Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis[1–3]

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ABSTRACT

Background: Multiple sclerosis (MS) risk is determined by both genes and environment. One of the most striking features of MS is its geographic distribution, particularly the pattern of high MS frequency in areas with low sunlight exposure, the main inducer of vitamin D synthesis. Recent epidemiologic, experimental, and clinical evidence support an effect for low environmental supplies of vitamin D in mediating an increased susceptibility to MS.

Objectives: We *1*) examined the association of serum 25-hydroxyvitaminD [25(OH)D] concentrations and MS status and *2*) assessed the genetic contribution to serum 25(OH)D concentrations and tested for its association with genetic variants in 2 candidate genes [vitamin D receptor and 1-α-hydroxylase (*CYP27B1*)].

Design: We used a twin study approach, comprising adult pairs identified from the longitudinal population-based Canadian Collaborative Project on Genetic Susceptibility to MS. Monozygotic (MZ; $n = 40$) and dizygotic (DZ; $n = 59$) pairs, both concordant and discordant for MS, were studied. End-of-winter serum 25(OH)D concentrations were measured by radioimmunoassay, and genotypes were assessed by single nucleotide polymorphism (SNP) assay.

Results: Serum concentrations of 25(OH)D were highly correlated in MS-concordant pairs ($r = 0.83$, $P < 0.001$), but they were not significantly associated with having the disease ($P = 0.4$) when analyzed by logistic regression. Intraclass correlation for 25(OH)D concentration was significantly greater in MZ pairs (MZ, r: 0.71 > DZ r: 0.32, $P = 0.006$). Significant associations of 2 *CYP27B1* SNP variants and 25(OH)D concentrations were observed.

Conclusion: The findings indicate important genetic influences on regulation of seasonal circulating 25(OH)D concentrations in MS twins. *Am J Clin Nutr* 2008;88:441–7.

INTRODUCTION

The cause of multiple sclerosis (MS) is unknown, but it is widely accepted as a complex trait that develops in genetically susceptible persons exposed to as yet undefined environmental risk factors (1). The strongest, consistently replicated, genetic susceptibility factor is the major histocompatibility complex human leukocyte antigen–dicloro-1-β-D-ribofuranosyl-benzimidazole 1 (HLA-DRB1) locus (1). The environmental component in MS susceptibility has attracted relatively little study in the past few decades. Genetic epidemiologic evidence strongly indicates that environment or gene-environment interactions in MS risk act at a broad population level (2–5). The most striking feature of MS epidemiology has long been the geographical distribution of prevalence (6), and its determinants are thought to be central to the environmental components in MS risk. The gradient of increasing MS risk with increasing latitude in both hemispheres is coincident with annual sunlight exposure and frequency of vitamin D deficiency (7). Several decades ago it was proposed that vitamin D adequacy mediates this ultraviolet radiation (UVR)–latitude effect (8). Inadequate concentrations of vitamin D has since reemerged as a prime candidate in MS susceptibility and pathogenesis (9), although strong evidence directly supporting this hypothesis has been difficult to establish (10).

Clinical observations include low serum concentrations of 25-hydroxyvitamin D [25(OH)D] in MS patients (11, 12), seasonal fluctuation in the timing of MS births (13, 14), and fluctuation in MS disease activity correlating with 25(OH)D seasonality (15, 16). Vitamin supplementation (17) and high concentrations of serum 25(OH)D in adulthood (18) were modestly associated with reduced risk of MS in 2 US cohort studies. In addition, sunlight exposure through outdoor activity has shown an inverse correlation with MS risk (19–22). Associations of various vitamin D receptor (*VDR*) gene variants and MS were also reported (23–25). In animal models for MS, prenatal hypovitaminosis D in rats has shown impaired nervous system development (26), and 1,25-dihydroxy-vitamin D inhibits development and progression of experimental allergic encephalomyelitis (27).

25(OH)D is the main circulating metabolite of vitamin D and is the best measure of vitamin D adequacy (28). Circulating 25(OH)D concentrations are predominantly influenced by ultraviolet B exposure from sunlight (9), although evidence suggests that genetic factors are also important (29, 30). Presumably, any

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genetic effect and elucidating genes involved would be most evident when ultraviolet exposure is inadequate or seasonally limited. This is the case in Canada, where the ultraviolet intensity is inadequate for cutaneous vitamin D synthesis from approximately October to March (31, 32).

Maintaining optimal concentrations of serum 25(OH)D is important to immune health and disease risk (9, 10). However, the relative contribution of genetic factors to maintaining 25(OH)D adequacy is unclear, and published studies elucidating genes involved are scarce. More systematic studies could shed light on underlying gene, gene-environment interactions, or both involved in MS. The aims of this study were to examine the vitamin D–MS relation and to investigate the genetic contribution to circulating 25(OH)D.

SUBJECTS AND METHODS

Concentrations of serum 25(OH)D were assayed in twin pairs concordant and discordant for MS. First, we examined the relation of disease concordance to 25(OH)D concentrations. We next assessed the association of circulating 25(OH)D and MS affection status. Monozygotic (MZ) and dizygotic (DZ) pairs were compared to examine the genetic influence on 25(OH)D concentrations. Finally, we tested for the association of candidate gene polymorphisms with both 25(OH)D and disease status. 25(OH)D was the only vitamin D metabolite measured in this study; for clarity, all references to vitamin D measurements within this study implicate this particular metabolite.

Study group

Twin pairs were identified from the database of the longitudinal, population-based Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS). The CCPGSMS ascertainment, database, and consenting process were described previously in detail (33). Procedures for recontact of the CCPGSMS subjects were followed in accordance with the study's institutional review board guidelines. Two hundred twenty persons participated by providing a blood sample and completing a short questionnaire (details below). Inclusion in the study required that both members of the twin pair agreed to participate. The twin cohort within the CCPGSMS has been followed for >20 y and has been described in detail (34). Zygosity was determined by self-reporting, and accuracy was subsequently verified in ≈10% of cases by microsatellite marker genotyping.

Sampling

Given the large seasonal dependence of serum 25(OH)D concentrations, all blood was drawn at the end of winter (March or April 2005) to minimize bias from environmental influence. Serum was extracted from the samples and stored at −80 °C. The study sample comprised 40 MZ and 59 DZ pairs, 18 of which were concordant for MS. The mean age of the sample was 53 y (range: 34–78 y), with an overall female-to-male sex ratio of ≈2.2:1. These characteristics are consistent with those of the entire CCPGSMS twin cohort previously reported (34).

To limit potential confounders, a short interview-based questionnaire about factors known to significantly affect 25(OH)D (9, 35) was administered by the health care professional doing the venipuncture. The participants were asked whether they were taking vitamin D supplements, had used a tanning bed, or had taken a sunshine vacation within the previous 6 wk. Exclusion criteria included taking supplements > 800 IU/d, use of a tanning bed, or vacation below the 38°N latitude within 6 wk before blood collection. If a participant met any of those criteria, their co-twin was also excluded.

Serum 25(OH)D assays

Radioimmunoassay was used to measure serum 25(OH)D. Serum samples frozen at −80 °C were measured with commercial immunoradiometric assay kits (DiaSorin Inc, Stillwater, MN) that detect $25(OH)D_2$ and $25(OH)D_3$ equally. Radioimmunoassay kit instructions were followed as outlined previously (36), and the antibody tracer was quantified with the use of a gamma counter (RIASTAR; Canberra Packard, Mississauga, Canada). The intraassay and interassay CVs are typically <16%; a within-run CV was <10%. Results consistently report within the central ± 1 SD of the method group mean in the international External Quality Assurance Survey (EQAS, Northwest Thames, United Kingdom).

Samples collected from all 220 twins were assayed for 25(OH)D concentration, but only 99 pairs met criteria for inclusion in the statistical analyses. Twenty-two persons were excluded because either the co-twin was unavailable ($n = 8$) or their answers to the interview questionnaire met exclusion criteria ($n = 14$). The mean concentration of serum 25(OH)D in the subjects excluded, based on questionnaire responses, was 167.1 ± 5.4 nmol/L.

Genotyping

DNA was extracted from blood as outlined in previous CCPGSMS studies (37). HLA-DRB1 types determined by microsatellite marker genotyping were available from past studies (34). The genotypes were used for stratification in analyses by the presence or absence of HLA-DRB1*15, given the known increased risk the alleles carry (37). In addition, 35 single nucleotide polymorphisms (SNPs) from 2 candidate genes [*VDR* and 1-α-hydroxylase (*CYP27B1*)] were assayed on the Sequenom MassARRAY platform (Sequenom, San Diego, CA). Genotyping was limited to 150 twins for whom DNA was available. To ensure full gene coverage, the SNP panels for *VDR* and *CYP27B1* were selected with the use of Haploview (38) and were designed to capture a minimum of 95% of all genetic variants listed in the National Center for Biotechnology Information Entrez SNP and Hapmap (Build 16) databases. Three variants failed the assay, and any SNP with total genotype counts < 10 in the heterozygous and rare homozygous groups combined ($n = 9$) was excluded from further analysis. The genotypes were analyzed for significant effects on prediction of MS susceptibility, 25(OH)D concentrations, or both.

Statistical analysis

25(OH)D concentrations are reported as mean ± SEM. For regression analysis, values were log-transformed to generate an approximate Gaussian distribution. Log 25(OH)D concentrations were tested for prediction of MS status with the use of logistic regression in STATA (39). Conversely, MS affection status was tested for the association with 25(OH)D concentration with linear regression in STATA (39). Age and sex were incorporated as covariates, because they are thought to be factors in

both MS risk and vitamin D metabolism. For all models, adjustment for the correlation between twin pairs was made with the use of Huber-White robust variance estimation (40).

In the region flanking a causal variant, it is not uncommon to see several SNPs associated with disease. This may be a result of linkage disequilibrium of all SNPs tested with a single causal variant, or it may be indicative of multiple causal variants. To distinguish between these possibilities, we carried out multilocus modeling with the use of a forward selection approach. We first assessed each SNP individually for association with log serum 25(OH)D concentrations, after adjustment for age, sex, and MS status. We next tested whether the genetic variants were predictors of MS affection status, after adjustment for age, sex, and log 25(OH)D concentrations. Multilocus models of association fully take account of the underlying linkage disequilibrium between SNPs, as well as the correlation in the effects of the SNPs; thus, it may be more powerful to detect the association than single-locus tests. All SNPs with single-locus effects of $P < 0.05$ were considered as potential predictors in an overall linear or logistic regression model to test each SNP for independent effects of significant association. At each stage, the most significantly associated SNP, after adjustment for the effects of all others in the model, is included. To allow for multiple testing (24 SNPs), we report results for a Bonferroni-corrected P value of 0.002.

RESULTS

Characteristics of twin data set

Analyses included 198 twin persons (130 women, 68 men) with a mean (±SD) age of 53.1 ± 9 y. This comprised 40 MZ and 59 DZ pairs, of which 16 pairs were concordant for MS (11 MZ, 5 DZ). The overall mean (±SEM) 25(OH)D concentration was 78.2 ± 2.4 nmol/L, and no significant differences were observed in the mean concentrations of men compared with women or MZ twins compared with DZ twins (**Table 1**). Among all pairs discordant for MS, the mean 25(OH)D concentration was similar in affected and unaffected subjects (Table 1). Several subjects (20.2%) had concentrations in the insufficiency range, defined as ≤45 nmol/L (31), 6 of which were ≤25 nmol/L. Rates of hypovitaminosis were not significantly influenced by disease status, MS concordance, sex, or age (data not shown).

25(OH)D concentrations and disease risk

Descriptively, it can be seen that 25(OH)D concentrations are more similar in MS-concordant pairs, but this may be affected by zygosity (Table 1; **Figure 1**). The intrapair difference is simply the absolute value of the difference between 25(OH)D concentration in twin 1 and twin 2. MS-concordant pairs were highly correlated for 25(OH)D concentrations (concordant pairs: $r = 0.83$, $P < 0.001$; discordant pairs: $r = 0.37$, $P = 0.01$; Table 1). The most dissimilar group was the DZ-discordant pairs (intrapair difference: 33.2 nmol/L; correlation: $r = 0.28$, $P = 0.380$), whereas MZ-concordant pairs were the most similar (intrapair difference: 20.4 nmol/L; $r = 0.86$, $P = 0.001$). The intrapair difference of same-sex compared with opposite-sex pairs was similar (26.6 and 28.3 nmol/L, respectively).

To test for any significant trends of lower or higher 25(OH)D concentrations in affected subjects, we used logistic regression, after adjustment for age and sex. 25(OH)D concentration was not a significant predictor of MS status in our sample ($P = 0.43$; **Table 2**). Stratification for the presence or absence of the HLA-DRB1*15 risk alleles did not influence these results (Table 2). Conversely, we examined whether MS status predicted 25(OH)D concentrations, and, again, no significant trends were observed (data not shown). Overall, although disease-concordant pairs were significantly correlated for 25(OH)D concentrations, this did not appear to be a result of low or high adult concentrations affecting the likelihood of MS affection status or vice versa.

Genetic contribution to 25(OH)D concentration

The mean intrapair difference for 25(OH)D concentration was lower in MZ twin pairs than in DZ twin pairs (Table 1). We assessed the significance of this intrapair variation with the use of correlation statistics and regression models. Comparison of MZ and DZ correlation coefficients for 25(OH)D concentrations showed higher similarity in MZ twins ($r = 0.71$, $P < 0.001$) than in DZ twins ($r = 0.32$, $P = 0.014$; Figure 1). The difference between these MZ-DZ correlation values was significant ($P < 0.006$). Both discordant and concordant pairs were included in the zygosity correlation. The heritability estimate for circulating 25(OH)D was calculated [$2 \times (r\text{MZ} - r\text{DZ})$], providing an estimate of 0.77. Finally, the assessment of the HLA-DRB1*15 genotype in the twins showed association (as expected) with MS (odds ratio: 1.63, $P = 0.01$) but not with concentrations of 25(OH)D (**Table 3**).

Genetic polymorphisms

Thirty SNPs of the *VDR* gene and 6 SNPs of the *CYP27B1* gene were genotyped in the 150 subjects for whom DNA was

TABLE 1
Characteristics of the twin data set with respect to 25-hydroxyvitamin D [25(OH)D] concentrations

	Subjects	25(OH)D	Intrapair difference of 25(OH)D[1]	Intraclass correlation (r)	P[2]
	n	*nmol/L*	*nmol/L*		
All twins	198	78.2 ± 2.4[3]	27.8 ± 3.3	—	
Females	130	79.6 ± 3.1	—	—	
Males	68	75.4 ± 3.6	—	—	
Affected-discordant	83	75.5 ± 3.6	—	—	
Unaffected-discordant	83	75.3 ± 3.5	—	—	
Concordant	32	79.9 ± 6.3	24.2 ± 4.1	0.83	<0.001
Discordant	166	75.4 ± 2.5	27.9 ± 3.8	0.37	0.010

[1] Absolute value of the difference between 25(OH)D of twin 1 and twin 2 for each pair.
[2] Significant correlation value.
[3] $\bar{x}$ ± SEM (all such values).

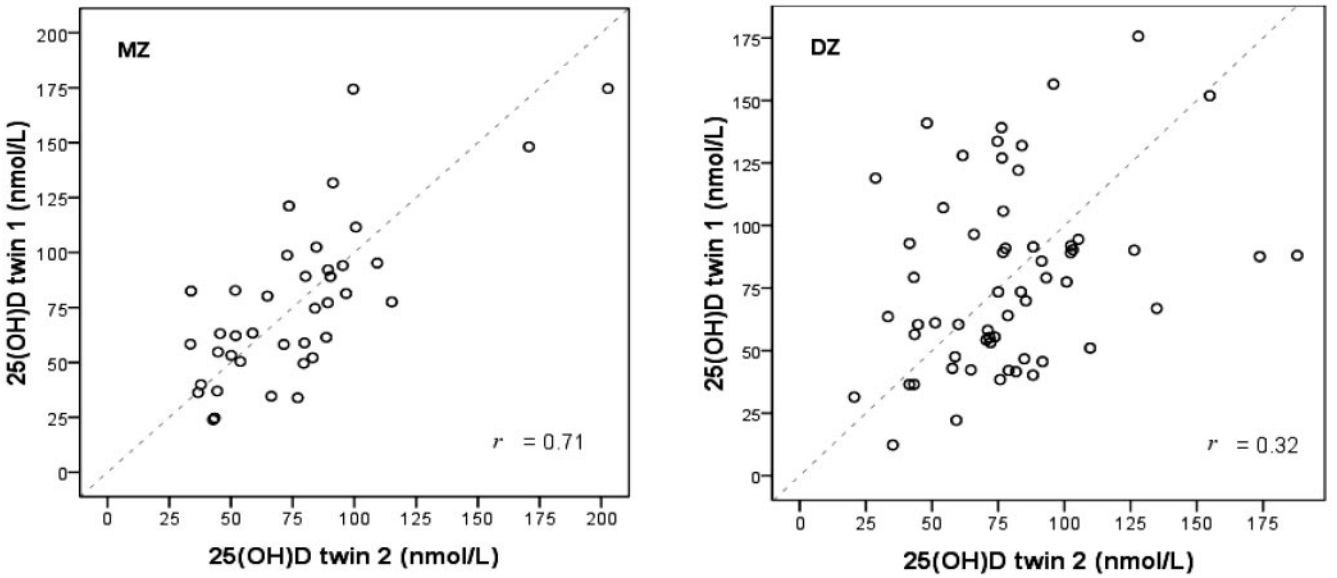

FIGURE 1. Intraclass correlation of serum 25-hyrdroxy-vitaminD [25(OH)D] concentration (in nmol/L) in monozygotic (MZ) and dizygotic (DZ) twin pairs. The Pearson correlation coefficients were $r = 0.71$ ($P < 0.001$) and $r = 0.32$ ($P = 0.014$) for MZ and DZ twins, respectively. The difference between the 2 correlation values was significant ($P < 0.006$). The dashed lines represent the theoretical axis for full correlation ($r = 1$) of 25(OH)D concentrations within each twin pair.

available. Multilocus modeling with the use of a forward selection approach was used. We first assessed each SNP individually for association with log serum 25(OH)D concentrations, after adjustment for age, sex, and MS status (Table 3). These covariates were not significant predictors of 25(OH)D concentration ($P > 0.05$); thus, they were dropped from the model. Next, we similarly tested whether the genetic variants were predictors of MS affection status, after adjustment for age, sex, and log 25(OH)D concentrations (Table 3). All SNPs with single-locus association of $P < 0.05$ were considered as potential predictors in the multilocus model. These included 2 *CYP27B1* variants and 3 *VDR* variants (Table 3). Results are not presented for the remaining 24 SNPs that showed no trend for association (uncorrected $P > 0.05$).

A Bonferroni correction for multiple testing was used ($P < 0.002$) for the multilocus model. *VDR* SNPs (rs2254210, rs98784) tested for the association with MS status did not reach significance ($P = 0.025$, $P = 0.137$, respectively; data not shown). The 2 *CYP27B1* SNPs (rs4646536, rs703842) both remained significant predictors of 25(OH)D concentrations ($P < 0.001$ for both; Table 3). Association of the *Fok*I (rs10735810) SNP and 25(OH)D fell slightly below significance ($P = 0.005$; **Table 4**). The mean concentration in subjects with the *Fok*I genotype (GG) coding for the shorter length VDR was 64.6 ± 5.6 nmol/L compared with 83.2 ± 3.9 nmol/L in subjects with the AA and AG genotype (Table 4). Mean concentrations were lower in subjects homozygous for the less frequent C allele in the *CYP27B1* SNPs (Table 4).

TABLE 2
Test for association of log 25-hydroxyvitamin D [25(OH)D] concentrations and multiple sclerosis (MS) affection status, with adjustment for age and sex ($n = 198$) as determined by logistic regression analysis and with stratification by the presence or absence of ≥1 DRB1*15 risk allele

MS status	Odds ratio	95% CI	*P*
25(OH)D	1.24	0.72, 2.13	0.432
Sex	1.55	0.93, 2.59	0.094
Age	0.99	0.97, 1.01	0.176
25(OH)D[1]			
DRB1*15-positive	1.50	0.70, 3.21	0.297
DRB1*15-negative	1.04	0.52, 2.09	0.907

[1] Adjusted for sex and age.

DISCUSSION

The evidence is compelling that MS is a disease strongly influenced by ≥1 environmental factors in genetically susceptible persons (1). Evidence from the CCPGSMS studies of adoptees (2), step-siblings (5), and half-siblings (3) indicates that the environmental component of MS risk is ubiquitous and acts at a broad population level, rather than within the shared family environment. A protective role for UVR exposure, mediated by vitamin D, remains the most attractive explanation for the latitude gradient of MS prevalence (6, 41). The active vitamin D metabolite, $1,25(OH)_2D$, is a potent immunomodulator that is important to immune function and development (42). It may be the imbalance or impairment of its immunologic functions that implicates vitamin D insufficiency in the pathogenesis of MS and other autoimmune diseases (42, 43). Chronic low concentrations, seasonal fluctuation, or acute effects are all plausible, but it remains likely that an underlying genetic predisposition to MS is required.

Recently, the evidence in support of the MS–vitamin D theory has mounted. This evidence includes genetic (23–25) and experimental (26, 27) studies, observational case-control and cohort studies (17, 19–22, 44), and clinical evidence (18, 45, 46). There are currently no clinical trials of vitamin D in MS prevention, but 2 treatment trials showed promising findings on reduction of exacerbation rates (47, 48). If MS susceptibility is associated with 25(OH)D concentrations, preventative measures could be

TABLE 3
Single-locus regression analyses for association of vitamin D receptor(*VDR*) and 1-α-hydroxylase (*CYP27B1*) gene variants with log 25-hydroxyvitamin D [25(OH)D] concentrations and multiple sclerosis (MS) affection status[1]

	25(OH)D concentration			MS status		
SNP; marker	Coefficient	95% CI	P^2	Odds ratio	95% CI	P^2
VDR						
rs2254210; A/G	0.03	−0.15, −0.20	0.742	1.78	1.04, 3.06	0.036
FokI rs10735810; A/G	−0.24	−0.40, −0.80	0.003	0.68	0.41, 1.15	0.152
rs98784; C/T	0.04	0.04, 0.17	0.596	1.53	1.01, 2.30	0.043
CYP27B1[1]						
rs4646536; C/T	0.17	0.03, 0.31	0.020	0.96	0.61, 1.49	0.854
rs703842; C/T	0.18	0.04, 0.33	0.015	0.47	0.56, 1.29	0.450
HLA DRB1						
DRB1*15; positive	0.08	−0.03, 0.20	0.136	1.63	1.12, 2.38	0.010

[1] SNP, single nucleotide polymorphism; *HLA-DRB1*, human leukocyte antigen–dicloro-1-β-D-ribofuranosyl-benzimidazole 1 gene.
[2] SNPs with associations that meet threshold for multilocus analysis ($P < 0.05$, uncorrected).

implemented easily and cost-effectively (49), especially given the known additional health benefits of vitamin D (9).

Despite numerous reports investigating the vitamin D–MS relation, most evidence is indirect, with relatively few studies measuring patients' vitamin D concentrations. This may be in part due to the challenge of studying environmental susceptibility factors in adult-onset diseases, especially those for which risk is believed to be acquired early in life, as is suggested for MS (13, 50). The timing of a possible 25(OH)D influence on MS susceptibility is unclear (ie, gestation, childhood, cumulative effects). Given that concentrations are to a certain extent heritable (30, 51), combined with behavioral consistencies, we reasoned that adult 25(OH)D concentrations potentially reflect prediagnosis concentrations, especially if certain persons are prone to insufficiency. Wintertime sampling was used to minimize current environmental effects because UVR wavelength is not effective for cutaneous vitamin D synthesis during the Canadian winter. Furthermore, a short questionnaire was used to reduce bias resulting from use of tanning beds, supplements, and sunshine vacations.

Serum 25(OH)D concentrations were analyzed in CCPGSMS twin subjects to examine the vitamin D–MS relation. We found a higher correlation of 25(OH)D concentrations in pairs in which both twins were affected. Presumably, this observation could be a result of 25(OH)D status influencing disease risk and, thus, the likelihood of concordance or conversely the disease affecting 25(OH)D concentrations. However, analysis of 25(OH)D and MS status (and vice versa) showed no significant associations. HLA-DRB1*15 stratification did not alter this result. Nevertheless, it should be noted that they were adult twin pairs; conclusive determination of 25(OH)D relation to MS risk may require sampling many years before disease onset or even sampling of mothers during gestation.

Several recent case-control studies in Finland (45, 52), Ireland (53), and Australia (46) also reported no difference in adult concentrations of 25(OH)D between MS-affected and MS-unaffected persons. Other studies have observed lower circulating 25(OH)D in MS patients (11, 12). In addition, the mean 25(OH)D concentration observed in our study was higher than previous findings (54, 55), albeit consistent with others (53, 56, 57). These conflicting findings may be a result of regional differences, interassay variation, or study design, including disease stage. Indeed, the Australian study found the trend for likelihood of 25(OH)D insufficiency only among MS patients with an Expanded Disability Status Scale score > 3 (46).

Because the correlation of 25(OH)D concentrations in concordant twin pairs was not a result of MS status, we examined the effects of zygosity on 25(OH)D concentrations. MZ and DZ twin comparisons can provide insight into the genetic influence on a particular trait (58). The limitation of equal environmental sharing in adult twins is acknowledged, and interpretation relies on the assumptions that differences will be similar for MZ and DZ twins and that environmental effect is minimized in the current study. The correlation for 25(OH)D concentrations in MZ twins

TABLE 4
Multilocus regression modeling to test for independent single nucleotide polymorphism (SNP) effects on association with log 25-hydroxyvitamin D [25(OH)D] concentration[1]

				25(OH)D[2]					
SNP	Coefficient	95% CI	P^3	*AA*	*AG*	*GG*	*CC*	*CT*	*TT*
				nmol/L					
VDR, *Fok*I	−0.21	−0.34, −0.06	0.005	100 ± 9[4]	80 ± 4	64 ± 6	—	—	—
CYP27B1, rs4646536	−0.90	−1.20, −0.60	<0.001	—	—	—	62 ± 7	73 ± 4	86 ± 5
CYP27B1, rs703842	1.02	0.83, 1.21	<0.001	—	—	—	59 ± 7	74 ± 4	86 ± 5

[1] SNP, single nucleotide polymorphism; *VDR*, vitamin D receptor gene; *CYP27B1*, 1-α-hydroxylase gene.
[2] For each genotype at the respective SNP locus.
[3] After Bonferroni correction for multiple tests ($n = 24$), the threshold for significance is $P < 0.002$.
[4] $\bar{x}$ ± SEM (all such values).

was more than double that in DZ twins, and the magnitude of the difference was significant. This suggests that gene or gene-environment factors influence the trait.

Evidence for genetic contribution to 25(OH)D concentrations was shown previously in an osteoarthritis twin cohort of mainly women (30), and a genome-wide linkage scan in asthma families reported a heritability index of 0.80 (51). Heritability estimates indicate the amount of phenotypic variance ascribed to genetic factors in a given population (58). They should not be mistaken as a genetic percentage and are both time- and population-dependent. The higher heritability index in the asthma and MS cohorts may be a reflection of 25(OH)D influence on disease susceptibility, such that the genetic effects are easier detected in these cohorts. Given these findings, we conducted a preliminary assessment of vitamin D–related gene region effects on 25(OH)D. Association with MS status was also assessed because this was previously reported (23, 24, 59). After Bonferroni correction, positive associations with 25(OH)D concentrations were observed for 2 *CYP27B1* SNPs. Effects of the *VDR Fok*I variant fell slightly below significance, although the trend is interesting, given its genotype-based differences in functionality (60). The resultant VDR isoforms differ structurally, and decreased activity was observed in the longer isoform (60). Although CYP27B1 and VDR actions are downstream of circulating 25(OH)D, causal variants could possibly alter their role in metabolic feedback loops or effect the speed at which 25(OH)D is metabolized. Speculation on the overall importance of these genes is limited by the sample size, and the findings merit follow-up.

Perhaps because of dominating environmental influences, there has been little investigation into the genes involved in regulation of serum 25(OH)D concentrations. Research about vitamin D genes is predominantly focused on SNP-disease associations, and most does not relate this to 25(OH)D or other metabolite concentrations. It is unclear the number, type, and interactions of genes that may be involved, although one quantitative trait locus scan has identified several regions of interest (51). The importance of genes in maintaining vitamin D adequacy is likely to be most significant in regions where UVR exposure is limited or is seasonally limited. It may be that some persons are genetically more susceptible to low 25(OH)D concentrations. There is widespread consensus that current recommended daily intake guidelines for vitamin D are not sufficient for optimal immune health (9, 49). A better understanding of the genetic contribution to 25(OH)D concentrations may have important implications in the debate for changing such guidelines.

Despite numerous studies that consider an association between vitamin D and MS risk, surprisingly few have directly measured serum concentrations of vitamin D [25(OH)D] in MS patients. Although we did not find an association of adult 25(OH)D concentrations and MS status in the current study, that does not rule out the possibility that such a relation exists. It may be that assessing childhood or even maternal concentrations is required to support or refute the vitamin D–MS theory, and the challenges of this are obvious. The present results support a significant genetic influence in determining serum 25(OH)D concentration, and further investigation to decipher the genes involved in its regulation is merited. If a particular genetic background alters 25(OH)D influence on immune function, these genetic factors could in turn affect susceptibility to MS.

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