Effects of Genetic and Nongenetic Factors on Total and Bioavailable 25(OH)D Responses to Vitamin D Supplementation

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Context: Little is known about how genetic and nongenetic factors modify responses of vitamin D supplementation in nonwhite populations.

Objective: To investigate factors modifying 25-hydroxyvitamin D [25(OH)D] and bioavailable 25(OH)D [25(OH)D_{Bio}] responses after vitamin D_3 supplementation.

Design, Setting, Participants, and Intervention: In this 20-week, randomized, double-blinded, placebo-controlled trial, 448 Chinese with vitamin D deficiency received 2000 IU/d vitamin D_3 or placebo.

Main Outcome Measures: Serum 25(OH)D, vitamin D-binding protein (VDBP), parathyroid hormone (PTH) and calcium were measured, and 25(OH)D_{Bio} was calculated based on VDBP levels. Six common polymorphisms in vitamin D metabolism genes were genotyped.

Results: Between-arm net changes were +30.6 ± 1.7 nmol/L for 25(OH)D, +2.7 ± 0.2 nmol/L for 25(OH)D_{Bio}, and −5.2 ± 1.2 pg/mL for PTH, corresponding to 70% [95% confidence interval (CI), 62.8% to 77.2%] net reversion rate for vitamin D deficiency at week 20 ($P < 0.001$). Only 25(OH)D_{Bio} change was positively associated with calcium change ($P < 0.001$). Genetic factors (*GC*-rs4588/*GC*-rs7041, *VDR*-rs2228570, and *CYP2R1*-rs10741657; $P \leq 0.04$) showed stronger influences on 25(OH)D or 25(OH)D_{Bio} responses than nongenetic factors, including baseline value, body mass index, and sex. An inverse association of PTH-25(OH)D was demonstrated only at 25(OH)D of <50.8 (95% CI, 43.6 to 59.0) nmol/L.

Conclusions: Supplemented 2000 IU/d vitamin D_3 raised 25(OH)D and 25(OH)D_{Bio} but was unable to correct deficiency in 25% of Chinese participants, which might be partially attributed to the effect of genetic modification. More studies are needed to elucidate appropriate vitamin D recommendations for Asians and the potential clinical implications of 25(OH)D_{Bio}. (***J Clin Endocrinol Metab*** **102: 100–110, 2017)**

Vitamin D deficiency (25-hydroxyvitamin D [25(OH)D] < 50 nmol/L) is one of the most common nutritional problems worldwide and has been linked to multiple unfavorable health consequences (1–5). There has been growing interest in routinely examining circulating 25(OH)D levels and taking supplementation in

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_{Bio}, bioavailable 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; DHCR7, 7-dehydrocholesterol reductase; GRS, genetic risk score; PTH, parathyroid hormone; SE, standard error; SNP, single nucleotide polymorphism; UL, upper intake level; VDBP, vitamin D–binding protein; VDR, vitamin D receptor.

recent years (5, 6). However, the current definition for vitamin D status and related recommendations are mainly based on evidence from white populations, whereas vitamin D metabolism and related health conditions may vary across different racial groups (6–9). For instance, low 25(OH)D was associated with a high risk of coronary heart disease or reduced bone mineral density in whites, but not in blacks (7, 10). Moreover, to achieve a target 25(OH)D of 50 nmol/L, the estimated daily recommended dietary allowance for vitamin D was 800 IU for whites (11), 1640 IU for blacks (12), and >2000 IU [the tolerable upper intake level (UL) in China] for Chinese in our previous trial (13). Thus, it is critically important to clarify whether the commonly used definition for vitamin D status and related recommendation are appropriate for nonwhite populations.

Vitamin D–binding protein (VDBP), encoded by the *GC* gene, binds to 85% to 90% circulating 25(OH)D and thereby regulates the vitamin D bioavailability (14). Bioavailable 25(OH)D [25(OH)D_{Bio}], the non–VDBP-bound portion, including albumin bound and free form, appears to be more biologically active in targeted tissues than in VDBP-bound 25(OH)D, according to the free hormone hypothesis (15, 16), which has been proposed as a universal mechanism for cellular uptake of steroid hormones and also applied to measurement of free forms of testosterone, cortisol, and thyroxine in clinical settings (17). With different combinations of common polymorphisms in *GC*, there were 3 major VDBP isoforms (Gc1F, Gc1S, and Gc2), and their concentrations varied according to measurement methods (18). For instance, VDBP levels were significantly lower in blacks than in whites when using the monoclonal antibody immunoassay, whereas no racial differences were detected when using polyclonal antibody immunoassay or proteomics (18–21). It was plausible that the monoclonal antibody immunoassay bound preferably to Gc1S and Gc2 rather than to Gc1F, a more highly prevalent isoform in blacks (92.7%) than in whites (6.0%) (18, 22). Compared with blacks and whites, Asians have a different VDBP isoform distribution (23), and it remains unknown how monoclonal and polyclonal antibody immunoassays influence VDBP levels.

Previous studies in Europeans and Chinese suggested that genetic variants in *GC*, *CYP2R1* (25-hydroxylase), and 7-dehydrocholesterol reductase (*DHCR7*) were associated with 25(OH)D concentrations (24–26). Clinical trials also indicated that nongenetic factors, including body mass index (BMI), baseline concentration, supplemental form, dose, and duration could influence 25(OH)D responses (11–13, 27). However, few studies have systematically evaluated how and to what extent genetic and nongenetic factors could modify the responses of 25(OH)D and 25(OH)D_{Bio} levels. Therefore, we conducted a 2-arm, randomized, double-blinded, placebo-controlled trial of vitamin D_3 in 448 Chinese with vitamin D deficiency for 20 weeks.

Subjects and Methods

Study design and participants

This randomized, placebo-controlled trial was conducted between January and May of 2014 for 20 weeks in Shanghai, China. After being recruited by an advertisement, a total of 1815 volunteers in Shanghai were screened by questionnaires and physical examination (Supplemental Fig. 1). Persons were eligible if they were Han Chinese, 20 to 45 years of age, with 25(OH)D between 12.5 and 50 nmol/L, BMI between 18.5 and 28 kg/m^2, and without taking vitamin D or calcium supplements in the previous month. Following daily administration of placebo capsules for 1 week, 448 persons were randomly assigned to either the placebo or 2000 IU/d vitamin D_3 arm. The randomization was performed according to block randomization of age, sex, BMI, and serum 25(OH)D by a statistician who was not involved in the trial. All participants provided written informed consent, and the study protocol was approved by the Ethics Committee of Huadong Hospital Affiliated to Fudan University, Shanghai.

To ensure the double blinding, the placebo and vitamin D_3 capsules had similar appearance and smell (provided by Sinopharm Xingsha Pharmaceuticals, Xiamen, China). An independent laboratory (Royal DSM China Campus, Shanghai, China) evaluated the vitamin D_3 contents of capsules 3 times. The average doses were 0 for placebo and 1940 IU for 2000 IU vitamin D_3 capsules. All participants were required to (1) maintain their habitual food intake and physical activity, (2) minimize sun exposure and vitamin D–rich foods such as fatty fish and cod liver oil as much as possible, and (3) avoid taking nontrial vitamin D supplements. The participants were asked to return all untaken capsules weekly, and adherence was assessed by capsule counts ([(supplied number − returned number)/supplied number] × 100%).

Data collection

A face-to-face interview was conducted by trained dieticians at weeks 0, 10, and 20 (13). The information on demographics, health status, lifestyles (physical activity, sun exposure, and dietary intake), family history of diseases, medical history, medication log, and intake of nutritional supplements was collected by using standardized questionnaires (13). Total physical activity levels were categorized as low, moderate, or high based on standardized protocols (28). Dietary intake was obtained by 3-day food records (2 weekdays plus 1 weekend day). Sun exposure levels were estimated by self-reported weekly outdoor hours from 10:00 AM to 3:00 PM (13). Sun exposure protection score was calculated according to the frequency of wearing hats, long sleeves, and using sunscreen within the previous month (13). Body weight, height, and blood pressure were measured by standardized procedures (13), and BMI was calculated as weight (kg)/height $(m)^2$.

Biochemical analyses

Overnight fasting blood samples were obtained at weeks 0, 10, and 20. Serum 25(OH)D was measured by liquid

chromatography–tandem mass spectrometry with deuterated internal standards (Sigma-Aldrich, St. Louis, MO), and the interassay and intraassay coefficients of variation (CVs) were ≤8.3%. VDBP was measured by (1) a monoclonal antibody ELISA kit (R&D Systems, Minneapolis, MN) with the interassay and intraassay CVs ≤ 7.2%; and (2) a polyclonal antibody ELISA kit (Immunodiagnostik, Bensheim, Germany) with the interassay and intraassay CVs ≤ 8.1%. Serum parathyroid hormone (PTH) was measured by an ADVIA Centaur XP immunoassay system (Siemens Healthcare, Erlangen, Germany) with the interassay and intraassay CVs ≤ 7.4%. Serum calcium, albumin, alanine transaminase, aspartate aminotransferase, γ-glutamyl transferase, creatinine, urea nitrogen, and uric acid concentrations were measured by an automatic biochemical analyzer (Hitachi 7080) using reagents purchased from Roche Diagnostics (Mannheim, Germany), and all interassay and intraassay CVs were ≤8.6%.

Genotyping

Based on the findings from our studies and those of others (25, 26, 29), *GC*-rs7041, *GC*-rs4588, vitamin D receptor (*VDR*)-rs2228570, *CYP2R1*-rs10741657, *DHCR7*-rs1790349, and *CYP24A1*-rs6013897 were selected and an effect allele was defined as 25(OH)D-raising allele, accordingly. The reproducibility of genotyping was 100% among 10% duplicated samples. Effect allele frequencies in our participants were similar to those in HapMap Han Chinese in Beijing, China, and no between-arm difference was observed (Supplemental Table 2).

Statistical analysis

Treatment effect was estimated by the intention-to-treat principle. Baseline characteristics between arms were compared using a Student *t* test or χ^2 test when appropriate. 25(OH)D_{Bio} was calculated using 25(OH)D, VDBP, and albumin concentrations based on the equations provided by Bhan *et al.* (30) (Supplemental Materials and Methods). Responses/changes were calculated by subtracting the baseline values from the values at week 10 or 20. Within-arm differences were analyzed using a mixed model (fixed effect, time; random effect, participant), followed by a Fisher's least significant difference multiple-comparisons test. Linear regression with adjustment for age, sex, BMI, and baseline values (except week 0) was used to evaluate: (1) between-arm differences at each visit time; (2) effects of each single nucleotide polymorphism (SNP) on 25(OH)D and 25(OH)D_{Bio} responses at week 20 under an additive model; and (3) effects of genotype–treatment interaction on relevant changes at week 20 (genotypes were coded as 0, 1, and 2 in a continuous form). A partial correlation coefficient was used to assess the correlation between change of calcium and change of 25(OH)D or 25(OH)D_{Bio} with adjustments for age, sex, and BMI. Backward stepwise regression analyses were used to select genetic and nongenetic variables to predict 25(OH)D and 25(OH)D_{Bio} responses (31). The adjusted R^2 was the overall variance explained in a given model. Subgroup analyses were performed according to baseline BMI, age, sex, and physical activity. Nonlinear associations between 25(OH)D and PTH at different levels were detected by locally weighted scatterplot smoothing. To test the PTH-based threshold effect of 25(OH)D, a mixed-effects model that accounted for repeatedly measured structure of our data was used to determine the associations for those participants with 25(OH)D below and above the identified breakpoint (32, 33). Data were analyzed using SAS, version 9.3 and R software, version 3.2.3. A 2-sided *P* of <0.05 was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics of participants were similar between the 2 arms (Table 1). Serum mean (±standard deviation) 25(OH)D and 25(OH)D_{Bio} were 32.8 ± 8.8 nmol/L and 2.7 ± 0.8 nmol/L, respectively. A total of 411 participants completed the 20-week trial with compliance rates of 98.9% in the vitamin D_3 arm and 97.6% in the placebo arm (P = 0.92). Using the monoclonal compared with polyclonal antibody immunoassay yielded significantly lower VDBP levels (165.3 ± 90.4 μg/mL vs 418.7 ± 99.0 μg/mL; $P < 0.001$). Because Chinese have a relatively higher frequency of Gc1F, a monoclonal antibody immunoassay would have underestimated VDBP levels; the current analyses therefore used polyclonal immunoassay–based VDBP concentrations, which were similar to previous studies using the same assay (21, 34).

Responses of 25(OH)D and 25(OH)D_{Bio}

Compared with the placebo arm at week 20, the net changes [mean ± standard error (SE)] in the vitamin D_3 arm were +30.6 ± 1.7 nmol/L for 25(OH)D, +2.7 ± 0.2 nmol/L for 25(OH)D_{Bio}, and −5.2 ± 1.2 pg/ml for PTH ($P_{between\text{-}arm} < 0.001$, Table 2). However, by the end of 20-week supplementation, 24.6% [95% confidence interval (CI), 19.0% to 31.2%) of participants were still classified as having vitamin D deficiency according to definitions of the Institute of Medicine or the Endocrine Society in the United States (6, 35) (Table 3). In the placebo arm, 25(OH)D and 25(OH)D_{Bio} increased and PTH decreased from winter to spring ($P < 0.001$). Meanwhile, sun exposure time was increased in both arms ($P < 0.001$). Mean (SE) VDBP at baseline was 418.7 ± 99.0 μg/mL and remained steady during the trial (week 20, 424.3 ± 108.1 μg/mL; P = 0.15). At baseline, serum calcium concentrations (albumin corrected) were positively associated with 25(OH)D_{Bio} (r = 0.11; $P < 0.001$) only. Notably, only change of 25(OH)D_{Bio} was positively associated with change of serum calcium (r = 0.22; $P < 0.001$) after adjusting for age, sex, and BMI (Supplemental Fig. 2).

Effect of genetic and nongenetic factors

At baseline, associations with 25(OH)D concentration were significant for rs4588 and rs7041 in *GC* ($P < 0.001$; Supplemental Table 3) and marginally significant for rs10741657 in *CYP2R1* and rs1790349 in *DHCR7*

Table 1. Baseline Characteristics

Characteristic	Overall (n = 448)	Placebo (n = 222)	Vitamin D_3 (n = 226)
Women, no. (%)	307 (69)	154 (69)	153 (68)
Age, y	30 (25, 39)	30 (25, 38)	31 (25, 40)
Education, no. (%)			
0–9 y	39 (9)	19 (9)	20 (9)
≥10 y	409 (91)	203 (91)	206 (91)
Current smoker, no. (%)	32 (7)	13 (6)	19 (8)
Alcohol drinker, no. (%)	134 (30)	66 (30)	68 (30)
Physical activity, no. (%)[a]			
High	136 (30)	72 (32)	64 (28)
Moderate	114 (26)	59 (27)	55 (25)
Low	198 (44)	91 (41)	107 (47)
Sun exposure, h/wk[b]	4.1 ± 2.4	4.1 ± 2.4	4.1 ± 2.5
Sun protection score[b]	4.9 ± 2.1	4.9 ± 1.9	5.0 ± 2.2
BMI, kg/m^2	22.1 ± 2.6	22.0 ± 2.7	22.2 ± 2.5
SBP, mm Hg	114 ± 14	114 ± 14	114 ± 13
DBP, mm Hg	74 ± 10	74 ± 10	75 ± 9
25(OH)D, nmol/L	32.8 ± 8.8	32.7 ± 8.7	32.9 ± 8.8
$25(OH)D_{Bio}$, nmol/L[c]	2.7 ± 0.8	2.7 ± 0.9	2.7 ± 0.8
VDBP, μg/mL			
Monoclonal assay	165.3 ± 90.4	165.2 ± 92.8	165.5 ± 88.2
Polyclonal assay	418.7 ± 99.0	425.2 ± 104.5	412.3 ± 93.2
PTH, pg/mL	39.2 ± 17.7	38.9 ± 18.1	39.5 ± 17.3
Calcium, mmol/L[d]	2.27 ± 0.18	2.27 ± 0.18	2.27 ± 0.17
Albumin, g/L	48.4 ± 3.9	48.3 ± 4.1	48.4 ± 3.7
ALT, IU/L	18 ± 13	17 ± 12	19 ± 14
AST, IU/L	19 ± 7	19 ± 6	19 ± 8
GGT, IU/L	20 ± 15	20 ± 16	20 ± 15
Estimated GFR, mL/min/1.73 m^2[e]	116.3 ± 10.9	116.0 ± 10.5	116.6 ± 11.3
Serum creatine, μmol/L	62.3 ± 13.4	62.9 ± 13.8	61.8 ± 13.0
Serum urea nitrogen, mmol/L	4.8 ± 1.2	4.8 ± 1.2	4.9 ± 1.1
Serum uric acid, μmol/L	281 ± 73	282 ± 74	279 ± 72

Data are mean ± standard deviation and n (%). There were no between-arm differences for any characteristic. To convert values for 25(OH)D from nanomoles per liter to nanograms per milliliter, multiply by 0.401.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; GFR, glomerular filtration rate; GGT, γ-glutamyl transferase; SBP, systolic blood pressure.

[a]Physical activity was categorized as 3 levels (high, moderate, and low) based on the International Physical Activity Questionnaire (28).

[b]Sun exposure time per week and sun protection score were assessed based on a questionnaire (13).

[c]$25(OH)D_{Bio}$ was calculated using the equations provided by Bhan *et al.* (30), based on polyclonal assay VDBP.

[d]Calcium levels were albumin corrected.

[e]The estimated GFR was calculated with the use of the chronic kidney disease epidemiology collaboration equation: GFR (mL/min/1.73 m^2) = $175 \times (\text{Creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$.

(P = 0.07 to 0.08). Notably, the rs4588-C allele was associated with higher VDBP and 25(OH)D concentrations ($P < 0.001$) with per C allele effect sizes (mean ± SE) of 38.5 ± 7.8 μg/mL and 2.2 ± 0.6 nmol/L, respectively (Supplemental Table 4).

At week 20, vitamin D_3 significantly interacted with *GC*-rs7041, *VDR*-rs2228570, and *CYP2R1*-rs10741657 on $25(OH)D_{Bio}$ response ($P_{interaction}$= 0.04, 0.02, and 0.003, respectively; Table 4), but none of the selected SNPs significantly interacted with treatment on the 25(OH)D response ($P_{interaction} \geq 0.07$). In response to vitamin D_3 treatment, rs4588-C, rs2228570-G, and rs10741657-A alleles were associated with a greater increase in 25(OH)D (P = 0.04, 0.009, and 0.04, respectively), whereas rs7041-G, rs2228570-G, and rs10741657-A alleles were associated with greater increases in $25(OH)D_{Bio}$ (P = 0.04, 0.01, and 0.003, respectively). No significant effect was detected for other SNPs (Supplemental Table 5). To determine combined effects of rs4588 [only 25(OH)D], rs7041 [only $25(OH)D_{Bio}$], rs2228570, and rs10741657 on both 25(OH)D and $25(OH)D_{Bio}$ responses, a genetic risk score (GRS) was calculated by counting the number of response-lowering alleles (risk alleles) from the aforementioned 3 SNPs, respectively (Fig. 1). At baseline, no significant association was observed between GRS and 25(OH)D or $25(OH)D_{Bio}$ concentrations (data not shown). However, significant interactions between GRS and treatment on both 25(OH)D and $25(OH)D_{Bio}$ responses were observed after adjustment for age, sex, BMI, and

Table 2. Serum Concentrations of 25(OH)D and 25(OH)D_{Bio}, VDBP, and PTH in the 20-Week Trial

Characteristic	Placebo (n = 204)[a]	Vitamin D_3 (n = 207)[a]	P[b]
25(OH)D, nmol/L			
Week 0	32.3 ± 8.7[b]	32.9 ± 8.9[b]	0.75
Week 10	31.8 ± 9.1[b]	67.2 ± 20.3[a]	<0.001
Week 20	36.3 ± 9.8[a]	67.3 ± 23.1[a]	<0.001
Change			
Week 10 − week 0	−0.6 ± 6.6	34.2 ± 21.1	<0.001
Week 20 − week 0	3.9 ± 7.3	34.4 ± 23.6	<0.001
$P_{time \times treatment}$[c]			<0.001
25(OH)D_{Bio}, nmol/L			
Week 0	2.7 ± 0.9[b]	2.8 ± 0.8[b]	0.24
Week 20	2.9 ± 1.0[a]	5.7 ± 3.0[a]	<0.001
Week 20 − week 0	0.3 ± 0.9	3.0 ± 3.0	<0.001
$P_{time \times treatment}$[c]			<0.001
VDBP (μg/mL)			
Week 0	424.1 ± 100.1	409.7 ± 91.4	0.13
Week 20	431.2 ± 99.4	417.6 ± 96.5	0.16
Week 20 − week 0	7.1 ± 75.6	7.8 ± 82.2	0.16
$P_{time \times treatment}$[c]			0.96
25(OH)D_{Bio}/25(OH)D, %[d]			
Week 0	8.3 ± 1.9	8.5 ± 1.8	0.25
Week 20	8.2 ± 2.0	8.6 ± 3.4	0.35
Week 20 − week 0	−0.1 ± 1.7	0.1 ± 3.2	0.35
$P_{time \times treatment}$[c]			0.29
PTH, pg/mL			
Week 0	39.2 ± 18.3[a]	39.8 ± 17.3[a]	0.52
Week 10	33.4 ± 15.7[b]	27.2 ± 13.4[b]	<0.001
Week 20	30.1 ± 16.0[c]	25.3 ± 11.1[b]	<0.001
Change			
Week 10 − week 0	−6.0 ± 18.5	−12.7 ± 15.9	<0.001
Week 20 − week 0	−9.2 ± 17.0	−14.5 ± 16.3	<0.001
$P_{time \times treatment}$[c]			<0.001

Data are means ± standard deviation. Within-arm differences (between weeks 0, 10, and 20) were explored by using a mixed model, followed by a Fisher's least significant difference multiple comparisons test when the difference among 3 time points was significant ($P < 0.05$). Values with different superscript letters indicate significant differences ($P < 0.05$).

[a]Only the participants who completed the trial with 3 blood samples were included in the analysis (vitamin D_3 arm, n = 207; placebo arm, n = 204).

[b]Between-arm difference at each visit time was compared by linear regression with adjustment for age, sex, BMI, and respective baseline value (except week 0).

[c]The *P* value for interaction (time × treatment) was obtained from a mixed effects model, the dose and visit time were included as fixed effects, and the participant was included as a random effect, adjusted for age, sex, and baseline BMI.

[d]Relative 25(OH)D_{Bio} (%) = [25(OH)D_{Bio} (nmol/L)/25(OH)D (nmol/L)] × 100%.

respective baseline value ($P_{interaction} \leq 0.04$). Moreover, significant linear negative trends between both responses and GRS were also observed ($P < 0.001$). Participants carrying 6 risk alleles experienced a reduced response in 25(OH)D (−13.2 ± 2.0 nmol/L) and 25(OH)D_{Bio} (−1.8 ± 0.3 nmol/L) than did those that carried a 0 or 1 risk allele.

As shown in Table 5, BMI was inversely associated with 25(OH)D response at week 20. Each unit increment of BMI reduced the response by 1.9 (95% CI, 1.0 to 2.8) nmol/L ($P < 0.001$). However, the 25(OH)D response was 12.8 (95% CI, 5.8 to 19.7) nmol/L [5.1 (2.3 to 7.9) ng/mL] greater in normal weight (BMI, 18.5 to 25 kg/m^2) participants than their overweight (BMI ≥ 25 kg/m^2) counterparts after 20-week supplementation ($P_{interaction}$ = 0.006; Supplemental Table 6). Meanwhile, lower baseline concentrations were associated with higher responses of 25(OH)D and 25(OH)D_{Bio} ($P < 0.001$). No other response-modifying effect was observed in the stratified analysis (Supplemental Table 6). Models including only supplement dose had an adjusted R^2 of 0.45 for 25(OH)D and 0.27 for 25(OH)D_{Bio} responses (Supplemental Fig. 3), and correspondingly increased to 0.51 and 0.36 after incorporating genetic and nongenetic factors in stepwise selection models. The joint effect of the 3 SNPs, measured by GRS, could explain a larger proportion than those combined nongenetic factors (baseline value, BMI, and sex) in response variations of both 25(OH)D (adjusted R^2, 0.05 vs 0.03) and 25(OH)D_{Bio} (0.08 vs 0.03).

25(OH)D threshold for PTH suppression

The nonlinear relationships between PTH and 25(OH)D and between PTH and 25(OH)D_{Bio} are depicted by locally weighted scatterplot smoothing model curves in Supplemental Fig. 4. Inverse associations were only observed when 25(OH)D < 50.8 (95% CI, 43.6 to 59.0) nmol/L or 25(OH)D_{Bio} < 5.8 (95% CI, 5.1 to 6.7) nmol/L, the concentration at which PTH began to level off, implicating a PTH-based threshold for vitamin D deficiency (36).

Discussion

In this study, we used a randomized trial to evaluate the effects of genetic and nongenetic factors on 25(OH)D and 25(OH)D_{Bio} responses in Chinese. Daily supplementation with 2000 IU vitamin D_3 for 20 weeks significantly raised total and bioavailable 25(OH)D concentrations, but it still left 25% of participants with uncorrected deficiency. Genetic factors exerted stronger impact than did nongenetic factors on both 25(OH)D and 25(OH)D_{Bio} responses.

After 20-week supplementation, 25(OH)D increased ~11.3-fold more than 25(OH)D_{Bio} (+30.6 vs +2.7 nmol/L), corresponding to the efficacy (the average increment per microgram of vitamin D_3) of 0.61 and 0.05 nmol/L/μg,

Table 3. Vitamin D Status in the 20-Week Trial

Arms	No.	Vitamin D Status [No. (%)][a]			P^b
		Deficiency	Insufficiency	Sufficiency	
Week 0					
Placebo	222	217 (97.7)	5 (2.3)	0 (0)	0.60
Vitamin D_3	226	218 (97.7)	8 (3.5)	0 (0)	
Week 10					
Placebo	206	202 (98.1)	4 (1.9)	0 (0)	<0.001
Vitamin D_3	214	46 (21.5)	85 (39.7)	83 (38.8)	
Week 20					
Placebo	204	189 (92.6)	15 (7.4)	0 (0)	<0.001
Vitamin D_3	207	51 (24.6)	78 (37.7)	78 (37.7)	

Data are no. (%). The percentage was calculated within each arm at weeks 0, 10, and 20.

[a]Vitamin D status was classified as sufficiency [25(OH)D ≥ 75 nmol/L], insufficiency [50 ≤ 25(OH)D < 75 nmol/L], or deficiency [25(OH)D < 50 nmol/L).

[b]Obtained from χ^2 test.

respectively. Although no existing trial evaluated the efficacy for 25(OH)D_{Bio}, the efficacy for raising 25(OH)D at the same dose was similar to that in our earlier trial (13), but lower than that in blacks (1.0 to 1.1 nmol/L/μg) (12, 37). Notably, the present trial found a positive association between change of 25(OH)D_{Bio} and change of serum albumin–corrected calcium levels only. Although few trial data were available and the physiological role of 25(OH)D_{Bio} remains controversial (30, 34, 38, 39), some of the cross-sectional studies suggested that associations of 25(OH)D_{Bio} with serum calcium, PTH, or bone mineral density status were stronger than those associations of 25(OH)D in healthy young people, white postmenopausal women, and hemodialysis patients (30, 38, 39). Nonetheless, it still remains to be elucidated whether 25(OH)D_{Bio} could provide additional information reflecting vitamin D physiologic function in clinical settings.

In the circulation, 85% to 90% of 25(OH)D is tightly bound to VDBP, with only 10% to 15% loosely bound to albumin, and <1% remains as free form (22). Thus, VDBP acts as a serum carrier and reservoir of circulating 25(OH)D to maintain its levels, facilitate its transportation to various tissues, and regulate its bioavailability (22). Based on different combinations of rs7041 and rs4588 in *GC* gene, 3 major isoforms of VDBP, namely Gc1F, Gc1S, and Gc2, are yielded with different binding affinities for 25(OH)D (Gc1F > Gc1S > Gc2) (40). Interestingly, Chinese homozygotes in our trial showed larger portions of phenotypes with low/medium binding affinity (28.5% Gc2/2 and 24.3% Gc1S/1S), whereas black homozygotes tend to carry the highest binding affinity form (92.7% Gc1F/1F) (22). Therefore, at a given 25(OH)D concentration, Chinese might have relatively higher non–VDBP-bound portions or higher 25(OH)D bioavailability than do blacks. Moreover, relatively higher frequency of Gc1F/1F in Chinese (47.2%) than whites (6.0%) may lead to underestimation of VDBP levels by using monoclonal antibody immunoassays (21). In line with previous multiethnic studies (18, 19, 21), we also observed a remarkably low VDBP concentration as measured by a monoclonal versus polyclonal antibody immunoassay in Chinese. Indeed, different VDBP concentrations between blacks and whites were only indicated by using the monoclonal antibody, but not by using the polyclonal antibody immunoassay or liquid chromatography–tandem mass spectrometry (19–21). Taken together, the polyclonal antibody immunoassay is the more appropriate method to assess VDBP and also 25(OH)D_{Bio} concentrations for the populations having a relatively higher frequency of the Gc1F/1F isoform, including blacks and Asians.

In response to vitamin D_3 treatment, we found that the polymorphisms in the genes involving the vitamin D metabolism pathway could modify the responses of 25(OH)D and/or 25(OH)D_{Bio} specifically. For instance, *GC*-rs4588 CC carriers with the highest VDBP concentration showed the highest response in 25(OH)D than in other genotypes. The specific effect of rs4588-C might be largely attributed to its raising VDBP level property, as well as higher binding affinity for 25(OH)D (≥1.7-fold) (40). In fact, the VDBP-bound 25(OH)D might have a relatively long half-life by avoiding being catabolized to inactive metabolites and therefore increasing 25(OH)D concentrations (41). Consistently, the rs4588-C allele was linked to a greater 25(OH)D response in Danish who received vitamin D_3–fortified bread and milk or UVB treatment (42), and also in Thais administered 400 IU/d vitamin D_3 plus calcium (41). Unlike in the case of *GC*-rs4588, *GC*-rs7041 significantly modified the 25(OH)D_{Bio} response, with the largest increase in rs7041-GG carriers, followed by GT and TT genotypes.

Table 4. Effects of SNPs on Responses of 25(OH)D and 25(OH)D_{Bio} at Week 20

		25(OH)D (nmol/L)		25(OH)D_{Bio} (nmol/L)	
SNP ID	**Genotype**	**Placebo**	**Vitamin D_3**	**Placebo**	**Vitamin D_3**
GC					
rs7041	TT	3.2 ± 0.7	32.8 ± 2.1	0.3 ± 0.1	2.8 ± 0.3[b]
	GT	5.3 ± 0.8	35.7 ± 2.6	0.3 ± 0.1	2.9 ± 0.3[b]
	GG	2.9 ± 1.7	38.7 ± 5.9	0.1 ± 0.2	5.0 ± 0.8[a]
	P_{trend}[a]	0.32	0.26	0.82	0.04
	$P_{interaction}$[b]		0.63		0.04
rs4588	AA	3.4 ± 1.6	28.2 ± 4.1[b]	0.3 ± 0.2	3.1 ± 0.6
	CA	4.0 ± 0.7	33.0 ± 2.4[a,b]	0.3 ± 0.1	2.7 ± 0.3
	CC	4.0 ± 0.7	37.4 ± 2.4[a]	0.2 ± 0.1	3.2 ± 0.3
	P_{trend}[a]	0.87	0.04	0.45	0.52
	$P_{interaction}$[b]		0.25		0.51
VDR					
rs2228570	AA	3.7 ± 1.1	28.2 ± 3.8[b]	0.3 ± 0.1	2.7 ± 0.5[b]
	GA	3.3 ± 0.7	33.8 ± 2.1[a,b]	0.3 ± 0.1	2.5 ± 0.3[b]
	GG	5.7 ± 1.0	39.6 ± 3.0[a]	0.4 ± 0.1	4.1 ± 0.4[a]
	P_{trend}[a]	0.14	0.009	0.62	0.01
	$P_{interaction}$[b]		0.10		0.02
CYP2R1					
rs10741657	AA	4.3 ± 1.4	39.7 ± 3.7[a]	0.2 ± 0.2	4.5 ± 0.5[a]
	GA	4.4 ± 0.8	30.1 ± 2.1[b]	0.4 ± 0.1	2.6 ± 0.3[b]
	GG	3.4 ± 0.7	32.5 ± 2.3[a,b]	0.2 ± 0.1	2.9 ± 0.3[b]
	P_{trend}[a]	0.77	0.04	0.41	0.003
	$P_{interaction}$[b]		0.07		0.003

Data are means ± SE. Responses were calculated by subtracting the baseline values from the values at week 20 (n = 409). A linear regression was used to analyze the pairwise genotype difference in the placebo and vitamin D_3 arms separately, with adjustment for age, sex, baseline BMI, and respective baseline value, and followed by a Fisher's least significant difference multiple comparisons test when the difference among the genotypes was significant ($P < 0.05$). Values with different superscript letters of indicate significant differences ($P < 0.05$). *GC* indicates VDBP.

[a]Obtained from a multiple linear regression model including age, sex, BMI, and baseline value. SNPs were treated as continuous terms based on the number of effect alleles.

[b]We used additive inheritance models (*e.g.*, *GC*-rs4588 genotype groups were coded as 0, 1, and 2 in continuous form for CC, CA, and AA) in the analyses. To test potential gene–treatment interactions, a genotype-by-treatment interaction term (*e.g.*, *GC*-rs4588 genotype × treatment/placebo group) was included in the models adjusted for age, sex, BMI, and respective baseline value.

Alternatively, the *VDR*-rs2228570 (Fok1) G allele modified responses in both 25(OH)D and 25(OH)D_{Bio}. *VDR* encodes the vitamin D receptor, which binds 1,25-hydroxyvitamin D to promote transcription, and it also regulates expression of vitamin D metabolism–related genes, such as *CYP27B1* and *CYP24A1*, as a feedback mechanism (1). Previously, *VDR*-rs7968585 and *CYP24A1*-rs6013897 (*CYP24A1*, encode 24-hydroxylase) were shown to modify 25(OH)D response in non-Hispanic whites receiving daily 1000 IU vitamin D_3 plus 1200 mg calcium for 12 months (43). Furthermore, we documented positive associations of the *CYP2R1*-rs10741657 A allele with both 25(OH)D and 25(OH)D_{Bio} responses. *CYP2R1* encodes an enzyme, 25-hydroxylase, which is responsible for the hydroxylation of vitamin D to 25(OH)D. Consistently, rs10741657 was reported to predict 25(OH)D responses in the aforementioned Danish study, as well as in a pooled analysis of 3 vitamin D_3 trials in Norway (42, 44). However, we could not find any significant effect of rs6013897 or rs1790349 on 25(OH)D response, which might be due to intervention types (vitamin D_3 with or without calcium, vitamin D_3 fortified bread and milk, UVB treatment), different doses, baseline vitamin D status, and different ethnic groups across studies (31, 42–44).

When both genetic (GRS) and nongenetic (baseline value, BMI, and sex) determinants are considered, genetic factors showed stronger impacts on 25(OH)D and 25(OH)D_{Bio} responses, particularly for 25(OH)D_{Bio}. Individuals carrying 6 risk alleles might need to take an additional amount of vitamin D than do their counterparts carrying no or 1 risk allele to achieve a targeted 25(OH)D level. In regard to the nongenetic determinants, we documented inverse associations of initial concentrations with 25(OH)D or 25(OH)D_{Bio} responses, which might result from regression to the mean (31, 44). Moreover, compared with normal weight persons, the 25(OH)D response was much lower in overweight participants, which might be attributed to the effect of a larger volume dilution for this fat-soluble vitamin (12, 44, 45). Other potential factors might also lead to various responses such as gastrointestinal absorption or more

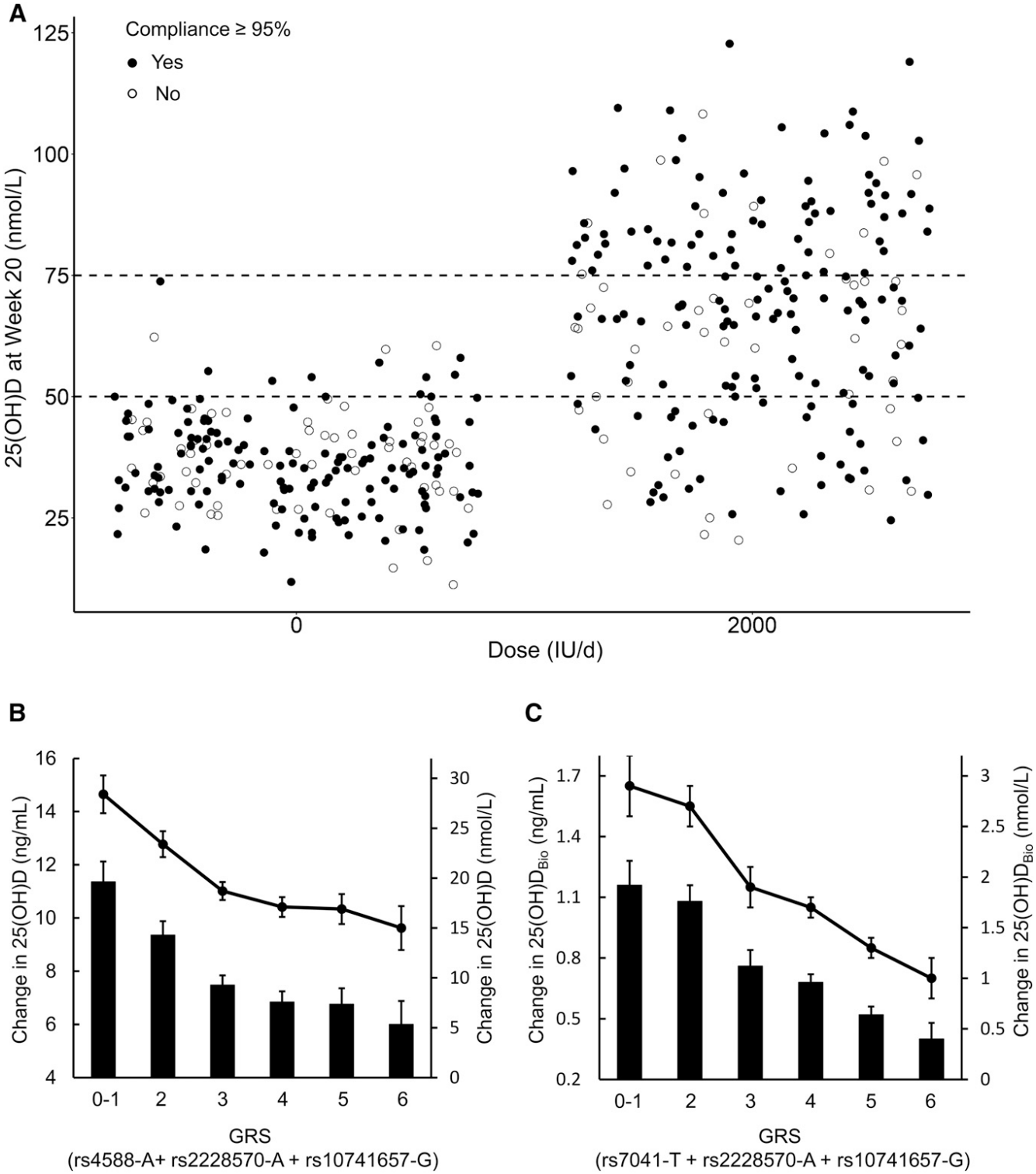

Figure 1. (A) Serum 25(OH)D levels according to arms at week 20. The filled circles and open circles represent the participants with compliance rates ≥95% and <95%, respectively. The horizontal dashed lines represent 75 and 50 nmol/L. No significant difference was observed between the intention-to-treat participants and the per-protocol participants (compliance rate ≥ 95%) regarding 25(OH)D levels or vitamin D status at week 20. (B and C) Adjusted mean (SE) changes in 25(OH)D and 25(OH)D_{Bio} concentration according to GRS category. Changes in nanograms per milliliter and nanomoles per liter are displayed by histograms with the left vertical axis and by lines with the right vertical axis, respectively. A GRS for 25(OH)D was calculated as the sum of the number of A alleles of rs4588, A alleles of rs2228570, and G alleles of rs10741657 (range, 0 to 6), and participants with GRS of 0 or 1 were combined to increase the group sample size (B). For 25(OH)D_{Bio}, a GRS was calculated as the sum of the numbers of T alleles of rs7041, A alleles of rs2228570, and G alleles of rs10741657 (range, 0 to 6), and participants with GRS of 0 or 1 were combined to increase the group sample size (C). Error bars show the SE.

Table 5. Genetic and Nongenetic Determinants of Responses of 25(OH)D and 25(OH)D_{Bio} Concentrations at Week 20

Variable	β (95% CI) (nmol/L)	*P*
25(OH)D		
Treatment, per 2000 IU/d	74.7 (46.6, 102.8)	<0.001
Baseline 25(OH)D, per 1 nmol/L	−0.4 (−0.5, −0.2)	<0.001
Sex, women vs men	−3.1 (−6.5, 0.3)	0.08
BMI, per 1 kg/m^2	−1.9 (−2.8, −1.0)	<0.001
GRS, per 1	−3.1 (−4.9, −1.3)	<0.001
Treatment × BMI	1.7 (0.5, 2.9)	0.006
Treatment × GRS[a]	2.4 (0.1, 5.4)	0.04
Adjusted R^2		*0.51*
25(OH)D_{Bio}		
Treatment, per 2000 IU/d	5.9 (4.3, 7.4)	<0.001
Baseline 25(OH)D_{Bio}, per 1 nmol/L	−0.4 (−0.7, −0.2)	<0.001
BMI, per 1 kg/m^2	−0.1 (−0.1, 0.0)	0.14
GRS, per 1	−0.7 (−1.0, −0.5)	<0.001
Treatment × GRS[b]	0.9 (0.5, 1.3)	<0.001
Adjusted R^2		*0.36*

Responses were calculated by subtracting the baseline values from the values at week 20 (n = 409). Backward stepwise regression analyses were used to select variables for model predicting responses. The initial model included age, sex, BMI, treatment, respective baseline concentrations, PTH and calcium, questionnaire (sun exposure time, sun exposure protection score, smoke, alcohol, and physical activity), and GRS and interaction term: treatment × GRS and treatment × BMI.

[a]A GRS for 25(OH)D was calculated as the sum of the number of A alleles of rs4588, A alleles of rs2228570, and G alleles of rs10741657 (range, 0 to 6).

[b]A GRS for 25(OH)D_{Bio} was calculated as the sum of the numbers of T alleles of rs7041, A alleles of rs2228570, and G alleles of rs10741657 (range, 0 to 6).

rapid metabolism of 25(OH)D. Additionally, vitamin D supplementation significantly interacted with GRS on both 25(OH)D and 25(OH)D_{Bio} responses, as well as with BMI on the 25(OH)D response. Therefore, effects of genetic and nongenetic factors on responses of 25(OH)D and 25(OH)D_{Bio} should take into account a more precise vitamin D assessment and intervention strategy.

It is noteworthy in our study that serum PTH concentration was maximally suppressed with 25(OH)D ≥ 50.8 nmol/L, which might serve as an alternative definition for vitamin D deficiency, because the relationship between optimal 25(OH)D and skeletal and nonskeletal health outcomes have not been established in Asians (36). This PTH-based 25(OH)D threshold was similar to the vitamin D deficiency definition (50 nmol/L) according to the Institute of Medicine and the Endocrine Society in the United States (6, 35). Vitamin D deficiency, accompanied with reduced absorption and circulating levels of calcium, could remarkably trigger PTH synthesis through a calcium-sensing receptor (1). Consequently, the elevated PTH promotes mineral release from bone and indirectly maximizes gut mineral resorption by increasing 1,25-hydroxyvitamin D synthesis (1). Note that the breakpoint of 25(OH)D 50.8 nmol/L in our study is comparable to 50.0 nmol/L in African Americans in the National Health and Nutrition Examination Survey (10), but lower than 72.5 nmol/L in whites (46), whereas no obvious 25(OH)D threshold was detected based on a cross-sectional analysis consisting of 312,962 clinically referred subjects (47). Therefore, the PTH-based optimal vitamin D levels might vary across ethnic groups. Moreover, an inverse PTH–25(OH)D_{Bio} association was shown only when 25(OH)D_{Bio}< 5.8 nmol/L, suggesting that 25(OH)D_{Bio} might also involve PTH regulation according to the free hormone hypothesis (15). Nonetheless, whether or to what extent the PTH-based 25(OH)D threshold could reflect bone and other health outcomes in Asians still needs to be clarified.

One of the major strengths of this trial is that we simultaneously studied efficacies of vitamin D_3 on elevating 25(OH)D and 25(OH)D_{Bio}, and we also quantitatively evaluated the relative contribution of genetic and nongenetic factors to both responses. Moreover, multiple biomarkers, including PTH, calcium, albumin, and VDBP concentrations, were measured at multiple time points to evaluate responses of different portions of 25(OH)D. In this study, we measured VDBP by 2 different assays in an Asian population. Additionally, potential confounding factors were minimalized with the randomized design, relatively large sample size, and high compliance rate, as well as closely monitored dietary intake and sun exposure levels. Admittedly, our study also has some limitations: (1) All participants were Chinese adults (20 to 45 years of age), and thus the findings might not generalize to other ethnicities or different age groups. (2) 25(OH)D_{Bio} concentrations were calculated rather than measured directly; however, a high correlation between the calculated and measured concentrations was reported previously (22). (3) We used only the current UL in China for supplementation, and thus the effects of other doses on responses after vitamin D_3 supplementation remain to be evaluated.

In conclusion, daily supplementation with a UL dose of vitamin D in China significantly raised 25(OH)D and 25(OH)D_{Bio} concentrations, but it was still unable to correct deficiency in 25% of participants. Genetic factors apparently exerted greater impact than did nongenetic factors on both responses. More studies are needed to elucidate appropriate vitamin D recommendation for Asians and potential clinical implications of 25(OH)D_{Bio}.

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