Etiology
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Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by the breakdown of the insulating myelin sheath that covers the nerve axons in the CNS and subsequent degeneration of axons. The process leads most commonly to intermittent neurological symptoms followed, over time, by progressive neurological symptoms in many patients. MS affects approximately 400,000 people in the USA and more than 2.1 million people worldwide, but the incidence has increased in the last five decades, particularly in women (3.6/100,000 person-years) compared to men (2.0/100,000 person-years) (Alonso & Hernan 2008; National Multiple Sclerosis Society 2012). While the etiology of MS is not understood in detail, it is unlikely to be the result of a single causative event. Instead, converging evidence suggests that MS is caused by an abnormal autoimmune response in genetically susceptible individuals after specific environmental exposures. Thus, it is not a heritable disease in the classic sense, but a complex disease that emerges from genes interacting with other genes and genes interacting with the environment. The factors thought to mediate the risk of MS are subject to intense ongoing research and include genetic, immunologic, infectious, and environmental contributors. The aim of this chapter is to review the current data on MS risk factors, with particular emphasis on those that may be modifiable on a personal or population level.

Genes

Familial aggregation is a well-recognized phenomenon in MS, and family and twin studies have long shown evidence for a strong genetic component underlying MS. This is illustrated by the 25–30% concordance among monozygotic twins, the 5% concordance among same-sex dizygotic twins, and the 3.5% concordance among nontwin siblings (Gourraud et al. 2012). However, the inheritance of MS cannot be explained by a simple genetic model, and
neither the familial recurrence rate nor twin concordance supports the presence of a Mendelian trait. Rather, susceptibility is polygenic, with each gene contributing a relatively small amount of the overall risk. More than likely, genetic heterogeneity (different susceptibilities among individuals) also exists. Additionally, epidemiological data strongly hint at a parent-of-origin effect in MS: maternal half-siblings having double the risk for MS compared to paternal half-siblings (2.35% vs. 1.31%), while the risk for MS in maternal half-siblings compared to their full siblings does not differ significantly (Gourraud et al. 2012). The mechanism of the increased risk conferred maternally remains to be elucidated, but epigenetic mechanisms such as DNA methylation or histone modification may play a role (Handel et al. 2010).

The first direct evidence for a relationship between genes and MS susceptibility came in 1972, when MS was shown to be associated with the human leukocyte antigen (HLA) on chromosome 6p21 (encoding proteins involved in presenting peptide antigens to T cells) (Gourraud et al. 2012). This association was later fine-mapped to a specific locus, HLA-DRB1 of the class II gene HLA-DRB1 (Gourraud et al. 2012). Although the HLA-DRB*1501 haplotype exerts the strongest genetic effect in MS (heterozygosity conferring an odds ratio (OR) of 2.7 and homozygosity of 6.7), the association is not straightforward. In fact, a number of HLA-DRB1 haplotypes are both positively and negatively associated with the disease, differ in magnitude of effect, and either act on their own or greatly alter risk in combination with another haplotype (Kallaur et al. 2011). For example, HLA-DRB1*08 only modestly increases MS risk, but in combination with HLA-DRB1*15, it more than doubles the risk associated with a single copy of the latter (Kallaur et al. 2011). On the other hand, HLA-DRB1*14 carries such a protective effect that it completely abrogates the increased risk of HLA-DRB1*15 (Kallaur et al. 2011). And whereas association of MS with HLA-DRB1*15 has long been known in Northern Europe, in other regions, such as Sardinia, HLA-DRB1*0301, HLA-DRB1*0405, and HLA-DRB1*1303 are more commonly associated with MS (Kallaur et al. 2011). In fact, the relative frequencies of susceptibility and protective HLA haplotypes, which vary between countries, may play important roles in determining the risk of the disease.

It has been estimated that the HLA locus accounts for 20–60% of the genetic susceptibility in MS, leaving a large portion of the genetic component of MS (still) to be explained. In 2007, the International Multiple Sclerosis Genetics Consortium (IMSGC) completed the first MS genome-wide association study (GWAS) using trios (an affected individual and both their parents) from the UK and the USA (Gourraud et al. 2012). In addition to the HLA-DRB1 region, two new risk loci were identified: the genes for interleukin-7 receptor alpha (IL-7RA) and interleukin-2 receptor alpha (IL-2RA), which have since been replicated. These genes code for the
alpha chain of the IL-7 or IL-2 receptors, which promote lymphocyte growth and differentiation. MS-associated variants in the IL-2RA gene contribute to the production of soluble IL-2RA, a biomarker of peripheral inflammation. The IL-7/IL-7RA interaction is important for memory T-cell maintenance and development and proliferation and survival of B and T cells; the protective haplotype is associated with less soluble IL-7RA; the risk allele thus likely produces a change in function (Gregory et al. 2007).

The most recent GWAS data from the IMSGC demonstrate at least 102 SNPs exerting a modest effect (OR, 1.06–1.22) (Gourraud et al. 2012). Most of the loci harbor genes with pertinent immunological roles, including several genes associated with other autoimmune disorders, consistent with the autoimmune hypothesis of MS etiology. Most notably, the results of the GWAS implicate genes coding for cytokine pathways (CXCR5, IL-2RA, IL-7R, IL-7, IL-12RB1, IL-22RA2, IL-12A, IL-12B, IRF8, TNFRSF1A, TNFRSF14, TNFSF14) and for costimulatory (CD37, CD40, CD58, CD80, CD86, CLECL1) and signal transduction (CBLB, GPR65, MALT1, RGS1, STAT3, TAGAP, TYK2) molecules of immunological relevance (Gourraud et al. 2012). Of interest, at least two genes (KIF1B, GPC5) not involved in the immune system but instead with neuronal growth and repair mechanisms may also be associated with MS. These genes may influence the potential of remyelination of lesions, and their discovery gives a hint to a disturbance of repair mechanisms in addition to autoimmune processes in MS.

Still relatively little is known about how the identified MS risk variants exert their effects at the molecular and cellular levels. Their incomplete penetrance and moderate individual effects probably reflect interactions with other genes, posttranscriptional regulatory mechanisms, or significant environmental and epigenetic influences. Further genetic and functional studies are required to pinpoint the functionally relevant genes and pathways, to understand how these influence risk, and to determine if the genes themselves, or the downstream effects thereof, can be modified to alter MS risk.

Gender effects: Genetic or biologic?
MS is more prevalent in females than males, and this female predominance appears to have increased markedly over the past 100 years. Interestingly, the preponderance of females among MS patients is even seen in the pediatric MS population, especially after about the age of 10 years. The mechanisms underlying these observations are still incompletely understood, and most investigations have focused on the role of gonadal hormones. However, several other factors may be of key relevance, such as intrinsic biological differences in the male and female immune system and CNS, genetic and epigenetic factors, maternal microchimerism, and differences in environmental exposures for males and females (e.g., higher numbers and changing roles of women in the workforce, outdoor activity, dietary habits, and alterations in menarche and in the age of childbearing).

The role of the environment
Genetic factors account only partially for MS susceptibility, as illustrated by the twin concordance data. Moreover, even among families, MS risk is known to be strongly influenced by location, season of birth, and the childhood environment. The environment thus appears to play an important role in setting thresholds for genetic penetrance. Further, recent increases in MS incidence are too rapid to be the result of genetic alterations and must, therefore, reflect differential exposure to environmental factors (Alonso & Hernan 2008). In particular, the rising worldwide incidence and increasing female to male preponderance have focused interest on environmental factors that may influence MS risk.

Environmental MS risk factors: The major players
All of the environmental factors involved in MS are not yet known, but accumulating evidence lends strong support to several candidates, most notably sunlight and/or vitamin D exposure, Epstein–Barr virus (EBV), and cigarette smoking (Ascherio & Munger 2007a, b), with unconfirmed
or hypothetical support for obesity, diet, and altered gut microbiota as risk factors. These factors could conceivably act to alter susceptibility to MS at any point in life from conception (or even before) to the onset of disease.

**Geography**

The uneven geographical distribution of MS is central to understanding the role of environment. The prevalence of MS increases with distance from the equator (Ascherio & Munger 2007b) and is greater in areas with temperate rather than tropical climates. Within regions of temperate climate, MS incidence and prevalence increase with latitude. Some of these observations may be explained by the non-random geographic distribution of racial/ethnic groups within these risk areas, such that what appears to be a latitudinal effect may be confounded by the genetic backgrounds of those who live in the various regions (i.e., racial/ethnic groups with a higher burden of risk alleles may be those who happen to live in regions of higher prevalence). However, migration studies demonstrate that moving from a region of high to low risk, or vice versa, leads to the adoption of the risk of the new region, especially if the migration occurred at a young age (Ascherio & Munger 2007b) such that at least part of the latitudinal gradient must be due to environmental differences.

One of the strongest correlates of latitude is the duration and intensity of sunlight. Thus, it is not surprising that an inverse correlation between MS prevalence and sunlight was already noted in early ecological studies; among US veterans, the average annual hours of sunshine and the average December daily solar radiation at place of birth were strongly inversely correlated with MS (Ascherio & Munger 2007b). Furthermore, several retrospective studies have demonstrated that sun exposure during childhood and adolescence as well as outdoor activity as an occupational exposure is inversely related to MS susceptibility (Ascherio & Munger 2007b). The protective effects of sunlight are thought to be mediated by ultraviolet radiation (UVR), possibly via vitamin D (see section Vitamin D).
mutations. Nevertheless, these data do not rule out a type of environmental imprinting, or that susceptibility (and resistance) could be entrained by cumulative exposures of (more than one) factors in the environment.

**Vitamin D**

It has become increasingly clear that vitamin D has a wide role in physiology and, importantly, also in disease. Evidence is mounting in support of vitamin D deficiency underlying risk for several autoimmune diseases. The pleiotropic actions of vitamin D, including immunomodulatory functions, lend strong support to the hypothesis that this hormone is important in the etiology of MS.

**SCIENCE REVISITED**

**Vitamin D**

The main source of vitamin D in humans is skin exposure to sunlight (hence its nickname, the *sunshine vitamin*), although it can also be obtained through the diet (e.g., through oily fish such as salmon, tuna, and mackerel, as well as cod liver oil) and from supplements. Previtamin D3 is formed in the skin upon exposure of 7-dehydrocholesterol to UVB radiation and is then converted to vitamin D3. Vitamin D from sun exposure and diet is hydroxylated (predominantly) in the liver to produce calcidiol (25(OH)D), the major circulating form of vitamin D. Since calcidiol is biologically inert, it requires further hydroxylation (predominantly) in the kidney to form the physiologically active form of vitamin D, calcitriol (1,25(OH)2D), a lipid-soluble secosteroid. Calcitriol is generally not used as an indicator of vitamin D status because it has a short half-life (15 h), and serum concentrations are closely regulated for purposes of calcium homeostasis. Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells and acts as a transcription factor that modulates gene expression. Vitamin D also affects the immune system, and VDRs are expressed in several cells involved in innate and adaptive immune responses, including monocytes, dendritic cells, and activated T and B cells.

For most people, skin exposure to sunlight is the major source of vitamin D and the most important predictor of vitamin D status. Several observations support that vitamin D insufficiency is a risk factor for MS: (1) MS prevalence increases as distance from the equator increases (corresponding with a decrease in sunlight exposure) (Ascherio & Munger 2007b); (2) those who migrate adopt the risk of the new area (Kurtzke *et al.* 1985); (3) UVB radiation (the main source of vitamin D) and skin cancer are inversely correlated with MS risk (Ascherio & Munger 2007b); (4) vitamin D intake significantly decreases the risk of MS (Munger *et al.* 2004); and (5) vitamin D levels inversely correlate with risk of MS later in life (Munger *et al.* 2006).

The strongest evidence for a role for vitamin D comes from a, nested case-control study among US military personnel showing that higher vitamin D levels conferred a lower subsequent risk of MS (Munger *et al.* 2006). Further evidence to support a protective effect of vitamin D on MS risk comes from the longitudinal Nurses’ Health Study: those with intake of vitamin D of at least 400 international units (IU)/day had a relative risk (RR) for MS of 0.59 compared with those who did not take supplemental vitamin D (Munger *et al.* 2004). Although confounding by unknown factors cannot be excluded, these cohort data strongly support a protective effect of vitamin D on MS risk. Ecological studies in coastal fishing areas in Norway have shown that inhabitants of these areas have lower MS prevalence than their neighbors dwelling in inland agricultural communities, which may be explained by their greater consumption of fatty seafood and cod liver oil, both rich in vitamin D (Kampman *et al.* 2007).
There is also functional evidence associating vitamin D and MS. There is a vitamin D response element (VDRE) close to the promoter region of HLA-DRB1, and calcitriol (the active form of vitamin D) modulates the expression of the particular allele most consistently associated with increased risk of MS, HLA-DRB1*1501 (Ramagopalan et al. 2009). While the in vivo functional consequence of this finding is yet to be determined, it does form a conceptual basis for an environment–gene interaction in the determination of MS risk. The HLA-DRB1*15 risk allele also interacts with the season of birth such that the reported relationship with risk of MS appears to be predominately driven by those carrying at least one copy of the DRB1*15 risk allele (Ramagopalan et al. 2009). In addition, a recent GWAS found association with genetic regions containing vitamin D metabolism genes—CYP24A1 and CYP27B1 (Gourraud et al. 2012)—providing more evidence for the potential role for vitamin D in MS. However, some data suggest that UV light may exert effects on MS risk independent of vitamin D status, such that some or all of the geographic distribution of MS thought to be due to UV-determined vitamin D levels could in fact be due to another UV-mediated mechanism.

Infection

That MS might be triggered by infection is supported by presence of high concentrations of a number of IgGs in the cerebrospinal fluid (CSF) of more than 90% of MS patients that are not present in the blood (oligoclonal bands), indicative of immune activation. Indirect support for a role of infection in MS is that viruses have been associated with other human and experimental demyelinating diseases. Although dozens of pathogens have been investigated as MS risk factors, it is still not clear which, if any, are definitively etiologic. That being said, there is strong support for EBV infection as important to disease risk in many MS patients.

Epstein–Barr virus

EBV, also known as human herpesvirus-4 (HHV-4), belongs to the gamma-herpesvirus family, which includes herpes simplex virus and cytomegalovirus. EBV is present in all populations and infects over 90% of individuals at some point in their life. Its discovery dates to the early 1960s, where it was isolated in lymphoma cells cultivated from tumor biopsies obtained from African children with jaw tumors. Primary infection usually occurs through contact with infected saliva and is asymptomatic in young children, but in up to 40% of adolescents and adults, it results in the symptomatic illness infectious mononucleosis (IM), an acute and usually self-limited lymphoproliferative disease. Since EBV preferentially infects B lymphocytes and persists lifelong in a transcriptionally quiescent state in circulating memory B cells, it goes largely undetected by the immune system. By immortalizing autoreactive B cells, which act as professional antigen-presenting cells, it is thought that EBV may drive persistent autoimmunity, possibly through antigen mimicry, immortalization of B-cell clones, and cytotoxic T-cell dysfunction against viral-infected B cells.
adolescence and young adulthood. In these countries, MS risk is two- to threefold higher among individuals with history of IM (Ascherio & Munger 2007a).

Although more than 90% of the general population appears to encounter EBV at some point in life, several lines of evidence highlight its possible role in the pathogenesis of MS. Large, independent studies have shown that nearly all (>99%) adults with MS are seropositive for antibodies directed against EBV, while the seropositivity rate is slightly lower in unaffected adults. The strongest evidence for the association with MS, however, comes from a nested case-control study of healthy individuals infected with EBV, whose subsequent MS risk increases by severalfold with increasing serum titers of anti-Epstein–Barr nuclear antigen (EBNA) complex and anti-EBNA-1 antibodies (Ascherio & Munger 2007a). These data show that EBV seroconversion predates MS onset. A history of EBV-induced IM increases the risk of developing MS, particularly in individuals who develop IM after the age of 15 years. Given the observation that EBV-negative individuals (likely to be exposed to the highest levels of hygiene) have the lowest risk of MS makes the hypothesis that good hygiene during childhood may predispose both to MS and to a later contact with EBV and therefore IM unlikely (Ascherio & Munger 2007a). However, whether the link between MS and EBV infection is actually causal or merely represents an association continues to be debated. In adults who are seronegative for EBV, there seems to be virtually no risk of developing MS (Ascherio & Munger 2007a). However, while a recent investigation of pediatric MS patients showed that EBNA-1 seropositivity is associated with an increased risk of developing MS, not all individuals with MS were positive for EBV, suggesting that infection with EBV is not necessary for all cases of MS (Waubant et al. 2011).

It is important to note that IM is also not sufficient to cause MS; since the large majority of individuals are infected with EBV, but only a relatively small percentage will ever get MS, other genetic and environmental factors must be critical for MS development. Indeed, the HLA-DRB1*1501 allele has been shown to interact with high levels of EBV antibodies in its association with greater risk of MS (De Jager et al. 2008). Evidence suggests that there may be a synergistic effect of vitamin D and IM on MS risk, possibly by an alteration of the initial education of the immune system or of the subsequent immune response to EBV infection in vitamin D deficient states or by EBV itself potentiating the effects of vitamin D deficiency, leading to autoimmunity.

Other viruses
While several studies of adult MS have attempted to link other viruses to MS risk, the results have been inconclusive. On the other hand, the pediatric MS study described earlier found that, independent of EBV status, remote infection with CMV was associated with a lower risk of developing MS and that HSV-1 status interacted with HLA-DRB1 in predicting MS, such that HSV-1 positivity was associated with a greater MS risk in those without a DRB1*15 allele and a reduced risk in those who were DRB1*15 positive (Waubant et al. 2011). These results need confirmation, but the totality of data suggests that there might be a complex interplay between various viral infections acquired during childhood and MS risk.

Smoking
Cigarette smoking has been shown to sizably increase susceptibility to MS in multiple studies (Ascherio & Munger 2007b). The most recent meta-analysis examining the effect of past or current smoking on MS susceptibility reported an RR between 1.3 and 1.8 associated with smoking (Ascherio & Munger 2007b). The smoking effect appears to be independent of gender (Hedstrom et al. 2009)) as well as of latitude and ancestry (Ascherio & Munger 2007b). The risk of MS increases with cumulative doses of cigarettes. Even children ever exposed to parental smoking have been found to have a higher risk of developing MS (Mikaeloff et al. 2007).
The mechanism relating cigarette smoking to MS risk is unclear. Smokeless tobacco (snuff) use has not been found to increase the risk of MS (Hedstrom et al. 2009), suggesting that the effect does not appear to be mediated solely by nicotine, but perhaps by components of the actual cigarette smoke, such as nitric oxide, which has putative roles in demyelination and axonal loss. Animal models have also indicated that smoke exposure affects several facets of the immune system, including innate immunity, B and T lymphocytes, and natural killer cells, so a direct impact of smoking on immune function is possible. Recent studies are just beginning to shed light on how smoking interacts with other factors in influencing MS risk.

Combining risk factors

While genetic and environmental risk factors clearly act together to influence MS risk, they have rarely been studied concomitantly, and much remains to be discovered about their respective contributions to or possible interplay in disease susceptibility. To date, the most comprehensive attempt at mathematically modeling risk factors to improve the prediction of MS was that by De Jager and colleagues, who attempted to combine 16 genetic risk loci, sex, smoking, and anti-EBNA-1 titers into a prediction model (De Jager et al. 2009). Overall, their data suggest that information obtained from MS susceptibility loci might provide useful if incorporated into clinical algorithms that contain other information, such as detailed immunological characterizations and environmental risk factors. More studies in large cohorts are needed to better understand the combined predictive power of risk factors.

Conclusion

Understanding the etiology of MS requires solving the complex genetics underlying the disease as well as advancing the understanding of the environmental components of its etiology. More information is needed on how the growing set of genetic susceptibility factors is affected by environmental risk factors such as EBV infection, smoking, and vitamin D status. Advances in genetics, immunology, and cell biology are greatly adding to the understanding of MS, and large national and international collaborations are underway to characterize the precise nature and extent of the multifaceted interactions between these known risk factors, as well as uncovering yet unknown ones. In recent years, the emphasis has increasingly been on identifying modifiable risk factors and translating these findings to the clinic. Thus, low circulating levels of vitamin D and cigarette smoking, clearly modifiable, are promising targets for the prevention and treatment of MS.

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References


