Common variants in *CYP2R1* and *GC* genes are both determinants of serum 25-hydroxyvitamin D concentrations after UVB irradiation and after consumption of vitamin D₃–fortified bread and milk during winter in Denmark[1–4]

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ABSTRACT

Background: Little is known about how the genetic variation in vitamin D modulating genes influences ultraviolet (UV)B–induced 25-hydroxyvitamin D [25(OH)D] concentrations. In the Food with vitamin D (VitmaD) study, we showed that common genetic variants rs10741657 and rs10766197 in 25-hydroxylase (*CYP2R1*) and rs842999 and rs4588 in vitamin D binding protein (*GC*) predict 25(OH)D concentrations at late summer and after 6-mo consumption of cholecalciferol (vitamin D₃)–fortified bread and milk.

Objectives: In the current study, called the Vitamin D in genes (VitDgen) study, we analyzed associations between the increase in 25(OH)D concentrations after a given dose of artificial UVB irradiation and 25 single nucleotide polymorphisms located in or near genes involved in vitamin D synthesis, transport, activation, or degradation as previously described for the VitmaD study. Second, we aimed to determine whether the genetic variations in *CYP2R1* and *GC* have similar effects on 25(OH)D concentrations after artificial UVB irradiation and supplementation by vitamin D₃–fortified bread and milk.

Design: The VitDgen study includes 92 healthy Danes who received 4 whole-body UVB treatments with a total dose of 6 or 7.5 standard erythema doses during a 10-d period in winter. The VitmaD study included 201 healthy Danish families who were given vitamin D₃–fortified bread and milk or placebo for 6 mo during the winter.

Results: After UVB treatments, rs10741657 in *CYP2R1* and rs4588 in *GC* predicted UVB-induced 25(OH)D concentrations as previously shown in the VitmaD study. Compared with noncarriers, carriers of 4 risk alleles of rs10741657 and rs4588 had lowest concentrations and smallest increases in 25(OH)D concentrations after 4 UVB treatments and largest decreases in 25(OH)D concentrations after 6-mo consumption of vitamin D₃–fortified bread and milk.

Conclusion: Common genetic variants in the *CYP2R1* and *GC* genes modify 25(OH)D concentrations in the same manner after artificial UVB-induced vitamin D and consumption of vitamin D₃–fortified bread and milk. The VitDgen study was registered at clinicaltrials.gov as NCT01741233. The VitmaD study was registered at clinicaltrials.gov as NCT01184716. *Am J Clin Nutr* 2015;101:218–27.

INTRODUCTION

Vitamin D deficiency is a common health problem in many countries (1). It is well recognized that vitamin D is important for maintaining bone health. Traditional clinical conditions linked to vitamin D deficiency are rickets in children and osteromalacia and osteroporosis in adults (1). A sufficient vitamin D status, which is measured as the 25-hydroxyvitamin D [25(OH)D][5] concentration in blood, may be associated with lower risk of several nonskeletal adverse health outcomes including autoimmune diseases, some cancers, risk of hypertension, and overall mortality (2, 3).

In humans, vitamin D can be obtained from the following 2 natural sources: *1*) the majority of vitamin D is synthesized in the skin after solar UVB exposure; and *2*) dietary intake contributes with a small amount of vitamin D because few natural foods contain significant amounts of vitamin D (1). Furthermore, vitamin D can be obtained from multivitamin tablets, vitamin D supplements, or fortified food products. In Northern countries, vitamin D concentrations follow the seasonal variation in UVB-fluence rates. Vitamin D cannot be synthesized in the skin during the winter months (from October to March) in latitudes above 40°N because of negligible UVB irradiation (4).

Several studies have indicated that the genetic variation at specific genes involved in vitamin D synthesis, transport, activation, or degradation may influence 25(OH)D concentrations appreciably (5). This effect may explain the observed interindividual variation in 25(OH)D concentrations, which seems to be independent of latitude (6). Two genome-wide association studies of vitamin D (7, 8) confirmed associations of common variants at 3 loci in vitamin D binding protein (*GC*; vitamin D transport), 25-hydroxylase [*CYP2R1*; hydroxylation of vitamin D to 25(OH)D] and 7-dehydrocholesterol reductase (*DHCR7*; involved in cholesterol synthesis from 7-dehydrocholesterol) genes. Risk of vitamin D insufficiency more than doubles for individuals carrying all risk alleles of all 3 loci (8), indicating that 25(OH)D concentrations do not only depend on vitamin D intake and UVB exposure but also on the genetic variation. A better understanding of how genetic variation influences 25(OH)D concentration after UVB exposure or consumption of vitamin D supplements is needed and may help to identify individuals who substantially elevated risk of developing vitamin D deficiency.

In the Vitamin D in genes (VitDgen) study [clinicaltrials.gov; NCT01741233], associations between 25 single nucleotide polymorphisms (SNPs) located in or near genes involved in vitamin D synthesis, transport, activation, or degradation and the increase in 25(OH)D concentration after a given dose of artificial UVB irradiation during a winter period of 10-d were examined in 92 healthy Danish adults. Furthermore, the effect of a genetic variation in *CYP2R1* and *GC* on 25(OH)D concentrations was compared for vitamin D acquired from artificial UVB irradiation (the VitDgen study) or from the food with vitamin D (VitmaD) study consumption of cholecalciferol (vitamin D₃)–fortified bread and milk (clinicaltrials.gov; NCT01184716).

SUBJECTS AND METHODS

Study population and design

The main focus of this article is on the VitDgen study, which analyzes the association between the increase in 25(OH)D concentration after a given dose of artificial UVB irradiation and 25 widely studied SNPs located in or near genes involved in vitamin D synthesis, transport, activation, or degradation. Second, the study aimed to determine whether genetic variations in *CYP2R1* and *GC* have similar effects on 25(OH)D concentrations after artificial UVB irradiation and supplementation by vitamin D₃–fortified bread and milk. Data from the VitmaD study were used to analyze the genetic effect on 25(OH)D after 6 mo of consumption of vitamin D₃–fortified bread and milk, which previously have been described (9–12).

VitmaD study

The VitmaD study, which was a double-blinded, randomized, placebo-controlled intervention trial, was conducted in the Gladsaxe Municipality in Denmark (latitude 56°N) from late summer to the end of winter (September 2010 to April 2011). The study design and methods were described in detail elsewhere (9–12), and thus, this article is not the first presentation of the 25(OH) response to vitamin D₃ fortification on the basis of the VitmaD study (12). In brief, healthy, ethnically Danish families were allocated either vitamin D₃–fortified bread (5.2 ± 0.3 μg vitamin D/100 g in wheat bread and 4.3 ± 0.3 μg vitamin D/100 g in rye bread) and milk (0.40 ± 0.01 mg/100 L) or placebo for 6 mo during the winter from September 2010 to April 2011 (**Supplemental Figure 1**). The study was conducted according to the guidelines in the Declaration of Helsinki, and the protocol was approved by the Danish ethics committee (H-4–2010-020). All participants gave written informed consent.

VitDgen study

The VitDgen study was an open and controlled clinical trial conducted at Bispebjerg University Hospital, Copenhagen, Denmark (latitude 56°N) during late winter and early spring (January to March 2013) when natural solar UVB irradiation is negligible (Supplemental Figure 1). Furthermore, the cold winter temperatures prevent solar exposure except on the face and hands. All recruited participants were healthy Danes (aged 18–60 y; men and women) with residence in Denmark. Power calculations indicated that a sample size of 78 participants should be sufficient to detect a mean difference of 20 nmol/L between a genetic outcome at the 5% significance level and with 80% power. There were 102 participants included, and 92 participants completed the study (**Supplemental Figure 2**).

Inclusion criteria were healthy Caucasians between 18–60 y of age. Exclusion criteria were the following: *1*) having a skin disease, *2*) taking a medication that influenced vitamin D metabolism or caused photosensitive skin, *3*) pregnancy or breastfeeding, *4*) having had a sun or ski vacation 3 mo before the study period, or *5*) having taken vitamin D supplements 3 mo before the study period. Participants were allowed to take a daily food supplement that contained ≤10 μg vitamin D. Participants were instructed not to use cosmetic makeup with UV filters or sunscreen when receiving UVB treatment. The study was conducted according to the guidelines in the Declaration of Helsinki, and the protocol was approved by the Danish ethics committee (H-4–2012-071). All participants gave written informed consent.

Skin type, pigmentation, and redness

In the VitDgen study, a skin reflectance meter (UV-Optimize, Scientific, Chromo-light) (13) was used to measure the percentage of redness (range: 0–100%) and the pigment protection factor (PPF; range: 1.0–24.0) on the forehead, shoulder (facultative pigmentations), and buttock (constitutive skin pigmentation) at baseline and 2 d after the last UVB treatment. This assessment was done to follow the skin response to UVB treatments. The percentage of redness reflects hemoglobin concentrations in the skin, and the PPF reflects melanin concentrations in the skin (14, 15).

Self-reported skin-type according to Fitzpatrick's classifications I–IV (16) was registered at baseline. Classifications of erythema and tanning reactions to first exposure in summer where skin type I represents always burn and never tan, skin type II represents usually burn and less tan than average (with difficulty), skin type III represents sometimes mild burns and tan about average, and skin type IV represents rarely burn and tan more than the average (with ease). There were 9 participants with skin type I, 29 participants with skin type II, 39 participants with skin type III, and 14 participants with skin type IV.

UVB exposure

While wearing underwear (underpants and bra for female participants), participants' body surfaces were equally exposed to UV radiation in a UV cabin (Waldmann UV1000L; Waldmann GmbH) equipped with a broadband UVB source consisting of 26 UV6 tubes (Waldmann GmbH) emitting radiation mainly between 290 and 350 nm. During the treatment period, the UV intensity was weekly controlled by using a Sola-Hazard spectroradiometer (Solatell).

A total of 92 participants completed the VitDgen study. During a 10-d period, participants received artificial UVB irradiation 4 times with a 2- or 3-d interval (Monday, Wednesday, Friday, and Monday). Standard erythema doses (SEDs) are a standardized measure of the accumulated erythemally weighted UV energy. One SED is equivalent to an erythemal effective radiant exposure of 100 Jm^{-2} at 298 nm by using the International Committee of Illumination erythema action spectrum and corresponds to a UV dose that causes perceptible erythema in the most–sun-sensitive individuals (17, 18). For example, 1.5 SEDs are equivalent to ∼15 min sun exposure in the middle of a clear summer day in Denmark (56°N). A total of 23 participants received a total dose of 7.5 SEDs (1 × 3 SEDs for the upper body and 3 × 1.5 SEDs for the whole body). After the first UVB exposure, 4 participants experienced erythema and withdrew from the study. Therefore, the SED dose was subsequently lowered to 1.5 SEDs and given on the whole body to minimize risk of erythema. Whole-body 1.5 SEDs were well tolerated, and none of the participants experienced erythema after these changes. Seventy-nine participants received a total dose of 6 SEDs (4 × 1.5 SEDs for the whole body). At the end of the study, an additional 6 participants withdrew from the study because of personal and other reasons (Supplemental Figure 2).

DNA extraction and genotyping

DNA was purified from buffy coats as described by Miller et al. (19). SNPs were genotyped by using a Sequenom platform and iPLEX Gold reaction. SNPs and the primers used are listed in **Supplemental Table 1**. Polymerase chain reaction (PCR) amplifications were carried out in 5-μL volumes containing the following: 10 ng genomic DNA, 0.5 U HotStart Taq (Qiagen), 1.25 × Enzyme Buffer (Qiagen), 3.5 mmol/L $MgCL_2$, and 1 mmol/L of each deoxynucleotide, and a final primer concentration of 500 mmol/L for each primer was added (Supplemental Table 1). PCRs were performed at the following cycling variables: a 15-min preheat to 94°C, 45 cycles (20 s at 94°C, 30 s at 56°C, and 1 min at 72°C) followed by 3 min at 72°C, and storage at −20°C. PCR products were treated with shrimp alkaline phosphatase, and the dephosphorylation of unincorporated de-oxyribonucleotide triphosphates and an extension with molecular weight-modified nucleotides were performed in accordance with the manufacturer's recommendations. PCR reactions were cleaned with resin and dispensed on SpectoCHIP bioarrays (Sequenom). The SpectroCHIP bioarrays were placed in a Matrix-assisted laser desorption/ionization Time of Flight mass spectrometer, and the results were analyzed by using MassARRAY Type 4.0 SNP genotyping (Sequenom) (9).

All SNPs analyzed were located in or near genes involved in vitamin D synthesis, transport, activation, or degradation. The following SNPs were selected because of evidence of a significant association in previous studies: *CYP2R1* (rs7116978, rs10741657, rs1562902, and rs10766197), 24-hydroxylase (*CYO24A1*) (rs6013897, rs4809960, rs2296241, rs17219315, and rs2426496), 1-α-hydroxylase (*CYP27B1*) (rs10877012), open-reading frame 88 on chromosome 10q26.13 (*C10orf88*) (rs6599638), 7-dehydrocholesterol reductase/nicotiamide adenine dinucleotide synthetase-1 (*DHCR7/NADSYN1*) (rs1790349 and rs12785878), *GC* (rs16846876, rs12512631, rs17467825, rs2882679, rs842999-triallelic, rs4588, rs222020, and rs2298849), and vitamin D receptor (*VDR*) [rs731236 (*TaqI*), rs757343 (*TruI*), rs10783219, and rs7139166). For the triallelic rs842999, there was a dose-dependent relation between 25(OH)D concentrations and carriers of no, 1, or 2 copies of the G allele, and genotypes are presented as GG, GX, and XX, where X represents C or A alleles (9). The linkage disequilibrium (LD) structure was evaluated by using Pearsons' *r* and the SNP Annotation and Proxy Search (SNAP) version 2.2 (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php).

Genotyping was successful in the 102 recruited participants. For quality control, 10%-duplicated samples were randomly placed throughout each of the 384-well plates, and the reproducibility was 100%. No deviation from the Hardy-Weinberg equilibrium was observed (chi-square test; Bonferroni *P* of 0.05/25 SNPs = 0.002).

Measurement of 25(OH)D concentrations

Blood samples were obtained without previous fasting, and sera were stored in aliquots at −20°C until analysis. Measurements of 25(OH)D concentrations relied on the determination of both 25(OH)D_2 and 25(OH)D_3 and were conducted by isotope-dilution liquid-chromatography–tandem mass spectrometry at the Clinical Biochemical Department, Holbæk Hospital, Holbæk, Denmark. 25(OH)D concentrations were measured at baseline and 48 h after the last UVB treatment.

Standard reference material, vitamin D in humans (SRM972), from the National Institute of Standards and Technology (United States) was used as the primary calibrator. The analytic quality of the 25(OH)D assay was assured by Vitamin D External Quality Assessment Scheme certification, and the mean bias was 5.7%.

Statistical analyses

Statistical analyses were performed with the SAS Enterprise Guide 6.1 application (SAS Institute Inc.). 25(OH)D concentrations were log transformed to approximate a normal distribution, and all means are presented as geometric means. A nominal *P* value of 0.05 was considered statistically significant. Data from the 2 study-populations VitDgen and VitmaD were analyzed in the same manner to compare how vitamin D status is affected by the genetic risk score (GRS) after UVB exposure or vitamin D supplementation.

In the VitDgen study, univariate models were performed to assess the association between baseline 25(OH)D concentrations and each of the following sun- and vitamin D–related variables: ski or sun vacation in the preceding 6-mo period (yes or no), sun preference (prefers sun, sometimes in the sun, or avoids the sun), sun bathing (yes, sometimes, or no), sunscreen use (always, most of the times, sometimes, or seldom/never), outdoor stay in light clothes (most of the time, often, sometimes, or seldom/never), outdoor transport to work (<15, 15–30, 30–60, or >60 min/d), preferring outdoor life (yes, sometimes, or no), working outdoor (always indoor, sometimes outdoor, or outdoor some of the day), sunbed use during the preceding year (yes or no), PPF buttock, Fitzpatrick's skin type (I–IV), and consuming fish (yes or no). Significant baseline ($P < 0.05$) sun- and vitamin D–related variables were included in a linear mixed model, with the following covariates: sex (male and female), age (18–58 y), BMI (underweight, normal weight, overweight, and obese) according to WHO international standards for adults (20), multivitamin use (yes or no), and vitamin D supplement use in the preceding 6 mo (yes or no). Several of the recruited participants were family members (couples: $n = 30$; parent/children: $n = 9$) and all linear mixed models were analyzed with family as a random factor to account for the nonindependency of these participants. Data on sun- and vitamin D–related variables and, in addition, age, sex, BMI, and multivitamin- and vitamin D–supplement use were obtained from a self-administered web-based questionnaire.

No difference in the increase in 25(OH)D concentrations after UVB treatments between the 2 different UVB treatment groups and sex ($P = 0.8871$, data not shown) was shown, and linear mixed models were combined for the 2 UVB treatment groups and adjusted for the following covariates: age, sex, BMI, family as a random factor, and baseline serum 25(OH)D concentration.

In both studies, a GRS was calculated as the sum of the number of risk alleles. The GRS (range: 0–4) was calculated as the sum of the number of G alleles of rs10741657 and A alleles of rs4588. A linear mixed model, which was adjusted for age, sex, BMI, baseline 25(OH)D concentration, and family as a random factor and, in addition, vacation, vitamin D intake, and vitamin D-supplementation use for the VitmaD study, was fitted to log 25(OH)D concentrations with the GRS as an explanatory factor. Adjusted mean concentrations of 25(OH)D were calculated for each GRS. For the VitmaD study, the GRS was calculated for the adult population (18-60 y) at baseline ($n = 414$) and end of the study only for the adult population who consumed vitamin D_3–fortified bread and milk ($n = 208$). The percentage decrease in vitamin D status in relation to the GRS was analyzed in the adult population who participated in the fortification group.

RESULTS

Out of a total of 102 recruited participants in the VitDgen study, 92 participants completed the study fully (submitted blood samples and genotypes and completed the questionnaire). Baseline characteristics of participants are shown in **Table 1**. At baseline, 51% of subjects had adequate concentrations of vitamin D (>50 nmol/L), 43% of subjects were vitamin D insufficient (25–50 nmol/L), and 5% of subjects were vitamin D deficient (<25 nmol/L). At the end of the study, 97% of subjects had adequate concentrations of vitamin D, 3% of subjects were

vitamin D insufficient, and none of the subjects were vitamin D deficient. On average, 25(OH)D concentrations increased 28 nmol/L (95% CI: 24.1, 31.1 nmol/L; data not shown) in response to the 4 UVB sessions.

In univariate models, the baseline 25(OH)D concentration was significantly associated with BMI ($P = 0.032$), multivitamin use ($P = 0.011$), and vitamin D–supplement use (supplementation ≤ 10 μg/d was allowed; $P = 0.0014$) and borderline significantly associated with outdoor stay in light clothes ($P = 0.063$), outdoor transport to work ($P = 0.051$), and sun bathing ($P = 0.051$). No associations were shown between baseline 25(OH)D concentrations and skiing or a sun vacation (compared with no vacation; $P = 0.23$), Fitzpatrick's skin-type classifications I–IV ($P = 0.78$), PPF buttock ($P = 0.60$), fish intake ($P = 0.34$), sunbed use ($P = 0.78$), sun preference ($P = 0.14$), sunscreen use ($P = 0.96$), working indoors ($P = 0.16$), employment ($P = 0.17$), or preferring outdoor life ($P = 0.27$), and thus, these variables were not included in the linear mixed models.

In a linear mixed model, there was no significant difference between the baseline 25(OH)D concentration in analyzed genotypes, except for rs12512631 in *GC,* after adjustment for the following variables: age, sex, BMI, use of multivitamin and vitamin D supplement, outdoor stay in light clothes, and sun bathing (**Table 2**). No significant difference was shown for age, sex, and outdoor transport to work for all analyzed genotypes (data not shown).

In a linear mixed model adjusted for age, sex, BMI, and baseline 25(OH)D, there was a significant association between end-of-study 25(OH)D concentrations and genotypes of rs10741657 in *CYP2R1* and rs16846876, rs17467825, rs2282679 and rs4588 in *GC* after 4 UVB treatments (Table 2). All 4 SNPs in *GC* were in strong LD. SNP rs4588 was in strong LD with rs2282679 (Pearson's $r = 0.99$, SNAP $R^2 = 0.98$, $D' = 1.00$) and rs17467825 (Pearson's $r = 0.99$, SNAP $R^2 = 1.00$, $D' = 1.00$). Furthermore, rs17467825 and rs2282679 (Pearson's $r = 1.00$, SNAP $R^2 = 1.00$, $D' = 1.00$) as well as rs2282679 and rs16846876 (Pearson's $r = 0.69$, SNAP $R^2 = 0.44$, $D' = 0.68$) were in LD. We previously showed that rs4588 had the strongest association with 25(OH)D concentrations (9). Additional analyses only included rs4588 in *GC* and rs10741657 in *CYP2R1*. None of the analyzed SNPs in *CYP24A1*, *CYP27B1*, *C10orf88*, *DHCR7/NADSYN1*, or *VDR* genes were significantly associated with the final 25(OH)D concentration.

For the rs10741657 polymorphism, highest end-of-study 25(OH)D concentrations were shown for participants carrying the rs10741657 AA genotype (93.7 nmol/L; 95% CI: 84.0, 104.6 nmol/L), intermediate concentrations were shown in participants carrying the rs10741657 GA genotype (81.9 nmol/L; 95% CI: 75.5, 88.9 nmol/L), and lowest concentrations were shown in participants carrying the rs10741657 GG genotype (77.0 nmol/L; 95% CI: 70.9, 83.5 nmol/L). For the rs4588 genotype, highest end-of-study 25(OH)D concentrations were shown in participants carrying the rs4588CC genotype (84.1 nmol/L; 95% CI: 78.3, 90.4 nmol/L), intermediate concentrations were shown in participants carrying the rs4588 CA genotype (83.5 nmol/L; 95% CI: 77.0, 90.6 nmol/L), and lowest concentrations were shown in participants carrying the rs4588 AA genotype (65.7 nmol/L; 95% CI: 54.5, 79.3 nmol/L) (Table 2).

To determinate combined effects of rs10741657 and rs4588 in the VitDgen study, a GRS was calculated as the sum of the

TABLE 1
Characteristics of the VitDgen study population[1]

	All (n = 92)		F (n = 60)		M (n = 32)	
	n	Value	n	Value	n	Value
Age, y	92	38.6 ± 12.0[2]	60	38.1 ± 11.6	32	39.6 ± 12.9
BMI,[3] kg/m^2						
Underweight (<18.5)	3	18.0 ± 0.4	2	18.0 ± 0.6	1	18.0
Normal weight (18.5–24.9)	57	22.1 ± 1.8	41	22.1 ± 1.9	16	22.3 ± 1.8
Overweight (25.0–29.9)	23	26.7 ± 1.3	11	26.8 ± 1.2	12	26.6 ± 1.5
Obese (>30.0)	9	33.6 ± 3.9	6	32.8 ± 4.1	3	35.5 ± 3.5
Baseline 25(OH)D, nmol/L						
>50	47	78.1 ± 21.8	32	80.6 ± 22.6	15	72.8 ± 19.6
25–50	40	38.2 ± 7.1	25	38.2 ± 7.2	15	38.2 ± 7.1
<25	5	20.4 ± 4.2	3	18.3 ± 4.4	2	23.5 ± 0.7
End 25(OH)D, nmol/L						
>50	89	86.5 ± 22.5	57	87.4 ± 25.7	32	84.8 ± 15.4
25–50	3	46.3 ± 2.9	3	46.3 ± 2.9	—	—
<25	—	—	—	—	—	—
Sun or ski vacation,[4] n (%)		45 (49)		31 (52)		14 (44)
Supplement users 6 mo before the intervention, n (%)						
Multivitamins		19 (21)		14 (23)		5 (16)
Vitamin D		8 (9)		5 (8)		3 (9)
Consuming fish, n (%)						
Yes, total		86 (95)		58 (97)		28 (90)
1–2 times/wk		60 (66)		40 (67)		20 (65)
≥3 times/wk		26 (29)		18 (30)		8 (26)
No		5 (5)		2 (3)		3 (10)
Fitzpatrick skin type, n (%)[5]						
I		9 (10)		6 (10)		3 (9)
II		29 (32)		20 (34)		9 (28)
III		39 (43)		25 (42)		14 (44)
IV		14 (15)		8 (14)		6 (19)
PPF[6]						
Forehead	92	5.5 ± 1.5	60	5.5 ± 1.6	32	5.5 ± 1.3
Shoulder	92	5.1 ± 1.4	60	5.4 ± 1.3	32	4.6 ± 1.4
Buttock	92	3.4 ± 1.1	60	3.6 ± 1.1	32	3.1 ± 1.0
Sunbed use in 2012, n (%)						
Did not use a sunbed		83 (90)		52 (87)		31 (97)
1–4 times		3 (3)		2 (3)		1 (3)
≥5 times		6 (7)		6 (10)		—

[1]PPF, pigment protection factor; VitDgen, Vitamin D in genes; 25(OH)D, 25-hydroxyvitamin D.
[2]Geometric mean ± SD (all such values).
[3]On the basis of WHO international standards for adults (20).
[4]Ski or sun vacation 6 mo before the study in places where dermal vitamin D production was expected.
[5]Fitzpatrick skin type categorization on the basis of sun-reactive types I–IV (16).
[6]PPF (range: 1.0–24.0) reflects melanin concentrations in the skin at baseline.

number of G alleles of rs10741657 and A alleles of rs4588 (range: 0–4) at baseline and final (**Figure 1**A). Coefficients of rs10741657 and rs4588 were very similar in a mixed regression model including both SNPs, and therefore, it was not necessary to weight risk alleles by the correlation coefficient (data not shown). At baseline, there were no associations between GRS and 25(OH)D concentrations ($P = 0.16$). At the end of the study, there was a linear negative trend between the 25(OH)D concentration and number of risk alleles (0-4 risk alleles; $P = 0.0045$). Overall, there was a mean difference in 25(OH)D concentrations of 20.9 nmol/L between carriers of no risk alleles and carriers of all 4 risk alleles. Furthermore, there was a significant linear negative trend between the increase in 25(OH)D concentration and the GRS ($P = 0.042$) (Figure 1B). The lowest increase in 25(OH)D concentrations was observed for carriers of all 4 risk alleles.

To evaluate the effect of rs10741657 and rs4588 on 25(OH)D concentrations at baseline and after 6 mo consumption of vitamin D$_3$–fortified bread and milk, data from the adult population of the VitmaD study (15, 20, 21) were used and analyzed in the same manner as previously described for the VitDgen study. At baseline (late summer; all adults: $n = 414$), there was a linear negative trend between the 25(OH)D concentration and carriage of 0–4 risk alleles ($P < 0.0001$) (Figure 1C). After a 6-mo consumption of vitamin D$_3$–fortified bread and milk (only adults in the fortification group: $n = 208$), there was still a linear negative trend between the 25(OH)D concentration and carriage of 0–4 risk alleles ($P = 0.0270$). With the use of a realistic vitamin D$_3$–fortification model, a decrease in 25(OH)D concentrations was observed during the winter, and the largest percentage decrease was observed for carriers of all 4 risk alleles (Figure 1D).

TABLE 2
Basic characteristics of individual SNPs and associations with 25(OH)D concentrations in the VitDgen study population ($n = 92$)[1]

SNP	HWE, P	MAF, %	M/m	Genotype	n	Baseline (day 0) 25(OH)D	P-adjusted[3]	End (day 10) 25(OH)D	Increase in 25(OH)D[2] 25(OH)D	P-adjusted[4]
CYP2R1										
rs7116978	0.11	39.5	C/T	CC	37	50.8 (43.8, 58.9)[5]	0.32	78.4 (72.3, 85.0)	22.6 (18.3, 27.8)	0.10
				CT	35	50.5 (43.4, 58.7)		80.5 (74.0, 87.5)	27.2 (21.8, 34.1)	
				TT	18	58.1 (47.0, 71.7)		93.4 (83.1, 104.9)	29.8 (21.7, 40.9)	
rs10741657	0.07	41.4	G/A	GG	36	50.2 (43.1, 58.4)	0.28	77.0 (70.9, 83.5)	21.7 (17.7, 26.8)	0.024[6]
				GA	36	50.2 (43.2, 58.5)		81.9 (75.5, 88.9)	28.6 (23.0, 35.5)	
				AA	20	57.9 (47.3, 71.0)		93.7 (84.0, 104.6)	30.7 (22.9, 41.2)	
rs1562902	0.35	43.8	T/C	TT	32	49.8 (42.4, 58.4)	0.84	79.0 (72.3, 86.3)	25.6 (20.4, 32.1)	0.32
				TC	40	49.8 (43.1, 57.4)		81.2 (75.0, 87.9)	26.8 (21.7, 33.2)	
				CC	20	59.9 (48.9, 73.3)		90.4 (80.1, 101.1)	24.6 (18.4, 33.0)	
rs10766197	0.39	48.9	G/A	GG	22	56.0 (46.1, 67.9)	0.40	87.3 (78.5, 97.1)	25.2 (19.0, 33.3)	0.13
				AG	49	52.5 (46.1, 59.7)		83.7 (77.9, 89.9)	26.5 (21.9, 32.1)	
				AA	21	46.4 (38.0, 56.5)		74.5 (66.8, 83.0)	25.3 (19.1, 33.7)	
CYP24A1										
rs6013897	0.83	20.5	T/A	TT	60	50.4 (44.8, 56.7)	0.07	81.9 (76.7, 87.5)	27.3 (23.1, 32.2)	0.70
				AT	28	53.6 (45.1, 63.7)		83.0 (75.4, 91.5)	25.2 (19.8, 32.0)	
				AA	4	61.1 (38.7, 96.5)		83.1 (83.1, 107.3)	12.3 (6.0, 25.2)	
rs4809960	0.11	23.7	T/C	TT	58	51.8 (46.0, 58.4)	0.45	82.5 (77.1, 88.2)	25.8 (21.7, 30.5)	0.26
				TC	31	53.1 (45.1, 62.6)		81.2 (74.1, 89.0)	24.4 (19.4, 30.9)	
				CC	8	39.9 (23.6, 67.6)		91.0 (67.8, 122.2)	49.6 (24.0, 102.5)	
rs2296241	0.26	46.0	G/A	GG	24	44.5 (37.1, 53.5)	0.16	77.5 (70.0, 80.1)	25.2 (19.5, 32.7)	0.39
				AG	52	55.7 (49.1, 63.0)		82.8 (77.2, 88.8)	24.5 (20.3, 29.4)	
				AA	16	51.5 (41.2, 64.5)		88.1 (77.6, 99.9)	31.8 (23.2, 43.6)	
rs17219315	0.78	2.8	A/G	AA	87	51.7 (46.8, 57.0)	0.53	82.0 (77.6, 86.6)	25.7 (23.3, 29.6)	0.29
				AG	5	54.4 (36.1, 82.0)		87.5 (69.6, 110.0)	29.2 (16.5, 51.7)	
rs2426496	0.29	23.3	G/T	GG	54	48.9 (43.2, 55.3)	0.44	78.8 (73.6, 84.3)	24.3 (20.4, 28.9)	0.25
				GT	35	56.7 (48.6, 66.1)		86.9 (79.9, 94.6)	27.8 (22.2, 34.8)	
				TT	3	51.8 (30.7, 87.4)		95.9 (71.9, 128.1)	37.4 (18.0, 77.7)	
CYP27B1										
rs10877012	0.97	35.2	G/T	GG	41	50.4 (43.7, 58.1)	0.38	81.3 (75.1, 88.1)	23.8 (19.4, 29.2)	0.91
				GT	40	50.7 (43.9, 58.6)		82.0 (75.7, 88.9)	28.3 (23.0, 34.7)	
				TT	11	61.9 (47.1, 81.4)		87.1 (74.7, 101.6)	25.8 (17.2, 38.5)	
C10orf88										
rs6599638	0.29	49.4	G/A	GG	20	52.5 (42.8, 64.5)	0.48	80.3 (71.7, 90.0)	23.3 (17.4, 31.2)	0.31
				GA	51	52.5 (46.2, 59.7)		84.2 (78.4, 90.4)	28.4 (23.6, 34.0)	
				AA	21	49.6 (40.6, 60.5)		79.7 (71.3, 89.0)	23.0 (17.3, 30.5)	
DHCR7/NADSYN1										
rs1790349	0.02	15.3	A/G	AA	69	50.6 (45.4, 56.5)	0.35	82.2 (77.3, 87.4)	26.5 (22.7, 31.1)	0.70
				GA	18	56.8 (45.8, 70.5)		84.7 (75.1, 95.5)	23.8 (17.3, 32.8)	
				GG	5	51.0 (33.9, 76.7)		76.0 (60.5, 95.5)	24.2 (13.7, 42.9)	
rs12785878	0.32	28.4	T/G	TT	49	51.2 (44.9, 58.3)	0.77	81.6 (75.9, 87.8)	27.4 (22.7, 33.1)	0.97
				GT	34	52.4 (44.7, 61.3)		83.3 (76.3, 91.0)	24.2 (19.3, 30.3)	
				GG	9	53.2 (39.2, 72.2)		82.2 (69.3, 97.5)	24.6 (16.1, 37.7)	
GC										
rs16846876	0.16	38.6	A/T	AA	32	58.8 (50.2, 68.8)	0.41	92.2 (84.6, 100.4)	26.6 (21.1, 33.4)	0.026[6]
				AT	50	50.0 (44.1, 56.8)		78.8 (73.6, 84.3)	25.5 (21.1, 30.8)	
				TT	10	41.2 (31.3, 54.6)		71.2 (61.2, 83.0)	25.7 (17.2, 38.6)	
rs12512631	0.07	31.6	T/C	TT	38	43.4 (37.7, 49.9)	0.025[6]	74.6 (69.1, 80.6)	26.3 (21.2, 32.5)	0.13
				TC	49	57.3 (50.6, 64.8)		86.1 (80.5, 92.1)	25.2 (20.9, 30.4)	
				CC	5	79.8 (50.9, 110.0)		111.3 (90.1, 137.5)	30.1 (17.0, 53.4)	
rs17467825	0.96	28.4	A/G	AA	49	53.0 (46.5, 60.4)	0.50	83.9 (78.2, 90.1)	24.4 (20.3, 29.4)	0.020[6]
				GA	36	51.3 (44.1, 59.8)		83.7 (77.1, 90.9)	29.1 (23.5, 36.2)	
				GG	7	46.2 (32.7, 65.3)		65.7 (54.5, 79.3)	20.9 (12.4, 35.0)	
rs2282679	0.96	28.4	A/C	AA	49	53.0 (46.5, 60.4)	0.50	83.9 (78.2, 90.1)	24.4 (20.3, 29.4)	0.020[6]
				CA	36	51.3 (44.1, 59.8)		83.7 (77.1, 90.9)	29.1 (23.5, 36.2)	
				CC	7	46.2 (32.7, 65.3)		65.7 (54.5, 79.3)	20.9 (12.4, 35.0)	

(Continued)

TABLE 2 (*Continued*)

SNP	HWE, P	MAF, %	M/m	Genotype	n	Baseline (day 0) 25(OH)D	P-adjusted[3]	End (day 10) 25(OH)D	Increase in 25(OH)D[2] 25(OH)D	P-adjusted[4]
rs842999	0.14	44.1	G/C/A	GG	25	54.3 (45.4, 65.1)	0.42	82.5 (74.4, 91.4)	24.0 (18.6, 30.9)	0.17
				GX[7]	50	53.1 (46.7, 60.3)		84.2 (78.3, 90.5)	25.8 (21.4, 31.1)	
				XX[8]	13	49.7 (38.7, 63.9)		75.7 (65.7, 87.3)	25.9 (17.9, 37.4)	
rs4588	0.84	29.0	C/A	CC	48	53.3 (46.7, 60.8)	0.57	84.1 (78.3, 90.4)	24.3 (20.1, 29.2)	0.020[6]
				CA	37	51.0 (43.9, 59.3)		83.5 (77.0, 90.6)	29.3 (23.6, 36.2)	
				AA	7	46.2 (32.7, 65.3)		65.7 (54.5, 79.3)	20.9 (12.5, 34.9)	
rs222020	0.84	22.2	T/C	TT	55	54.7 (48.5, 61.6)	0.068	86.2 (80.7, 92.0)	27.2 (22.8, 32.5)	0.31
				TC	33	45.1 (38.7, 52.6)		74.4 (68.4, 81.0)	24.2 (19.3, 30.4)	
				CC	4	77.7 (50.0, 120.9)		100.6 (78.9, 128.2)	22.4 (11.8, 42.3)	
rs2298849	0.80	25.3	T/C	TT	51	53.4 (47.0, 60.6)	0.31	85.5 (79.8, 91.7)	29.0 (24.2, 34.8)	0.33
				CT	35	47.7 (40.9, 55.5)		76.5 (70.3, 83.2)	22.2 (17.8, 27.6)	
				CC	6	65.4 (45.2, 94.6)		91.2 (74.4, 11.8)	24.3 (14.6, 40.6)	
VDR										
rs731236	0.08	42.6	T/C	TT	34	52.2 (44.6, 61.0)	0.35	83.4 (76.5, 91.0)	24.9 (19.9, 31.2)	0.66
				TC	38	49.3 (42.5, 57.1)		79.5 (73.2, 86.4)	27.3 (22.0, 33.9)	
				CC	20	56.4 (46.0, 69.1)		86.8 (76.6, 96.1)	25.0 (18.8, 33.3)	
rs757343	0.98	10.8	G/A	GG	74	52.8 (47.5, 58.8)	0.76	83.2 (78.4, 88.2)	26.8 (23.0, 31.3)	0.56
				AG	17	47.8 (38.3, 59.7)		79.1 (69.9, 89.6)	22.3 (16.4, 30.4)	
				AA	1	47.6 (19.1, 118.7)		74.9 (45.0, 124.7)	27.3 (17.7, 97.5)	
rs10783219	1.00	36.9	A/T	AA	36	53.0 (45.5, 61.8)	0.82	82.1 (75.5, 89.4)	25.4 (20.4, 31.6)	0.69
				TA	43	50.5 (43.9, 58.1)		81.2 (75.2, 87.8)	25.6 (20.9, 31.2)	
				TT	13	52.8 (41.0, 68.1)		86.4 (75.0, 99.5)	28.5 (19.7, 41.2)	
rs7139166	0.24	40.3	C/G	CC	37	53.6 (46.1, 62.3)	0.53	84.4 (77.7, 91.8)	26.1 (21.0, 32.4)	0.81
				CG	37	51.6 (44.4, 59.9)		82.1 (75.5, 89.3)	25.6 (20.6, 31.7)	
				GG	18	48.7 (39.3, 60.5)		78.5 (69.6, 88.5)	26.2 (19.2, 35.7)	

[1]*CYP2R1*, 25-hydroxylase; *CYP24A1*, 24-hydroxylase; *CYP27B1*, 1-α-hydroxylase; *C10orf88*, open-reading frame 88 on chromosome 10q26.13; *DHCR7/NADSYN1*, 7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase-1; *GC*, vitamin D binding protein; HWE, Hardy-Weinberg equilibrium in the unrelated population; MAF, minor allele frequency for the unrelated population; M/m, major/minor alleles; SNP, single nucleotide polymorphism (ordered by position); *VDR*, vitamin D receptor; VitDgen, Vitamin D in genes; 25(OH)D, 25-hydroxyvitamin D.

[2]Increase in 25(OH)D concentration after 4 UVB treatments with a total of 6 or 7.5 standard erythema doses during a 10-d period.

[3]Linear mixed models with family as a random factor adjusted for age, sex, BMI, use of multivitamin and vitamin D supplementation, outdoor stay in light clothes, outdoor transport to work, and sun bathing.

[4]Linear mixed models with family as a random factor adjusted for age, sex, BMI, and baseline serum 25(OH)D concentration.

[5]Raw serum 25(OH)D concentrations were log transformed to approximate a normal distribution and are presented as geometric means (nmol/L); 95% CIs in parentheses (all such values).

[6]Significant P value (<0.05).

[7]GX, GC/GA.

[8]XX, CC/CA/AA.

DISCUSSION

To our knowledge, this is the first study to evaluate the increase in 25(OH)D concentrations after artificial UVB treatments in relation to *GC* and *CYP2R1* genotypes. There was a gene-dose–dependent relation between the UVB-dependent increase in serum 25(OH)D concentrations and the GRS. Genetically predisposed individuals carrying all 4 risk alleles of rs10741657 and rs4588 had the lowest baseline mean 25(OH)D concentration and the smallest increase in 25(OH)D concentrations after 4 UVB treatments during the winter compared with those of carriers of a lower GRS. Furthermore, there was a gene-dose–dependent relation between the percentage decrease in the 25(OH)D concentration and GRS after a 6-mo consumption of vitamin D$_3$–fortified bread and milk. The largest percentage decrease in 25(OH)D concentrations was also observed in individuals carrying all 4 risk alleles of rs10741657 and rs4588 compared with carriers of a lower GRS. Nimitphong et al. (21) also observed a significantly smaller increase in 25(OH)D$_3$ and total 25(OH)D concentrations after oral intake of 400 IU vitamin

D$_3$/d (10 μg vitamin D$_3$/d) for 3 mo in individuals carrying CA or AA genotypes of rs4588.

This study is important for public health recommendations and vitamin D food-fortification programs because it showed that the genetic predisposition in the *CYP2R1* and *GC* genes may have a large impact on 25(OH)D concentrations. During winter, individuals carrying all 4 risk alleles of rs10741657 and rs4588 benefitted the least from either UVB treatments or the consumption of vitamin D$_3$–fortified bread and milk. In agreement with our findings, Engelman et al. (22) performed a GRS encompassing rs4588 in *GC* and rs2060793 in *CYP2R1* and showed that the mean 25(OH)D concentration was highest in the group with no copies of rs4588 and rs2060793 risk alleles who also had high external sources of vitamin D (>10 μg/d). Furthermore, Engelman et al. (22) showed that the lowest mean 25(OH)D concentration was shown in the group with 3 risk alleles and low external sources of vitamin D (<10 μg/d) or 4 risk alleles regardless of the external sources of vitamin D.
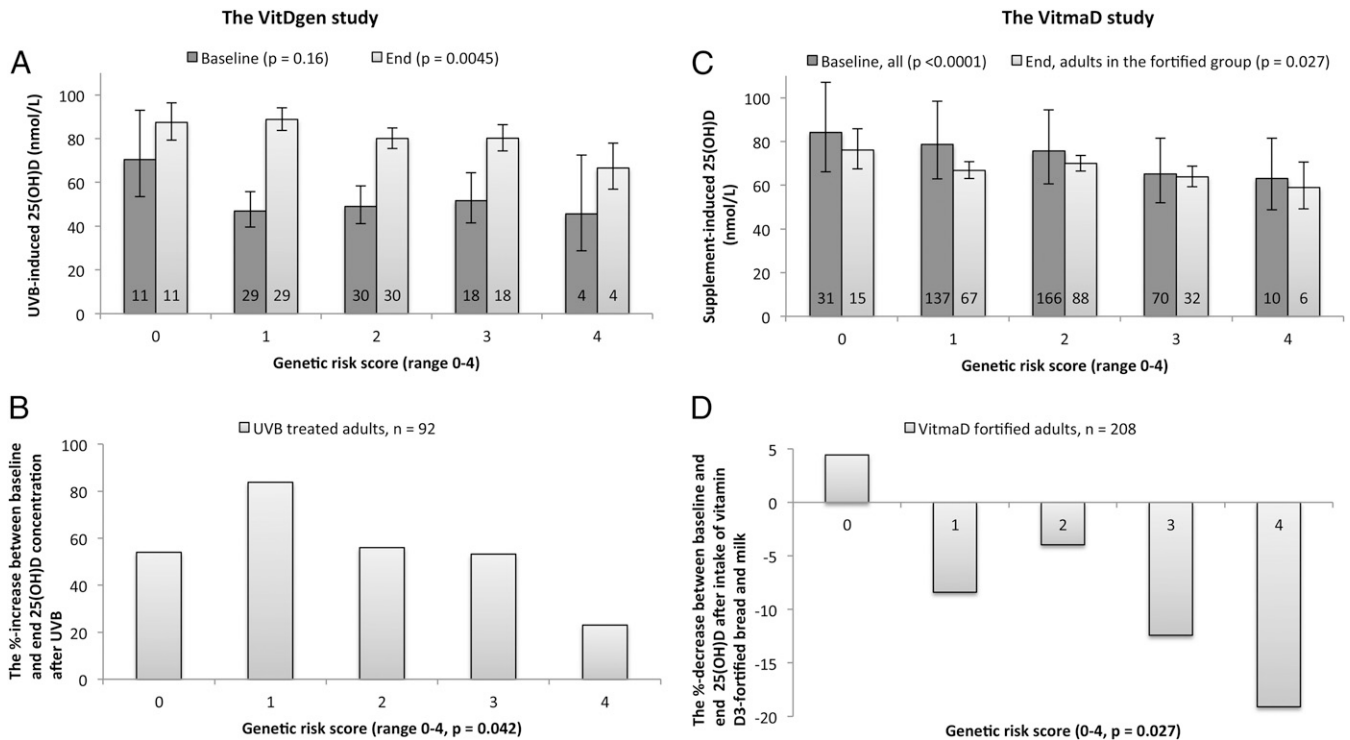
FIGURE 1 Adjusted mean (95% CI) 25(OH)D concentrations at baseline and end of the study were calculated for each GRS category of rs10742657 and rs4588 stratified by UVB treatment in the VitDgen study (A) or by consumption of vitamin D_3–fortified bread and milk in the VitmaD study (C). The GRS (range: 0–4) was calculated as the sum of the number of G alleles of rs10741657 and A alleles of rs4588. The percentage increase in 25(OH)D concentrations after UVB treatment in the VitDgen study (B) or percentage decrease in 25(OH)D concentration after a 6-mo consumption of vitamin D_3–fortified bread and milk during winter in the VitmaD study (D) for each GRS category of rs10742657 and rs4588. The percentage decrease in vitamin D status in relation to the GRS was analyzed in the adult population who participated in the fortification group ($n = 208$) in the VitmaD study. In both studies, linear mixed models were adjusted for age, sex, BMI, baseline 25(OH)D concentration, and family as a random factor and, in addition, for ski and sun vacations, vitamin D intake, and supplementation for the VitmaD study. Linear mixed models were fitted to log 25(OH)D concentrations with the GRS as an explanatory factor. For the VitmaD study, the GRS was calculated for the adult population (18–60 y) at baseline ($n = 414$) and at the end of the study only for the adult population who consumed vitamin D_3–fortified bread and milk ($n = 208$). Numbers in the columns present total numbers of participants carrying the GRS. Error bars indicate 95% CIs. GRS, genetic risk score; VitDgen, Vitamin D in genes; VitmaD, Food with vitamin D; 25(OH)D, 25-hydroxyvitamin D.

Our study indicated that individuals carrying a high GRS may need a longer UVB-exposure time or a higher amount of vitamin D supplementation to achieve a given 25(OH)D concentration than do individuals carrying a lower GRS, or perhaps the results suggest that there is variability in the physiologically normal range of 25(OH)D concentration. Regardless of the method used to increase or maintain a serum 25(OH)D concentration during winter, the effects of UVB treatments or vitamin D supplementation on 25(OH)D concentrations seemed remarkably similar. This study emphasizes the findings that individuals with genetically determined low 25(OH)D concentrations may need different health recommendations to improve their vitamin D status or that there is physiologic variation in the normal range of 25(OH)D concentration, showing that a one-size-fits-all approach may not work well for vitamin D. If the genetically determined low 25(OH)D concentration poses health risk, then carriers of all 4 risk alleles of rs10741657 and rs4588 should be at increased risk of developing vitamin D deficiency or at risk for adverse health outcomes associated with vitamin D deficiency or insufficiency. The genetic variation in rs10741657 has been associated with risk of type 1 diabetes (23). Several studies have reported an association between *GC* genotypes rs7041 and rs4588 and adverse health outcomes including premenopausal bone fracture, postmenopausal breast cancer, endometriosis, diabetes, severity of obstructive pulmonary disease, asthma susceptibility, and rheumatic fever (24–28).

At baseline, there was no significant difference between 25(OH)D concentrations for the analyzed SNPs except for rs12512631 in *GC*. The association between rs12512631 and 25(OH)D concentrations disappeared after 4 UVB treatments. For every 20 statistical tests made for associations with 25(OH)D concentrations at baseline, it was expected to have one false-positive result at the $P < 0.05$ concentration, which the rs12512631 finding may have been. Otherwise, our findings are in agreement with those of previous studies that showed no effects of genetic variation on 25(OH)D concentrations during winter (12, 21, 22). During winter, the vitamin D stored during summer is used, and thus, the genetic variation in biosynthesis genes cannot predict 25(OH)D concentrations.

At the end of the VitDgen study, rs10741657 in *CYP2R1* and rs4588 in *GC* predicted the UVB-induced 25(OH)D concentration. The same polymorphisms have previously been shown to predict 25(OH)D concentrations at late summer and after a 6-mo consumption of vitamin D_3–fortified bread and milk in the VitmaD study (9, 12). In contrast, 2 other polymorphisms, rs10766197 in *CYP2R1* and rs842999 in *GC*, did not predict the UVB-induced 25(OH)D concentration at the end of the VitDgen study, whereas both polymorphisms were associated with 25(OH)D concentrations at late summer and after a 6-mo consumption of vitamin D_3–fortified bread and milk in the VitmaD study (12). The lack of replication of the 2 SNPs in the VitDgen study was likely due to the small sample size.

A strength of the VitDgen study design was that it was conducted in presumably healthy Caucasians aged 18–60 y, and thus, the potential impact of diseases was minimized. Moreover, the increase in 25(OH)D concentration was well controlled by using an artificial UVB source. All blood samples were drawn within a 10-d period during the winter, when the solar influence was minimized. Vitamin D status relied on a single measurement of 25(OH)D concentrations and was analyzed in a single batch-with isotope-dilution liquid-chromatography–tandem mass spectrometry. A disadvantage was that some of the known predictors of 25(OH)D concentrations were quantified by using self-reported questionnaires. It would have been interesting to have measured parathyroid hormone concentrations to assess if there was a recessive effect of rs4588 AA on parathyroid hormone concentrations after UVB treatment as observed after vitamin D supplementation in the VitmaD study (12) and by Pekkinen et al. (29). Moreover, it would have been interesting to analyze possible effects of rs7041 and rs4588 on free and bioavailable 25(OH)D concentrations because genetic differences in the vitamin D binding protein gene may affect the binding of 25(OH)D and, thereby, the amount of free and bioavailable 25(OH)D (30, 31).

In conclusion, common genetic variants in *CYP2R1* and *GC* are predictive of 25(OH)D concentrations in a healthy Caucasian population. Carriers of all 4 risk alleles of rs10741657 in *CYP2R1* and rs4588 in *GC* had the lowest baseline mean 25(OH)D concentration, smallest increase in 25(OH)D concentrations after 4 UVB treatments, and largest percentage decrease in 25(OH)D concentrations after consumption of vitamin D_3–fortified bread and milk during winter compared with in carriers of no risk alleles. This study is important for public health recommendations and vitamin D–food fortification programs because it shows that a genetic predisposition in *CYP2R1* and *GC* genes may have a large impact on 25(OH)D concentrations. Genetic variability may be associated with different response to UVB exposure or vitamin D supplementation perhaps suggesting that some individuals may need different health recommendations to improve their vitamin D status or that there is a physiologic variability in the normal range of 25(OH)D concentrations.

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