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Genetic predictors of 25-hydroxyvitamin D levels and risk of multiple sclerosis

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Abstract

Risk of multiple sclerosis (MS) decreases with increasing plasma levels of 25-hydroxyvitamin D [25(OH)D]. If this association reflected a protective effect of vitamin D, MS risk should be lower among individuals carrying genetic variants that predict high 25(OH)D levels. The aim of the study was to determine whether individuals with genotypes predicting higher 25(OH)D levels have decreased MS risk. Logistic regression was used to assess the association between single nucleotide polymorphisms (SNPs) associated with 25(OH)D levels and MS risk in 1,655 cases and 6,349 controls. Analyses were further stratified by *HLA-DR15* status, assessed by genotyping a single SNP strongly correlated with the *HLA DRB1*1501* risk haplotype, and complemented by

considering a SNP near *CYP27B1*. SNPs in *GC* were predictors of 25(OH)D levels, but not MS risk, in either *HLA-DR15* negative or *HLA-DR15* positive individuals. In contrast, there was a suggestion of a difference in the effect of a *CYP2R1* allele dependent on *HLA-DR15* genotype. The 'A' allele of *CYP2R1* rs10741657 was associated with increased 25(OH)D levels and a non-significant reduced MS risk among *HLA-DR15* negative (OR = 0.89, 95% CI: 0.79, 1.01) that was not apparent in *HLA-DR15* positive individuals. The 'C' allele of *CYP27B1* rs703842 was inversely associated with MS risk; this association appeared stronger among *HLA-DR15* negative (OR = 0.79, 95% CI: 0.69, 0.90) compared to *HLA-DR15* positive individuals (OR = 0.91, 95% CI: 0.80, 1.04). This preliminary finding suggests the possibility that the putative beneficial effect of vitamin D on MS risk maybe attenuated in individuals carrying the *HLA-DR15* MS risk allele.

Keywords

Multiple sclerosis; Genetics; *HLA-DR15*; Vitamin D; 25-hydroxyvitamin D

Introduction

In longitudinal studies, both higher vitamin D intake [1] and high serum levels of 25-hydroxyvitamin D (25(OH)D) [2], which is a good biomarker of availability of vitamin D to tissues, have been found to be inversely related to MS risk. These observations, and experimental evidence that vitamin D may modulate autoimmune responses [3], support the hypothesis that high vitamin D levels may protect against MS [4]. On the other hand, a conserved vitamin D responsive element (VDRE) was recently found to be present in the promoter region of the *HLA DRB1*1501* MS risk haplotype (*HLA-DR15*) [5]. This finding suggests that the effect of vitamin D on MS risk may be dependent on an individual's *HLA-DR15* status.

Ideally, the relation between 25(OH)D levels and MS risk should be studied directly in large longitudinal studies. These studies, however, are difficult to conduct, because they involve the collection of serial blood samples from large numbers of healthy individuals. An alternative approach, sometimes referred to as 'Mendelian randomization' [6], involves using genetic factors that predict the exposure of interest as a proxy for the exposure itself. If vitamin D had a protective effect, individuals with genotypes associated with higher 25(OH)D would be expected to have a reduced MS risk, unless the same genotypes also increase MS risk through independent mechanisms. Although reliance on the latter assumption is an important limitation, this may be in part offset by the advantage of having a measure of exposure that does not vary in time, is easy to measure, and may reflect long term differences between individuals from birth up to the time of disease onset. The latter is particularly important in investigations of vitamin D levels, which vary considerably with age and season, so that levels at a single point in time may have only a modest effect on MS risk.

We, therefore, undertook to examine the relationship between genetic predictors of 25(OH)D levels, identified by conducting a genome-wide association study of 25(OH)D levels, and MS risk in the presence or absence of *HLA-DR15*.

Methods

Human subjects

This work was approved by the appropriate ethics committee.

Identification of genetic predictors of 25(OH)D

Study population—The study population included 872 women (436 women with breast cancer and 436 healthy control women) enrolled in the Nurses' Health Study (NHS). These women were selected because they had participated in a genome-wide association study (GWAS), the Cancer Genetic Markers of Susceptibility (CGEMS) study [7], and in a study of breast cancer risk that involved measurement of plasma levels of 25(OH)D [8]. All participants were also included in a concurrent collaboration with investigators at the NCI to perform a meta-analysis of genome-wide association with 25(OH)D levels [9].

Methods—Genotyping in the CGEMS study was performed using the Illumina HumanMap 500 array which includes 528,173 SNPs [7]. To assess the association between 25(OH)D levels and individual SNPs, we first created an adjusted vitamin D level for each woman by regressing the plasma 25(OH)D concentration on age at blood draw, season (using a sin/cos function [2]), body mass index, physical activity, laboratory batch, state of residence, and vitamin D intake from supplements and food sources, and taking the residuals of this regression. Linear regression was then performed by regressing the adjusted residual 25(OH)D levels on each SNP in the GWAS (coded by number of minor alleles). To address potential population stratification, models were adjusted for the top four principal components of variation, as previously described [10].

Further investigation of gene regions of interest was conducted through imputation of SNPs using the computationally efficient Hidden Markov Models implemented in MACH 1.0 [11] for SNPs categorized in the HapMap but not in the Illumina 500 K panel. The adjusted 25(OH)D was regressed on these SNPs using an expectation–substitution approach to account for uncertainty in imputed genotypes [12, 13].

SNP markers were considered for further study in relation to MS risk if they were either (1) significant at genome wide significance level ($p \leq 5 \times 10^{-8}$) or (2) were suggestive of association at whole genome level (defined as $p \leq 1 \times 10^{-4}$) and a priori candidates based on a well described role in the vitamin D metabolic pathway. We considered as candidates the main enzymes involved in the metabolism of vitamin D (*CYP27B1*, *CYP2R1*, *CYP27A1*, *CYP24A1*), the vitamin D binding protein (*GC/DBP*), and the vitamin D receptor (*VDR*).

Association with MS in the IMSGC

Study population—SNPs identified to be positive hits for predicting 25(OH)D using the criteria described above were examined in relation to MS risk in data from the participants in two MS genetics studies. First, we included participants in the International Multiple Sclerosis Genetics Consortium (IMSGC), [14] which includes MS cases from the United Kingdom and United States, and unrelated, healthy control subjects. We also analyzed an unpublished dataset of MS cases and controls enrolled in the Brigham and Women's Hospital (BWH) Genetics Collection, recruited at the Partners MS Center in Boston, Massachusetts. All MS cases met McDonald criteria [15]. Further details on demographic and clinical characteristics of participants in the BWH and IMSGC are described in a recent genome-wide meta-analysis for MS which utilized the same data [16]. For all individuals in the BWH Genetics Collection ($n = 860$ cases and 1,720 controls) and participants in the IMSGC in the US ($n = 342$ cases and 1,679 controls) and UK ($n = 453$ cases and 2,950 controls), we obtained direct genotype data (Affy 500 K) as well as all imputed SNPs using the CEU phase 2 HapMap data [17] for a total study population of 1655 cases and 6349 controls. A subset of the IMSGC controls was also included as part of the recent ANZgene study which identified two novel MS susceptibility loci including a SNP upstream of *CYP27B1* [18].

Methods—Main effects of SNPs identified from the GWAS of 25(OH)D were assessed via unconditional logistic regression models adjusting for gender and study. SNPs were coded as an additive or allelic model assessing the effect associated with an additional minor allele. Interactions with *HLA-DR15* were considered by including an interaction term in the logistic regression models which was the cross-product of the number of minor alleles for the 25(OH)D SNP and the number of minor alleles for *HLA-DR15*. The *HLA-DR15* genotype was determined by genotyping rs3135388, a SNP which is highly correlated ($r^2 = 0.97$) with *HLA DRB1*1501 (HLA-DR15)* [19].

Study specific estimates were pooled to determine a combined OR and 95% CI using inverse variance weights [20]. Fixed effect model estimates were used as all tests of heterogeneity were non-significant at the $\alpha = 0.05$ level.

Results

GWAS of 25(OH)D

In the GWAS of 25(OH)D levels including only the 872 women from the CGEMS study, no SNPs reached genome wide significance ($p < 5 \times 10^{-8}$). However, in the meta-analysis including these women as well as 3,629 men and women from four additional cohorts and an additional 4,378 replication samples from the NHS and HPFS, we identified genome-wide significant associations with rs2282679 in *GC* ($p < 1 \times 10^{-29}$) and rs10741657 in *CYP2R1* ($p < 10^{-15}$) [9]. No other markers met our selection criteria.

In our study population of the 872 CGEMS women, with an average 25(OH)D of 81.9 nmol/l, each additional 'C' allele of *GC*rs2282679 was associated with a 7.8 nmol decrease in 25(OH)D, and each additional 'T' allele of *GC*rs7041 (a frequently studied non-synonymous coding exon polymorphism in linkage disequilibrium with rs2282679) was associated with a 5.5 nmol/decrease in 25(OH)D. The finding of an association between the SNPs in *GC*, particularly our top hit rs2282679, and 25(OH)D levels is consistent with a recent publication in the PLCO Cancer Screening Trial [21]. In the CGEMS women, each additional 'A' allele of *CYP2R1* rs10741657 was associated with a 5.3 nmol/L increase in 25(OH)D level. This SNP was of particular interest because of the previous report of its association with type 1 diabetes and 25(OH)D among diabetic patients [22].

Established MS susceptibility loci and 25(OH)D levels

We also investigated whether recently confirmed and newly identified MS susceptibility loci [16, 18] were associated with 25(OH)D levels in our population. Fifteen of 19 of these SNPs, or proxies with $r^2 > 0.8$, were identified in our data (*HLA-DRB1*, *HLA-B*, *CD58*, *CLEC16A*, *IL7R*, *TNFRSF1A*, *CD6*, *CSCR4*, *PTGER2*, *MPHOSPH9*, *IL12A*, *OLIG3*, *TNFAIP3*, *CYP27B1*, *CD40*). None were associated with 25(OH)D levels (all $p > 0.1$). Because of differences in genotyping platforms used, suitable proxies were not available for the SNPs in *IL2RA*, *TNFRSF1A* (rs4149584), *IRF8*, *ZMIZ1* and *RGS1*.

Association with risk of MS

We tested the hypothesis that variants associated with increased plasma levels of 25(OH)D are inversely related to risk of MS in the combined BWH/IMSGC data. We also considered a SNP (rs703842) upstream of *CYP27B1*, recently identified in a GWAS of MS [18] and replicated in a more recent study [23]. *CYP27B1* encodes the enzyme that catalyzes the conversion of 25(OH)D to its active form, 1, 25(OH)₂D, and variations in *CYP27B1* may thus contribute to tissue availability of vitamin D.

None of the *GC* SNPs were associated with MS risk (OR = 0.96, 95% CI: 0.88, 1.05; for rs2282679) and this effect did not vary by *HLA-DR15* genotype.

For *CYP2R1* rs10741657, the association with MS risk was not significant, but the results were suggestive of an inverse association between the alleles that predict higher 25(OH)D levels and MS risk (Table 1). The interaction with *HLA-DR15* was not significant. However, in stratified analyses, the minor 'A' allele of rs10741657 was suggestive of an association with a lower MS risk among *HLA-DR15* negative ($p = 0.06$), but not *HLA-DR15* positive individuals (Table 1).

Similar to the previous report, which includes a sub-set of the controls used in the current study [18], rs703842, near *CYP27B1* was significantly associated with risk of MS (Table 1). The interaction between this SNP and *HLA-DR15* was not significant, though the protective effect appeared to be stronger in *HLA-DR15* negative compared to *HLA-DR15* positive individuals (Table 1).

Discussion

To assess whether higher vitamin D levels may reduce the risk of MS, we identified genetic variants that predict high serum 25(OH)D levels, and then compared the risk of MS across the corresponding genotypes. Single nucleotide polymorphisms in *GC*, encoding the vitamin D binding protein, and *CYP2R1*, a key 25-hydroxylase that catalyzes the conversion of vitamin D to 25(OH)D [24], were identified as the strongest determinants of 25(OH)D levels [9]. No associations were found between *GC* SNPs and MS risk, whereas there was evidence that the *CYP2R1* genotype that results in the highest 25(OH)D levels was associated with a reduced risk of MS in *HLA-DR15* negative individuals, but not in *HLA-DR15* positive individuals. We also observed a significant association between *CYP27B1* SNP rs703842, consistent with previous findings [18, 23], and MS risk, although this SNP was not associated with 25(OH)D levels, nor were any other MS susceptibility SNPs for which we had data available.

DBP transports vitamin D metabolites from the liver and kidneys to target tissues [25]. Greater than 85% of 25(OH)D and 1,25(OH)₂D are bound to DBP, with 25(OH)D showing a ten fold higher affinity [26]. We identified SNPs in *GC* that were significantly associated with 25(OH)D levels. The strongest predictor of 25(OH)D, rs2282679, was also previously identified in a study among 781 participants in the PLCO Cancer Screening trial [21] using a candidate gene/SNP approach and in a recent GWAS including our NHS CGEMS data [9]. For rs2282679, we observed a 20% reduction in 25(OH)D levels for those carrying the variant 'AA' compared to those with 'CC' genotype (70 vs. 87 nmol/L in NHS CGEMS women). Despite this notable difference in 25(OH)D levels, we did not observe an association between this or any of the *GC* SNPs and risk of MS. Further stratification of risk estimates by *HLA-DR15* positivity did not reveal any interactions or suggestion of differential risk. The results of two previous studies of *GC/DBP* SNPs and MS risk, one including 236 Canadian sib-pairs and one among 107 Japanese cases and controls, also found no significant associations [27, 28].

The fact that MS risk was not increased among individuals carrying *GC* variants associated with lower 25(OH)D levels is apparently inconsistent with the hypothesis that vitamin D reduces MS risk. It remains possible, however, that variations in 25(OH)D due to differences in DBP are poorly correlated with the availability of 25(OH)D to target tissues, perhaps because of concomitant changes in the DBP affinity for vitamin D metabolites. In particular, a polymorphism in the non-synonymous coding exon SNP (rs7041) that is in linkage disequilibrium with rs2282679, may reduce the DBP affinity for 1,25(OH)₂D [29],

and may thus result in relatively higher levels of free 1,25(OH)₂D and maintained biological activity in spite of lower circulating 25(OH)D levels.

The other significant predictor of 25(OH)D levels, *CYP2R1*, rs10741657 is one of several SNPs in strong linkage disequilibrium found to be associated with circulating 25(OH)D levels [9]. We chose to study rs10741657 because it was consistently associated with 25(OH)D levels in two previous studies, one among 133 individuals with type I diabetes in Germany [22], and one among German asthmatic families [30]. In our study of NHS CGEMS participants, the 'A' allele of rs10741657 predicted a 7% increase in 25(OH)D levels relative to a mean of 81.9 nmol/L in our population. Based on the results of the only prospective study of 25(OH)D and MS risk [2], and applying a linear interpolation, a 4.5 nmol/L increase in serum 25(OH)D (the increase associated with each additional 'G' allele of *CYP2R1* rs10741657 in our GWAS) would predict a relative risk of MS of only 0.95, which is the same relative risk of MS that we observed for an additive model. This consistency suggests that any observed association with *CYP2R1* is likely mediated through the effect on 25(OH)D levels. The fact that our finding was not significant may be due to our relatively small sample size given the predicted modest effect on MS risk.

No polymorphisms in the *CYP27B1* region were associated with 25(OH)D levels, consistent with some studies [21, 30], but not another in MS twins [31].

The relation between the variant *CYP2R1* and *CYP27B1* alleles and MS risk is possibly dependent on the presence of the *HLA-DR15* risk allele—among *HLA-DR15* negative individuals, the *CYP2R1* 'A' and *CYP27B1* 'C' alleles were associated with a 11 and 21% lower risk of MS, whereas among *HLA-DR15* positive individuals a weaker inverse association or no association was observed. An interaction between the effect of a polymorphism associated with circulating levels of 25(OH)D or bioavailability of vitamin D to target tissues and the *HLA-DR15* haplotype could be related to the recent findings of a highly conserved VDRE in the promoter region of the *HLA DRB1*1501* haplotype. In ex vivo experiments, the conserved VDRE was found to be functional and bound VDR at a higher affinity than other VDREs. Stimulation with 1,25(OH)₂D of cells transiently transfected with gene constructs including the consensus *HLA-DR15* sequence increased *HLA-DRB1*1501* expression 1.6 folds, while no change was seen for constructs bearing sequences specific to other DR haplotypes [5]. An increased expression of *HLA-DRB1*1501* could theoretically offset or dampen an otherwise beneficial effect of high levels of vitamin D.

There are certain limitations to our analysis. Though the number of subjects for the GWAS was relatively small, our results were consistent with the finding from the larger collaboration [9] and with a more recent large GWAS for 25(OH)D [32]. We also had only one measurement of 25(OH)D for each individual and, therefore, there is some misclassification of long term 25(OH)D status. However, in a subset of participants in the NHS, we have previously shown that a one time measure of 25(OH)D is a reasonable classification of exposure for 3 years (intraclass correlation over 3 year period = 0.7) [33]. Finally, in the analyses relating genotype to MS risk, we could not account for UVB exposure and other environmental determinants of circulating 25(OH)D. Because genetic variations contribute only modestly to determine 25(OH)D levels, the expected associations between 25(OH)D-related SNPs and MS risk are modest, and could be obfuscated by variations in 25(OH)D due to environmental factors.

Overall, the results of this study provide only modest support to the hypothesis that genetically determined increases in serum 25(OH)D levels are associated with a reduced MS risk, and suggest that the possible protective effect of vitamin D may be restricted to

individuals who do not carry the *HLA-DR15* MS risk allele. However, this finding requires replication and, because genotype was used as a proxy for actual 25(OH)D levels, the implicated effect needs to be confirmed in a study population where prospectively collected 25(OH)D levels can be directly measured.

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Table 1Odds ratios of multiple sclerosis according to *HLA-DR15* genotype

	Genotype	Cases (<i>n</i> = 1,655)	Controls (<i>n</i> = 6,349)	OR (95% CI) ^a
<i>HLA-DR15</i>	CC/CT/TT (%)	43/48/9	72/25/2	3.06 (2.76, 3.38)
<i>GC</i> , rs2282679	CC/CA/AA (%)	51/40/8	51/40/9	0.96 (0.88, 1.05)
<i>CYP2R1</i> , rs10741657	GG/GA/AA (%)	40/45/15	37/48/15	0.95 (0.88, 1.04)
<i>CYP27B1</i> , rs703582	TT/TC/CC (%)	50/42/8	46/44/10	0.87 (0.80, 0.95)
Distribution of <i>GC</i> rs2282679 genotypes in MS cases and controls				
Among <i>HLA-DR15</i> −	CC/CA/AA (%)	52/40/8	51/41/9	0.91 (0.80, 1.04)
Among <i>HLA-DR15</i> +	CC/CA/AA (%)	51/41/9	52/39/9	1.02 (0.89, 1.16)
<i>P</i> for interaction		0.10		
Distribution of <i>CYP2R1</i> rs10741657 genotypes in MS cases and controls				
Among <i>HLA-DR15</i> −	GG/GA/AA (%)	42/42/15	36/49/15	0.89 (0.79, 1.01)
Among <i>HLA-DR15</i> +	GG/GA/AA (%)	38/48/15	38/47/14	1.05 (0.93, 1.19)
<i>P</i> for interaction		0.06		
Distribution of <i>CYP27B1</i> rs703842 genotypes in MS cases and controls				
Among <i>HLA-DR15</i> −	TT/TC/CC (%)	53/40/6	46/44/10	0.79 (0.69, 0.90)
Among <i>HLA-DR15</i> +	TT/TC/CC (%)	47/43/9	45/44/11	0.91 (0.80, 1.04)
<i>P</i> for interaction		0.10		

Adjusted for gender and study

^aOR for an additional minor allele