Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women[1–3]

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ABSTRACT

Background: Vitamin D status, determined on the basis of 25-hydroxyvitamin D [25(OH)D] concentrations, is associated with the risk of several diseases. Vitamin D binding protein (DBP) is the major carrier of vitamin D and its metabolites, but the role of DBP single nucleotide polymorphisms (SNPs) on 25(OH)D concentrations is unclear.

Objective: The objective was to evaluate the association of 2 DBP gene SNPs with 25(OH)D concentrations and explore whether such association varies according to the amount of vitamin D that needs to be transported.

Design: This cross-sectional study included 741 premenopausal white women, mostly of French descent. Plasma 25(OH)D concentrations were measured by radioimmunoassay. *DBP*-1 (rs7041) and *DBP*-2 (rs4588) were genotyped with a Sequenom MassArray platform. Associations and interactions were modeled by using multivariate linear regression.

Results: *DBP*-1 and *DBP*-2 SNPs were in strong linkage disequilibrium and were both associated with 25(OH)D concentrations. An additional copy of the rare allele of *DBP*-1 or *DBP*-2 was associated with lower 25(OH)D concentrations ($\beta = -3.29$, P for trend $= 0.0003$; $\beta = -4.22$, P for trend < 0.0001, respectively). These DBP polymorphisms explained as much of the variation in circulating 25(OH)D as did total vitamin D intake ($r^2 = 1.3\%$ for *DBP*-1, $r^2 = 2.0\%$ for *DBP*-2, and $r^2 \leq 1.2\%$ for vitamin D intake).

Conclusion: Circulating 25(OH)D concentrations in premenopausal women are strongly related to *DBP* polymorphisms. Whether *DBP* rare allele carriers have a different risk of vitamin D–related diseases and whether such carriers can benefit more or less from dietary interventions, vitamin D supplementation, or sun exposure need to be clarified.

INTRODUCTION

Higher vitamin D intakes or circulating concentrations are associated with a lower risk of several chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular diseases (reviewed in 1, 2). A better understanding of vitamin D biology may prove useful in clarifying these effects and designing effective interventions.

The principal circulating vitamin D metabolite, 25-hydroxyvitamin D [25(OH)D], is recognized (3) as the best short-term biomarker of total exposure to vitamin D (ingested from food or dietary supplements and produced by the skin after sun exposure). More than 99% of 25(OH)D is bound to plasma protein (4), of which ≈90% is bound to the vitamin D binding protein (DBP) (5). DBP, also known as Gc-globulin, is a member of the albumin (*ALB*) and alpha-fetoprotein (*AFP*) gene family. It is mainly synthesized in the liver, where 25(OH)D is also produced. Serum concentrations of DBP range between 4 and 8 μmol/L. DBP has a short half-life of 2.5–3 d compared with 1 to 2 months for 25(OH)D (reviewed in 6–8). The precise role of DBP in vitamin D action is still incompletely understood, but DBP concentrations do not seem to be influenced by vitamin D sterols or other calciotropic hormones or by seasonal variation (reviewed in 7). DBP is significantly elevated during pregnancy and estrogen therapy (9–11) and is low after liver diseases (4, 12), nephrotic syndrome, and malnutrition, probably because of a diminished synthesis rate or excessive protein loss (reviewed in 7).

Until now, little has been known about the influence of *DBP* polymorphisms on circulating 25(OH)D concentrations, but differences in the coding of amino acids could affect the concentration of the binding protein or its affinity for vitamin D metabolites. In this study we assessed the association of 2 single nucleotide polymorphisms (SNPs) located on exons of the vitamin D binding protein gene (rs7041 and rs4588) with plasma 25(OH)D concentrations and explored whether these associations vary according to the amount of vitamin D that needs to be transported.

SUBJECTS AND METHODS

Study population and recruitment procedures

The study design and methods were published previously (13, 14). Briefly, 783 premenopausal women who underwent screening mammography between February and December 2001 were recruited at the Clinique Radiologique Audet (Québec, Canada). Eligible women had no personal history of cancer or breast surgery, had no endocrine diseases, never took selective estrogen-receptor modulators, and had not used hormonal derivatives in the 3 mo before blood sampling. Of the 783 eligible women, 741 provided written informed consent to use their blood samples for assays other than those planned at recruitment (14) and had DNA available (15). This study was approved by the Research Ethics Review Board–Hôpital du Saint-Sacrement du CHA de Québec.

Data collection

Anthropometric measurements and blood samples were taken at recruitment. Breast cancer risk factors were documented by telephone interview, including reproductive and menstrual history, family history of breast cancer, personal history of breast biopsies, past use of exogenous hormones, smoking status, alcohol intake, education, and physical activity. Finally, diet was assessed with a self-administered 161-item semiquantitative food-frequency questionnaire (97GP copyrighted at Harvard University, Boston, MA).

Assessment of plasma 25(OH)D

At the time of collection, blood constituents were rapidly stored in aliquots at −80°C until analyzed. Plasma 25(OH)D concentrations were measured between November 2005 and January 2006 by radioimmunoassay after acetonitrile extraction (DiaSorin Inc, Stillwater, MN). The intrabatch and between-batch CVs were 7.3% and 8.8%, respectively (4 blinded duplicates on average for each of the 24 batches), and the results met the performance target set by the international 25-hydroxyvitamin D External Quality Assessment Scheme (DEQAS) Advisory Panel in 2004–2005.

DNA extraction and SNP genotyping

Genotyping procedures were described previously (15). Briefly, DNA was extracted from the buffy coat by using the PUREGene DNA extraction kit (Gentra Inc, Minneapolis, MN) following the manufacturer's protocol, and DNA samples were then blindly genotyped. The rs7041 and rs4588 SNPs, resulting in a T-to-G transversion [an aspartic acid (Asp: GAT) to a glutamic acid (Glu: GAG)] and a C-to-A transversion [a threonine (Thr: ACG) to a lysine (Lys: AAG)], respectively, in exon 11 of *DBP* (16) were assessed by using the Sequenom MassArray (Sequenom Inc, San Diego, CA) genotyping platform according to the manufacturer's protocol. Each 96-well plate included negative (no DNA) and positive controls to ensure genotyping accuracy. Genotyping call rates were 98.7% for both *DBP*-1 (rs7041) and *DBP*-2 (rs4588). The protocol can be provided on request. In this study, concordance of genotyping from the new Sequenom MassArray platform was compared with Fluorescent Polarization–Single Base Extension platform on 10% of the samples; concordance was 100%.

Statistical methods

Crude and adjusted associations of plasma 25(OH)D concentrations with 6 continuous and 3 categorical potential explanatory variables (reviewed in 3, 17) were estimated by using generalized linear models (GLMs). Season of blood collection and leisure-time physical activity [metabolic equivalent (MET)-h/wk] in the past year (proxy variables for sun exposure), total vitamin D (IU/d) and total calcium (mg/d) intakes from food and supplements in the past year, body mass index (BMI; in kg/m^2), smoking status, education, and age (y) were included in our analyses. Total energy intake (kcal/d) in the past year was also included in the models.

Deviation from the Hardy-Weinberg equilibrium was assessed for each SNP by a chi-square test with one df, and linkage disequilibrium strength was evaluated with r and Lewontin's D' statistic between SNPs. Univariate and multivariate-adjusted mean circulating plasma 25(OH)D concentrations by category of genotypes under codominant mode of inheritance were estimated by using GLM models. Trends between the number of copies of the rare allele, entered as a continuous variable (0, 1, or 2), and concentrations of 25(OH)D were evaluated by linear regression models where the β coefficients represent the per allele variation in nmol/L of plasma 25(OH)D concentration. The strength of associations of genotypes to 25(OH)D concentrations among women with a high vitamin D load was compared with that among women with a low vitamin D load by using the above models to which an interaction term was added. The P value of these interaction terms between vitamin D loads (low or high) and the genotypes under the codominant mode of inheritance were used to assess the effect modification of vitamin D load. Periods of low and high vitamin D loads were chosen based on the seasonal variation in 25(OH)D seen in this cohort (13). Data collected throughout year 2001 were dichotomized in two 6-mo periods during which the vitamin D load is expected to be high (May to October; median 25(OH)D concentration = 68.6 nmol/L) or low (November to April; median 25(OH)D = 54.1 nmol/L).

Partial r^2 values, generated from the adjusted GLM models as the ratio of type II sum of squares on the total sum of squares, were mutually adjusted and interpreted as the independent contribution of each variable in the model to the explanation of the variation in 25(OH)D concentrations.

Assumption of normality of residuals from these analyses was met with untransformed variables. Neither multicollinearity nor influential observation was detected. All tests were 2-sided and carried out by using SAS version 9.1 (SAS Institute Inc, Cary, NC), and a nominal P value of 0.05 was considered statistically significant.

RESULTS

Determinants of 25(OH)D concentrations

Characteristics of the study population are described in detail elsewhere (14). Briefly, 741 premenopausal women in the present study had a mean (±SD) age of 46.8 ± 4.6 and plasma 25(OH)D concentration of 64.9 ± 19.6. The mean (±SD) leisure-time physical activity and BMI were 27.1 ± 22.2 MET-hour/wk and 25.2 ± 4.6, respectively. The mean (±SD) total daily vitamin D, total daily calcium, and total daily energy intakes were of 284 ± 232 IU, 969 ± 433 mg, and 1907 ± 515 kcal, respectively. The percentage of university and college compared with

secondary school degree or less and of nonsmokers compared with smokers and former smokers were 61.9% and 44.9%, respectively.

Several variables were associated with 25(OH)D concentrations (**Table 1**). Mean plasma 25(OH)D was much higher among women recruited in summer than in those recruited in winter (difference = 21.7 nmol/L; an increase of 38%). Leisure-time physical activity was positively related to 25(OH)D concentrations ($P < 0.001$), as were total vitamin D ($P = 0.0014$) and total calcium ($P < 0.001$) intakes. BMI was negatively associated with 25(OH)D concentrations ($P < 0.001$), as were total energy intake and education (both $P < 0.05$). Smokers tended to have higher 25(OH)D concentrations than nonsmokers, although this difference was statistically significant only for ex-smokers. Concentrations of 25(OH)D tended to decrease as age increased in this population of premenopausal women, although this association was not statistically significant ($P = 0.12$).

Vitamin D binding protein SNPs and 25(OH)D concentrations

No significant deviation from Hardy-Weinberg expectations was observed for polymorphisms *DBP*-1 ($P = 0.19$) and *DBP*-2 ($P = 0.48$). Linkage disequilibrium between these SNPs was almost complete ($D' = 1.00$, $r = 0.74$). In crude or adjusted models, rare allele carriers had lower circulating 25(OH)D concentrations than did homozygotes for the common allele (**Table 2**). Each additional rare allele was associated with a reduction in 25(OH)D concentrations of 3.29 and 4.22 nmol/L for *DBP*-1 and *DBP*-2, respectively, with a gene dosage compatible with a codominant mode of inheritance (P for trend = 0.0003 and <0.0001, respectively).

Vitamin D load and strength of DPB SNP effects

The amount of vitamin D needed for transportation (''vitamin D load'') tends to vary with season as reflected by seasonal variations in circulating 25(OH)D. Thus, vitamin D binding protein polymorphisms could have different effects according to vitamin D load. The effect of both SNPs on 25(OH)D concentrations was more apparent when the amount of vitamin D to be transported was high (**Table 3**). Indeed, associations were stronger in May to October than in November to April for *DBP*-1 [$\beta = -3.78$ (P for trend = 0.0018) compared with $\beta = -1.74$ (P for trend = 0.21)] as well as for *DBP*-2 [$\beta = -5.73$ (P for trend < 0.0001) compared with $\beta = -3.03$ (P for trend = 0.037)], although the interaction was not statistically significant (P for interaction = 0.27 and 0.16 for *DBP*-1 and *DBP*-2, respectively).

Contribution to variation of 25(OH)D concentrations

Relative contributions of *DBP*-1 or *DBP*-2 and other variables to the variation in plasma 25(OH)D concentrations are shown in **Table 4**. The adjusted model without any SNP explained 30.3% of the variation in 25(OH)D. Adding either polymorphism to this model improved its explanatory capacity to 31.2% for *DBP*-1 (partial $r^2 = 1.3\%$) and to 31.9% for *DBP*-2 (partial $r^2 =$

TABLE 1
Relation between plasma 25-hydroxyvitamin D [25(OH)D] concentrations and potentially explanatory variables in premenopausal women

	Crude models		Adjusted models[1]	
Explanatory variables	$\beta \pm$ SE[2]	P value	$\beta \pm$ SE[2]	P value
Season at time of blood collection				
Spring ($n = 251$)	4.72 ± 2.26	0.037	4.51 ± 2.07	0.030
Summer ($n = 186$)	21.34 ± 2.36	<0.001	21.74 ± 2.18	<0.001
Fall ($n = 218$)	5.26 ± 2.31	0.023	5.06 ± 2.12	0.017
Winter ($n = 86$)	—	—	—	—
Leisure-time physical activity (MET-h/wk) ($n = 740$)	0.18 ± 0.03	<0.001	0.17 ± 0.03	<0.001
Total vitamin D intake (IU/d) ($n = 736$)	1.82 ± 0.30	<0.001	1.06 ± 0.33	0.0014
Total calcium intake (mg/d) ($n = 736$)	1.19 ± 0.16	<0.001	0.92 ± 0.20	<0.001
Total energy intake (kcal/d) ($n = 736$)	0.23 ± 0.14	0.11	−0.27 ± 0.14	0.045
BMI (kg/m^2) ($n = 741$)	−0.67 ± 0.16	<0.001	−0.51 ± 0.14	<0.001
Education, degree completed				
University ($n = 262$)	−2.72 ± 3.10	0.38	−8.55 ± 2.64	0.0013
College ($n = 197$)	−3.96 ± 3.18	0.21	−6.35 ± 2.71	0.020
Secondary ($n = 235$)	−3.88 ± 3.13	0.22	−5.70 ± 2.64	0.031
Less than secondary ($n = 47$)	—	—	—	—
Smoking status				
Smoker ($n = 111$)	1.51 ± 2.14	0.48	2.06 ± 1.87	0.27
Former smoker ($n = 296$)	3.13 ± 1.56	0.045	3.40 ± 1.33	0.011
Nonsmoker ($n = 334$)	—		—	
Age (y) ($n = 741$)	−0.22 ± 0.16	0.16	−0.21 ± 0.14	0.12

[1] Adjusted for all variables in the table. $n = 735$ because of 6 missing values. MET, metabolic equivalent.

[2] Values are derived from linear regression models and represent absolute mean differences in plasma 25(OH)D concentrations (nmol/L) for increments of one MET-h/wk of leisure-time physical activity, 100 IU vitamin D intake, 100 mg total calcium intake per day, 100 kcal of energy intake per day, 1 unit of BMI (kg/m^2), and 1 y of age. For categorical variables, β values represent absolute mean difference in plasma 25(OH)D concentrations (nmol/L) in blood collected in the spring, summer, or fall as compared with in the winter (crude and adjusted means: 56.4 nmol/L); for university, college, and secondary school degrees compared with less than secondary; and for smokers and former smokers compared with nonsmokers.

TABLE 2
Relation between plasma 25-hydroxyvitamin D [25(OH)D] concentrations and vitamin D binding protein (DBP) genotypes in premenopausal women

				Plasma 25(OH)D concentration			
Single nucleotide polymorphism	rs No.	Genotype	Subjects[1]	Crude mean ± SE[1]	*P* value[2]	Adjusted mean ± SE[3]	*P* value[2]
			n (%)	*nmol/L*		*nmol/L*	
DBP-1[4,5]	rs7041	*GG*	228 (31.1)	67.3 ± 1.3	—	67.5 ± 1.1	—
		GT	377 (51.4)	65.0 ± 1.0	0.16	64.5 ± 0.8	0.034
		TT	128 (17.5)	60.2 ± 1.7	0.0010	60.8 ± 1.5	0.0003
DBP-2[4,6]	rs4588	*CC*	370 (50.5)	67.2 ± 1.0	—	67.2 ± 0.9	—
		CA	296 (40.4)	63.2 ± 1.1	0.0081	63.2 ± 0.9	0.0018
		AA	67 (9.1)	59.0 ± 2.4	0.0016	58.4 ± 2.0	<0.0001

[1] $n = 733$ women because of 8 missing values for genotype.
[2] The Wald test was used to compare mean concentrations with reference genotype (common homozygote) level.
[3] Adjusted for all variables presented in Table 1; $n = 727$ because of 6 missing values.
[4] $\beta \pm$ SE values from linear regression models represent the absolute mean difference in plasma 25(OH)D concentrations (nmol/L) for increments of one rare allele. The Wald test was used to derive *P* for trend values between genotypes entered as a continuous variable and plasma 25(OH)D concentrations [ie, test of the linear decrease in plasma 25(OH)D concentrations for increments of one rare allele].
[5] Crude value: $\beta \pm$ SE $= -3.37 \pm 1.05$, *P* for trend $= 0.0014$; adjusted value: $\beta \pm$ SE $= -3.29 \pm 0.89$, *P* for trend $= 0.0003$.
[6] Crude value: $\beta \pm$ SE $= -4.06 \pm 1.09$, *P* for trend $= 0.0002$; adjusted value: $\beta \pm$ SE $= -4.22 \pm 0.93$, *P* for trend $= <0.0001$.

2.0%), but did not substantially change the relative contribution of the other variables. *DBP*-1 explained as much variation in 25(OH)D concentrations as did total vitamin D intake, BMI, and education. *DBP*-2 explained as much variation as did total calcium intake and more than any other variable except for those related to sun exposure (ie, season and leisure-time physical activity). The importance of *DBP*-1 and *DBP*-2 as explanatory variables of the variation in 25(OH)D concentrations was even greater in May to October when the amount of vitamin D to be transported was high (partial $r^2 = 1.6\%$ and 3.7% for *DBP*-1 and *DBP*-2, respectively). During this period, both SNPs explained 25(OH)D concentrations as much or even more than any other variable, except for leisure-time physical activity. In contrast, in November-April, when the amount of vitamin D to be transported was low, both SNPs explained less of the 25(OH)D variation, whereas total vitamin D intake, total calcium intake, and leisure-time physical activity became the major explanatory variables of plasma 25(OH)D.

DISCUSSION

At the DNA level, this is the first study to our knowledge to show that plasma 25(OH)D concentrations decrease when the number of rare alleles of *DBP*-1 or *DBP*-2 increases in healthy

TABLE 3
Relation between plasma 25-hydroxyvitamin D [25(OH)D] concentrations and genotypes of vitamin D binding protein (DBP) by vitamin D load in premenopausal women

			Adjusted plasma 25(OH)D concentration[1]			
			Low vitamin D load[2]		High vitamin D load[2]	
Single nucleotide polymorphism	rs No.	Genotype	Subjects	Mean ± SE	Subjects	Mean ± SE
			n (%)	*nmol/L*	*n* (%)	*nmol/L*
DBP-1[3,4]	rs7041	*GG*	86 (27.9)	58.4 ± 1.8	140 (33.4)	73.4 ± 1.4
		GT	162 (52.6)	55.6 ± 1.3	214 (51.1)	71.4 ± 1.1
		TT	60 (19.5)	55.1 ± 2.1	65 (15.5)	64.8 ± 2.1
DBP-2[3,5]	rs4588	*CC*	155 (50.3)	58.2 ± 1.3	212 (50.6)	74.1 ± 1.1
		CA	126 (40.9)	54.8 ± 1.5	169 (40.3)	69.3 ± 1.3
		AA	27 (8.8)	52.7 ± 3.2	38 (9.1)	61.5 ± 2.7

[1] Adjusted for all variables presented in Table 1, except season. $n = 308$ and 419 in low- and high–vitamin D load periods, respectively.
[2] Corresponding to periods of low (November to April) and high (May to October) vitamin D loads.
[3] $\beta \pm$ SE values from linear regression models represent the absolute mean difference in plasma 25(OH)D concentrations (nmol/L) for increments of one rare allele. The Wald test was used to derive *P* for trend values between genotypes entered as a continuous variable and plasma 25(OH)D concentrations [ie, test of the linear decrease in plasma 25(OH)D concentrations for increments of one rare allele]. For the *P* for interaction, the Wald test was used to compare difference in β values between low and high vitamin D loads.
[4] *P* for interaction $= 0.27$. Low vitamin D load: $\beta \pm$ SE $= -1.74 \pm 1.38$ (*P* for trend $= 0.21$); high vitamin D load: $\beta \pm$ SE $= -3.78 \pm 1.20$ (*P* for trend $= 0.0018$).
[5] *P* for interaction $= 0.16$. Low vitamin D load: $\beta \pm$ SE $= -3.03 \pm 1.45$ (*P* for trend $= 0.037$); high vitamin D load: $\beta \pm$ SE $= -5.73 \pm 1.24$ (*P* for trend < 0.0001).

TABLE 4
Relative contribution of single nucleotide polymorphisms (SNPs) of vitamin D binding protein (DBP) genes *DBP-1* or *DBP-2* and other variables to the variation in plasma 25-hydroxyvitamin D [25(OH)D] concentrations globally and stratified by vitamin D load in premenopausal women

	Model without *DBP*-1 or *DBP*-2[1]	Models including *DBP*-1[1]			Models including *DBP*-2[1]		
			Vitamin D load[2]			Vitamin D load[2]	
Explanatory variables	Global Partial[3] r^2	Global Partial[3] r^2	Low[1] Partial[3] r^2	High[1] Partial[3] r^2	Global Partial[3] r^2	Low[1] Partial[3] r^2	High[1] Partial[3] r^2
Season at time of blood collection	15.7[4]	15.4[4]	—	—	15.3[4]	—	—
Leisure-time physical activity (MET-h/wk)	3.7[4]	3.9[4]	2.8[5]	4.2[4]	4.0[4]	2.9[5]	4.4[4]
Total vitamin D intake (IU/d)	1.0[5]	1.2[4]	3.9[4]	<0.1	1.0[5]	3.4[4]	<0.1
Total calcium intake (mg/d)	2.1[4]	1.8[4]	3.0[4]	2.2[5]	2.0[4]	3.4[4]	2.3[4]
Total energy intake (kcal/d)	0.4[5]	0.4[5]	1.9[5]	<0.1	0.5[5]	2.1[5]	<0.1
BMI (kg/m^2)	1.3[4]	1.3[4]	0.6	1.6[5]	1.3[4]	0.7	1.6[5]
Education	1.1[5]	1.2[5]	1.8	0.2	1.1[5]	1.6	<0.1
Smoking status	0.6[5]	0.7[5]	0.3	1.5[5]	0.7[5]	0.2	1.7[5]
Age	0.2	0.1	<0.1	0.9[5]	0.1	<0.1	0.9[5]
DBP-1 (rs7041) SNP	—	1.3[4]	0.7	1.6[5]	—	—	—
DBP-2 (rs4588) SNP	—	—	—	—	2.0[4]	1.4[5]	3.7[4]
Total r^2 (%)	30.3	31.2	22.7	17.1	31.9	23.5	19.2

[1] Adjusted for all variables presented in each model. $n = 735$ in the adjusted model without SNP, and $n = 727$ in the adjusted model with either *DBP*-1 or *DBP*-2. MET, metabolic equivalent.

[2] Corresponding to periods of low (November to April) and high (May to October) vitamin D loads. $n = 308$ and 419 for the low- and high–vitamin D load periods, respectively.

[3] Partial r^2 represents the independent adjusted contribution of each variable in the model and therefore does not add up to total r^2.

[4] $P < 0.001$.

[5] $P < 0.05$.

premenopausal women. In a recent study, serum 25(OH)D concentrations in population-based postmenopausal control women also decreased significantly by Gc genotypes (defined by *DBP*-1 and *DBP*-2) (18). The *DBP*-2 SNP has been shown to be associated with lower 25(OH)D concentrations, but only in a group of Polish Graves' Disease cases (16). Whereas season remains the most important explanatory variable of 25(OH)D concentrations, *DBP* polymorphisms explain 25(OH)D variation as much as do vitamin D intake, calcium intake, and BMI. Each *DBP* SNP explains even more of the 25(OH)D variation when the amount of vitamin D to be transported is high.

Our results suggest that both *DBP* SNPs generate functionally different proteins and that such differences affect circulating 25(OH)D concentrations. The rare allele of *DBP*-1 codes for the aspartic acid residue at amino acid position 416 of the DBP protein, whereas *DBP*-2 codes for the lysine residue at position 420, which allows for differentiation of 3 major DBP protein phenotypes (19). Being rare homozygote for both *DBP*-1 and *DBP*-2 characterize the glycosylation pattern of the protein phenotype Gc2-2, which has been shown to be associated with low mean serum DBP protein concentrations (20) and recently with low mean serum 25(OH)D concentrations in postmenopausal women (18). These results suggest that rare alleles of *DBP*-1 and *DBP*-2 are associated with lower 25(OH)D concentrations, at least in part because of the lowering effect on DBP protein concentrations. Whether the variation in DBP protein concentrations stems from different protein production or degradation rates associated with different *DBP* genotypes and phenotypes is unclear, but Lauridsen et al (21) suggest that, on the basis of glycosylation patterns, DBP phenotypes related to a low vitamin D concentration should be metabolized faster. This would in turn decrease the half-life of 25(OH)D, increase its conversion to inactive metabolites, and consequently reduce 25(OH)D concentrations, as shown in *DBP* KO mice after tritium-labeled 25(OH)D injection (22).

Few studies have examined *DBP* polymorphisms and risk of vitamin D–related diseases. A nonsignificant increased risk of breast cancer was associated with the *DBP*-2 rare homozygote (23). The rare homozygote for both *DBP* SNPs was associated with a reduction in postmenopausal breast cancer (18). The haplotypes of 3 SNPs on the promoter of the *DBP* gene suggest a nonsignificant association with prostate cancer in Americans of European descent (24). The (TAAA)n-Alu repeat polymorphisms of the *DBP* gene was associated with fracture risk (25, 26). *DBP* SNPs in linkage disequilibrium with *DBP*-2 were associated with bone mineral density in Japanese postmenopausal women (27). *DBP*-2, but not *DBP*-1, was associated with Graves' disease (16) in Poland but not in nuclear families from Germany and Italy, although the *DBP* polymorphism (TAAA)n-Alu repeat was (28). Associations with type 2 diabetes mellitus and obesity-related traits have been observed, but the results have been inconsistent (reviewed in 7). *DBP* SNPs can possibly influence bioactive 25(OH)D concentrations through changes in the ratio of free to bound hormones (29), by differential affinity (30), or through effects on levels of the DBP/25(OH)D complex that can be internalized by receptor-mediated endocytosis and activate the vitamin D receptor pathway, as recently shown in mammary cells (31). In addition *DBP* SNPs could have effects on carcinogenesis through activation of tumoricidal macrophages and antiangiogenic effects of DBP-macrophage activating factor (reviewed in 7, 32). Overall, evidence suggests that further studies between *DBP* SNPs and health outcomes are needed.

The relation of both *DBP* SNPs to 25(OH)D seems to be more apparent when the amount of vitamin D in need of transportation is

high (ie, May to October compared with November to April). Differences in 25(OH)D concentrations were also more apparent under normal rather than deficient diets in experiments with *DBP* knockout mice (22). Our observations appear consistent with those experimental works. Indeed, if we had assessed this association only during winter, when the vitamin D load is the lowest, no association or trend would have been detected. The effect modification by vitamin D load was not statistically significant though and should be ascertained in other studies. Nevertheless, these results stress the potential importance of considering baseline concentrations of 25(OH)D or recruitment season when studying the association of *DBP* SNPs with circulating 25(OH)D.

A strength of this study was that recruitment took place over one full calendar year in a relatively small geographic area over which the population experiences a large seasonal variation in sun exposure. This design facilitated the assessment of the effects of *DBP* genotypes on 25(OH)D concentrations, but other personal characteristics and variations in their effects according to vitamin D load could also be investigated. Leisure-time physical activity predicts greater 25(OH)D concentrations during a high vitamin D load and putatively shows that outdoor activity can improve vitamin D status, even when solar radiation and meteorological characteristics are less favorable. Even though this variable did not take into account the actual amount of outdoor exercise, level of clothing, time of day, and use of sunscreen, the results further support the idea that leisure-time physical activity is a surrogate of exposure to solar ultraviolet-B, as suggested in a recent prospective study by Giovannucci et al (33). The explanation capacity of 25(OH)D concentrations by BMI during a high vitamin D load is no longer significant during a low vitamin D load. This suggests that, in this population, possible sequestration of vitamin D into the subcutaneous fat mass reservoir (34) could be more important from early May to the end of October, when more vitamin D_3 is synthesized in the skin. Total vitamin D intake only contributes to the variation in 25(OH)D concentrations under conditions of a low vitamin D load, whereas total calcium intake remains significant in both periods, which suggests that calcium's effect on 25(OH)D concentrations is independent of vitamin D intake even though both nutrient intakes are strongly correlated. Age is becoming a significant negative predictor of 25(OH)D only during high vitamin D load, which possibly reflects a reduction in 7-dehydrocholesterol (ie, precursor of vitamin D_3) in the skin with aging (reviewed in 35).

Our study had some limitations. False-positive results are common in studies of the association between genetic markers and outcomes, but because we only analyzed 2 polymorphisms of the vitamin D binding protein in relation to circulating 25(OH)D concentrations, we believe that type 1 errors were not likely to explain our findings. Moreover, if we had used Bonferroni-corrected *P* values, the results would have remained statistically significant. Population stratification can be a concern with this type of study (36, 37), although this problem was likely not as important as anticipated in North American white populations (38–40). In our study, most of the women were from the Quebec City area, white (99.7%), and of French descent (87.7%) (15), which suggests that the associations we found were not due to population stratification. Confounding was considered; however, we do not think that this was a major concern because most key variables known to be associated with vitamin D status were accounted for in the analysis. Estrogen concentrations, which were not assessed in the present study, are known to be associated with DBP; nevertheless, adjustment for past hormone derivative use did not affect our results.

In conclusion, the number of rare alleles of *DBP*-1 and *DBP*-2 polymorphisms is inversely related to 25(OH)D concentrations in premenopausal women, and their effects on variation in 25(OH)D concentrations are comparable with those of total vitamin D intakes. *DBP* SNPs are inherited, which suggests that the reduction in 25(OH)D concentrations found within the rare allele carriers would persist over a lifetime. Lifelong low vitamin D concentrations may have an impact on health, and additional studies of the association between *DBP* polymorphisms and clinical outcomes are needed. Whether rare allele carriers of *DBP* rs7041 and rs4588 SNPs could benefit more or less from dietary intervention, supplementation, or sun exposure warrants additional attention.

The authors' responsibilities were as follows—CD, MS, SB, MP, and JB: involved in the study design; CD, SB, and JB: supervised the data collection; and MS: performed the statistical analyses and wrote the manuscript. All authors contributed to the drafts of the manuscript and approved the final version. None of the authors had a personal or financial conflict of interest.

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