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Efficacy of vitamin D supplementation according to vitamin D-binding protein polymorphisms

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ABSTRACT

Objectives: The aim of this study was to determine the influence of vitamin D–binding protein (DBP) gene polymorphisms in vitamin D metabolites before and after vitamin D supplementation.
Methods: In all, 234 participants (126 women; 108 men) with vitamin D deficiency [25(OH)D <50 nmol/L] were given 50 000 IU of vitamin D supplements for 8 wk followed by daily maintenance of 1000 IU for 4 mo. Two single-nucleotide polymorphisms (rs4588 and rs7041) in DBP coding gene were assessed.
Results: Baseline 25(OH)D was significantly in higher in participants with homozygous major genotype of rs7041 than other genotypes ($P = 0.02$). Postsupplementation 25(OH)D was significantly higher in participants with homozygous major genotypes of either rs4588 and rs7041 than other genotypes ($P < 0.001$). Participants with the minor allele of either rs4588 or rs7041 were 2.9 (1.9–4.5) times and 3.7 (2.1–6.6) times, respectively, more likely to be non-responders (postsupplementation 25 OHD <50 nmol/L) than those homozygous for the major allele at these locations ($P < 0.001$). Furthermore, participants with homozygous minor and heterozygous genotype of rs7041 were 6.2 and 4.2 times more likely to be non-responders than those with the homozygous major genotype ($P < 0.001$) even after adjustments for age, sex, body mass index, baseline 25(OH)D concentration, and other alleles. Participants with homozygous minor and heterozygous genotypes of rs4588 were 4.1 and 12.4 times more likely to be non-responders than those with homozygous major genotypes. These significant risks, however, were lost after adjustment.
Conclusions: rs7041 and rs4588 variants of the DBP gene are associated with variations in 25(OH)D levels and efficacy of response to vitamin D supplementation in Saudi Arabian adults.

Introduction

Vitamin D (cholecalciferol) is essential for overall bone health and its deficiency has been linked to bone-related diseases such as rickets in children and osteomalacia or osteoporosis in adults [1]. Vitamin D deficiency is a global health problem affecting >1 billion people worldwide [2]. It is more common in the Middle East and North African countries than other geographic regions [3]. In humans, the majority of vitamin D is synthesized by the body after exposure to sunlight (ultraviolet B radiation) and a smaller portion from natural foods or as dietary supplements [2]. Vitamin D obtained from skin synthesis or diet undergoes two hydroxylations to become biologically active metabolite. The first hydroxylation by enzyme 25-hydroxylase occurs in the liver, producing 25-hydroxyvitamin D (25[OH]D), the main circulating form of vitamin D. The next hydroxylation occurs in the kidney by enzyme 1-hydroxylase to produce the biologically active 1,25-dihydroxyvitamin D (1,25[OH]D) [4]. The 25(OH)D binds to DBP and is transported from the liver to other organs and tissues of the body. The

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majority (85–90%) of the total circulating 25(OH)D binds to DBP, 10% to 14% binds to albumin (bioavailable), and ≤1% is found in free form [5]. The presence of vitamin D receptors in most human tissues indicate the extraskeletal role of vitamin D. Numerous studies have shown an association between vitamin D deficiency and increased risk for 15 different types of cancers [2], cardiovascular diseases [6,7], diabetes [8,9], autoimmune diseases [10], and overall mortality [11].

Circulating 25(OH)D is used as a clinical biomarker for vitamin D status. In recent years, awareness about vitamin D deficiency has increased greatly, and vitamin D supplementation is currently considered one of the best approaches for vitamin D repletion. However, changes in serum 25(OH)D relative to vitamin D supplementation vary widely among individuals. Variations to vitamin D supplementation response can be explained by demographic, genetic, and environmental factors [12,13]. Previous studies involving different cohorts suggest that genetic variants in DBP are strong determinants of circulating 25(OH)D [14–16]. The DBP group-specific component (*GC*) gene has two well-known common single-nucleotide polymorphisms (SNPs) at exon 11; rs7041 T > G transversion results in aspartate (GAT) to glutamate (GAG); rs4588 C > A transversion results in threonine (ACG) to a lysine (AAG). The diplotypes of these SNPs result in protein isoforms *GC1 F*, *GC1 S*, and *GC2*, which are electrophoretically distinguishable and differ in their binding affinity for vitamin D [17,18]. Studies that investigate the effect of genetic variants in DBP gene on circulating 25(OH)D levels are lacking in the Saudi Arabian population, despite having a vitamin D deficiency prevalence of >80% [19]. Thus, we investigated whether the common genetic variants in DBP influence variations in the levels of total serum and bioavailable and free 25(OH)D, and efficacy in response to vitamin D supplementation, in a cohort of vitamin D–deficient Saudi Arabians.

Methods

Participants

In all, 234 Saudi adults (126 women and 108 men) with serum 25(OH)D levels <50 nmol/L were recruited for this study between December and May 2016 (the cold season in Riyadh). All the participants were outpatients of King Khalid University Hospital (KKUH, Riyadh, Saudi Arabia). They were supplemented by physicians with weekly 50 000 IU (VitaD50000 Synergy Pharma, Dubai, UAE) for 8 wk, followed by daily maintenance of 1000 IU (VitaD1000 Synergy Pharma) for 4 mo. To monitor compliance, all participants were asked to return once every 2 mo to surrender any unused tablets and to get a fresh refill. All individuals were asked to complete a generalized, prestructured questionnaire that included present and past medical history. All participants underwent physical examination and submitted written informed consents before inclusion. Patients taking multivitamins, calcium, cortisone, or other steroids; products with mineral oil, regular antacids, diuretics, phenytoin, and phenobarbital medicatons; or weight loss drugs were excluded from the study. In addition, patients with gallbladder or gastrointestinal disorders and liver problems and those with evidence of metabolic disease (Paget's disease or osteomalacia), renal stone disease, hyperparathyroidism, abnormal levels of calcium, alkaline phosphatase, and phosphorous were excluded. All methods of sampling and protocols were approved by the Ethics Committee and Institutional Review Board of the College of Medicine, King Saud University in Riyadh, Saudi Arabia. All methods were performed in accordance with the relevant guidelines of the Helsinki Declaration. At the end of the intervention, participants were classified as either responders (those whose serum 25[OH]D increased to ≥50 nmol/L) or non-responders (those whose serum 25[OH]D did not reach 50 nmol/L).

Anthropometry and blood withdrawal

All participants were requested to fast for ≥8 h before blood extraction and anthropometrics collection. Anthropometrics included weight, height, hip and waist circumference (cm), and systolic and diastolic blood pressure. Body mass index (BMI) was calculated (kg/m^2). Blood samples were collected in Ethylenediamine tetraacetic acid (EDTA) as well as in tubes with no anticoagulant. EDTA samples were used for genomic DNA extraction; whereas serum was isolated from other samples for serologic examination. Serum samples were aliquoted into plain tubes and stored at −20°C until further use.

Biochemical analysis

Fasting glucose and lipid profiles were measured using a chemical analyzer (Konelab, Finland). Serum 25(OH)D was estimated using enzyme-linked immunosorbent assay (ELISA; IDS Ltd., Boldon Colliery, Tyne & Wear, UK). The inter- and intraassay variabilities were 5.3% and 4.6%, respectively. Serum DBP levels were measured using ELISA (R&D Systems, R&D Systems, Minneapolis, MN, USA) with interassay coefficients of variability (1.6–3.6%) and recovery of 98% to 103%. Free and bioavailable 25(OH)D levels were calculated using the formula by Powe et al. [20]:

$$\text{free25(OH)D} = \frac{\left[-b + \sqrt{\left(b^2 - 4\,ac\right)}\right]}{2\,a} \quad (1)$$

$a \quad k_{alb} * albumin + K_{dbp}$

$b \quad (kdbp * DBP) - (Kdbp * 25(OH)D + (k\_alb * albumin) + 1$

Kdpb affinity constant between 25(OH)D and DBP
Kalb affinity constant between 25(OH)D and albumin

$$Bioavailable\ 25(OH)D = (k_{alb} * albumin + 1) * (free25(OH)D) \quad (2)$$

Genetic analyses for VDBP *polymorphisms*

Genomic DNA was extracted from whole blood using DNeasy blood and tissue kits (Qiagen, Hilden, Germany). DNA purity (260:280 ratio) and concentrations were measured using Nano-drop spectrophotometer. The two DBP SNPs—rs4588 and rs7041—were evaluated using real-time polymerase chain reaction (PCR) with predesigned TaqMan genotyping assays (Applied Biosystems, Foster City, CA, USA). The polymerase chain reaction program was heated at 95°C for 10 min followed by 45 cycles of 94°C for 15s and 60°C for 1 min; fluorescence detection occurred at 60°C. All genotyping was performed in 10 μL reactions, using TaqMan Genotyping Master Mix in 96-well plates in an ABI 7000 instrument (Applied Biosystems).

Statistical analysis

Data were analyzed using SPSS version 21 (IBM, Armonk, NY, USA). Variables were expressed as mean ± SD. Normality of data was tested using Kolmogrov–Smirnov test. Non-normal variables were transformed logarithmically. Paired *t* test was used to compare pre- and postsupplementation differences. Analysis of variance (ANOVA) was used to compare genotype groups. Post hoc analysis was done using Tukey's test. Mantel–Haenzsel test (χ^2 linear-by-linear association) was used to determine P_{trend}. Bonferroni correction was applied to adjust for multiple comparisons among genotypes and diplotypes. Odds ratios and 95% confidence intervals (CIs) were calculated using multinomial logistic regression using dominant genotypes or alleles as the factor with age, sex, BMI and baseline 25(OH)D levels as covariates. $P < 0.05$ was considered statistically significant.

Results

Participants' characteristics pre- and postsupplementation

In all, 234 patients (126 women and 108 men) participated in this study. Participants had a mean age of 44.9 ± 11.5 y and a mean BMI of 30.4 ± 6.1 kg/m^2. Serum 25(OH)D levels increased significantly (baseline 34.1 nmol/L [24–46.8] to follow-up 55.3 nmol/L [40.4–73.8]; $P < 0.001$) along with free 25(OH)D (21.5 [11.7–33.2] to 31.6 [13.7–50.9]; $P < 0.001$) and bioavailable 25(OH)D (17 [9.2–28.5] to 26.6 [11.7–42.1]; $P < 0.001$). Both calcium and albumin concentrations increased significantly after intervention ($P = 0.02$ and 0.04, respectively). No significant changes were observed in serum DBP levels nor in glucose and lipid profiles after supplementation (Table 1). All participants claimed full compliance (100% prescribed dose consumed) and none surrendered unused supplements during the refill periods of treatment monitoring.

Genotypic characteristics of participants

Baseline clinical and anthropometric characteristics of the participants according to rs4588 and rs7041 SNPs were presented in

Table 1
Clinical and anthropometric characteristics of participants at pre- and postsupplementation

Parameters	All		*P*-value
	Pre	Post	
N (M/F)	234 (108/126)		
Age (y)	44.9 ± 11.5		
Body mass index (kg/m^2)	30.4 ± 6.1		
Waist-to-hip ratio	0.96 ± 0.10		
Systolic blood pressure (mm Hg)	125.9 ± 14.1		
Diastolic blood pressure (mm Hg)	79.3 ± 9.4		
Glucose (mmol/L)	7 ± 3	7.3 ± 3	0.25
Triacylglycerols (mmol/L)*	1.5 (1.1–2.1)	1.6 (1.1–2.1)	0.60
Total cholesterol (mmol/L)	4.9 ± 1.1	5 ± 1.2	0.30
HDL cholesterol (mmol/L)	1 ± 0.3	1 ± 0.4	0.20
LDL cholesterol (mmol/L)	3.1 ± 0.9	3.2 ± 0.9	0.21
Calcium (mmol/L)	2.2 ± 0.2	2.2 ± 0.3	0.02
Albumin (g/L)	36.7 ± 4.4	37.6 ± 5.4	0.04
25(OH) D (nmol/L) #	34.1 (24–46.8)	55.3 (40.4–73.8)	<0.001
Vitamin D-binding protein (μg/mL) #	19.6 (11.5–49.5)	19.8 (11–80.7)	0.10
Free 25 (OH)D (nmol/L) #	21.5 (11.7–33.2)	31.6 (13.7–50.9)	<0.001
Bio-free available (OH)D (nmol/L) #	17 (9.2–28.5)	26.6 (11.7–42.1)	<0.001

25(OH) D, 25-hydroxyvitamin D; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data presented as mean ± SD and median (first to third) percentiles for normal and non-normal variables, respectively.
*Non-normal variables; significant at $P < 0.05$.

Table 2. In rs4588, the waist-to hip ratio in participants with homozygous minor (*AA*) genotype was significantly higher than heterozygous (*CA*) and homozygous major (*CC*) genotype carriers, respectively ($P < 0.001$). Similarly, the waist-to hip ratio in the homozygous minor (TT) genotype group was significantly higher than the homozygous major (*GG*) genotype group in rs7041 ($P = 0.031$). In rs4588, baseline albumin level in the homozygous minor genotype (*AA*) group was significantly lower than homozygous major (*CC*) genotype group ($P = 0.02$). Also, serum 25(OH)D in the homozygous major (*CC*) genotype group was higher compared with heterozygous (*CA*) and homozygous minor (*AA*) genotype groups, respectively. However, this difference was not statistically significant ($P = 0.07$). In rs7041, median 25(OH)D levels in participants with homozygous major (*GG*) genotype was significantly higher than their homozygous minor (*TT*) genotype counterparts ($P = 0.02$). No significant differences were observed in other parameters, including lipids, glucose, calcium, DBP, free 25-(OH) D and bioavailable 25(OH)D among groups (Table 2).

Vitamin D metabolites according to DBP SNPs postsupplementation

Postsupplementation, participants in the homozygous major genotype group in either rs4588 and rs7041 DBP SNPs showed the highest increase in serum 25(OH)D levels ($P < 0.001$; Table 3). No significant differences in DBP, free 25(OH)D, and bioavailable 25 (OH)D were observed in rs4588 (Table 3). However, in rs7041, free 25(OH)D levels in homozygous minor (*TT*) genotype group (18.9 nmol/L [10.6–34.9]) was significantly lower than participants with heterozygous (*TG*) genotype (35.6 nmol/L [19.7–45.7]) and homozygous major (*GG*) genotype (37.4 nmol/L [15.3–58.8]). In addition, free 25(OH)D level in homozygous major (*GG*) genotype group significantly increased by 93.8% postsupplementation compared with baseline (23.5%) in participants with homozygous minor (*TT*) genotype ($P = 0.04$; Table 3). Moreover, participants with homozygous major (*GG*) genotype had an increase in DBP by 94.4% compared with a 2.6% increase in both heterozygous (*TG*) and homozygous minor (*TT*) genotypes, but these were not statistically significant ($P = 0.08$; Table 3). Postsupplementation, the participants in *GC1S/S (GG:CC)* diplotype group showed the highest increase in serum 25(OH)D, with the lowest being the *GC1F/F (TT: CC)* diplotype ($P < 0.001$; Fig. 1 and Table 4). No significant differences were observed in levels of DBP, free 25(OH)D, and bioavailable 25(OH)D among DBP diplotypes.

Baseline and follow-up characteristics of participants according to diplotypes are presented in Supplementary Table 1. Percentage changes in parameters measured according to diplotypes are presented in Supplementary Table 2.

Risk for non-response to vitamin D correction

Allelic distribution according to response in vitamin D intervention is presented in Figure 2. It should be noted that among the

Table 2
Baseline characteristics of participants according to rs4588 and rs7041

Parameters (rs4588)	rs4588			*P*-value	rs7041			*P*-value
	Homozygous major *CC*	Heterozygous *CA*	Homozygous minor *AA*		Homozygous major *GG*	Heterozygous *TG*	Homozygous minor *TT*	
N (M/F)	169 (76/93)	59 (29/30)	6 (3/3)		94 (38/56)	88 (47/41)	52 (23/29)	
Age (y)	44.5 ± 11.8	45.2 ± 11.1	49.7 ± 8.4	0.72	43.6 ± 11.8	45.5 ± 10.9	46.3 ± 11.9	0.34
Body mass index (kg/m^2)	29.9 ± 6	31.6 ± 6.1	31.2 ± 6.2	0.14	29.5 ± 5.9	31.2 ± 6.7	30.6 ± 5.1	0.21
Waist-to-hip ratio	0.9 ± 0.10	1 ± 0.1	1.2 ± 0.2 [AB]	<0.01	0.94 ± 0.10	0.96 ± 0.10	0.99 ± 0.12 [C]	0.03
Systolic BP (mm Hg)	125.9 ± 14.1	126.8 ± 12.8	139.3 ± 3.3	0.08	123.4 ± 14.5	127.4 ± 13.3	127.9 ± 14.3	0.14
Diastolic BP (mm Hg)	78.5 ± 9.8	80.5 ± 8.1	86.6 ± 7.1	0.08	77.7 ± 9.2	79.8 ± 9.9	81.1 ± 8.9	0.16
Glucose (mmol/L)	6.8 ± 2.8	7.5 ± 3	9.5 ± 6.1	0.23	6.6 ± 2.6	7.2 ± 2.9	7.5 ± 3.6	0.29
Triacylglycerols (mmol/L)*	1.5 (1.1–2.1)	1.5 (1.2–2)	2.2 (1.3–2.7)	0.85	1.5 (1.1–2.2)	1.7 (1.3–2.1)	1.6 (1.4–2.1)	0.11
Total cholesterol (mmol/L)	4.8 ± 1.1	5.2 ± 1.2	4.5 ± 0.5	0.07	4.8 ± 1	5.1 ± 1.2	4.9 ± 1	0.13
HDL cholesterol (mmol/L)	1 ± 0.3	1.1 ± 0.3	1 ± 0.3	0.57	1 ± 0.4	1 ± 0.3	1 ± 0.3	0.34
LDL cholesterol (mmol/L)	3 ± 0.9	3.3 ± 0.8	2.7 ± 0.8	0.06	3 ± 0.9	3.2 ± 0.9	3.05 ± 0.9	0.36
Calcium (mmol/L)	2.2 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	0.73	2.2 ± 0.3	2.2 ± 0.2	2.2 ± 0.2	0.99
Albumin (g/L)	36.9 ± 4.1	36.6 ± 4.7	31.8 ± 6.3 [A]	0.02	36.8 ± 4.3	36.9 ± 4.2	35.8 ± 5.1	0.69
25(OH)D (nmol/L)*	35.9 (25–48)	27.4 (22–40)	25.2 (22–33)	0.07	36.7 (25–51)	32.6 (25–45)	29.4 (21–38)[C]	0.02
DBP (μg/mL)*	19.4 (10–54)	19.8 (16–25)	19.2 (19–20)	0.67	19.3 (10–128)	19.2 (11–32)	21.5 (19–96)	0.11
Free 25(OH)D (nmol/L)*	19.3 (10–40)	22.5 (15–31)	16.8 (15–25)	0.99	19.3 (10–48)	25.3 (14–34)	15.3 (6–26)	0.09
Bio 25(OH)D (nmol/L)*	16.9 (8–34)	18.1 (13–26)	12.5 (11–18)	0.97	15.3 (8–36)	20.3 (12–31)	13.7 (6–21)	0.08

25(OH) D, 25-hydroxyvitamin D; BP, blood pressure; DBP, vitamin D-binding protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data presented as mean ± SD and median (first to third) percentiles for normal and non-normal variables respectively. Superscripts A and B denote significant differences compared with *CC* and *CA* for rs4588; superscript C denotes significance compared with *GG* for rs7041. Significant at $P < 0.05$.
*Non-normal variables.

Table 3
Follow-up characteristics of participants according to rs4588 and rs7041

Parameter	rs4588			*P*-value	rs7041			*P*-value
	Homozygous major *CC*	Heterozygous *CA*	Homozygous minor *AA*		Homozygous major *GG*	Heterozygous *TG*	Homozygous minor *TT*	
N (M/F)	169 (76/93)	59 (29/30)	6 (3/3)		94 (38/56)	88 (47/41)	52 (23/29)	
Glucose (mmol/L)	7.1 ± 3.1	7.8 ± 3.1	6.1 ± 0.5	0.28	6.9 ± 2.9	7.8 ± 3.4	7.2 ± 2.6	0.14
	4.4	4	−35.8	0.69	4.5	8.3	−4	0.42
Triacylglycerols (mmol/L)*	1.5 (1.1–2.2)	1.6 (1.3–1.9)	2.5 (1.3–3)	0.10	1.5 (1.1–2.1)	1.6 (1.2–2)	1.7 (1.12–2.2)	0.72
	0	6.7	13.6	0.28	0	−5.3	6.2	0.05
Total cholesterol (mmol/L)	5 ± 1.2	5.2 ± 1.1	5 ± 1.1	0.39	5 ± 1.2	5 ± 1.1	5.2 ± 1.2	0.57
	4.2	0	11.1	0.80	4.2	−2	6.1	0.28
HDL cholesterol (mmol/L)	1 ± 0.4	0.9 ± 0.4	10 ± 0.5	0.55	1 ± 0.4	1 ± 0.4	1 ± 0.4	0.33
	0	−18.2	0	0.53	0	−4.8	−3.8	0.31
LDL cholesterol (mmol/L)	3.1 ± 0.9	3.4 ± 0.9 [A]	2.7 ± 0.8	0.08	3 ± 0.8	3.2 ± 0.9	3.4 ± 10 [C]	0.03
	3.3	3	0	0.89	0	0	11.5	0.26
Calcium (mmol/L)	2.2 ± 0.3	2.2 ± 0.3	2.1 ± 0.2	0.70	2.2 ± 0.3	2.2 ± 0.2	2.1 ± 0.3	0.67
	0	−4.3	−4.5	0.90	0	0	−4.5	0.41
Albumin (g/L)	38.2 ± 5.3	36.4 ± 6	35.4 ± 2.9	0.10	38.6 ± 5.2	37.4 ± 5.3	36.2 ± 5.8	0.08
	3.5	−0.5	11.3	0.08	4.9	1.4	1.1	0.54
25(OH)D (nmol/L)*	61.2 (46–77)	43 (34–60)[A]	42.2 (39–47)	<0.001	66.6 (53–84)	51.6 (38–70)[C]	44.9 (33–63)[C]	<0.001
	70.5	56.9 [A]	67.5	<0.001	81.5	58.3 [C]	52.7 [C]	<0.001
DBP (μg/mL)*	20 (11–127)	20.1 (12–80)	19.2 (19–20)	0.66	19.8 (10–145)	19.7 (11–49)	41.8 (20–163)	0.07
	3.1	1.5	0	0.69	2.6	2.6	94.4	0.08
Free 25 (OH) D (nmol/L)*	34.9 (14–54)	26.6 (14–44)	31.1 (30–34)	0.67	37.4 (15–59)	35.6 (20–46)	18.9 (11–35)[C]	0.05
	80.8	18.2	85.1	0.85	93.8	40.7	23.5 [C]	0.04
Bio 25(OH)D (nmol/L)*	31.3 (13–47)	23.6 (12–33)	23.3 (22–27)	0.52	32.7 (13–50)	28.7 (16–36)	16.9 (10–27)[C]	0.01
	85.2	30.4	86.4	0.79	113.7	41.4	23.4 [C]	0.02

25(OH) D, 25-hydroxyvitamin D; DBP, vitamin D-binding protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data presented as frequencies for N; mean ± SD (mean % change) and median (quartile 1 to quartile 3) (median % change) for normal and non-normal variables respectively; superscript A denotes significant difference compared with *CC* for rs4588; superscript C denotes significance compared with *GG* for rs7041; significant at $P < 0.05$.
*Non-normal variables; significant at $P < 0.05$.

SNPs, there was a deviation in Hardy–Weinberg equilibrium in rs7041 ($P < 0.001$), whereas equilibrium was observed in rs4588 ($P > 0.05$).

The prevalence of rs7041 minor (*T*) allele among non-responders was higher than responders (59.3% versus 33.2%; Table 5). Thus, participants with minor (*T*) allele were 2.9 (1.9–4.5) times more likely to be non-responders than those with major (*G*) allele ($P < 0.001$). Similarly, the prevalence of rs4588 minor (*A*) allele was higher in non-responders than responders (28.7% versus 9.7%). Hence, participants with minor (*A*) allele were 3.7 (2.1–6.6) times

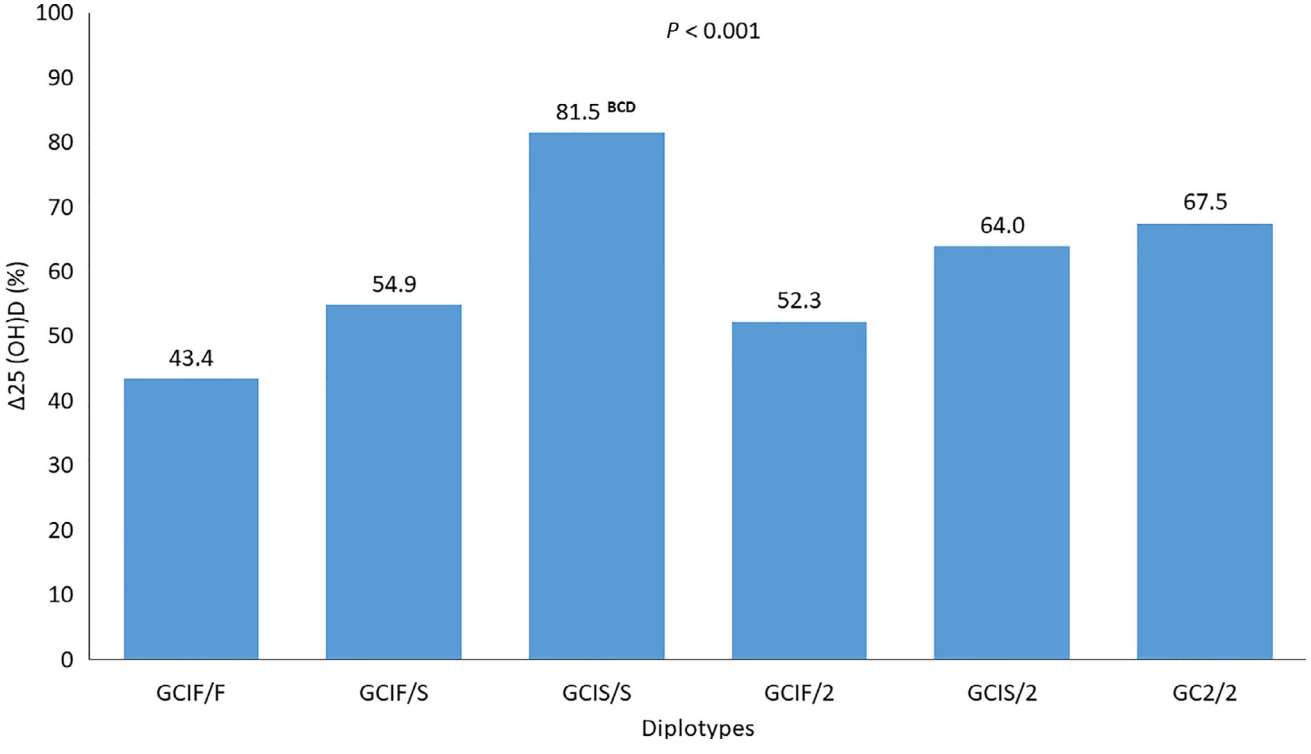

Fig. 1. Percentage change in 25-hydroxyvitamin D levels after vitamin D supplementation. Superscripts B, C, and D indicate that *GC1S/S* is significantly higher than *GC1F/2*, *GC1F/F* and *GC1S/2*, respectively.

Table 4
Baseline and follow-up characteristics of participants according to diplotypes

Parameters		*GC1F/F TT:CC*	*GC1F/S TG:CC*	*GC1S/S GG:CC*	*GC1F/2 TT:CA*	*GC1S/2 TG:CA*	*GC2/2 TT:AA*	*P*-value
N (M/F)		25 (12/13)	51 (27/24)	94 (38/56)	21 (8/13)	37 (20/17)	6 (3/3)	0.59
25(OH)D (nmol/L)	Baseline	33.5 (19–38)	36.1 (29–47)	36.7 (26–51)	25.8 (23–38)	28.3 (23–44)	25.2 (22–33)	0.057
	Follow-up	51.9 (34–63)	59.2 (45–74)	66.6 (53–84)^{ABC}	37 (32–64)	43.1 (37–52)	42.2 (39–47)	<0.001
	Δ Change	43.4	54.9	81.5 ^{ABC}	52.3	64.0	67.5	<0.001
DBP (μg/mL)	Baseline	720 (20–212)	19 (10–37)	19.3 (10–128)	22 (20–79)	19.7 (14–23)	19.2 (19–20)	0.27
	Follow-up	91.4 (22–252)	19.8 (12–50)	19.8 (10–145)	31 (20–107)	19.7 (11–23)	19.2 (19–20)	0.09
	Δ Change	40.9	26.9	2.6	0.0	4.2	0	0.15
Free 25(OH)D (nmol/L)	Baseline	7.9 (5–34)	24.6 (14–38)	16.8 (15–25)	19.8 (11–25)	27.5 (16–32)	16.8 (15–25)	0.26
	Follow-up	13.3 (9–20)	39.6 (20–47)	37.4 (15–59)	22.4 (14–46)	27.6 (24–41)	31.1 (30–34)	0.20
	Δ Change	13.1	68.4	122.6	0.4	61	85.1	0.10
Bio 25(OH)D (nmol/L)	Baseline	6.4 (4–27)	20.2 (12–34)	12.5 (11–18)	16.8 (10–21)	20.4 (12–27)	12.5 (11–18)	0.24
	Follow-up	10.7 (8–19)	31.6 (16–43)	32.7 (13–50)	21.9 (11–33)	24.5 (16–33)	23.3 (22–27)	0.12
	Δ Change	30.4	67.2	161.6	20.1	56.4	86.4	0.08

25(OH) D, 25-hydroxyvitamin D; DBP, vitamin D-binding protein; *GC1F/2*, xxxx; *GC1F/F*, xxxx; *GC1F/S*, xxxx.
Data presented as median (quartile 1 to quartile 3) and Δ change; superscripts A, B and C indicate significant differences compared with *TT:CC*, *TT:CA*, and *TG:CA* respectively; significant at $P < 0.05$.

more likely to be non-responders than those with major (*C*) allele ($P < 0.001$; Table 5). This significance persisted even after adjustments for age, sex, BMI, baseline 25(OH)D concentration, and other alleles. The prevalence of rs7041 homozygous major (*GG*) genotype was lowest among non-responders at 17.3% followed by homozygous (*TT*) minor and heterozygous (*TG*) genotype at 46.7% and 36%, respectively. The significant ORs (6.2; 95% CI, 2.7–14.4 and 4.2 95% CI, 2–8.9) indicate that participants with homozygous minor (*TT*) and heterozygous (*TG*) genotype were 6.2 and 4.2 times more likely to be non-responders than those with the homozygous major (*GG*) genotype ($P < 0.001$), respectively (Table 5). The prevalence of rs4588 homozygous minor (*AA*) genotype was 0.9% in responders compared with homozygous major (*CC*) and heterozygous (*CA*) genotypes with 81.4% and 17.7%, respectively. Last, participants with heterozygous (*CA*) and homozygous minor (*AA*) genotypes were 12.4 (1.4–11) and 4.1 (2.1–8) times more likely to be non-responders than those with homozygous major (*CC*) genotype, respectively (Table 5). The significance persisted even after adjustments for age, sex, BMI, and baseline 25(OH)D levels.

Discussion

The present study examined the efficacy of vitamin D supplementation according to DBP gene polymorphisms (rs4588 and rs7041) in a cohort of vitamin D–deficient Saudi Arabian participants. The associations of DBP SNPs with baseline circulating total, free, and bioavailable 25(OH)D levels and other serologic parameters were evaluated. The major finding of the present study indicated that certain genotypes or diplotypes of the rs7041 and rs4588 alleles significantly increases the risk for non-response to vitamin D correction even at higher doses. This has significant clinical implications because carriers of such genotypes or diplotypes render current vitamin D management schemes ineffective, predisposing such individuals to a higher risk for vitamin D deficiency and its related diseases.

The *GC* gene encodes the 52 to 59 kDA DBP protein, which regulates bioavailability and plays a key role in transporting vitamin D and its metabolites to various tissues. *GC* gene polymorphisms might therefore explain variations in total serum, free, and bioavailable 25(OH)D levels. Several studies have reported the association of more than ten SNPs in *GC* gene and serum 25(OH)D levels, and the most consistent association was detected with two nonsynonymous SNPs 4588 (Thr → Lys) and rs7041 polymorphisms (Asp → Glu) [1,2,6]. Different combinations of rs7041 and rs4588 polymorphisms in GC gene result in three major isoforms of DBP, namely *Gc1 F*, *Gc1 S*, and *Gc2* phenotypes with different binding affinities for 25(OH)D (*Gc1 F*>*Gc1 S*>*Gc2*) [11]. Several studies have reported the association between *GC* gene polymorphisms and serum 25(OH)D concentration. One of the studies involving 3210 Han Chinese found a significant association between rs4588 and rs7041 polymorphisms with serum 25(OH)D levels [8]. Similarly, Engelman et al. also showed a significant association between

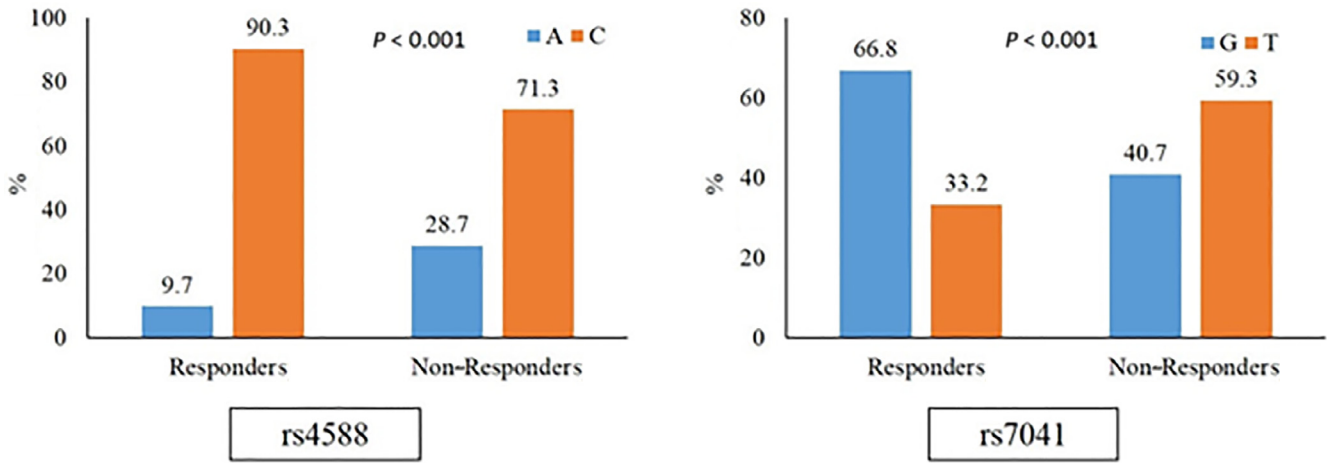

Fig. 2. Allelic distribution according to response to vitamin D intervention.

Table 5
Risk of non-response to Vitamin D supplementation according to DBP alleles and genotypes

	Responders	Non-responders	OR (95% CI)	*P*-value	Adjusted OR*	*P*-value*
Allele						
rs4588						
C (major allele)	204 (90.3)	107 (71.3)	1			
A (minor allele)	22 (9.7)	43 (28.7)	3.7 (2.1–6.6)	<0.001	3.4 (1.8–6.3)	<0.001
rs7041						
G (major allele)	151 (66.8)	61 (40.7)	1			
T (minor allele)	75 (33.2)	89 (59.3)	2.9 (1.9–4.5)	<0.001	3.2 (2–5.3)	<0.001
Genotype						
rs4588						
CC (homozygous major)	92 (81.4)	37 (49.3)	1			
CA (heterozygous)	20 (17.7)	33 (44)	12.4 (1.4–110)	0.023	5.7 (0.6–57)	0.14
AA (homozygous minor)	1 (0.9)	5 (6.7)	4.1 (2.1–8)	<0.001	2 (0.9–4.4)	0.10
rs7041						
GG (homozygous major)	57 (50.4)	13 (17.3)	1			
TG (heterozygous)	37 (32.7)	35 (46.7)	4.2 (1.9–8.9)	<0.001	3.2 (1.3–8.2)	0.01
TT (homozygous minor)	19 (16.8)	27 (36)	6.2 (2.7–14.4)	<0.001	4.7 (1.7–13.2)	0.003

Data presented as frequencies (%) and OR with 95% CI.
*P-value adjusted for age, sex, body mass index, baseline 25-hydroxyvitamin D concentration, and other alleles; significant at $P < 0.05$.

these *GC* gene polymorphisms and 25(OH)D levels [9]. The rs7041 polymorphism has been observed to be significantly associated with 25(OH)D in other ethnic groups such as Kuwaiti Arabs and South Asians [10]. In agreement with these studies, the results from present study indicated that homozygous major genotypes of rs7041 and rs4588 SNPs are associated with increased 25(OH)D concentrations. Similarly, *GC1S/S* diplotypes showed a trend toward higher baseline 25(OH)D levels than other diplotypes. These significant associations persisted even after adjusting for age, sex, and BMI.

Although the association between DBP genotypes and serum 25 (OH)D concentration was already identified in several studies, few have examined the effects of these genotypes in terms of efficacy of response to vitamin D supplementation. The present analysis revealed that post-supplementation 25(OH)D levels were significantly higher in participants with homozygous major genotype than with heterozygous and homozygous minor genotypes of rs4588, respectively. The overall increase in 25(OH)D levels in homozygous major genotype group was 70.5% from the baseline, whereas the heterozygous genotype increase was 56.9% from baseline. Similarly, postsupplementation 25(OH)D levels in participants with the homozygous genotype was significantly higher than heterozygous and homozygous minor genotype in rs7041. The increase in 25(OH)D levels in the homozygous major genotype was 81.5% from baseline compared with 58.3% and 52.7% increase in participants with heterozygous and homozygous minor genotypes, respectively. Furthermore, participants with heterozygous or homozygous minor variants had less effective responses to supplementation. Consequently, participants with normal genotypes (homozygous major) showed better responses. Thus, we speculate that individuals with these polymorphisms may be at higher risk for chronic vitamin D deficiency owing to allelic variations.

The association of DBP genotypes with higher 25(OH)D levels are shown to be related to DBP concentrations. DBP genotype-based variations in serum DBP concentrations have been reported previously, but the results were not consistent. A study in the United States showed that individuals with *GC1S* homozygotes had the highest serum DBP concentration, whereas those with *GC1F* had the lowest [4,21]. On the other hand, other studies have detected lower DBP concentrations in *GC2* homozygotes [5,17]. Similarly, Powe et al. showed that T allele at rs7041 was associated with decreased DBP levels, which in turn was associated with serum 25(OH)D levels among black Americans [20]. The same study reported that the high prevalence of DBP gene variants among black Americans resulted in lower levels of DBP and total 25(OH)D but have similar calculated bioavailable 25-(OH) D to their white counterparts [20]. Other investigators have challenged this view owing to concerns about the accuracy of DBP measurement because differences in serum DBP levels among blacks and whites using a monoclonal assay were absent with the use of polyclonal antibodies or liquid chromatography with tandem mass spectrometry methods [22,23]. In contrast, no significant effects of DBP genotypes or diplotypes either at baseline or post-supplementation were found on serum DBP levels in the present study. The current results can be expanded by using polyclonal assays or mass spectrometry because the use of monoclonal ELISA in the present study could be one factor that explains the lack of association between DBP genotypes and serum DBP levels.

Other factors including L-cysteine and glutathione (GSH) have been recently observed to influence DBP status [24]. GSH levels can modify DBP status as improvements in GSH via L-cysteine supplementation upregulates DBP levels [24, 25]. The observed null effect of DBP genotypes or diplotypes on DBP levels in the present study could be partially explained by the GSH status, which was not measured in the studied participants.

Oral vitamin D supplementation is an easy and cost-effective strategy to correct vitamin D deficiency. However, there is an ongoing debate regarding the recommended vitamin D dose for the general population [26]. It has been reported that there is a wide interindividual variation in terms of response to a given dose of vitamin D [27]. A systematic review indicated that 34.5% of variations in serum 25(OH)D levels post-vitamin D supplementation can be explained by body weight, 9.8% by type of supplement (D_2 or D_3), 3.7% by age, 2.4% by calcium intake, 1.9% by baseline 25(OH) D concentrations, and the remaining 50% by unknown factors [28]. In a recent randomized trial, Yao et al. demonstrated that genetic factors exert more effect than non-genetic factors in 25(OH)D response to vitamin D supplementation [29]. In the present study, almost 20% of the participants remained vitamin D deficient even after receiving different doses of vitamin D for 6 mo, suggesting that DBP gene variants can modify 25(OH)D responses to vitamin D supplementation. In agreement with our results, Nissen et al. demonstrated that carriers of rare risk alleles at rs4588 had the smallest increase in serum 25(OH)D among Danes receiving vitamin D–fortified bread and milk or ultraviolet B treatment [30]. Similarly, Nimitphong et al. showed that individuals with the rs4588 homozygous major genotype had the highest response in 25(OH)D than other genotypes [31]. Given the results of the present study

and previous observations, genetic screening for common DBP SNPs and other genes associated with vitamin D deficiency may be performed before prescribing mega doses of vitamin D supplementation to non-responsive but compliant patients.

The present study had some limitations. Findings should be interpreted with caution regarding rs7041 because Hardy–Weinberg equilibrium was not achieved. This could have been due to sample size limitations. To our knowledge, the study nevertheless is the first to document in a homogenous Arab population that DBP SNPs significantly influence efficiency of response to vitamin D therapy.

Conclusions

Data from the present study suggest that SNPs in the *GC* gene are associated with variations in baseline 25(OH)D levels and response to vitamin D supplementation in the Saudi Arabian population. Carriers of rare alleles of rs4588 and rs7041 GC SNPs showed the lowest baseline 25(OH)D levels and exhibited the lowest increase in serum 25(OH)D levels after vitamin D supplementation. This suggests that a higher dose of vitamin D may be needed to achieve sufficient levels because patients carrying homozygous variants exhibit a less efficient response to vitamin D supplementation

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.02.003.

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