Published in final edited form as:
*Mult Scler*. 2010 February ; 16(2): 133. doi:10.1177/1352458509355069.

# Polymorphisms in vitamin D metabolism related genes and risk of multiple sclerosis

**K Claire Simon**[1], **Kassandra L Munger**[1], **Xing Yang**[1], and **Alberto Ascherio**[1,2,3]

[1]Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

[2]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

[3]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

## Abstract

**Background—**The extent to which potential genetic determinants of vitamin D levels may be related to MS risk has not been thoroughly explored.

**Objective—**To determine whether polymorphisms in *VDR, CYP27B1, CYP24A1, CYP2R1* and *DBP* are associated with risk of MS and whether these variants may modify associations between environmental or dietary vitamin D on MS risk.

**Methods—**Nested case-control study in two, large cohorts of US nurses, including 214 MS cases and 428 age-matched controls. Conditional logistic regression models were used to calculate relative risks (RR) and 95% confidence intervals (CIs) and to assess the significance of gene-environment interactions.

**Results—**No associations were observed for any of the SNPs in *VDR, CYP27B1, CYP24A1, CYP2R1* or *DBP* (p>0.05 for all). We did observe an interaction (p=0.04) between dietary intake of vitamin D and the VDR Fok I polymorphism on MS risk. The protective effect of increasing vitamin D was evident only in individuals with the 'ff' genotype (RR=0.2, 95% CI: 0.06, 0.78; p=0.02 for 400IU/day increase).

**Conclusion—**This does not support a role for the selected SNPs involved in vitamin D metabolism in the etiology of MS. The finding of a marginally significant gene-environment interaction requires replication in larger datasets, but suggests future genetic studies may benefit from considering relevant environmental context.

## Introduction

Previous work suggests a role for higher vitamin D providing protection against multiple sclerosis (MS). Vitamin D intake, decreasing latitude, increased sun exposure, and high serum vitamin D levels have all been shown to be associated with decreased risk of MS [1].

The metabolism of vitamin D is carried out through a series of hydroxylation reactions in the liver and kidneys catalyzed by members of the cytochrome p450 family.[2] CYP2R1 is the primary enzyme responsible for the metabolism of vitamin D to 25-hydroxyvitamin D (25(OH)

Corresponding author: K Claire Simon – ksimon@hsph.harvard.edu, Harvard School of Public Health, Dept of Nutrition, 665 Huntington Avenue, Building 2, Rm 322, Boston, MA 02115, Ph: 617-432-7153, fax: 617-432-2435.

D) [3], which is further metabolized to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) via the action of CYP27B1. The primary bioactive metabolite $1,25(OH)_2D$ exerts its effect through association with the vitamin D receptor (VDR), which is found on a variety of cell types, including cells in the immune system[4].

It is plausible that genes that are involved in vitamin D metabolism, transport or activity may be related to risk of MS or modify the association between environmental or dietary exposure to MS. Polymorphisms in the vitamin D receptor have been the most studied in relation to MS, but findings have been inconsistent. Some have reported a significant association between individual SNPs (ApaI, TaqI, FokI and BsmI) and risk of MS,[5–7] while others found no significant association.[8–11]. The vitamin D binding protein (DBP) which is involved in transport and binding of vitamin D metabolites has been investigated in two MS studies, both finding no association with MS[7,8].

There is one study of interaction between potential genetic determinants of vitamin D metabolism and vitamin D intake or environmental exposure as it relates to MS. Dickinson et al., recently observed an interaction between the VDR Cdx-1 polymorphism and sun exposure at ages 6–10. They found there was an increased MS risk associated with the 'G' allele among those with low sun exposure at ages 6–10, but not in those with high exposure during that time period [12].

Additionally, possible modification by the HLA DRB1* 1501 risk haplotype has not been adequately explored. The study mentioned above found no significant interaction between VDR polymorphisms and HLA DR15 genotype, but no other studies have investigated this gene-gene interaction. Notably, recent experimental work has shown that the DRB1*1501 risk haplotype contains a highly conserved vitamin D responsive element, whereas considerable variability exists in this region of the non-risk DRB1 haplotypes. This difference was found to have a functional impact with increased DR15 expression in cells expressing DRB1*1501 upon administration of $1,25(OH)_2D$ that was not observed in other DRB1 haplotype bearing cells [13].

We, therefore, conducted a nested case-control study within the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS II) to investigate the relationship between SNPs related to vitamin D metabolism and risk of MS, as well as gene-environment and gene-gene interactions in the vitamin D pathway as they relate to MS risk.

## Methods

### Study population

Participants in this study were women who provided blood from amongst those enrolled in the NHS and NHS II. The NHS began in 1976 when 121,700 nurses aged 30–55 returned mailed questionnaires regarding lifestyle factors and disease history. The NHS II began in 1989 when 116,671 women aged 25–42 returned similar questionnaires. Biennial questionnaires are mailed to update information on risk factors and disease occurrence.

All participants were invited to provide blood samples for investigations of biomarkers and disease outcomes. Blood was collected from women between 1989 and 1990 in NHS (32, 826 women) and from 1996 to 1999 in NHS II (29, 613 women).

### Case ascertainment

The ascertainment of MS cases in these cohorts has been previously described [14]. Briefly, participants who reported a new diagnosis of MS were asked permission to contact their neurologists and review their medical records. After obtaining permission, neurologists were

sent a questionnaire to determine certainty of the diagnosis (definite, probable, possible, or not MS), the date of onset of neurological symptoms related to MS, other aspects of the clinical history, and laboratory test results. Since 93% of all definite and probable diagnoses conformed to the Poser criteria for diagnosis of MS when applied to the clinical and laboratory data provided in the questionnaire, we classified as cases women who had a diagnosis of definite or probable MS according to their neurologists.

A total of 217 incident cases of MS were documented (149 with blood and 68 with buccal cell), of which 214 cases and matched controls had relevant data for analysis. For each case, we randomly selected 2 women without MS, matched by year of birth and study cohort. Over 90% of the women included in the study reported having a white ancestry.

## Laboratory analysis

SNPs in *VDR*, *CYP27B1*, *CYP24A1*, *CYP2R1* and *DBP* were chosen based on information from previous literature and minor allele frequency greater than 10%. The following SNPs were identified for inclusion: *VDR*-rs1544410(BsmI), rs7975232(ApaI), rs731236(TaqI), rs10735810(FokI), rs11568820(Cdx2); *CYP27B1*-rs703426 and rs10877012; *CYP24A1*-rs2296241; *CYP2R*1-rs10500804, rs12794714; *DBP*- rs7041 and rs4588. Genotyping was performed on genomic DNA extracted from buffy coat with QIAmp (Qiagen Inc., Chatsworth, CA) using the TaqMan assay on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Concordance of blinded quality control samples was 100%.

A single SNP, rs3135005, was used to assess HLA DRB1*1501 as previously described [15].

## Covariate assessment

Total dietary vitamin D intake was assessed via validated food frequency questionnaires as previously described[16]. Ethnicity and residence at birth, age 15 and age 30 were asked on the biennial questionnaires as part of the general cohort follow-up. From state of residence, latitude (North, Middle, South) was determined as previously described[14]. Measurements of anti-EBNA antibodies were used in a prior study in these cohorts as previously described [17].

## Statistical analysis

The assumption of Hardy-Weinberg equilibrium was tested for all SNPs using a $\chi^2$ test comparing observed to expected genotype frequencies. Given our sample size, we estimate that we have >_80% power to detect an odds ratio of 1.8 for a minor allele frequency of 0.17 (the lowest reported minor allele frequency in this study).

Conditional logistic regression models were used to calculate relative risks (RRs) and 95% confidence intervals (CIs) assessing the relationship between individual SNPs and risk of MS. To test for effects of genotype, we used likelihood ratio tests, comparing a model including genotype (coded as 0, 1, or 2 according to number of minor alleles) to the same model without genotypes.

To investigate possible interactions, interaction terms were created which were the cross-product of number of minor alleles of the SNP and vitamin D intake (continuous), latitude (categorized) or HLA DR15(additive for number of minor alleles). Further, for those SNPs which suggested significant heterogeneity (p for interaction < 0.05), estimates of the association between vitamin D intake, latitude and DR15 and risk of MS were generated within strata of the relevant genotype.

## Results

Tests of HWE did not suggest significant deviations for any of the genotyped SNPs. Among controls, the wild type genotype of the two DBP SNPs(rs7041-GG and rs4588-AA) was more common in women reporting Scandanavian or other white ancestry compared to those reporting Southern European or non-white ancestry. Otherwise, no significant associations were observed for association between anti-EBNA Ab titers, ethnicity or latitude of residence and any vitamin D related SNP (data not shown). Similarly, no associations were observed between any of the individual SNPs and risk of MS (Table 1). Further adjustment for the HLA DR15 resulted in similar effect estimates and pair-wise tests of the interaction between individual vitamin D SNPs and HLA DR15 were non-significant.

We did, however, observe a significant interaction between vitamin D intake and the VDR FokI polymorphism (p for interaction=0.04; Table 2). Stratifying by genotype showed that among women with the common 'FF' genotype, no association between vitamin D intake and risk of MS was observed. In contrast, among those with the variant 'ff' genotype, there was a significant 80% reduced risk of MS for an increase of 400 IU/day of vitamin D. This relationship appeared to be dose dependent and the risk in women carrying the 'Ff" genotype was intermediate. Though not significant (p=0.19), a similar trend for an interaction between latitude of residence at age 15 and VDR FokI genotype was observed with a stronger protective effect of living further South seen among women with the 'ff' genotype (Table 2).

## Discussion

These findings do not support a role for an independent effect of the vitamin D related gene polymorphisms investigated and risk of MS. This is consistent with some investigations showing no association [8–11], but not others in which one of the SNPs of VDR was significantly associated with risk of MS [5–7]. The finding of no association with the two SNPs in DBP is also consistent with the two previous studies of this gene and MS risk [7,8].

We did, however, observe a significant interaction between vitamin D intake and the VDR FokI polymorphism as it relates to MS risk, but not the previously reported interaction with Cdx-1[12]. The interaction effect is similar, though the SNPs are not in LD with one another, in that the effect of the polymorphism in both cases appears to be restricted to those with low vitamin D exposure (as measured by intake in our study or by past sun exposure in the Dickinson et al. paper). This interaction has been explored in other diseases, but findings are not consistent. For example, a genetic effect only in those with low vitamin D exposure is consistent with four studies of prostate cancer risk in which VDR polymorphisms were associated with disease risk only among those with the low serum 25(OH)D[18–21]. However, two other studies of prostate cancer risk found stronger associations among those with high sunlight exposure [22,23]. Similarly, the relationship between VDR FokI and vitamin D intake is in contrast to other diseases, such as type 1 diabetes in which a significant interaction was found, but in the opposite direction, with increased protection of UVR among women with the 'F' allele [24]. In our study, the protective association of dietary or environmental vitamin D appeared stronger among women with the 'f' allele.

The VDR FokI polymorphism is a C/T polymorphism in the translation initiation codon of VDR. The variant T (or 'f') results in the presence of a FokI restriction enzyme site and translation of a 3 amino acid longer VDR protein than the C (or 'F') allele. The wild type, shorter VDR, is associated with increased transcriptional activity [25]. Our findings, therefore, suggest that there may be some threshold level of transcriptional activity necessary to maintain downstream cellular signaling pathways in such a way as to prevent changes that are related to development of MS. Specifically, increased exposure to vitamin D may rescue any decreased

target cell activity, due to decreased transcription, that may result in altered immunologic profiles or activity that contribute to MS risk. In contrast, among women with increased target cell activity, minimal amounts of environmental or dietary exposure to vitamin D may be sufficient to surpass this threshold and maintain a healthy immunologic environment.

There are limitations to the present investigation. First, in relation to the findings of the main effects of individuals SNPs and MS risk, this was not an exhaustive examination of variants in these genes and the selected SNPs did not provide full tagging coverage as assessed by the HapMap data [26]. Therefore, we cannot exclude the possibility that other gene regions may be important. Second, because of the small sample size, we were underpowered to detect modest effect sizes, therefore, these findings only provide evidence against strong effects of these genes. Lastly, we identified the two CYP2R1 SNPs using information from previous literature and minor allele frequencies. It seems unlikely that the two SNPs chosen are variants that result in functional changes as one is located in an intronic region and the other a synonymous coding exon polymorphism. Therefore, if there is a true effect, it is likely due to a polymorphism in linkage disequilibrium with the two chosen here.

The finding of a significant interaction could be due to chance and requires replication in larger datasets. The consistency of this finding considering vitamin D intake and latitude of residence supports that this is not due to chance as it is unlikely these two factors are correlated and therefore, supports the notion that vitamin D from exogenous sources provides varying levels of protection against MS dependent on an individual's genetic variation. Notably, there are few populations with biological samples for genetic analysis and prospectively collected data, which are necessary to test many gene-environment hypotheses, such as those related to diet, in an unbiased manner.

It is clear that MS is a multifactorial disease and this finding supports the notion that risk factors may only be relevant in a proportion of the population with underlying genetic susceptibility. Further investigations are necessary to replicate this finding and explore biological underpinnings of the plausibility of a gene-environment interaction as it relates to vitamin D and MS risk.

## Acknowledgments

The authors would like to thank Eilis O'Reilly, Pati Soule and Hardeep Ranu for technical and laboratory support. The work presented here is the sole content of the authors and does not necessarily represent the view of the National Institutes of Health.

**Funding**

This work was funded in part by the National Institutes of Health/National Institute of Neurological Disorders and Stroke [R01 NS47467]. Dr. Simon is supported a National Institute of Health/National Research Service Award grant [T32 ES016645-01].

## References

1. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol 2007;61(6):504–513. [PubMed: 17492755]
2. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998;78(4):1193–1231. [PubMed: 9790574]
3. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A 2004;101(20):7711–7715. [PubMed: 15128933]
4. Bikle D. Nonclassic actions of vitamin d. J Clin Endocrinol Metab 2009;94(1):26–34. [PubMed: 18854395]

5. Tajouri L, Ovcaric M, Curtain R, Johnson MP, Griffiths LR, Csurhes P, et al. Variation in the vitamin D receptor gene is associated with multiple sclerosis in an Australian population. J Neurogenet 2005;19 (1):25–38. [PubMed: 16076630]
6. Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Hamada T, Miyasaka K, et al. Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. J Neurol Sci 1999;166(1):47–52. [PubMed: 10465499]
7. Niino M, Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Tashiro K. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. J Neurol Sci 2000;177(1):65–71. [PubMed: 10967184]
8. Steckley JL, Dyment DA, Sadovnick AD, Risch N, Hayes C, Ebers GC. Genetic analysis of vitamin D related genes in Canadian multiple sclerosis patients. Canadian Collaborative Study Group. Neurology 2000;54(3):729–732. [PubMed: 10680811]
9. Yeo TW, Maranian M, Singlehurst S, Gray J, Compston A, Sawcer S. Four single nucleotide polymorphisms from the vitamin D receptor gene in UK multiple sclerosis. J Neurol 2004;251(6):753–754. [PubMed: 15311355]
10. Smolders J, Damoiseaux J, Menheere P, Tervaert JW, Hupperts R. Fok-I vitamin D receptor gene polymorphism (rs10735810) and vitamin D metabolism in multiple sclerosis. J Neuroimmunol 2009;207(1–2):117–121. [PubMed: 19178954]
11. Partridge JM, Weatherby SJ, Woolmore JA, Highland DJ, Fryer AA, Mann CL, et al. Susceptibility and outcome in MS: associations with polymorphisms in pigmentation-related genes. Neurology 2004;62(12):2323–2325. [PubMed: 15210908]
12. Dickinson J, Perera D, van der Mei A, Ponsonby AL, Polanowski A, Thomson R, et al. Past environmental sun exposure and risk of multiple sclerosis: a role for the Cdx-2 Vitamin D receptor variant in this interaction. Mult Scler 2009;15(5):563–570. [PubMed: 19383647]
13. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton S, Dyment DA. Expression of the multiple sclerosis-associated MHC class II allele HLADrB1* 1501 is regulated by vitamin D. PLoS Genet 2009;5(2):e1000369. [PubMed: 19197344]
14. Hernán MA, Olek MJ, Ascherio A. Geographic variation of MS incidence in two prospective studies of US women. Neurology 1999;53(8):1711–1718. [PubMed: 10563617]
15. De Jager PL, Simon KC, Munger KL, Rioux JD, Hafler DA, Ascherio A. Integrating risk factors: HLA-DRB1*1501 and Epstein-Barr virus in multiple sclerosis. Neurology 2008;70(13 Pt 2):1113–1118. [PubMed: 18272866]
16. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology 2004;62:60–65. [PubMed: 14718698]
17. Ascherio A, Munger KL, Lennette ET, Spiegelman D, Hernán MA, Olek MJ, et al. Epstein-barr virus antibodies and risk of multiple sclerosis: A prospective study. JAMA 2001;286(24):3083–3088. [PubMed: 11754673]
18. Ahn J, Albanes D, Berndt SI, Peters U, Chatterjee N, Freedman ND, et al. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. Carcinogenesis 2009;30(5):769–776. [PubMed: 19255064]
19. Mikhak B, Hunter DJ, Spiegelman D, Platz EA, Hollis BW, Giovannucci E. Vitamin D receptor (VDR) gene polymorphisms and haplotypes, interactions with plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, and prostate cancer risk. Prostate 2007;67(9):911–923. [PubMed: 17440943]
20. Li H, Stampfer MJ, Hollis JB, Mucci LA, Gaziano JM, Hunter D, et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med 2007;4 (3):e103. [PubMed: 17388667]
21. Ma J, Stampfer MJ, Gann PH, Hough H, Giovannucci E, Kelsey KT, et al. Vitamin D receptor polymorphisms, circulating vitamin D metabolites and risk of prostate cancer in United States physicians. Cancer Epidemiol Biomarkers Prev 1998;7:385–390. [PubMed: 9610787]
22. Bodiwala D, Luscombe CJ, French ME, Liu S, Saxby MF, Jones PW. Polymorphisms in the vitamin D receptor gene, ultraviolet radiation, and susceptibility to prostate cancer. Environ Mol Mutagen 2004;43(2):121–127. [PubMed: 14991752]

23. John EM, Schwartz GG, Koo J, Van Den Berg D, Ingles SA. Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer. Cancer Res 2005;65(12):5470–5479. [PubMed: 15958597]

24. Ponsonby AL, Pezic A, Ellis J, Morley R, Cameron F, Carlin J, et al. Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels: a meta-regression analysis. Am J Epidemiol 2008;168(4):358–365. [PubMed: 18552362]

25. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338(2):143–156. [PubMed: 15315818]

26. The International HapMap Consortium. The International HapMap Project. Nature 2003;426(6968): 789–796. [PubMed: 14685227]

## Table 1

Risk of multiple sclerosis associated with vitamin D related SNPs

| SNP | Genotype | Case (%) | Control (%) | RR (95% CI)* |
|---|---|---|---|---|
| *VDR - rs1544410 (BsmI)* | TT/TC/CC | 39/49/13 | 34/47/19 | 1.1 (0.93, 1.31) |
| *VDR - rs7975232 (ApaI)* | TT/TG/GG | 29/45/26 | 28/50/22 | 0.96 (0.81, 1.15) |
| *VDR – rs731236 (TaqI)* | TT/TC/CC | 40/50/10 | 36/48/16 | 1.11 (0.92, 1.33) |
| *VDR-rs10735810 (FokI)* | CC/CT/TT | 36/45/19 | 41/44/15 | 0.93 (0.78, 1.09) |
| *VDR-rs11568820 (Cdx2)* | GG/GA/AA | 65/31/5 | 65/32/3 | 0.97 (0.79, 1.21) |
| *CYP27B1-rs703842* | TT/TC/CC | 50/42/8 | 47/41/12 | 1.07 (0.90, 1.27) |
| *CYP27B1-rs10877012* | GG/GC/CC | 52/40/8 | 48/40/12 | 1.11 (0.93, 1.32) |
| *CYP24A1-rs2296241* | AA/AG/GG | 29/48/23 | 31/46/23 | 0.98 (0.83, 1.16) |
| *CYP2R1-rs10500804* | GG/GT/TT | 28/49/23 | 30/51/19 | 0.94 (0.79, 1.11) |
| *CYP2R1-rs12794714* | AA/AG/GG | 29/48/22 | 30/51/19 | 0.96 (0.81, 1.15) |
| *DBP-rs7041* | GG/GT/TT | 33/51/16 | 30/51/19 | 1.07 (0.90, 1.26) |
| *DBP-rs4588* | AA/AC/CC | 54/41/5 | 53/39/9 | 1.06 (0.88, 1.27) |

* For increasing number of minor alleles

**Table 2**

Risk of multiple sclerosis associated with vitamin D intake and latitude of residence at age 15 according to VDR FokI genotype

| VDR_Fok I genotype | Vitamin D intake* | | | Latitude of residence** | | |
|---|---|---|---|---|---|---|
| | *N* | *RR (95% CI)* | *p-value* | *N* | *RR (95% CI)* | *p-value* |
| *F/F* | | | | | | |
| Cases | 43 | 1.18 (0.60, 2.31) | 0.63 | 71 | 0.82 (0.53, 1.28) | 0.38 |
| Controls | 151 | REF | | 153 | REF | |
| *F/f* | | | | | | |
| Cases | 55 | 0.77 (0.43, 1.36) | 0.36 | 87 | 0.56 (0.35, 0.91) | 0.02 |
| Controls | 163 | REF | | 164 | REF | |
| *f/f* | | | | | | |
| Cases | 21 | 0.21 (0.06, 0.78) | 0.02 | 33 | 0.50 (0.21, 1.20) | 0.12 |
| Controls | 60 | REF | | 55 | REF | |

Vitamin D intake prior to date of onset of first symptoms was available for 119 cases. Numbers may not sum to total number of cases and controls due to missing values.

*For a 400IU/day increase

**Trend across three categories of decreasing latitude (North, Middle, South)