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XVI European Charcot Foundation Lecture: Nutrition and environment, can MS be prevented?

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Abstract

Multiple sclerosis is a relatively common debilitating neurologic disease that affects people in early adulthood. While the characteristic pathology of MS has been well described, the etiology of the disease is not well understood, despite decades of research and the identification of strong genetic and environmental candidates for susceptibility. A question central to all diseases, but posed specifically for MS at the XVI European Charcot Foundation Lecture, was 'Can MS be prevented?' To address this question, we have evaluated the available data regarding nutritional and environmental factors that may be related to MS susceptibility and suggest the extent to which a potential intervention may reduce disease burden. It is our opinion that intervention, particularly supplementation with vitamin D, could have a dramatic impact on disease prevalence. Understanding that any intervention or behavioral modification will surely act in the context of genetic susceptibility and unidentified stochastic events, it is likely that not all MS is 'preventable'. Epidemiologic observation has provided key insights into environmental and nutritional factors that may alter one's susceptibility to MS, however, there are still many questions in unraveling the etiology of this complex disease.

Keywords

Multiple sclerosis; epidemiology; EBV; smoking; vitamin D

Introduction

Multiple sclerosis is a relatively common, debilitating neurologic disease affecting young adults, likely autoimmune in origin. With most individuals affected in their early 30's and marked disability eventually developing in most patients, there is a compelling need to understand whether modifiable factors may alter disease risk, as well as disease course. The lifetime risk of MS is approximately 1/200 for women in high risk areas, [1, 2] with women

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Conflicts of Interest

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being affected approximately 2–3 times more commonly than men, and evidence that this disparity is increasing. [3, 4] Several lines of epidemiologic evidence support the notion that environmental and lifestyle factors may modify future MS risk. [5, 6] This suggests that interventions could be developed to prevent some proportion of MS cases. MS is most certainly heterogeneous; multiple factors determine disease risk and these risk factors will also likely vary based on individual characteristics, both genetic and environmental. The existence of a genetic susceptibility to MS has been long known and a family history is the strongest identified MS risk factor. Siblings of MS patients are at a 30-fold increased risk of MS compared to the general population, [7] and the concordance rate in monozygotic twins is approximately eight to ten folds higher than in dizygotic twins. [8, 9] That clustering of MS within families is due to genetic rather than environmental factors is further supported by the observation that the MS risk of half-siblings and adoptees is related to the MS history only of their biological relatives. [10] The strongest contributor to genetic susceptibility is the Major Histocompatibility Complex (MHC); the *HLA-DRB1*1501* haplotype, in particular, shows a strong association with MS risk in Caucasians [10]. Many other susceptibility candidates have now been identified, though effects are generally modes. [11–14] The genetics of the MHC region, however, is complex with potential epistatic interactions between DR haplotypes and heterogeneity according to ethnicity. [15] The existence of a strong genetic factor clearly predisposes some individuals to MS and this may outweigh any nutritional, behavioral or environmental factors. That being said, for a substantial number of people who are susceptible to MS, it does appear that non-genetic factors may play a role in whether they develop the disease. In this paper, a review of the presentation at the ‘XVI European Charcot Foundation Symposium’, we evaluate the evidence for nutrition and environmental factors in MS etiology noting points of convergence as well as inconsistencies and suggest recommendations related to modifiable factors with strong evidence for potential prevention.

1. Geographic distribution and migration

Geography and latitude

MS is a relatively common disease in Europe, the United States, Canada, New Zealand and southern Australia. MS is generally rare in equatorial regions and on the Asian continent. [16] The existence of a latitude gradient in temperate regions has been extensively described with a lower MS incidence for populations nearer the equator [17] and recent data suggest that the relationship between latitude and MS risk may be dependent on gender. [18] Notable exceptions exist; however, it is generally accepted that a latitude gradient has existed in regards to MS prevalence and the extent to which it is evident may be dependent on the genetic loading for MS (eg Sardinia) in certain populations or region specific environmental exposures. Regardless, the existence of a latitude gradient could provide evidence of a genetic factor that is tightly correlated with this geographic distribution or an environmental factor, whose distribution similarly varies by geographic region and latitude. Genetic predisposition, however, cannot explain the remarkable differences in MS risk associated with individuals of common ancestry migrating between areas of low and high risk, [19] the differences in MS risk for children of immigrants compared to those remaining in their home country, [20, 21] and the more recent observation of a disappearance of the latitude gradient in countries where it previously existed, such as the United States. [22, 23]

Migration studies

An environmental factor more readily explains the findings from migrant studies, which, in addition to providing argument for an environmental trigger, provide evidence of a potential age-specific susceptibility in childhood/adolescence. Individuals migrating from areas of high risk to low risk tend to adopt the risk of their new country if they emigrated in

childhood or early adolescence, whereas little change in risk is seen for migration in adulthood. Those migrating from areas of low risk to high risk show a reverse pattern, however the difference is not as striking. [5, 19] It is important to note that migration studies have several limitations, making inference somewhat tenuous. Immigrants are generally not representative of the place from which they emigrated, on average, tending to be younger and healthier. Additionally, access to heath care and services that are requisite for a diagnosis may be different for immigrants compared to native inhabitants. [19] Overall, however, it seems unlikely that these biases would produce such a consistent observation if one did not exist. Strong data come from records of United States enlisted military personnel where one could argue that many of the immigration biases would not exist. Kurtzke and colleagues found that enlistees who were born in the Northern US and who then resided in the Southern US at the time of enlistment reduced their MS risk by 50 % compared to those who remained in the North. Similarly, they found a non-significant 20% increased risk of MS for those who were born in the South and enlisted in the North compared to those were born and subsequently enlisted from a Southern state. Risk associated with the Middle tier of the US was intermediate. [17]

As alluded to above, there are, however, newer data showing a possible attenuation of the latitude gradient. [22, 23] In the US, there is reasonable evidence in women to suggest that this disappearance of geographic differences is due to a relative increase in incidence in the South. [22, 24] Also of interest, there is consistent evidence that age at migration is important in determining subsequent MS risk. In general, studies have shown that the protection afforded by migration from a high to low risk area is only evident if this migration occurs by age 15, [19] although newer data are not consistent with this cut-off. [25] Suffice it to say that there is a good evidence of geographic variation in MS, overall supported by a latitude gradient suggesting an environmental factor that may act in childhood/early adolescence.

Season of birth

Some newer findings provide further support for the importance of early life exposures with strong data showing a relationship between season of birth and subsequent MS risk. Pooling data from more than 40,000 MS patients in Scotland, Denmark, Sweden and Canada, Willer and colleagues found an excess of MS cases born in May, who, *in utero*, would have had the lowest annual sun exposure. [26] These findings are consistent with the results of large investigations in Sweden, where an excess of June births [27] was observed, and Australia, where the opposite pattern was observed with a relative paucity of MS cases born in May-June. [28] These data, taken together, provide strong evidence of an environmental factor operating in MS. What these factors might be and how they might inform preventative strategies is the subject of the following section.

2. Modifiable factors

Smoking

Consistent epidemiologic evidence shows an approximate 40–50% increased risk of MS associated with a history of ever smoking and a dose-response between number of pack-years of smoking and increased MS risk, estimated to be greater than a two-fold increased risk in long term smokers as compared to never smokers. [6, 29] More recent work suggests that the increased risk of MS associated with smoking may be stronger in men than women. [30, 31] In the context of population trends of smoking, it is interesting to consider whether population level changes in prevalence of smoking and MS could contribute to the increasing female to male ratio in MS incidence. [3, 4, 22, 32, 33] International country-specific smoking data show that the male to female ratio for smoking has dramatically

decreased in the course of the past century. For example, for individuals born in the early 1900's, the prevalence of men smoking was approximately 2.5 times that of women. In contrast, for those born in the 1950's and 1960's, the percentage of men smoking almost equaled the percentage of women smoking. These changes in smoking behavior would be expected to result in a decline in MS incidence in men, but not in women, and could thus explain a large proportion of the increase in the female to male ratio in MS incidence. [34] However, it appears that the increasing sex ratio difference is due to an increased incidence of MS among women rather than to a decline among men. A possible explanation to reconcile these observations is the existence of another environmental factor which is increasing incidence in both sexes, but is offset in men by decreasing smoking behavior. Although it is not clear as to what this factor might be, increasing levels of hygiene have been implicated as potentially explaining the general worldwide increase in MS incidence.

EBV infection and infectious mononucleosis

Hygiene hypothesis and MS—A correlation between 'hygiene', as defined by early life exposure to infectious agents, and MS risk has long been noted. The 'hygiene hypothesis' for MS evolved from the observations of Poskanzer and colleagues who suggested that a virus may exist that increases MS risk when acquired in late childhood or adulthood, but confers immunologic protection if acquired in infancy or early childhood. [35] The suggested mechanism underlying the benefit of acquiring infections at a young age is that low exposure to infectious agents in childhood favors the development of a Th1 pro-inflammatory cellular immune response and subsequently high MS risk, whereas exposure to multiple infections in childhood modulates the immune response towards Th2 and regulatory T cells resulting in decreased susceptibility to MS. This hypothesis, however, does not implicate a particular pathogen, [36–38] but rather suggests MS is an autoimmune disease triggered by multiple microorganisms in genetically susceptible individuals. Early observations of an association between increased MS incidence and increased sanitation in Israel [39] provided support for an association between 'high hygiene' and increased MS risk. The hygiene hypothesis could explain some characteristic epidemiologic findings including a lower incidence of MS in developing countries, [17] increased MS associated with increasing education/SES [40–42] and the association between a history of infectious mononucleosis (IM) and MS. [43, 44] IM is a manifestation of late acquisition of EBV infection. More than 95% of the world's population has acquired EBV infection, generally at a young age. In developing countries, the seroprevalence by age 6 has been estimated to be near 90%, while in the Northeastern U.S. prevalence rates are closer to 30%. [45] In the continental U.S., a study of U.S. military enrollees showed an EBV seropositivity gradient that mirrored the latitude gradient for MS with seropositivity in the Southeast estimated at 80% and lower in the North-West and New England at approximately 50% for young adults. [46] For those individuals who acquire EBV infection after childhood, IM is a common sequelae occurring in up to 40% of infected individuals. [47] Therefore, a history of IM can be seen as a marker of a more hygienic environment. The similarities between the epidemiology of MS and IM, including similar geographic distributions, associations with SES and increased prevalence in whites compared to blacks and Asians, were noted more than 20 years ago. [48, 49]

Hygiene hypothesis paradox—Although the observation of increased risk of MS with a late age at infection with EBV (and manifestation as IM) tends to support the hygiene hypothesis in MS, there is a paradox in that those who completely escape EBV infection have very low MS risk (OR=0.06 for seropositivity versus seronegativity) as evidenced by a meta-analysis of published studies on EBV serology and MS risk. [5] If the hygiene hypothesis were true, these individuals would be expected to have a very high risk of MS

assuming a lack of EBV infection, a pathogen that is nearly ubiquitous, is a marker of a highly hygienic environment.

Longitudinal studies of EBV and MS—Strong support for a causal interpretation of an association between EBV itself, and not other infectious agents (which have been the subject of numerous investigations), [5] and MS risk would come from prospective studies of healthy individuals who are seronegative for EBV infection who are then followed for documentation of new onset of MS. To address this hypothesis, we conducted a nested case-control study of MS in military personnel with samples deposited in the Department of Defense Serum Respository (DoDSR). The DoDSR includes over 40 million blood samples taken from over 8 million military personnel since 1990. Cases are identified via Physical Disability Agencies and are matched to controls by age, sex, race/ethnicity and dates of blood collection. Blood is collected at the time of enrollment in active duty and periodically throughout service. Of these healthy individuals, approximately 3% are EBV-negative, resulting in the prospective follow-up of almost 200,000 EBV-negative individuals. From the total study population of 8 million people, we identified 305 individuals who developed MS and matched them to 610 controls. EBV serology was measured in samples taken before onset of MS in cases and control samples date matched to cases. In this casecontrol study, nested in a prospective cohort, at baseline, 10 MS cases (3%) and 32 controls (5%) were seronegative and all MS cases and 28/32 controls had at least one additional follow-up blood sample. During the follow-up, all 10 of the initially EBV seronegative MS cases became seropositive, while only 10/28 controls seroconverted. This provides compelling evidence that EBV infection precedes onset of MS, with seroconversion estimated to occur approximately 5 years before onset. [50] Given it is difficult to prove a causal association, what could be alternative explanations of this finding? Although possible, common genetic susceptibility seems an unlikely explanation. Given the data above, there are a small percentage of individuals who remain EBV negative into adulthood and it could be hypothesized that are resistant to infection. However, for those seronegative individuals who develop MS, all seroconvert prior to onset. This suggests that they are, in fact, susceptible to EBV infection, and that their risk of MS changes dramatically following EBV infection. [50] This change in risk in the same individuals following EBV infection and the lack of MS without EBV infection strongly support EBV as a requisite factor in the etiology and makes it unlikely the observed association is due to a common genetic determinant. Other non-causal explanations include laboratory assay artifacts, confouding or reverse causation. An artifact due to an increased proportion of cases showing seropositivity or a relative decrease in controls having EBV detected seems unlikely given the high sensitivity and specificity of EBV serology. [47] To explain the observed odds ratios would require an unlikely amount of differential misclassification. Although always possible, the existence of a confounder that could explain such a strong association and has yet to be identified seems unlikely. Reverse causation also seems unlikely as EBV seropositivity measured several years before onset is associated with MS risk, [51, 52] and, as previously mentioned, most EBV infection is acquired early in life. Nontheless, there could still be alternative explanations for this finding and more compelling evidence would come from the identification of a definitive causal mechanism.

In addition to an association between seropositivity and MS risk, there are consistent data showing a dose-response relationship between titers to anti-EBV IgG antibodies, particularly Epstein-varr virus nuclear antigen-1 (EBNA-1), and MS risk. [51–55] Following primary infection, anti-EBNA1 IgG antibodies appear tend to remain stable over one's life [47] and the epidemiology mirrors this finding in that strong associations are seen between antibody titers to EBV and MS risk when measured before or after onset, though pre-onset titers are clearly more important in establishing temporality and causality. Updating the previous analysis in the U.S. military and including additional newly diagnosed cases (total n=222

cases and 444 matched controls), we have now shown that individuals with anti-EBNA IgG Ab titers of >320 have a 36-fold higher MS risk than those with anti-EBNA Ab titers <20 ($p<10-9$). [55] Collectively, the evidence strongly supports a role for EBV in MS etiology and, although EBV clearly invokes a strong immune response, evidence suggests that this effect on MS risk is independent of the effect of *HLA-DRB1*1501.* [56–58]

Implications for prevention—It is clear that there is likely underlying genetic susceptibility in all individuals who develop MS. There will also likely be some cases of MS that occur in the absence of EBV, though we estimate this number is small, roughly 10% of cases may develop unrelated to EBV. If we then assume that EBV is necessary in at least 90% of MS cases (in addition to other diverse causes with varying population distributions) and the EBV association is truly causal, an EBV vaccine could prevent (with caveats) over 80% of MS cases. There are, however, epidemiological aspects of MS that are not explained by EBV. As reviewed elsewhere[5], the straightforward causal association between EBV infection and MS risk cannot explain the reported occurrence of an epidemic in the Faroe Islands [59] or the decreased risk in migrants moving from high to low risk areas. A possible explanation is that different EBV strains confer different MS susceptibility as we have previously hypothesized. [5] Differences in strain prevalence have been identified that correlate with the geographic distribution of other diseases, such as nasopharyngeal carcinoma in parts of South East Asia [60] and Burkitt's lymphoma in sub-Saharan Africa. [61–63] The possibility that variations in the EBV genome explain the geographical specificity of EBV related diseases is intriguing. In the case of MS, this possibility could explain the Faroe island epidemic and the change in risk with migration. There is, however, little direct evidence in favor or against this possibility, because the few studies conducted have only considered selected gene regions. [64–67]

Vitamin D

The observation of increased MS prevalence at higher latitudes and the strong inverse correlation between increasing latitude and decreased sunlight intensity and duration provided early speculation that vitamin D insufficiency may be a risk factor for MS. [68] Empirically, MS prevalence is tightly correlated not only with latitude, but also with annual ultraviolet radiation. [69–72] Ultraviolet B (UVB) radiation from sunlight exposure is the main source of an individual's vitamin D (vitamin D3; cholecalciferol), with much smaller contributions from dietary sources (such as fortified foods and dark fish) and vitamin supplements. A typical multivitamin generally has 400IU of cholecalciferol, whereas 20 minutes of whole body sun exposure in the summer is equivalent to approximately 10,000 IU. [73, 74] Upon UVB exposure, cutaneous 7-dehydrocholesterol is converted to pre-vitamin D3 and spontaneously isomerizes to cholecalciferol. In the liver, a hydroxylation reaction results in the production of 25-hydroxyvitamin D (25(OH)D) from either vitamin D3 or ingested vitamin D2. The final hydroxylation occurs primarily in the kidneys, but some extra-renal cells can also perform this function, to produce the bioactive form of the hormone, 1,25-dihydroxyvitamin D (1,25(OH)D). Average serum concentrations of 25(OH)D are between 30 and 150nmol/L and it is considered a good biomarker of vitamin D availability and nutritional status as its formation is not tightly regulated and has a relatively long half life of 20–60 days, unlike 1,25(OH)D which is under tight homeostatic control and shows little variation in concentration. [75, 76] Although vitamin D's primary role is in maintaining calcium homeostasis, a role for vitamin D in immune regulation is supported by several lines of evidence. [77]

Experimental evidence implicating a protective role for vitamin D—In animal models, calcitriol (1,25(OH)D) has been shown to protect against the development and progression of experimental autoimmune encephalomyelitis (EAE). [78–83] It appears that

this effect is mediated through promotion of regulatory T-cell function as opposed to direct effects on Th1 or Th2 cells. [77, 84] However, prevention of EAE with cholecalciferol dietary supplementation has been shown to be specific to female mice with adequate estrogen, with male mice and oophorectimized female mice showing no effect. [81, 85, 86] The latter finding is inconsistent with observational studies showing a latitude gradient that is present in both sexes and the protective effect of vitamin D in both sexes to be discussed later, which highlights the need to be cautious in extrapolating animal data to humans.

Ecologic and case-control studies of vitamin D and MS—Although the idea that MS is due to a lack of sunlight was proposed almost 40 years ago[87], strong epidemiologic studies supporting this hypothesis have been lacking until the last decade. As reviewed extensively elsewhere, [68] early ecological studies correlated vitamin D related exposures, such as fish intake, and MS prevalence, [88, 89] however, these population based correlations are subject to confounding, and ideally one would compare varying exposures within the same population. Mortality/co-morbidity studies have provided some evidence for a role of vitamin D, finding lower risk of MS among those reporting outdoor occupations. [90, 91] Similarly, a history of skin cancer has been associated with decreased MS risk in one study, [92] but not confirmed in others. [93–96] However, these findings could be explained by ‘reverse causation’. Individuals with MS may be less likely to have physical, outdoor occupations. Similarly, individuals with MS may be less likely to spend time outdoors and, therefore, may be less likely to develop skin cancer. Well conducted case-control studies may be less susceptible to reverse causation, but still may produce biased results because cases may tend to over or under report certain behaviors compared to those without MS – recall bias. Previous case-control studies have produced generally consistent results showing an association between increased sun exposure, particularly in childhood, and decreased MS risk. [97–99] A study in Israel, [100] however, found the opposite, but as mentioned above, these studies are prone to recall bias and could produce spurious results. Similarly, a study in northern Norway (where diet is the primary source of vitamin D given the high latitude, 66°–71°N) found a decreased risk of MS associated with frequent fish consumption, [99] however, a study in Canada [101] did not support this finding. Two studies of vitamin D supplement use in adolescence found no statistical association with MS risk, [98, 102] though one provided suggestive evidence of decreased MS risk with use of >=400 IU/day. [102] Using a more objective marker of sun exposure, an Australian study found an association between increased actinic damage and decreased MS risk. [98] Considering all the studies together provides modest evidence for an association between vitamin D and MS. As alluded to, there are several limitations of these studies, particularly in assessing the association between vitamin D and MS because there are significant changes in lifestyle, including reduced physical activity and time spent outdoors among patients with MS that may result in decreases in vitamin D/sun exposure as a consequence of the disease. Of particular importance to studies where affected and unaffected individuals self-report exposure history is the possibility of recall bias. To better understand the extent to which recall bias might influence observed associations, Giovannucci and colleagues conducted a study of dietary fat and breast cancer risk in the Nurses’ Health Study, a prospective cohort of more than 121,000 registered U.S. nurses. Women with breast cancer and matched controls were identified for whom dietary fat had been collected before their disease onset and they were then re-contacted after the onset of their cancer to ask about previous dietary fat consumption from the same period that was assessed prospectively. In that study, there was a 40% increased risk of breast cancer for the highest versus lowest quintile of fat intake when diet was assessed retrospectively, whereas no association was seen with prospectively assessed fat, the accurate assessment of true intake. [103]

Prospective studies of vitamin D and MS risk—Ideally, associations between dietary or behavioral factors and risk should be assessed prospectively. The exposure should be measured well before disease onset to ensure temporality and validity. In the case of self-reported exposures, this eliminates recall bias as it is highly unlikely that people who are destined to develop MS in the future (but are still healthy at the time of the self-report) will have a differential recall of dietary or behavioral habits. With respect to biomarkers, this ensures that the measurement precedes disease onset and is a not a consequence of the disease itself or changes in behaviors (such as reduced outdoor activities) as a consequence of disease onset. Because of the importance of establishing temporality, we have meticulously documented incident cases of MS in two, large, ongoing prospective cohorts-the Nurses' Health Study and Nurses' Health Study II (NHS/NHS II), where every two years since 1980 for NHS I and 1991 for NHS II, dietary information (obtained via a food frequency questionnaire) has been reported. From baseline until 2001, we identified 173 MS cases with onset of first MS symptoms after baseline and after the collection of dietary information and found that use of >=400IU/day vitamin D supplement prior to disease onset compared to no supplement use was associated with a significant 40% decreased MS risk and there was a significant trend for increasing vitamin D supplement use. [104] Similarly, we found that vitamin D use during pregnancy was associated with decreased MS risk in the offspring. Specifically, women whose mothers were in the highest quintile of dietary vitamin D intake during pregnancy had a 43% lower MS risk than those whose mothers were in the lowest quintile (RR=0.57; 95% CI:0.35–0.91; p-trend=0.002). [105] The potential importance of maternal vitamin D deficiency and subsequent MS risk was previously suggested by Willer and colleagues [26] as an explanation for the season of birth findings previously discussed.

Serum 25(OH)D and MS risk—To more convincingly implicate vitamin D, we undertook a nested case-control study of 25(OH)D and MS risk [106] among U.S. military personnel with serum samples banked at the DoDSR, as described above. We identified 257 MS cases and matched them to 514 controls to determine if 25(OH)D levels prior to MS onset were associated with risk of MS. Most participants had 3 blood samples prior to the onset date and, because we also had a post-onset blood sample, we were able to show that 25(OH)D levels do, in fact, decrease after diagnosis, so that 25(OH)D measures taken from patients are likely to reflect changes in behavior as a result of the disease and are not informative for inferring a causal association with MS risk. In the prospective analysis of 25(OH)D and MS risk, among Whites, we found a 60% reduced MS risk for those in the highest quintile of 25(OH)D (>99.1 nmol/L) compared to those in the lowest quintile (15.2–63.2 nmol/L). This association was adjusted for latitude at entry into the military, so this effect of 25(OH)D is independent of latitude. Similarly, using *a priori* cut points, there was a 50% reduced MS risk for those with serum 25(OH)D levels greater than 100nmol/L compared to those with serum 25(OH)D of < 75nmol/L. We did not find, however, evidence of an interaction between 25(OH)D and gender, as suggested by the experimental animal literature. The associations with MS risk were similar in men and women, though this is based on relatively small numbers (n=74 female cases). Interestingly, we did not find an association between 25(OH)D and MS risk among African-Americans. However, most African-Americans had 25(OH)D levels below 74 nmol/L and none had levels greater than 100nmol/L. Because of this and the small sample size, these findings are equivocal as to whether the same association between 25(OH)D and MS risk exists in African-Americans. Our overall interpretation of these data is that high 25(OH)D is protective for MS among whites, as the risk is significantly lower in those with 25(OH)D in the highest quintile compared to the lowest. If this relationship is linear across our observed range, this translates into a 41% decrease in MS risk for a 50nmol/L increase in 25(OH)D. However, the risks of MS in the middle quintiles were not significantly different from each other

suggesting a possible threshold effect. Considering this data alone would suggest a threshold around 100nmol/L. The main limitations for providing recommendations based on these findings are that we were underpowered to thoroughly address the question of a threshold effect and we cannot extrapolate beyond the observed data upper limit of 150 nmol/L. Although we believe there is little risk of toxicity from 25(OH)D, the misuse of epidemiologic evidence to design clinical trials has, in the past, resulted in apparently conflicting results that dampen the public's enthusiasm for potentially beneficial interventions and unnecessarily deem hypotheses uninformative. The overall evidence for a protective effect of vitamin D on MS risk appears strong but there are still several uncertainties regarding whether there is a critical age and what it might be, whether a dose-response exists, and the biological mechanism of action.

Implications for prevention—If the epidemiologic evidence is true, there are important implications for prevention. Assuming the optimal level is in the range of 100–150nmol/L, this can be safely obtained with 1,000–4,000IU/day supplements. [107–110] Because population surveys suggest a large majority of adults (other than lifeguards in sunny climates) may be below this level, [111, 112] supplementation could have a dramatic effect on MS incidence.

Although large, randomized trial of healthy individuals would be required to determine if this relationship is causal, we would suggest, based on the best evidence available, that 100nmol/L is the optimal target for MS prevention.

Vitamin D and recent epidemiologic observations—Returning to some of the newer observations from MS epidemiology, can vitamin D explain the month of birth results? If vitamin D is responsible for the decreased risk of MS associated with being born in November, then this implies that maternal vitamin D nutrition during the last two trimesters is important since November babies would have spent their first twelve weeks *in utero* in periods when, on average, maternal 25(OH)D levels would be the lowest based on population data for fluctuations in 25(OH)D [112]. Can vitamin D explain the increasing female to male ratio? It seems unlikely since the findings of decreased risk of MS associated with 25(OH)D were observed in both sexes [106] and, in the U.S., there is no apparent decrease in average 25(OH)D in adults. [111] So, clearly, some questions remain and this may be partly explained by biological interactions between MS risk factors. The etiology of MS surely involves a complex interplay between factors and, therefore, understanding susceptibility will likely involve the simultaneous consideration of several components. The study of such interactions is complex and requires large sample sizes with comprehensive data, but recent evidence supports the importance of potential interactions that may underlie some of the observational findings in MS. For example, Ramagopalan and colleagues found evidence of regulation of *HLA-DRB1* 1501* by vitamin D, showing the promoter region of *HLA-DRB1*1501* contains a conserved vitamin D responsive element (VDRE), the presence of which resulted in increased *HLA-DRB1*1501* expression upon stimulation with $1,25(OH)^2D$ that was that not observed with other non-conserved VDREs present on other DR haplotypes. [113] Additionally, a role of vitamin D in autoimmune diseases is supported by the finding that vitamin D receptor (VDR) binding sites are significantly enriched in genes associated with autoimmune diseases. [114]

BMI and MS risk

Environmental exposures during childhood and adolescence are thought to contribute to MS etiology and overweight/obesity during these life periods may increase risk of MS. Excess adipose tissue may disrupt vitamin D metabolism and availability and/or modulate immune system function in ways that promote autoimmunity. Several studies have shown that obese

individuals have lower 25(OH)D levels as compared to normal weight individuals, [115–120] likely due to increased sequestering of vitamin D_3 by subcutaneous fat. [119] Leptin is an adipokine secreted by adipose tissue and high levels have been associated with reduced regulatory T cell activity in MS patients, [121] and in experimental studies, leptin deficient mice do not develop EAE [122] and leptin increases clinical severity of EAE [122, 123]. One Canadian case-control study [101] reported a 31% reduced risk of MS with every 5 kg/m^2 increase in body mass index (BMI), but the cases and controls may not be comparable as cases were asked about their weight in the year prior to diagnosis and controls for the year prior to interview, and this association could reflect weight loss among the cases after MS onset. In the only prospective study to date,[124] women in the Nurses' Health Studies (NHS/NHSII) reported their body size at ages 5, 10, and 20, adult height, and weight at age 18 and at baseline (1976—NHS; 1989—NHSII). Women who were obese at age 18 (BMI ≥30 kg/m^2) had a greater than 2-fold increased risk of MS as compared to women with a healthy weight BMI between 18.5 and 20.9 kg/m^2 (RR=2.25, 95%CI: 1.50–3.37). BMI at baseline, however, was not associated with MS risk. Women reporting a large body size at ages 5, 10, and 20 also had an increased risk of MS, however, after adjusting for body size at all three ages, only a 2-fold increased risk remained with large body size at age 20 (RR=1.96, 95%CI:1.33–2.89). Collectively, these results suggest that obesity in late adolescence/young adulthood, rather than in childhood or adulthood, may be an important determinant of MS risk.

3. Web of causation

The true biology underlying the development of MS involves multiple factors acting simultaneously, though how different factors combine to determine MS risk remains uncertain. Preliminary evidence suggests the possibility of interesting interactions between smoking, EBV, and vitamin D insufficiency, and between these factors and genetic susceptibility, [55, 57, 58, 125–135] but larger investigations will be needed to confirm or disprove these findings. Although we often model the effect of a risk factor, while keeping other conditions constant, diseases are almost always the result of multiple contingencies and no single cause can be identified. Considering the work of MacMahon and Pugh, we consider the practical aim of epidemiology should not be to determine 'the' cause of, but rather 'a' cause of disease. [136] And a practical definition of causality could follow that a factor is considered causal when its perturbation results in a change in the frequency of disease, although the mechanism underlying the observation may not be known at the time. A classic example illustrating this point is John Snow's well-documented observation that contaminated water caused cholera. The actual causal agent underlying this association was the microorganism, *Vibrio cholerae*. Although we do not understand the mechanism of action, for example of vitamin D, the current evidence should compel us to consider recommendations for prevention. Given the available evidence, it seems likely that MS is a rare complication of EBV infection in susceptible individuals. A substantial proportion of cases could be prevented by smoking avoidance or cessation, promoting EBV infection in childhood (until a suitable vaccine becomes available), and by maintaining good vitamin D status. Although changes in environmental and nutritional factors would surely not eradicate MS completely, they could account for a large number of cases and have a dramatic impact on the occurrence of MS.

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