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Vitamin D Status Is Associated with Relapse Rate in Pediatric-Onset Multiple Sclerosis

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Objective: We sought to determine if vitamin D status, a risk factor for multiple sclerosis, is associated with the rate of subsequent clinical relapses in pediatric-onset multiple sclerosis.
Methods: This is a retrospective study of patients with pediatric-onset multiple sclerosis or clinically isolated syndrome who were consecutively recruited into a prospective cohort at their clinical visit at the pediatric multiple sclerosis center of University of California, San Francisco or State University of New York at Stony Brook . Of 171 eligible patients, 134 (78%) with multiple sclerosis/clinically isolated syndrome were included in the cohort; a further 24 were excluded from this analysis due to lack of available serum (n = 7) or lack of follow-up (n = 17). Serum 25-hydroxyvitamin D_3 levels were measured and were adjusted to reflect a deseasonalized value. The adjusted serum 25-hydroxyvitamin D_3 level was the primary predictor in a multivariate negative binomial regression model in which the main outcome measure was the number of subsequent relapses.
Results: Among the 110 subjects, the mean unadjusted 25-hydroxyvitamin D_3 level was 22 $\pm$ 9ng/ml. After adjustment for age, gender, race, ethnicity, disease duration, disease-modifying therapy, and length of follow-up, every 10ng/ml increase in the adjusted 25-hydroxyvitamin D_3 level was associated with a 34% decrease in the rate of subsequent relapses (incidence rate ratio, 0.66; 95% confidence interval, 0.46–0.95; $p = 0.024$).
Interpretation: Lower serum 25-hydroxyvitamin D_3 levels are associated with a substantially increased subsequent relapse rate in pediatric-onset multiple sclerosis or clinically isolated syndrome, providing rationale for a randomized controlled trial of vitamin D supplementation.

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Vitamin D insufficiency, which appears to be a risk factor for several systemic autoimmune diseases, such as systemic lupus erythematosus and type I diabetes,[1,2] has likewise emerged as a risk factor for susceptibility to multiple sclerosis (MS).[3] It is uncertain, however, if vitamin D status influences the prognosis of individuals who have already developed MS. Although vitamin D supplementation improves clinical outcomes in an animal model of MS,[4] well-designed studies in humans are lacking. Recently, a vitamin D response element was reported in the promoter region of *HLA-DRB1*15* haplotypes.[5] *HLA-DRB1*1501* is the main susceptibility allele for MS, particularly in those of Northern European descent, and *HLA-DRB1*1503* is also associated with an increased risk of the disease, particularly in African Americans.[6] The identification of this vitamin D response element therefore raises the possibility that vitamin D supplementation could be harmful for at least some of those with a known diagnosis of MS, particularly because the active form of vitamin D increases *HLA-DRB*15* expression in vitro.[5]

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We therefore sought to address the question of whether vitamin D status affects the risk of subsequent relapse in a prospectively acquired cohort of patients with pediatric-onset MS.

Patients and Methods

The study was approved by the institutional review boards of the University of California, San Francisco (UCSF) and State University of New York (SUNY) Stony Brook. The study was conducted at each institution's Regional Pediatric Multiple Sclerosis Center of Excellence, representing 2 of the 6 multidisciplinary referral centers in the United States sponsored by the National Multiple Sclerosis Society. Patients with MS or clinically isolated syndrome (CIS)[7] with symptom onset at age ≤18 years who are seen at these centers are consecutively invited to participate in a longitudinal cohort study in which blood samples are collected at baseline, and demographic and clinical data are captured at baseline and throughout the period of follow-up (since April 2004 at SUNY Stony Brook and September 2006 at UCSF). Data acquired at the time of blood collection include age at onset, disease duration, gender, race, ethnicity, relapse history, Expanded Disability Status Scale (EDSS) score, and use of disease-modifying therapies (DMTs). Self-reported race was divided into 3 categories: white, mixed white/nonwhite, and nonwhite. Self-reported ethnicity was coded as non-Hispanic, partially Hispanic, or Hispanic. These variables were captured because it is thought that race and ethnicity influence the clinical severity of MS.[8] A patient was considered as being on a given DMT if he or she had received it continuously for at least 90 days, because it is thought that there is a lag between the initiation of a therapy and the onset of its effectiveness.[9,10] Furthermore, a therapy's effect was considered to remain for a period of 90 days after its discontinuation. Because *HLA-DRB1*15* is known to be the most powerful susceptibility locus for MS,[11] appears to affect the severity of the disease,[12,13] and has been shown to have a vitamin D response element in its promoter region,[5] we also performed *HLA-DRB1* genotyping as described previously.[11]

In the follow-up period, relapses, treatment status, and other data were documented at the patient's pediatric MS center visit; when a visit did not occur, follow-up details were provided by telephone contact with the patient's parent or by the patient's local physician. Relapses, or exacerbations, were defined as new or recurring neurologic symptoms referable to the central nervous system lasting for at least 48 hours after a remission of 30 days or more since the previous event. Pseudoexacerbations (symptoms occurring in the presence of fever or illness) were excluded from the analyses.

The primary predictor was the 25-hydroxyvitamin D_3 level (ng/ml). Levels in stored baseline serum samples were assessed by batched chemiluminescent assay (ARUP Laboratories, Salt Lake City, UT) for all but 8 samples, which were measured at a later time. The within-batch coefficient of variation for the test at ARUP Laboratories is between 4.5% and 10.1% (ARUP Laboratories, personal communication). 25-Hydroxyvitamin D_3 was chosen as a marker of vitamin D status because its concentration has a longer half-life than 1,25-dihydroxyvitamin D_3,[14] the active form to which the former is converted. Further, extrarenal (eg, macrophage) production of 1,25-dihydroxyvitamin D_3 is responsive to changes in the serum 25-hydroxyvitamin D_3 level.[15] We used baseline levels to represent typical levels during the follow-up period, because measurement during follow-up would be more likely to reflect a spurious effect-cause relationship due to increased disability causing reduced sunlight exposure. Serum 25-hydroxyvitamin D_3 levels are known to fluctuate according to season, and seasonal fluctuations may differ based on skin tone (eg, a white individual, who is more likely to be able to absorb ultraviolet radiation than an African American individual, may also be more likely to experience fluctuations in 25-hydroxyvitamin D_3 levels based on the season).[16] To make baseline values better represent typical levels, we therefore created deseasonalized values, stratified by race and ethnicity. We first categorized partial or full Hispanic white individuals as white Hispanics and full or partial non-white individuals (regardless of ethnicity) as nonwhite. We then created a 3-level variable to reflect skin tone: white non-Hispanic, white Hispanic, and nonwhite. For each level of that variable, we generated sine and cosine terms to model the influence of the date of blood draw (sine term = sine[day of year/365 · 2 · 3.14159]; cosine term= cosine[day of year/365 · 2 · 3.14159]). This led to the generation of 6 terms (white sine, white cosine, nonwhite sine, etc). These terms were added as predictors into a single linear regression model, along with the nonwhite and white Hispanic terms, in which the unadjusted 25-hydroxyvitamin D_3 level was the outcome. For each individual, a deseasonalized 25-hydroxyvitamin D_3 level was calculated as their predicted level on January 1 given their observed level, the day of the year it occurred, and their race/ethnicity category. The results of vitamin D testing were not given to the patients before the end of the study, because testing was done on stored specimens long after collection.

Statistical Analyses

The primary outcome of the study was the number of relapses occurring from the time of the blood draw to the time of last follow-up. The primary predictor for the analyses was the adjusted serum 25-hydroxyvitamin D_3 level.

As follow-up time was not uniform, and because the assumptions of the Poisson model were violated, a multivariate negative binomial regression model, adjusted for length of follow-up, was used to assess the impact of adjusted serum 25-hydroxyvitamin D_3 levels on the number of subsequent attacks, generating an incidence rate ratio (IRR) and corresponding 95% confidence intervals (CIs). Assessment of the linearity assumption for 25-hydroxyvitamin D_3 by inclusion of a quadratic term did not produce strong evidence of nonlinearity ($p = 0.32$). We included potential covariates of interest, including age at blood collection, gender, race, ethnicity, and disease duration. We also assessed whether *HLA-DRB1*1501/1503* status confounded or interacted with adjusted 25-hydroxyvitamin D_3 levels. To ensure no confounding by center of origin (UCSF vs SUNY Stony

Brook), year of blood draw, baseline EDSS, or number of relapses prior to baseline, we assessed the effect of adding those variable to the models. Finally, DMT was considered in the analyses. Because several patients started DMT after the date of blood collection, for the multivariate negative binomial regression models, the percentage of time that a patient was on treatment was added as a covariate. We also explored the impact of dichotomizing therapy exposure (yes/no for any treatment exposure during the study period). Because confounding by indication can severely distort the apparent effect of therapy, we also examined models that did not include therapy in the model.

Statistical analyses were performed using Stata 10.0 (StataCorp, College Station, TX).

Results

Of 171 patients seen at the pediatric MS centers, 134 (78%) enrolled in the cohort; 24 patients were excluded from this analysis because serum (n = 7) or clinical follow-up (n = 17) was not available. Genotyping of *HLA-DRB1*1501/1503* was not available for 6 patients; race (or race and ethnicity) could not be determined for 2 patients. Demographic and clinical data at the time of blood collection are presented in Table 1. Median follow-up during the study was 1.7 years (interquartile range, 0.2–4.0). In the follow-up period, 57 (52%) patients had another attack; 33 (30%) had 1 attack, 11 (10%) had 2 attacks, and 13 (12%) had ≥3 attacks. Whereas 80 (73%) were treated with DMTs during the study, only 23 (21%) were on therapy during the entire period of follow-up.

The mean (±standard deviation) unadjusted serum 25-hydroxyvitamin D_3 level for the cohort was 22 (±9) ng/ml. When a normal level was defined as ≥30ng/ml,[14] only 16 (15%) patients had normal unadjusted serum 25-hydroxyvitamin D_3 levels. The fitted 25-hydroxyvitamin D_3 levels for each race/ethnicity category are presented in the Figure.

In the univariate model, every 10ng/ml increase in the adjusted serum 25-hydroxyvitamin D_3 level was associated with a 34% decrease in the rate of subsequent attacks (IRR, 0.66; 95% CI, 0.49–0.90; $p = 0.009$). The results of the multivariate negative binomial regression analysis are presented in Table 2. For every 10ng/ml increase in the adjusted serum 25-hydroxyvitamin D_3 level, there was an estimated 34% decrease in the rate of subsequent attacks (IRR, 0.66; 95% CI, 0.46–0.95; $p = 0.024$). The estimates did not meaningfully change when treatment was removed from the model or when treatment was added as a binary covariate. Further, adding center (UCSF vs SUNY Stony Brook), year of blood draw, number of relapses before blood draw, baseline

TABLE 1: Demographic and Clinical Features of Patients at Time of Blood Collection

Characteristic	All Patients (n=110)	SUNY Stony Brook (n=43)	UCSF (n=67)
Age at blood collection, mean yr ± SD	15 ± 3	15 ± 3	15 ± 4
Disease duration at blood collection, median yr (IQ range)	1.0 (0.1–8.3)	0.9 (0.2–5.3)	1.1 (0.1–7.3)
Females, No. (%)	71 (65)	29 (67)	42 (63)
Nonwhite or partially nonwhite, No. (%)	29 (26)	9 (21)	20 (30)
Hispanic or partially Hispanic, No. (%)	48 (43)	16 (37)	32 (48)
*HLA-DRB*1501/1503* positive, No. (%)[a]	51 (49)	23 (56)	28 (44)
Relapses prior to blood draw, No. (%)			
1	33 (30)	13 (30)	20 (30)
2	35 (32)	16 (37)	19 (28)
≥3	42 (38)	14 (33)	28 (42)
Expanded Disability Status Scale score, median (IQ range)	1.75 (0–6.5)	2 (0–4)	1.5 (0–6.5)
Unadjusted serum 25-hydroxyvitamin D_3 in ng/ml,[b] mean ± SD	22 ± 9	22 ± 10	22 ± 9

[a]Missing for 6 people.
[b]Conversion factor to SI units=2.496.
SUNY = State University of New York; UCSF = University of California, San Francisco; SD = standard deviation; IQ = interquartile.

TABLE 2: Rate of Subsequent Relapse in Multivariate Negative Binomial Regression Analysis

Predictor	IRR	95% CI	*p*
25-Hydroxyvitamin D_3 level (per 10ng/ml increase)[a]	0.66	0.46–0.95	0.02
Age (per 5-year increase)	0.89	0.58–1.37	0.61
Disease duration (per 1-year increase)	0.95	0.83–1.09	0.45
Female	1.09	0.59–2.01	0.79
Nonwhite race			
Partial (n = 13)	0.71	0.26–1.95	0.51
Full (n = 16)	1.64	0.72–3.75	0.24
Hispanic ethnicity			
Partial (n = 14)	3.96	1.57–9.93	0.003
Full (n = 34)	1.65	0.82–3.31	0.16
On DMT for whole study	1.47	0.67–3.24	0.34

[a]Adjusted for season, by race/ethnicity; conversion factor to SI units = 2.496.
IRR = incidence rate ratio; CI = confidence interval; DMT = disease-modifying therapy.

EDSS, or **1501/*1503* status to the model did not meaningfully change the estimates. Only Hispanic ethnicity appeared to be independently associated with an increased risk of relapse, although the confidence intervals surrounding some of the other estimates, particularly for the number of relapses prior to blood draw and race, were too wide to exclude an important independent association. There was no meaningful change in the estimates when the 8 samples that were measured in a batch at a different time point from the main batched analysis were removed from the analyses.

We explored interactions between the adjusted serum 25-hydroxyvitamin D_3 level and *HLA-DRB1*1501/1503* in a more parsimonious model that dropped other covariates with $p > 0.05$ and included race and ethnicity as the only additional covariates. The p value associated with the interaction term was 0.58. Among the *DRB1*1501/1503*-negative patients, a 10ng/ml greater adjusted serum 25-hydroxyvitamin D_3 level was associated with an estimated IRR of 0.69 (95% CI, 0.45–1.08; $p = 0.102$). For those patients who were *DRB1*1501/1503* positive, a 10ng/ml greater adjusted serum 25-hydroxyvitamin D_3 level was associated with an estimated IRR of 0.57 (95% CI, 0.32–0.99; $p = 0.046$).

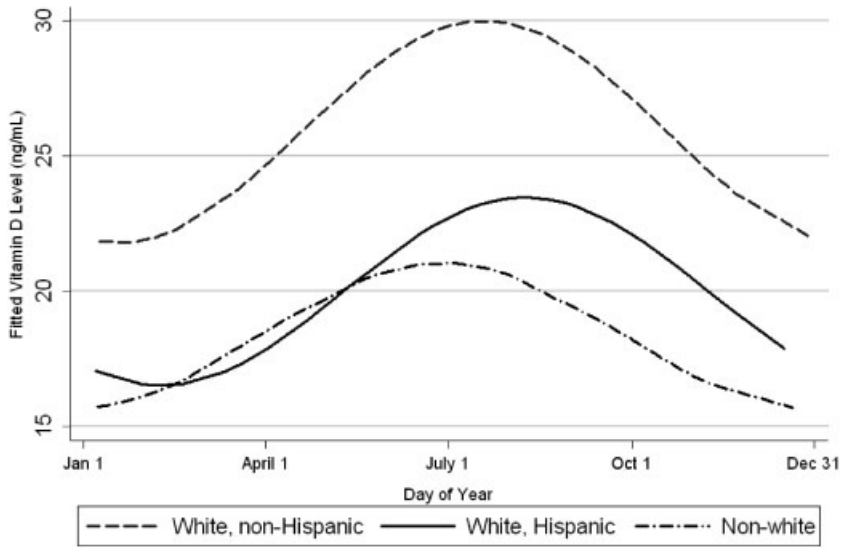

FIGURE: Fitted values from the model of vitamin D levels in terms of racial/ethnic category and the day of the year on which the sample was taken (day 0 = January 1). This model was used to create a deseasonalized vitamin D level for each individual.

Discussion

In patients with pediatric-onset MS/CIS, each 10ng/ml increase in the adjusted 25-hydroxyvitamin D_3 level was associated with a $\frac{1}{3}$ reduction in the rate of subsequent relapse. This finding suggests that interventions increasing serum vitamin D stores have the potential to make an impact on the course of MS, even after accounting for DMT.

There have been previous attempts to determine whether vitamin D status impacts the clinical course of MS, but these were limited by study design or sample size. One group concluded that serum vitamin D levels are inversely associated with relapse rate and disability, but the serum level of 25-hydroxyvitamin D_3 was measured after the period in which the clinical outcomes had occurred, so reverse causality (ie, MS itself leading to a subsequent decrease in vitamin D levels, presumably due to sun avoidance) could explain the results.[17] In another study, vitamin D levels were correlated with disability score, but this result was also potentially an effect-cause relationship, because the more disabled patients reported obtaining less sun exposure.[18] Although relapse timing appeared to correlate with ambient ultraviolet light levels in an additional study, the analyses were not adjusted for important covariates; further, regional averages, rather than the patients' own levels of ultraviolet light exposure, were used as predictors.[19] A few pilot studies of oral vitamin D supplementation suggested, but did not definitively confirm, a possible positive effect on the disease course.[20–22] We addressed the possibility of reverse causality by using vitamin D levels measured before the outcome measures and by confirming that the association persists when controlling for previous disease course (prestudy attacks and baseline EDSS score), which also reduces concern for confounding by an unknown underlying process common to both vitamin D status and disease course. We also avoided some other limitations of previous studies by including a larger number of patients, by considering a proxy for skin tone and date of year drawn to create an adjusted vitamin D level, and by accounting for important potential covariates.

The active form of vitamin D, 1,25-dihydroxyvitamin D_3, has several immunomodulatory properties. Some of its identified actions include downregulating differentiated dendritic cells (eg, dendritic cells pretreated with vitamin D less effectively induce a mixed lymphocyte reaction and express less CD83) and preventing dendritic cell differentiation and migration to lymph nodes, thereby contributing to dendritic cell tolerance.[23] Furthermore, toll-like receptor activation of 1α-hydroxylase in macrophages, leading to targeted killing of intracellular bacteria, requires the presence of 1,25-dihydroxyvitamin D_3.[24] 1,25-dihydroxyvitamin D_3 prevents activated B-cell proliferation and enhances apoptosis thereof.[25] In mice, vitamin D receptor agonists reduce the proinflammatory cytokine interleukin-17,[26] which modulates experimental autoimmune encephalomyelitis, and inhibit the development of the proinflammatory T_H1 cell.[4] Finally, vitamin D receptor agonists promote the induction and function of $CD4+CD25+T_{REG}$ cells. [27] Although 1 study in patients with MS demonstrated similar results in that 1,25-dihydroxyvitamin D_3 inhibited CD4+ and myelin basic protein-specific T cells and enhanced $CD4+CD25+T_{REG}$ cells, the precise *in vivo* immunomodulatory properties underlying its influence on susceptibility to and prognosis of MS are unclear.[28]

In this study, *HLA-DRB1*1501/1503* status did not appear to be independently associated with relapse rate, nor did it substantially change the association of relapse rate and vitamin D status. However, our analyses do not exclude a potential interaction between 25-hydroxyvitamin D_3 levels and *HLA-DRB1*15*, as the estimated reduction in relapse rate associated with 25-hydroxyvitamin D_3 was slightly less in those who were *DRB1*1501/1503* negative. One group demonstrated that *DRB1*15* expression is increased in the presence of 1,25-dihydroxyvitamin D_3[5]; if the increased MS severity associated with the *DRB1*15* allele is mediated by its expression,[12,13] we might have expected the direction of an interaction to be the opposite of what we found. Larger studies will be useful to explore this interaction; if it exists, more vitamin D supplementation may be required in those who are negative for the allele to achieve the same amount of risk reduction. Alternatively, the results may suggest that those who are *DRB1*1501/1503* positive would have a greater benefit from supplementation than those who are negative.

Hispanic and partially Hispanic individuals, regardless of race, vitamin D status, and other covariates, had a much greater relapse rate than non-Hispanic patients (see Table 2), suggesting that factors other than vitamin D are important contributors to the risk of relapse in those of Hispanic ethnicity. Although nonwhite race did not appear to be associated with relapse risk, the confidence intervals do not exclude a relationship.

The study has several limitations. The seasonal adjustment may not perfectly account for differences in the geographical regions in which our patients live, particularly as the catchment areas for the 2 referral centers are large. It is also possible that nonseasonal changes occurred in the subjects' vitamin D levels in the follow-up period. Few studies have evaluated whether a meaningful longitudinal change in vitamin D status occurs, except as in-

duced by season. In a group of healthy elderly individuals, the mean springtime 25-hydroxyvitamin D_3 levels were similar over 1 year.[29] In older adults, there was a very mild decrease in winter and spring, but not summer, 25-hydroxyvitamin D_3 levels over 16 years.[30] Multivitamin and vitamin D supplementation data were not available for all patients included in this study, but it is unlikely that patients changed their supplement use during the course of the study as an intervention for MS, as interest in vitamin D as a modifier of the course of MS is very recent. Further, the American Academy of Pediatrics only changed its recommended daily intake of vitamin D from 200IU to 400IU in October 2008[31]; even 400IU may be an inadequate dose to lead to a substantial increase in a person's vitamin D status.[32] It therefore seems unlikely that our patients experienced a substantial change in vitamin D status during the study. The most recent follow-up information for some patients was obtained by telephone contact with their local physicians or the family. Although it is preferable to have similar types of follow-up for all patients, we decided to include these patients, because to have left them out may have introduced bias. Our ability to fully adjust for use of DMT may be limited, as there are likely different indications to initiate therapy for which we cannot account. Nevertheless, no matter which way we modeled therapy, the association of vitamin D status and subsequent relapse rate remained essentially unchanged. Although we were only crudely able to account for skin tone by using race/ethnicity as a proxy, future studies may benefit from performing quantitative skin tone measurements.

Our results raise several questions that should be addressed before systematically recommending 25-hydroxyvitamin D_3 repletion in individuals with MS/CIS. Although there was little indication of nonlinearity in our models, our patients had a limited range of vitamin D values; it is important to determine whether there is an optimal serum 25-hydroxyvitamin D_3 range in MS and whether there is a threshold level above which no meaningful decrease in relapse risk can be expected. It is also unclear which type (oral vitamin D_3 versus exposure to ultraviolet light) is appropriate. Finally, although our findings do not prove that altering the 25-hydroxyvitamin D_3 level in a person with MS or CIS modifies the risk of an exacerbation, they provide strong rationale for a randomized clinical trial of vitamin D supplementation in MS. If such a trial demonstrates that vitamin D supplementation reduces the risk of an MS exacerbation, it will likely be ubiquitously recommended as an inexpensive, relatively safe add-on to already-approved therapies in patients with the disease.

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Potential Conflicts of Interest

Nothing to report.

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