
<tr>
<td>rs1805009 (MCIR)</td>
<td>8.2 (0.6, 15.8)</td>
<td>.03</td>
</tr>
<tr>
<td>rs10877012 (CYPIA2)</td>
<td>2.4 (-0.4, 5.2)</td>
<td>.09</td>
</tr>
<tr>
<td>rs2228570 (VDR)</td>
<td>2.5 (-0.1, 5.2)</td>
<td>.06</td>
</tr>
<tr>
<td>rs1408799 (TYRP1)</td>
<td>-2.3 (-4.9, 0.3)</td>
<td>.08</td>
</tr>
<tr>
<td>rs182549 (MCIA)</td>
<td>2.7 (-0.1, 5.4)</td>
<td>.06</td>
</tr>
<tr>
<td>rs1667394 (H(ERC2))</td>
<td>-3.2 (-6.6, 0.2)</td>
<td>.07</td>
</tr>
<tr>
<td>Adjusted P value (% CI)</td>
<td>0.30 (0.23, 0.41)</td>
<td>.03</td>
</tr>
</tbody>
</table>

60 000 IU (n = 180)

<table>
<thead>
<tr>
<th>Variableb</th>
<th>Regression Coefficient (95% CI)</th>
<th>P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement dose (per 30 000 IU)</td>
<td>-0.5 (-0.7, -0.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>BMI (per 5 kg/m²)</td>
<td>-3.1 (-6.2, -0.1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Self-reported health status</td>
<td>9.1 (4.4, 17.9)</td>
<td>.04</td>
</tr>
<tr>
<td>Ambient UVR (per 200 J/m²)</td>
<td>-0.2 (-0.8, 0.4)</td>
<td>.52</td>
</tr>
<tr>
<td>rs10766197 (CYPIA1)</td>
<td>-3.0 (-7.0, 0.9)</td>
<td>.13</td>
</tr>
<tr>
<td>rs12203592 (IFIT4)</td>
<td>2.6 (1.9, 7.1)</td>
<td>.26</td>
</tr>
<tr>
<td>rs1805009 (MCIR)</td>
<td>2.3 (0.6, 3.9)</td>
<td>.01</td>
</tr>
<tr>
<td>rs10877012 (CYPIA2)</td>
<td>1.7 (-2.8, 6.1)</td>
<td>.46</td>
</tr>
<tr>
<td>rs2228570 (VDR)</td>
<td>2.7 (-1.4, 6.7)</td>
<td>.19</td>
</tr>
<tr>
<td>rs1408799 (TYRP1)</td>
<td>-2.0 (-6.0, 0.2)</td>
<td>.33</td>
</tr>
<tr>
<td>rs182549 (MCIA)</td>
<td>3.1 (-1.0, 7.3)</td>
<td>.14</td>
</tr>
<tr>
<td>rs1667394 (H(ERC2))</td>
<td>-4.2 (-9.4, 1.1)</td>
<td>.12</td>
</tr>
<tr>
<td>Adjusted P value (% CI)</td>
<td>0.17 (0.08, 0.30)</td>
<td>.24</td>
</tr>
</tbody>
</table>

P values are not Bonferroni-adjusted. P values .05 (before rounding) are shown in bold.

95% CIs were estimated using percentiles from 500 bootstrap samples.
Some studies have found an inverse relationship between BMI and change in serum 25(OH)D level (16–20, 34), with others finding no association (21, 23, 26). Perhaps one of the most comprehensive explorations of this issue was a study in which postmenopausal women with baseline serum 25(OH)D ≤ 50 nmol/L were randomized to receive placebo or 1 of 7 different vitamin D doses for 1 year (20). Although women with a normal BMI experienced a greater response to supplementation than both overweight and obese women, the dose-response curves for these 3 groups were parallel. The authors have interpreted this as suggesting that volumetric dilution is the factor underpinning the apparently lower response to supplementation in overweight people.

The association between self-reported fair or poor health status and a greater response to supplementation independent of baseline 25(OH)D level is surprising. Given the challenges in measuring circulating 25(OH)D (35), this finding might reflect residual confounding by health status and a greater response to supplementation.

Two GWASs have identified loci associated with serum 25(OH)D levels at the genome-wide significance level (24, 25). Both GWASs found significant associations for rs2282679, rs7041, and rs1155563 in the CYP2R1 gene, which we confirmed in our sample, although only rs2282679 was significantly associated with baseline 25(OH)D level after adjustment for multiple testing. Of the 3 other SNPs in GC/DBP to show an association in our study, 1 (rs4588) was in LD with both rs2282679 and rs1155563. Both GWASs also found associations in SNPs in or near CYP2R1. Although not significant after adjustment for multiple testing, we found associations with 2 SNPs near CYP2R1, one of which was found in both GWASs (rs2060793), whereas the other (rs10766197) was in LD with an SNP found in a GWAS (rs12794714) (25). We did not find associations with SNPs in NADSYN1/DHCR7 or CYP24A1, which were found in at least 1 of the GWASs (24, 25). A number of SNPs in FTO, MCM6, SLC24A4, TPCN2, and TYRP1 were associated with 25(OH)D in our sample (at P < .05), but the significance level for these SNPs were not published in the GWASs, and they may be a chance finding in our data.

In our study, the only significant SNP in model 2 after Bonferroni adjustment was rs10766197, located in the 5’-flanking region of CYP2R1. People who were homozygous for the major allele of rs10766197 experienced a greater increase in serum 25(OH)D level than those with other genotypes at this location. They also had a higher mean baseline serum 25(OH)D level. Our results are consistent with another study that found an association between rs10741657 (CYP2R1) and increase in serum 25(OH)D level (19). CYP2R1 encodes an enzyme (CYP2R1) that is most likely responsible for the hydroxylation of vitamin D to 25(OH)D (36), suggesting that this finding is biologically plausible. Because rs10766197 is located in the promoter region of CYP2R1, it may affect gene transcription. Alternatively, it might be in LD with unidentified causal SNPs.

In support of other findings (19), we found that people who were homozygous for the minor allele of rs2282679 in GC/DBP had a lower increase in serum 25(OH)D than those with at least 1 major allele, but in our study, this was not significant. We could not establish the association between rs4588 (GC/DBP) and change in serum 25(OH)D as previously reported (26, 27). Earlier investigations of rs4588 were limited by small sample sizes, used different vitamin D doses, and included mostly females aged less than 60 years (26) or only Thai participants (27).

Among Caucasians, rs1805009 (MC1R) is associated with red hair, fair skin, and sun sensitivity (37). In this analysis, which did not include any subjects with 2 copies of the risk allele, we noted an interaction between rs1805009 and dose. Although this may be a real finding, we generated 12 models that included an interaction term, and it is not unexpected that 1 interaction term should be significant simply due to chance.

This study has some limitations, the most important being the small sample size and consequent limited power. Second, although we used an immunoassay that has been widely used in previous studies, the mean baseline

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**Table 3. Characteristics of the SNPs Selected in the Stepwise Regression Model**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene (Nearby)</th>
<th>Chromosome Position</th>
<th>Region</th>
<th>Functional Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10766197</td>
<td>(CYP2R1)</td>
<td>14 921 880</td>
<td>5’-Flanking</td>
<td>Intron variant</td>
</tr>
<tr>
<td>rs12203592</td>
<td>IGF4</td>
<td>396 321</td>
<td>Intron</td>
<td>Missense</td>
</tr>
<tr>
<td>rs1805009</td>
<td>MC1R</td>
<td>89 986 546</td>
<td>Coding</td>
<td>Missense</td>
</tr>
<tr>
<td>rs10877012</td>
<td>(CYP27B1)</td>
<td>58 162 085</td>
<td>5’-Flanking</td>
<td>Upstream variant</td>
</tr>
<tr>
<td>rs2228570</td>
<td>VDR</td>
<td>48 272 895</td>
<td>Coding</td>
<td>Missense</td>
</tr>
<tr>
<td>rs1408799</td>
<td>(TYRP1)</td>
<td>12 672 097</td>
<td>Intergenic</td>
<td>Intron variant</td>
</tr>
<tr>
<td>rs182549</td>
<td>MCM6</td>
<td>136 616 754</td>
<td>Intron</td>
<td>Intron variant</td>
</tr>
<tr>
<td>rs1667394</td>
<td>HERC2</td>
<td>28 530 182</td>
<td>Intron</td>
<td>Intron variant</td>
</tr>
</tbody>
</table>

25(OH)D level was somewhat lower than expected. However, our results should be internally valid because the same assay was used pre- and post-intervention. Another limitation is that we did not have data available to explore the possible influence of medications or specific pathologies. The absorption of supplements in the gastrointestinal tract might be affected by certain medications, and this possibility should be explored in future studies. A familial predisposition to vitamin D deficiency might also affect a person’s response to supplementation. Given that we would not expect many of our participants to report such a condition, we would not have been likely to find any significant associations even if such data had been collected. However, this line of investigation warrants further consideration in a larger trial. Finally, because study participants were from an older, predominantly (95%) Caucasian population, our results may not be generalizable.

Our study also has strengths. They include being population-based, albeit with a low response rate, and randomization allocated to supplement doses. High rates of retention, adherence, and follow-up strengthen the internal validity of our analysis. Our participants came from a wide range of latitudes (from tropical to cool temperate), allowing us to test for the possible effect of ambient UVR better than many other studies. We also included a wide range of genetic variants in relevant candidate genes.

These results highlight the importance of supplement dose, baseline serum 25(OH)D level, and BMI in determining response to vitamin D supplementation. Importantly, we have also shown that genetic variability is associated with response, perhaps suggesting that some people might need a higher dose to reach optimal 25(OH)D levels or that there is variability in the level of 25(OH)D that is physiologically normal. Further research is needed to verify our findings and to elucidate the physiological basis for the genetic associations.

Acknowledgments

We acknowledge the contribution of Ivan Hanigan who assisted with the derivation of the satellite UVR data.

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This project was funded by the National Health and Medical Research Council (NHMRC) of Australia (Grant 613655). The investigational product was supplied free of charge by Sanofi-Aventis Healthcare Pty Ltd trading as Sanofi Consumer Healthcare. P.M.W., R.E.N., and A.V. are supported by Fellowships from the NHMRC. D.C.W. is supported by a Future Fellowship from the Australian Research Council. R.M.L. is supported by an NHMRC Career Development Fellowship. M.G.K. is supported through a Cancer Council Queensland Senior Research Fellowship.

B.T. and M.W. performed the statistical analysis and wrote the manuscript. R.N. coordinated the study, contributed to writing the manuscript, and provided overall supervision. All authors contributed to study design and data interpretation and read and approved the final version of the manuscript.

Disclosure Summary: P.R.E. has received prior funding from Sanofi-Aventis Healthcare. All other authors have no conflicts of interest to disclose.

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