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Multiple Sclerosis Risk Factors and Pathogenesis

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ABSTRACT

PURPOSE OF REVIEW: This article summarizes recent advances in the identification of genetic and environmental factors that affect the risk of developing multiple sclerosis (MS) and the pathogenic processes involved in acute relapses and relapse-independent disability progression.

RECENT FINDINGS: The number of single-nucleotide polymorphisms associated with increased risk of MS has increased to more than 200 variants. The evidence for the association of Epstein-Barr virus infection, vitamin D deficiency, obesity, and smoking with increased risk of MS has further accumulated, and, in cases of obesity and vitamin D deficiency, the evidence for causal association has strengthened. Interactions between genetic and environmental factors have been studied more extensively. Dietary factors and changes in the gut microbiota are emerging as possible modulators of the disease risk. Several processes important to MS pathogenesis have been newly investigated or investigated more comprehensively, including the role of B cells, innate immune cells, meningeal inflammation, cortical and gray matter demyelination, and early axonal and neuronal loss.

SUMMARY: MS is a complex disease in which the interaction between genetic and environmental factors causes a cascade of events, including activation of the adaptive and innate immune system, blood-brain barrier breakdown, central nervous system demyelination, and axonal and neuronal damage with variable degrees of repair. These events manifest as potentially reversible focal neurologic symptoms or progressive nonremitting physical and cognitive disability, or both. Advances in the understanding of the risk factors and pathogenic mechanisms of MS have resulted in improved therapeutic strategies. The results of ongoing or future studies are needed to successfully and fully translate these advances into clinical practice.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic disease characterized by inflammation and demyelination of the central nervous system (CNS) associated with variable degrees of axonal and neuronal damage. It usually presents with recurrent, subacute, focal neurologic symptoms and signs that improve (at least to some extent) over several weeks or months. The disease may initially present with (less

often) or evolve into (more common) a relentless progressive decline of neurologic functioning, commonly affecting locomotion, bladder function, and cognition.[1]

Although there is no doubt that immune mechanisms and inflammation are pivotal to the pathogenesis of MS,[2] it is still debated whether the inflammation is the initial event in the cascade of pathophysiologic events or is a secondary response to a yet-unknown infectious agent or intrinsic/primary CNS degeneration. Although MS does not have some of the characteristics typical of autoimmune disease,[2,3] most MS investigators believe that self-reactive immune cells gaining access to and attacking components of the myelin sheath in the CNS is the primary pathogenic event. A breakdown in immune tolerance is posited to result in the persistence of autoreactive lymphocytes. However, it is less clear how these cells become activated, proliferate, access the CNS, and orchestrate the pathologic events that lead to neurologic dysfunction. A complex interaction among genetic and environmental factors and stochastic events affects not only the probability of these pathogenic events but also the strength of the CNS repair mechanisms, functional plasticity, and physical and cognitive reserve. These factors all affect the probability of a person becoming symptomatic from the inflammatory demyelination and thus being diagnosed with MS.

Unlike several other common neurologic diseases (such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis), no undisputed mendelian form of MS has thus far been reported.[4,5] Also, despite decades of research to evaluate infectious agents as the cause of MS, no single pathogen has been determined to be necessary for the development of MS. Data from preclinical and observational studies (including cohort and case-control studies) have resulted in the discovery of associations between many genetic loci and several environmental factors with the risk of developing MS. However, demonstrating causality of these associations is more difficult, particularly because performing experimental (interventional) studies in healthy humans is extremely difficult/infeasible, costly, or unethical. Also, because, in most patients, MS has a long subclinical period (as evidenced by the presence of silent lesions on MRI at the time of clinical onset and subtle deficits on clinical testing years before the onset of symptoms),[6] the specific environmental factors that predisposed a given person to developing MS were probably present many years before the clinical onset. This makes identifying these factors more difficult. Despite these hurdles, identifying environmental and genetic risk factors provides mechanistic insights that can be examined as putative complementary treatments for MS. Discovering modifiable risk factors also allows for consideration of studies or interventions related to disease prevention.

KEY POINT

- **Unlike several other common neurologic diseases (such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis), no mendelian form of multiple sclerosis has thus far been reported.**

OVERVIEW OF MULTIPLE SCLEROSIS PATHOGENESIS

MS, like many other chronic neurologic diseases, has a complex etiopathogenesis. Both genetic susceptibility and environmental exposures have been incriminated.[7] The concordance rate of MS for northern European monozygotic twins (with close to 100% genetic similarity) is only 20% to 30%.[8] Although much higher than the risk of MS in the general population (only 0.1% to 0.2% in northern Europeans), suggesting strong evidence for high degree of heritability of the disease, this provides evidence that environmental exposures or nonmendelian genetics must play an important role in MS pathogenesis. Some experts have proposed that MS develops in a genetically susceptible individual

who experiences a "sufficient set" of environmental factors. This "sufficient set" can be different for persons with different genetic backgrounds,[9] and even the constituents of the set may differ from one person to the next. Early life events in the presence of this predisposing genotype and the interaction of some of these environmental and genetic factors are hypothesized to result in defective immune tolerance, with persistence and activation of autoreactive adaptive immune cells that access the CNS and set off pathologic events that result in demyelination, axonal and neuronal damage, and gliosis. Over time, incomplete repair, activation of innate immunity in the CNS, oxidative damage, abnormal energy metabolism, and possibly the development of lymphoid follicle–like structures in the meninges are thought to propagate the injury and exhaust the compensatory mechanisms, resulting in relentless neurologic worsening.[10]

No single autoantigen, autoantibody, or infectious agent has thus far been unequivocally associated with MS. However, as many MS susceptibility genes are shared by other autoimmune disorders and as immunosuppressive therapies modify the disease course, most researchers consider MS a primary organ-specific autoimmune disease. An increased risk of several autoimmune diseases (including autoimmune thyroid disease, inflammatory bowel disease, and psoriasis) also exists in patients with MS.[11]

Autoreactive lymphocytes that gain access to the CNS start a pathogenic cascade that culminates in demyelination, neuroaxonal degeneration, synaptic loss, dying-back oligodendrogliopathy, and, eventually, tissue loss and astrogliosis.[12] Relapses are thought to be caused by the subacute development of inflammatory aggregates in an eloquent area of the CNS. These inflamed areas, which are also associated with blood-brain barrier breakdown, appear as gadolinium-enhancing lesions on MRI around the time they develop.[13] Downstream immunopathologic events lead to the development of confluent demyelinated areas in both white and gray matter, indicating the loss of oligodendrocytes, loss of myelin sheaths, and astrocytic scars, producing the pathologic markers of MS: demyelinated plaques.[12] Demyelination in MS is not confined to the white matter. Cortical and deep gray matter demyelination can be detected pathologically and is present even in early stages of the disease.[14]

Historically, MS was thought to be a T-lymphocyte–mediated disease. Animal models of MS had provided ample evidence for the role of T cells in the pathogenesis of neuroinflammation.[15,16] However, as clinical trials in patients with MS demonstrated unequivocal efficacy of B-lymphocyte–depleting antibodies in decreasing the disease activity and even some effects on slowing disability accumulation in progressive disease,[17,18] it seems that B cells also play an important role in disease pathogenesis.[19,20] The rapid response to B-cell–depleting antibodies suggests that antigen presentation and production of proinflammatory chemokines and cytokines by B lymphocytes (as compared to their role in antibody production) might be more relevant to MS pathogenesis.[21]

Although demyelination is the hallmark of the disease, axonal injury is present, even from the earliest stages of MS, and appears to be an important contributor to symptoms and disability.[22,23] In mouse models of demyelination, CD8+ T cells have been shown to damage the axons by releasing perforin and granzyme.[24] Other mechanisms underlying neuronal and axonal injury include glutamatergic excitotoxicity,[25] cytokine release,[26] hypoxia, and generation of

reactive oxygen and nitrogen species.[27,28] These events lead to calcium influx into the axons and neurons, mitochondrial dysfunction, and oxidative stress. Ultimately, ion channel redistribution and energy deficiency result in cell swelling, activation of degrading enzymes, and eventual apoptotic and necrotic neuronal damage.[10]

In progressive stages of the disease, when the acute inflammatory infiltrates develop less frequently or completely cease to happen, it is not clear what drives the progressive neurologic disability. Some argue that age-related changes in an already-injured brain and spinal cord underlie the progression. The more recent discovery of meningeal lymphoid follicle–like aggregates,[29] which can be imaged with MRI,[30] has raised the possibility that ongoing sequestered intrathecal inflammation might be a factor contributing to progression. These lymphoid aggregates are not accessible to systemically administered anti-inflammatory and immunosuppressive therapies. Another proposed mechanism of continued neurodegeneration in the absence of apparent adaptive immune activity is activation of CNS innate immunity, particularly microglial cells.[10] The development of effective therapies for progressive MS requires better understanding of the relative contribution of each pathogenic mechanism, an understanding of when in the disease it is important to disrupt each mechanism, and the development of medications that cross the blood-brain barrier and target the mechanisms effectively. Alternatively, medications that protect mitochondria or target ion channels or antioxidative agents may prove to be beneficial in slowing the progressive stages of the disease.

GENETIC RISK FACTORS

Genetic contributions to MS risk were known to exist decades ago. Familial clustering of the disease, with increased risk dependent on the degree of genetic similarity to the proband, and higher prevalence of MS in some racial groups are strong evidence for a genetic basis of the disease.[31] Family and population data suggested that multiple DNA variants that are relatively frequent in the population are the basis of MS heritability.[32]

For decades, only several variants of HLA antigen were known to affect MS risk. Genes in the HLA antigen locus have the strongest effect on the risk of MS. Carrying HLA-DRB1*1501 is associated with about threefold greater odds of developing MS, while carrying HLA-A*02 is associated with meaningfully reduced odds of developing MS.[33]

In the past 15 years, international collaborations for performing genome-wide association studies have studied tens of thousands of MS patients and controls and provided strong evidence for the association of approximately 200 autosomal susceptibility variants outside of the major histocompatibility complex gene complex. In addition, one variant in chromosome X and 32 independent variants in the extended major histocompatibility complex region were discovered.[34] Almost all these variants are located in the noncoding regions of the genome, and many of them are in intergenic regions. These variants are thought to affect regulatory mechanisms and gene activity. Interestingly, many MS-associated genetic variants are located close to the genes that regulate innate or adaptive immunity and are shared by several other autoimmune diseases. All these variants only explain 20% to 30% of MS heritability, suggesting the remainder of heritability is likely related to epigenetic factors and gene-gene or gene-environment interactions.[35]

KEY POINTS

- No single autoantigen, autoantibody, or infectious agent has thus far been unequivocally associated with multiple sclerosis.
- Autoreactive lymphocytes that gain access to the central nervous system start a pathogenic cascade that culminates in demyelination, neuroaxonal degeneration, synaptic loss, dying-back oligodendrogliopathy, and, eventually, tissue loss and astrogliosis.
- Demyelination in multiple sclerosis is not confined to the white matter, and cortical and deep gray matter demyelination can be detected pathologically and is present even in early stages of the disease.
- Both T lymphocytes and B lymphocytes, as well as innate immune mechanisms, participate in multiple sclerosis pathogenesis.
- Although demyelination in the central nervous system is the hallmark of multiple sclerosis, axonal injury is present from the earliest stages of the disease and is a major contributor to physical and cognitive disability.
- More than 200 genetic variants have been discovered to be associated with modifying the risk of multiple sclerosis.

ENVIRONMENTAL RISK FACTORS

Dozens of environmental factors and exposures have been reported to be associated with the risk for developing MS. Only a few, however, have been studied in adequately powered and less biased studies; even fewer of those findings have been replicated consistently. This article reviews the factors with the strongest incriminating evidence for a role in MS, including low sunlight exposure, vitamin D deficiency, obesity, and smoking, with a focus on those that have more recently been implicated (TABLE 1-1[36] and CASE 1-1).

Epstein-Barr Virus

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus. EBV seropositivity, or the presence of antibodies indicating prior exposure to EBV, has been consistently shown to be associated with adult- and pediatric-onset MS in people of different races and ethnicities.[37,38] In fact, almost all adult patients with MS have serologic evidence of prior EBV infection. People with MS have higher titers of antibodies to Epstein-Barr nuclear antigen 1 (EBNA1).[39] In a nested case-control study, all adults with MS who were initially EBNA1 antibody negative had become antibody positive before the disease onset.[40] While childhood infection with EBV is usually asymptomatic, infection with this virus later in life can be associated with clinical infectious mononucleosis. The risk of MS in people with a clinical history of infectious mononucleosis is more than twice that of the general population.[41] In fact, it has been suggested that EBV infection during adolescence or adulthood, perhaps more so than during childhood, increases the risk of MS.[42]

TABLE 1-1 **Environmental Risk Factors for Multiple Sclerosis**[a]

Risk Factor	Odds Ratio (Approximate)	Interaction With HLA Antigen Gene	Strength of Evidence
Epstein-Barr virus seropositivity	3.6	Yes	+++
Adolescent obesity	2	Yes	+++
Smoking	1.6	Yes	+++
Vitamin D deficiency	1.4	Probably	+++
Low sunlight exposure	2	Probably	++
Shift work	1.7	No	++
Infectious mononucleosis	2	Yes	++
Passive smoking	1.3	Unknown	+
Oral tobacco	0.5	Unknown	+
Coffee	0.7	Unknown	+
Alcohol	0.6	Unknown	+

[a] Modified with permission from Olsson T, et al, Nat Rev Neurol.[36] © 2016 Springer Nature.

CASE 1-1

An otherwise healthy 27-year-old woman presented to the clinic with blurred vision in her right eye that had started 5 days earlier. She had no significant past medical or family history. On examination, she had visual acuity of 20/200 in her right eye and a right afferent pupillary defect. Brain and cervical spine MRI showed T2-hyperintense lesions in the juxtacortical and periventricular white matter and cervical spinal cord in a pattern suggestive for inflammatory demyelination. Some of the brain lesions enhanced after gadolinium administration. Her CSF analysis was notable only for more than five oligoclonal bands that were not present in the serum.

She was diagnosed with relapsing-remitting multiple sclerosis (MS). During a clinic visit, she asked about the risk of MS in her 3-year-old son and 30-year-old sister and if anything could be done to reduce their risks of developing MS.

COMMENT

Relatives of a person with MS are at increased risk of developing MS. The risk depends on the degree of genetic similarity to the patient. First-degree relatives have about 2% to 5% chance of developing MS. Many environmental risk factors for MS seem to be more important in young adults. Although preventive studies have not been conducted to date, it stands to reason that, in theory, modifying environmental factors and exposures might have a more profound effect on MS risk in younger people (eg, for this patient's 3-year-old son, as opposed to her 30-year-old sister). In other words, decreasing the chance of MS development by reducing exposure to MS risk factors will probably be more effective in children than older adults.

Despite the lack of interventional data to support lifestyle changes to prevent MS, avoiding smoking and the prevention and treatment of obesity and vitamin D deficiency can be considered by most or all those who are at higher risk of developing MS as these are unlikely to be harmful. In particular for children, the decision to initiate vitamin D supplementation with the goal of reducing the risk of MS should be made in concert with the primary care provider. Ultraviolet radiation exposure, because of its carcinogenic effects, cannot be routinely recommended. No effective intervention for prevention of Epstein-Barr virus infection is known. Not enough evidence exists for other environmental exposures (such as diets, dietary factors, or nutrients) to be routinely recommended for MS prevention. Genetic testing (eg, for HLA antigen MS risk alleles) is not recommended.

A mendelian randomization study has shown that genetic factors associated with higher EBNA1 titers are positively associated with MS risk (BOX 1-1).[46] An additive interaction also exists between EBNA1 titer (or a self-reported history of infectious mononucleosis) and HLA antigen MS risk genetic variants as well as between these EBV markers and obesity. Specifically, having a higher EBNA1 titer or history of infectious mononucleosis is a bigger risk factor in those who carry HLA antigen MS risk alleles, or among those who are obese, than in those who do not possess those features.[47,48] These results are interpreted as the evidence for causal association between EBV infection and MS risk. In pediatric MS, EBV antibodies had a multiplicative interaction with race.[49] Positive EBV antibody is more strongly associated with the odds of pediatric MS development in whites than in African Americans. Despite the robust epidemiologic evidence for the importance of EBV infection in the development of MS, the mechanism by which EBV increases MS risk is not clear. Several mechanisms have been proposed, including direct infection of the CNS by the virus, molecular mimicry, and cross-reaction between the virus and human antigens and latent infection of B cells.

Unlike EBV, the evidence for the association of cytomegalovirus (CMV) and herpes simplex virus is not compelling. Although positive CMV serology was reported to be associated with decreased risk of pediatric MS,[50] a larger study did not replicate this result.[49] A case-control study also reported a negative association between CMV seropositivity and MS risk in adults in Sweden,[51] but a 2017 study in California showed an association between CMV infection and decreased risk of MS in Hispanics but not in non-Hispanic whites or African Americans.[52] The results were interpreted as a sign of noncausal association. Among white children, positive herpes simplex virus serology has been shown to be associated with higher pediatric MS risk only in those who are negative for the HLA antigen MS risk allele (HLA-DRB1*15:01).[50] These results require replication.

Ultraviolet Radiation Exposure and Vitamin D

MS incidence and prevalence are higher in higher latitudes.[53] This observation was the basis of many studies looking at the effects of sun exposure and vitamin D on the risk of MS. Ultraviolet radiation synthesizes vitamin D in the skin, so it is difficult to study the independent effects of these variables on the odds of developing MS. However, it seems that both ultraviolet radiation and vitamin D are associated with lower odds of MS and that not all of the effect of ultraviolet radiation may be entirely explained by its contribution to vitamin D synthesis.[54]

Multiple studies have shown the negative association of ultraviolet radiation exposure and MS, when assessed at the time of MS onset or diagnosis. This association remains even after adjusting the statistical models for serum vitamin D levels.[55,56] These results are in line with preclinical studies, in which ultraviolet radiation exposure, independent of vitamin D, is protective in an animal model of MS. These effects might be mediated by the effects of ultraviolet radiation on regulatory T cells and antigen-presenting dendritic cells.[57]

Vitamin D, in part, may mediate some of the protective effects of ultraviolet radiation on MS. If the effect of ultraviolet radiation was completely independent of vitamin D, then oral intake of vitamin D should have no effect on the risk of MS. However, it has been shown that a diet high in fatty fish (a good source of vitamin D) is associated with lower odds of MS in people with low sun exposure.[58]

Further, the apparent association between serum vitamin D levels and MS risk remains even when measures of ultraviolet radiation exposure were included in the models.[56] Higher serum vitamin D levels (particularly in young adulthood) have been shown to be associated with lower risk of developing MS later in life, although these analyses did not account for ultraviolet radiation exposure.[59] On the other hand, the effect of vitamin D deficiency of a mother during pregnancy on the MS risk of offspring is controversial.[60,61]

Several mendelian randomization studies have demonstrated the association of genetic variants affecting serum vitamin D levels and the risk of MS. These studies are the strongest evidence of a causal association of vitamin D status and MS.[62,63] However, these conclusions are not undisputed. For example, the association between serum vitamin D levels and MS has been only shown in whites and was not seen in African Americans or Hispanics.[54] The absence of association in some racial or ethnic groups has cast some doubt on the causal association between vitamin D and MS risk, at least as a universally important factor.

Obesity

Several cohort and case-control studies have demonstrated the association between obesity and MS risk.[64–66] Similar to many other environmental risk factors, obesity in adolescence and young adulthood, but not later in adult life, seems to be associated with subsequent risk of MS.[64] The risk is highest in overtly obese individuals, although a milder degree of being overweight may also associated with greater risk.

By showing an association between genetic variants affecting the body mass index and the risk of MS, mendelian randomization studies have provided stronger evidence for causality of the association of obesity and MS.[67,68] Similar to adult-onset disease, obesity has been shown to be associated with pediatric-onset MS.[69] Obesity may also interact with HLA antigen MS risk variants: in one study, obesity was a stronger risk factor in individuals carrying HLA-DRB1*1501.[64]

An interaction between EBV infection and obesity may also exist. In one cohort, while obesity and EBV infection were each associated with about twofold greater odds of MS, the co-occurrence of both risk factors in the same person increased the odds 14-fold.[48] These interactions are interpreted as providing stronger evidence for causality of these environmental risk factors.

Smoking

A large case-control study and pooled analysis of several smaller studies have shown that smoking is a risk factor for MS.[70,71] This association is dose dependent: smoking more cigarettes a day is associated with more substantial MS risk. Even passive smoking has been incriminated as a risk factor, including in pediatric-onset MS. In addition to self-reported smoking, elevated serum cotinine levels (a marker of smoking) in samples obtained before developing MS has been shown to be associated with increased MS risk.[72]

One study showed that the use of oral tobacco was associated with lower odds of MS, particularly among concomitant cigarette smokers.[73] This observation has led to the conclusion that the lung irritation from inhalation of cigarette smoke is the mediator of association between smoking and MS risk. This is similar to several other autoimmune diseases, such as rheumatoid arthritis, in

KEY POINTS

- Low sunlight exposure, vitamin D deficiency, obesity, and smoking are factors with strong evidence for association with multiple sclerosis risk.
- Many infectious agents have been reported to be associated with multiple sclerosis risk; however, only Epstein-Barr virus infection has been consistently shown to be a risk factor.
- Exposure to several risk factors for developing multiple sclerosis (including Epstein-Barr virus infection and obesity) during adolescence appears to be more detrimental than exposure in adulthood.
- Statistical interactions between risk factors and mendelian randomization studies have provided evidence for the causal association of several environmental factors and the risk of multiple sclerosis.
- Lung irritation from inhalation of cigarette smoke is likely the mediator of association between smoking and multiple sclerosis risk.

BOX 1-1 Instrumental Variables, Mendelian Randomization Studies, and Statistical Interaction

Observational studies can provide evidence for the association between an exposure (eg, a potential risk factor) and an outcome (eg, the development of multiple sclerosis [MS]). However, to prove causality, randomized controlled studies are usually needed. Although randomized controlled trials to demonstrate the efficacy of an intervention for the purpose of treatment are feasible and commonly performed, randomized studies of disease risk factors are extremely difficult to undertake, may be unethical, and often are not feasible. Using instrumental variables in observational studies has become a rising trend in epidemiology to demonstrate causal association. An instrumental variable is a factor that is associated with the exposure of interest but is not associated with the outcome of interest, either directly or through another pathway. If these assumptions are correct, then showing the association between the instrumental variable and the outcome of interest demonstrates the causal association of the exposure of interest and the outcome.[43] For example, when

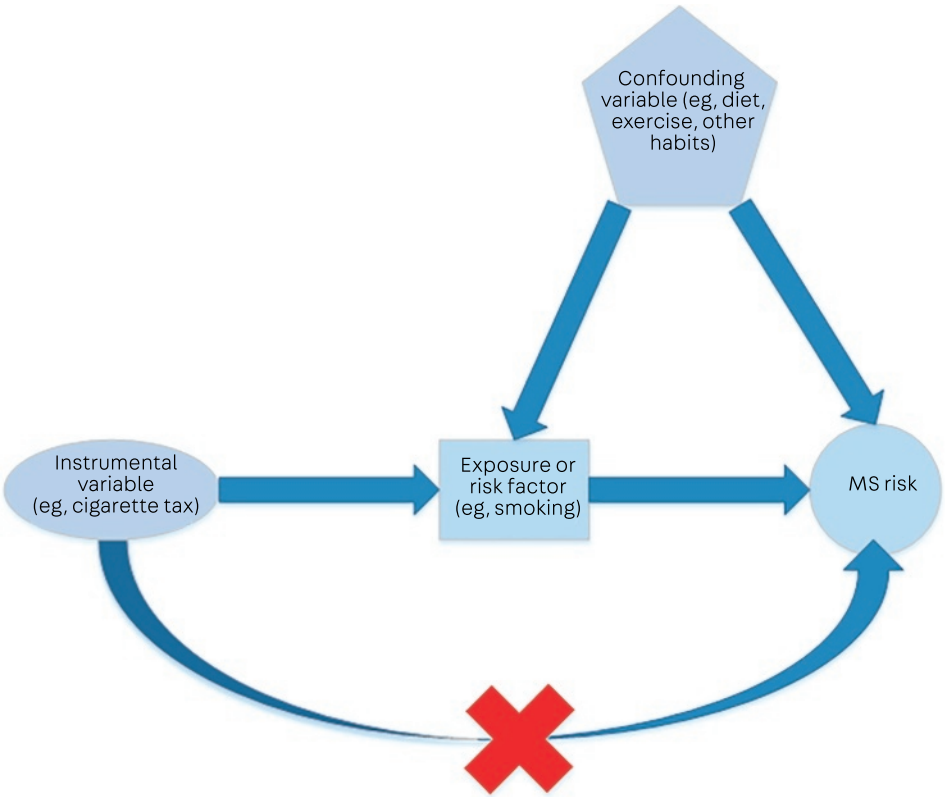

FIGURE 1-1
Schematic representation of the instrumental variable concept. In this hypothetical example, if it were demonstrated that imposing a cigarette tax in a geographical area was associated with decreased MS incidence in that area, since a cigarette tax cannot be associated with multiple sclerosis risk through any other mechanism aside from changing (probably reducing) smoking habits, it would provide strong evidence for the causal association between smoking and multiple sclerosis.

considering the role of smoking on the risk of MS, case-control or cohort studies have shown a clear association of smoking with MS. However, confounding variables might explain this association. Using an instrumental variable can remove the effect of potential confounders. In this case, if some areas of the country start imposing a very high tax on cigarettes and a subsequent decrease in the incidence of MS were observed in those areas, cigarette tax, as an instrumental variable, would demonstrate causal association between the smoking and risk of MS (FIGURE 1-1).

Mendelian randomization is one method of using instrumental variables. Random segregation of alleles during meiosis provides an opportunity to conduct instrumental variable studies for many different risk factors.[44] For example, if the heterogeneity in a gene is shown to be associated with a trait (such as height) and no plausibility exists that the gene can be associated with MS risk through any other pathway, showing the association of the heterogeneity of this gene and MS risk is suggestive of a causal association between height and MS risk (FIGURE 1-2).

In epidemiology, the effects of an independent variable on an outcome may depend on the level of another (independent) variable, a phenomenon known as interaction. Interaction can be additive or multiplicative.[45] In the additive interaction of two risk factors, the risk of an outcome in a person exposed to both factors is higher (or lower) than the sum of the effects of each factor in the absence of the other one. For example, if the baseline risk of MS in an individual not exposed to risk factors A and B is x, an individual exposed to only risk factor A is x + a and in an individual exposed only to risk factor B is x + b; if an additive interaction exists between A and B, the risk of MS in an individual exposed to both A and B will be x + a + b + c, where c is the interaction effect. Multiplicative interactions between a risk factor and demographic factor(s) may also exist. For example, factor A might be a risk factor for MS in the general population, but it may have a multiplicative interaction with race. For example, on further analysis, a researcher might find that factor A is only a risk factor in one race but not other races. The concepts of additive and multiplicative interaction are discussed, when appropriate, in the following sections.

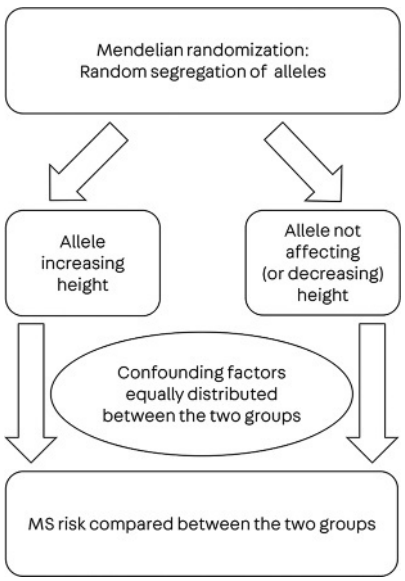

FIGURE 1-2
Mendelian randomization. Mendelian randomization is the use of random segregation of alleles that affect a trait as an instrumental variable to demonstrate the causal association of that trait and a specific outcome. In this hypothetical example, if genetic variability that is known to affect height were to be demonstrated to be associated with the risk of multiple sclerosis, it would be evidence for a causal association between height and the risk of multiple sclerosis.

which lung irritation can trigger inflammatory responses and autoimmunity. In the mouse model of MS (experimental autoimmune encephalomyelitis), lymphocytes that attack the CNS acquire their migratory abilities in the lungs, enabling them to enter the CNS.

Similar to EBV infection and obesity, one unreplicated study found that smoking interacts with HLA antigen MS risk alleles.[74] Smoking and harboring HLA-DRB1*15:01 together increase the odds of developing MS by about 14 times, much higher than the sum of the absolute effect of each risk factor alone. A similar interaction was reported with a non–HLA antigen gene (*NAT1*).[75] These gene-environment interactions increase the likelihood that the association between smoking and MS is causal.

LIFESTYLE RISK FACTORS

Multiple biases (including recall bias, measurement bias, and, particularly, confounding) can affect the study of association between different nutrients or dietary factors and the risk of a disease. It is particularly difficult to convincingly show the causality of the observed associations. Randomized controlled trials of diets, nutrients, and dietary factors may be unethical or infeasible. Nonetheless, the more commonly studied dietary factors are briefly reviewed here.

Diet

In vitro and experimental autoimmune encephalomyelitis (a mouse model of MS) studies demonstrated that high salt conditions induce a proinflammatory state and worsening of the disease in the animal model.[76] Among those with existing MS, a study in Argentina reported increased clinical and radiologic disease activity in patients with higher salt intake,[77] an observation that was not replicated in subsequent studies.[78,79] A pediatric case-control study also did not show an association between higher dietary salt intake and MS risk.[80] Thus, the evidence for salt intake being a major contributor to MS risk (or prognosis) is still lacking.

Two population-based studies reported that high coffee consumption reduces the risk of MS.[81] This observation is in line with the preclinical observation of the neuroprotective and anti-inflammatory activity of caffeine. Similarly, a case-control study demonstrated that alcohol, in a dose-dependent fashion, decreases the risk of MS.[82] However, a prospective study did not show an association between alcohol or coffee consumption and MS.[83] Unknown and unmeasured confounders, such as conditions that are often comorbid with MS (eg, migraine) influencing intake of caffeine or alcohol, could readily explain the disparity in the results of these studies.

Shift Work

At least two studies have suggested that shift work increases the risk of MS.[84,85] Melatonin has been implicated in inhibiting pathogenic T-cell differentiation and reducing the disease severity in the experimental autoimmune encephalomyelitis model. Dysregulation of melatonin may be the mediator of the effects of shift work on the risk of MS.

MICROBIOTA

The observation that germ-free mice are resistant to the development of neuroinflammation[86] sparked an interest in studying the association between

the human microbiota and MS. About 10^{14} microbes live on or in the human body.[87] Rapid advances in nucleic acid sequencing technology have allowed identification of these microorganisms. Most studies have focused on the most abundant microbial population in the body: the gut microbiota. Preliminary studies in both adult and pediatric populations have reported differences in the composition of the gut microbiota between MS patients and healthy controls.[88,89] It is not clear if changes in the gut microbiota had a causal role in development of MS or if having MS causes changes in the gut microbiota. Nonetheless, a strong scientific rationale exists for more definitive studies. Several mechanisms have been proposed for this association, including the effect of the gut microbiota on the immune system, production of neuromodulatory and immunomodulatory compounds and metabolites by these organisms, and altered gut permeability. Several ongoing studies of the human microbiota, including of the respiratory and other site-specific microbiota, are expected to shed light on the role of these microorganisms in the pathogenesis of MS in upcoming years.

CONCLUSION

MS is a disease with a complex etiopathogenesis. Despite tremendous advances in the discovery of genetic and environmental risk factors of the disease, the mechanisms by which these factors change the risk of MS remain largely unknown. Understanding these mechanisms may lead to the discovery of pathogenic pathways and new and more specific treatment targets. Many of the incriminated genetic variants and environmental factors are thought to affect the immune system, highlighting the importance of peripheral immune response in initiating the neuroinflammation. The formidable tasks ahead of MS epidemiologists are studying how the known genetic and environmental factors interact to change the MS risk, studying how the gut microbiota and human epigenetics affect the risk of the disease, and determining how to translate these data into preventive strategies, particularly for those at high risk of developing MS.

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