Environmental Risk Factors for Paediatric Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a common neurological disorder that is likely the result of aberrant immune responses to key childhood environmental exposures in genetically predisposed individuals. This article discusses the current understanding of environmental risk factors implicated in MS with a focus on paediatric research. The study of children with MS is a powerful means of understanding MS biology and is highly relevant to the development of disease prevention strategies.

Keywords

Multiple sclerosis, children, environmental risk factors, Epstein–Barr virus, vitamin D

Multiple sclerosis (MS) is a chronic inflammatory and degenerative disorder of the central nervous system (CNS). Up to 10 % of adults with MS recall experiencing their first symptoms before 18 years of age, and between 2.5 and 5 % of MS patients are formally diagnosed during childhood or adolescence.[1–7] The pathobiologic mechanisms underlying MS remain incompletely understood. However, there is increasing evidence that MS may be due to aberrant immunological responses to environmental exposures experienced during childhood in genetically predisposed individuals.[8] Owing to their young age, children with MS have a shorter time window than adult patients between exposure to an environmental trigger and clinical disease onset. Appreciation of the key environmental factors is therefore vital to developing preventative strategies. In this article, we discuss the current understanding of environmental risk factors implicated in childhood-onset MS, with a focus on paediatric MS-based research.

Geography and Migration

The worldwide geographic distribution of adult-onset MS suggests a relationship between MS prevalence and increasing latitude[9–11] (see *Figure 1*). Latitude gradients may be observed even within a single country as reported in Australia, France, and the US.[12–14] MS in children has been reported in many countries worldwide;[1–4,15–27] however, large-scale international collaborations will be required to determine whether global geographic patterns observed in adult-onset disease hold true in the paediatric population.

Place of residence early in life may have a significant influence on future MS risk. The prevalence of MS in individuals immigrating to England from India or Pakistan was found to be highly dependent on age at immigration, with MS being much more common in those who immigrated before 15 years of age.[28] A Canadian study examining country of birth, place of residence during childhood, and self-reported ancestry in 44 paediatric-onset and 573 adult-onset MS patients found that paediatric MS patients were more likely to report Caribbean (maternal p=0.0177; paternal p=0.0007) or Asian (maternal p<0.0001; paternal p<0.0001) ancestry compared with adult-onset MS patients, reflecting recent immigration patterns.[29] In contrast to differences in parental ancestry, there was a striking similarity in the proportion of adult-onset and paediatric-onset MS patients born in North America and in the proportion of MS patients who had spent some of or all of their childhood in Canada, suggesting that place of residence during childhood is a profound determinant of MS risk.

Vitamin D
Background
Geographical variations in MS prevalence, including observed latitude gradients, may be explained by ambient sunlight exposure and vitamin D status. In humans, the primary source of vitamin D is via cutaneous conversion of 7-dehydrocholesterol to vitamin D3, a process requiring ultraviolet B (UVB) radiation. Vitamin D3 can also be obtained through the diet, predominantly through ingestion of oily fish and fortified foods. In the liver, vitamin D3 is converted to 25-hydroxyvitaminD3 [25(OH)D], the main circulating form of vitamin D and the most commonly used serum marker of vitamin D status.[30,31]

Epidemiology
Seasonal variations in vitamin D status may explain the month of birth relationship observed in MS patients.[32,33] In a pooled analysis of 44,045 adult-onset MS patients in Canada, the UK, Denmark, and Sweden, more MS patients than expected, relative to population controls, were born in May (4,056 observed versus 3,717 expected; p<0.0001) and fewer MS patients than expected were born in November (2,974 observed versus 3,252 expected; p<0.0001).[34] A similar disproportionate relationship between winter and spring or summer births has been independently reported in adult MS patients in Sardinia, France, Sweden and Scotland.[35–38] A comparison of 96 paediatric-onset MS patients from Canada, the US, South America,

Figure 1: Worldwide Multiple Sclerosis Prevalence
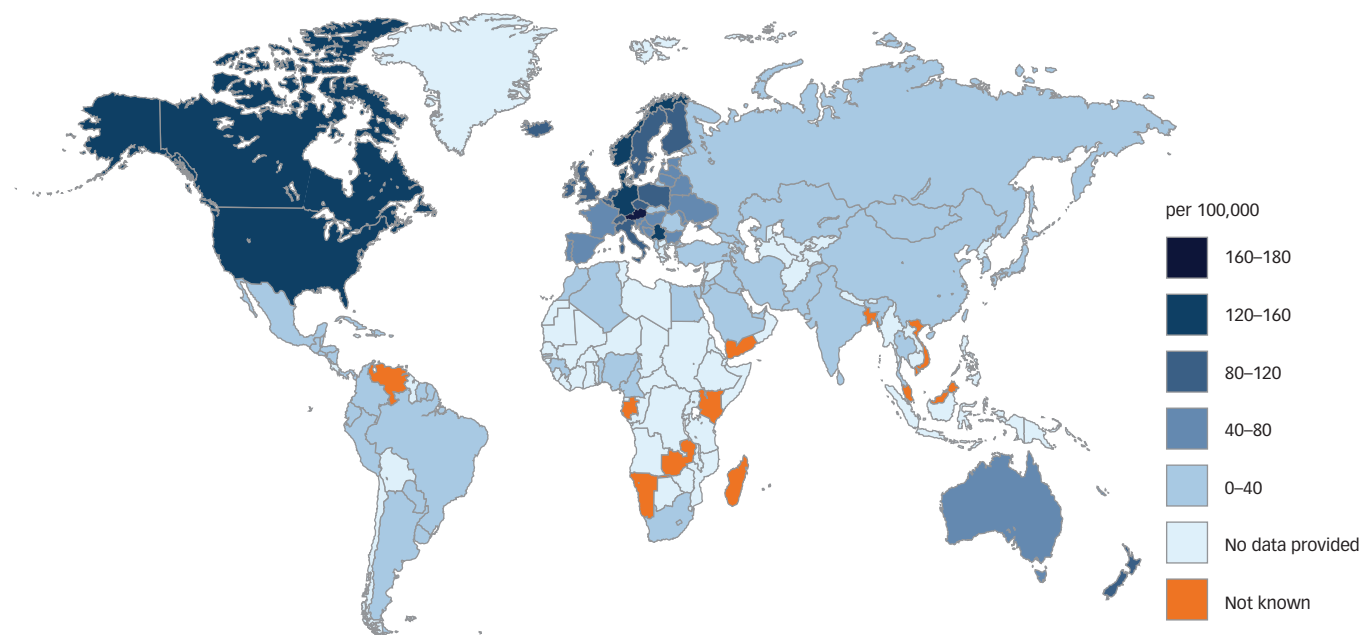
Source: www.atlasofMS.org

and Europe with 96 control children did not show a difference in birth month, although owing to the small number of participants the study may have had limited power to detect such a relationship.[39]

The timing of sun exposure may be important, as evidenced by a population-based study of 137 people with MS and 272 matched controls from Tasmania, in which higher sun exposure between the ages of six and 15 years was associated with a decreased risk for MS diagnosis in adulthood (adjusted odds ratio [OR] 0.31, 95 confidence interval [CI] 0.16–0.59).[40]

In a Norwegian study of 152 people with MS and 402 population controls, increased time spent engaging in summertime outdoor activities, particularly between 16 and 20 years of age, was associated with a reduced risk for MS diagnosis (OR 0.55, 95 % CI 0.39–0.78).[41] A study of 193 adult-onset MS patients and 358 matched controls from Cuba, Martinique and Sicily found that self-reported sun exposure before 15 years of age conferred a small but statistically significant reduction in MS risk.[42] In this study, there appeared to be a dose–response relationship with lower odds of MS being observed with greater number of hours spent outdoors. In a study of 79 monozygotic twins discordant for MS diagnosis, MS-affected twins reported lower exposure to nine sun-related activities compared with unaffected co-twins.[43]

Dietary vitamin D ingestion may also be of importance. A study of dietary vitamin D intake in a cohort of almost 200,000 US nurses found that women in the highest quintile of daily dietary vitamin D intake at the time of entry into the cohort study had a 33 % reduction in subsequent likelihood of MS compared with those in the lowest quintile (OR 0.67, 95 % CI 0.40–1.12). Women reporting ingestion of >400 IU per day in the form of supplements had a 40 % lower risk for MS than those reporting no supplement ingestion (OR 0.59, 95 % CI 0.38–0.91).

Questionnaire-based evaluation of vitamin D status is inherently limited by patient recall and challenged by limited knowledge of the actual vitamin D dose conferred by outdoor activity, by the content and bioavailability of vitamin D in foods, and by accurate recollection of compliance with supplements. Vitamin D exposure through sunlight and diet can be evaluated by measurement of serum 25(OH)D levels, which provide a direct reflection of vitamin D status in the weeks preceding sample procurement and are often interpreted as an estimate of general vitamin D status. A nested case-control study of US military personnel found that among Caucasian individuals (148 cases and 296 controls) the likelihood of future MS diagnosis declined by nearly 40 % for every 50 nmol/L increase in 25(OH)D level (OR 0.59, 95 % CI 0.36–0.97).[44] This relationship was particularly notable for military personnel sampled before 20 years of age when those with high 25(OH)D levels (>100 nmol/L) were substantially less likely to be diagnosed with MS (OR 0.09, 95 % CI 0.01–0.75). One could hypothesise that the vitamin D levels obtained in these younger individuals more closely reflected their vitamin D status during childhood, the time period when MS risk is thought to be determined.

Vitamin D levels may not only influence MS risk and serve as a biomarker for MS susceptibility, but serum vitamin D concentrations may also influence MS disease course. In a study of 80 female MS patients, the mean 25(OH)D level was 43 nmol/L, which is in the insufficient range for bone health.[45] A Finnish study reported that mean 25(OH)D levels during the summer (June through September) were significantly lower in newly diagnosed MS patients compared with healthy individuals living in the same region (58 ± 3 nmol/L versus 85 ± 8 nmol/L; p=0.022).[46] Three studies have reported that 25(OH)D levels in adult MS patients are lower at times of relapse compared with periods of clinical disease remission.[46–48] An Australian study of 145 adult patients with relapsing–remitting MS (RRMS) showed a small but statistically significant inverse relationship between serum 25(OH)D level and risk for relapse in the subsequent six months with a hazard ratio of 0.91 (95 % CI 0.85–0.97) for every 10 nmol/L increase in serum 25(OH)D.[49] A study of 110 children with either a first attack of demyelination or established MS, reported a mean 25(OH)D level in this cohort of 57.3 nmol/L (22.9 ng/L) and found

that each 25 nmol/L increase in adjusted 25(OH)D level was associated with a 34 % reduction in number of subsequent relapses (incidence rate ratio of 0.66, 95 % CI 0.46–0.95).[50] Finally, vitamin D concentrations have also been variably linked to MRI evidence of MS disease activity.[47,51]

Pathobiological Insights

The effects of vitamin D in the experimental autoimmune encephalomyelitis (EAE) animal model of MS provide insight into the role of vitamin D in MS biology. In this model, injection of 1,25-dihydroxyvitamin D (1,25[OH]2D) prior to disease induction can prevent the clinical manifestation of symptoms and the development of characteristic pathologic lesions.[51,52] If given at the onset of clinical symptoms, treatment with 1,25(OH)2D resulted in an improvement of clinical symptoms,[53] with more of an effect in female animals.[54] The mechanisms that underlie these observations may involve vitamin D-mediated interleukin-10 (IL-10) cellular signaling pathways,[55] reduced monocyte CNS entry or accumulation,[56,57] or induction of inflammatory cell apoptosis.[58] There have been conflicting reports of the ability of UV light exposure prior to EAE induction to prevent clinical symptoms in this model.[59,60]

In human cell cultures, 1,25(OH)2D modulates monocyte functions, including antigen presentation, inhibits autoreactive T-cell activation by dendritic cells, and induces the action of regulatory T cells.[61–63] Production of pro-inflammatory cytokines such as interferon gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha), and IL-2 are inhibited by 1,25(OH)2D, whereas levels of anti-inflammatory cytokines such as IL-10 are enhanced.[48,61,63–69]

Vitamin D-related Genetics

Several MS susceptibility loci have been identified in the human genome, with the most robust association being with specific alleles of the human leukocyte antigen (HLA) locus on chromosome 6.[70] Recently, a vitamin D response element (VDRE) was found in the promoter region of HLA-DR1 that was absolutely conserved in HLA DRB1*15 haplotypes, the major genetic risk haplotype for MS.[71] Vitamin D receptor binding sites have now been identified in 2,776 locations across the human genome, affecting the expression of 229 genes, including loci implicated as risk genes for MS.[72] These studies provide an interesting conceptual means whereby genetic susceptibility and environmental exposures may interact to increase MS risk.

Viral Exposures

Background

The powerful impact of infection on host immune behaviour, the frequent similarity in structure of microbial proteins and human tissue antigens, and the noted difference in host response to specific pathogens as a function of age at exposure has led to many hypotheses linking MS and infection. The general upregulation of immune responses to any infection has been considered as potentially important in increasing the likelihood of MS relapse.[73] However, it could be argued that the most compelling link between MS and a specific pathogen exists for Epstein–Barr Virus (EBV).

EBV is a DNA virus in the herpes group that has a widespread geographic distribution and infects more than 90 % of individuals by adulthood.[9,74,75] Primary infection is often asymptomatic in younger children, but when infection occurs later in childhood or adolescence

the result is clinical infectious mononucleosis (IM) in 40–50 % of cases. Following a primary infection, EBV remains latent in B cells and persists throughout life. In tonsils, the virus can periodically become reactivated, resulting in viral shedding and transmission may then occur to other individuals through infected saliva. EBV expresses viral capsid antigen (VCA) and early antigen (EA) during active infections, and the EBV nuclear antigen (EBNA) 1–6 and latent membrane protein (LMP) 1, 2a, and 2b during chronic infections.[9,76]

Epidemiology

Similarities in the geographic distribution, age at onset, association with socioeconomic status (SES), and predilection for individuals of particular ethnicities between IM and MS first raised the idea that EBV could be involved in MS pathogenesis.[9,74,77]

EBV seropositivity appears to increase greatly the risk for adult-onset MS. A nested case-control study of US military personnel identified 10 initially seronegative patients with an eventual diagnosis of MS and 32 seronegative controls who did not develop MS. During the follow-up period, all 10 individuals who developed MS became seropositive for EBV prior to onset of clinical symptoms of MS, with an estimated mean time between primary EBV infection and MS diagnosis of 5.6 years (range 2.3 to 9.4 years). In this study, no person who remained seronegative for EBV developed MS in adulthood.[78]

Four prospective studies have shown that in EBV seropositive adults, the likelihood of future MS diagnosis increases as a function of higher concentrations of anti-EBNA complex and EBNA1 antibody titers.[79–82]

A recent meta-analysis that included a total of 18 case-control and prospective cohort studies found that the risk for adult-onset MS is particularly high for individuals that have experienced clinical IM with a combined relative risk for 2.17 (95 % CI 1.97–2.39).[83] Similarly, in a Canadian cohort of 14,362 individuals with MS and 7,671 spousal controls, people with MS were twice as likely to report a history of clinical IM than their spousal controls (OR 2.06, 95 % CI 1.71–2.48).[84] A model has been proposed in which adult-onset MS risk is negligible in individuals not exposed to EBV, is intermediate in those with serologic evidence of remote EBV infection without clinical signs, and highest in those individuals who have experienced IM.[85]

A higher prevalence of anti-EBV-VCA antibodies in adult MS patients, as compared to healthy controls, was described in a US study in 1976.[86] Although this initial study did not reach statistical significance, an expansion of the study to include 157 adult-onset MS patients and 81 control subjects found anti-EBV-VCA antibodies to be significantly more prevalent in MS patients (98.7 %) compared with controls (93.8 %), with higher geometric mean titers of EBV-VCA antibodies significantly elevated in MS patients.[87] Many subsequent studies have reported that adult MS patients are more likely than control subjects to have both anti-EBV-VCA and EBNA antibodies with elevated titers.[82,88–95] Seroprevalence studies show that nearly 100 % of adult-onset MS patients compared with 95 % of controls have antibodies for one or more EBV antigens with an odds ratio for MS in seronegative compared with seropositive individuals of 0.06 (95 % CI 0.03–0.13).[9]

In childhood, three case-control studies have shown that EBV seropositivity is more common in paediatric-onset MS patients than in healthy age-matched and regionally matched children. A single-centre Canadian study reported that serologic evidence of

remote EBV infection was present in 83 % of children with MS (n=30) compared with 42 % of healthy age-matched controls (n=90, OR 8.7, 95 % CI 2.5–30.3; p<0.01).[96] A multinational study that enrolled children with MS and matched controls from Canada, the US, South America, and Europe reported serologic evidence of remote EBV infection in 86 % (108 of 126) of children with MS compared with 64 % (61/96) of control participants (p=0.025).[39] Among seropositive children, mean EBNA1 titers in 73 children with MS were significantly higher than in 54 EBV-positive children without MS (187.4 ± 59.5 versus 152.5 ± 70.1; p=0.006).[39] The association of remote EBV infection and paediatric MS was further strengthened by a German study of 147 children with MS and 147 paired sex- and age-matched controls in which remote EBV infection was more common in MS patients than in healthy controls (84 versus 56 %; p=0.0033).[97] Median anti-EBV-VCA and anti-EBNA1 antibody titers were significantly higher in seropositive MS patients compared with seropositive control children (anti-EBV-VCA 62 versus 44; p=0.0002; anti-EBNA1 36 versus 14; p=0.003). To date, none of the paediatric MS studies have found differences in seroprevalence rates for other common childhood viruses studied, including herpes simplex virus, cytomegalovirus, varicella zoster virus, or Parvovirus B19.

In adults with established MS two studies have demonstrated active EBV replication during relapses but not during periods of clinical quiescence;[98,99] however, two others have not.[88,100] A multinational study of childhood MS found no correlation between mean relapse rates and EBV seropositivity (mean relapse rate in EBV positive patients of 1.18 ± 0.7 versus mean relapse rate in EBV negative patients of 1.01 ± 0.6; p=0.49), but these results may have been confounded by age.[39]

Anti-EBV antibody titers may correlate with disease activity on MRI. A recent study of 50 adults with MS showed that anti-EBV-VCA antibody titers were inversely correlated with MRI measures of gray-matter atrophy in the following three years.[101] A study from the UK of 50 patients with a clinically isolated syndrome (CIS), 25 patients with relapsing–remitting MS (RRMS), and 25 patients with primary-progressive MS showed that participants with at least one gadolinium-enhancing lesion on MRI had higher median EBNA1 titers than those who did not (791 [95 % CI 414–1704] versus 251 [95 % CI 82–599]; p<0.001). There was also a positive linear correlation between EBNA1 IgG concentration and number of gadolinium-enhancing lesions (Spearman r=0.33; p<0.001).[102]

Pathobiological Insights

The underlying biological mechanisms responsible for the observed epidemiological associations between MS risk and EBV infection remain to be fully explained. Several studies in adults have shown elevated levels of intrathecal anti-EBV-VCA antibodies,[91] anti-EBNA1 antibodies,[103] and antibodies directed against the EBV protein BRRF2 in MS patients.[104] EBV may influence MS pathogenesis through cellular immune mechanisms. A study of 20 EBV seropositive MS patients and 20 seropositive healthy controls showed an increased frequency of memory CD4+ T cells, enhanced proliferation of CD4+ cells, and increased production of the proinflammatory cytokine interferon gamma in samples from MS patients.[105] Several studies have noted T-cell cross reactivity between EBV antigens and autoantigens, including myelin basic protein.[106–109] These results would support the hypothesis that there is cross-reactivity between EBV viral epitopes and self-antigens.[110]

Other Viruses

There has been interest in other potential viral aetiologies for MS, including varicella zoster virus (VZV) infection.[9,111,112] Several case-control studies in children have largely shown no differences in the presence of anti-VZV antibodies in serum or CSF between children with MS and controls,[39,96,113,114] but the young age of VZV acquisition leads to a high seroprevalence rate even in healthy children. A French study of 137 children with clinically definite MS and 1061 matched control children found that a history of clinical chickenpox was present in fewer children with MS compared with healthy controls (76.6 versus 84.9 %; adjusted OR 0.58, 95 % CI 0.36–0.92).[115] We would hypothesise that the lower frequency of VZV infection in paediatric MS patients indicates limited exposure to childhood infections and that delayed exposure to common infectious agents could render an individual particularly prone to aberrant immune responses to other key environmental triggers such as EBV. Recent VZV vaccination also confounds studies of VZV infection rates in paediatric MS.

Cigarette Smoke
Epidemiology

Increased risk for adult-onset MS in individuals who report a history of smoking has been found in several case-control and prospective studies[116–121] and the relative risk for MS in patients with a past or current smoking history versus those with no smoking history has been estimated to be 1.2 (95 % CI 0.9–1.6) and 1.4 (95 % CI 1.2–1.7), respectively.[11] A meta-analysis of six retrospective and prospective studies reported a pooled odds ratio of 1.34 (95 % CI 1.17–1.54) for MS in smokers versus non-smokers.[122]

A French study of 129 children diagnosed with MS before 16 years of age and 1,038 matched population controls reported exposure to parental smoking in 62 % of children with MS compared with 45.1 % of control children (adjusted relative risk for MS 2.12, 95 % CI 1.43–3.15).[123] When stratified by age, adjusted relative risk for children over 10 years of age (2.49, 95 % CI 1.53–4.08) was higher than that for children under 10 years of age (1.47, 95 % CI 0.73–2.96), which may reflect a longer duration of exposure.

In a Swedish study of 143 patients with MS, 36 of whom were diagnosed before 16 years of age, and 1,730 matched controls, maternal smoking during pregnancy was not associated with increased risk for MS diagnosis in either childhood (adjusted OR 1.12, 95 % CI 0.50–2.51) or early adulthood (adjusted OR 0.91, 95 % CI 0.57–1.46).[124] This study did not assess exposure to smoking by other family members and had limited power to detect relevant differences in MS rates in the 'under 16-years-old' age group.

Studies in adult patients with MS have suggested that a personal history of smoking may be related to a greater risk for conversion to clinically definite MS after a first demyelinating event, may hasten progression to secondary progressive MS in those initially diagnosed with RRMS, and may be related to T2 lesion accumulation on MRI.[118,125,126]

Pathobiological Insights

Cigarette smoke exposure may affect MS biology in several ways. Nicotine may alter blood-brain barrier permeability, and thus allow immune cells to enter the nervous system more readily.[127] Cyanide, a component of cigarette smoke, may be directly toxic to CNS white matter.[128] Exposure to cigarette smoke in childhood may also increase

Table 1: Environmental Risk Factors for Multiple Sclerosis

Risk Factor	Putative Mechanisms
Vitamin D deficiency	Altered antigen presentation, altered T-cell activation, increased central nervous system monocyte entry, increased pro-inflammatory cytokine production, altered gene expression
Viral exposures (ex: EBV)	Increased CD4^{+} T-cell production, increased pro-inflammatory cytokine production, cross-reactivity with autoantigens, including myelin basic protein
Cigarette smoke	Altered blood–brain barrier permeability, direct myelin toxicity, altered cytokine profile, more frequent exposure to viral respiratory infections

EBV = Epstein-Barr virus.

frequency of viral infections.[129] Cigarette smoke may lead to altered cytokine profiles, as demonstrated by studies of immune cell cytokine expression in people who smoke.[130]

Hepatitis B Vaccination

A single case-control study from the UK reported an increased risk for MS diagnosis in adults associated with hepatitis B vaccination in the preceding three years.[131] However, numerous other studies have shown no such association.[132–135] A French population-based case-control study of 349 children with a first presentation of acquired CNS demyelination (ADS) and 2941 matched controls found no significant difference in hepatitis B vaccination rates between the two groups in the three years prior to presentation with an adjusted OR of 0.74 (95 % CI 0.54–1.02).[136]

In a study of 143 children with confirmed MS and 1,122 matched population control children, hepatitis B vaccination rates in the three years before first presentation of neurological symptoms was not significantly different between the children with MS and the matched controls (adjusted OR 1.03, 95 % CI 0.62–1.69).[137] Furthermore, there has been no change in childhood MS incidence in British Columbia, Canada, following implementation of a school-based vaccination program in 1992.[138] The combined results of these studies suggest that exposure to the hepatitis B vaccine does not confer increased risk for demyelinating symptoms in children or adults.

Conclusions

There are marked variations in worldwide MS prevalence. While genetic predisposition no doubt plays a role, environmental factors such as ambient sunlight exposure, dietary vitamin D intake, age and frequency of viral infection, and exposure to cigarette smoke also likely contribute to regional differences in MS risk (*Table 1*). The increasing incidence of MS, particularly in women,[139,140] may reflect the rising number of women in the workplace and the associated decreased time spent outdoors, increased sunscreen use, decreased nutritional value of the modern diet in industrialized nations, and the marked increase in smoking rates among women. If childhood is a vulnerable time period for MS development, then the study of children with MS will provide important insights into the timing of key environmental exposures. Collaborative, international efforts are essential to determine important environmental triggers relevant across diverse populations and to define regional risk factors with a goal of developing novel strategies for primary disease prevention. ■

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