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Chapter 11

The epidemiology of multiple sclerosis: insights to a causal cascade[†]

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Abstract

MS-pathogenesis involves both genetic-susceptibility and environmental determinants. Three (or more) sequential environmental-factors are implicated. The first acts near birth, the second acts during childhood/adolescence, and the third acts subsequently. Two candidate factors (vitamin D deficiency and Epstein-Barr viral infection) seem particularly well-suited to the first two environmental-events but other factors (e.g., obesity and smoking behavior) seem also to be involved in the causal scheme.

MS-pathogenesis can be modeled by incorporating both the environmental and genetic-factors into a causal scheme, which can then help to explain some of the changes in MS-epidemiology (e.g., increasing disease-prevalence, changing sex-ratio, and regional-variations in monozygotic-twin-concordance-rates), which have been taking place recently. This model suggests that genetic-susceptibility is overwhelmingly the most important determinant of MS and that, at least, 92.5% of individuals (and likely much more) are, essentially, incapable of developing MS, regardless of their specific environmental-exposures. Nevertheless, the genetics is complex and the contribution of any specific gene to MS-susceptibility seems to be quite modest. Thus, even for the DRB1*1501 allele (the strongest known MS-susceptibility marker), most carriers are not in the genetically-susceptible group. Moreover, 45–50% of individuals with MS lack this allele entirely and some of the haplotypes that carry this allele don't also confer any disease–risk.

Finally, because the prevalence of genetic-susceptibility seems to be so similar throughout North America and Europe, and despite the crucial importance of a person's genetic make-up to disease pathogenesis, it is the environmental-factors, which largely responsible for the observed regional variations in disease-characteristics. Thus, despite MS being more common in women, men are more likely to be genetically-susceptible. This apparent paradox seems to relate to the fact that women are much more responsive than men to the recent changes in environmental-exposure (whatever these have been). These gender-differences may help to explain changes in the sex-ratio and the increasing disease-prevalence, which have both been observed recently. The potential importance of these conclusions regarding the role of environment in MS-pathogenesis is that they open the door to the possibility of pursuing strategies for primary primary disease prevention in the future.

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OVERVIEW

Multiple sclerosis (MS) is a chronic recurrent inflammatory disease of the central nervous system (CNS) and it is one of the most disabling neurologic diseases of young adults. Unpredictable inflammatory episodes, lasting days to months, result in injury to the myelin sheaths, to the oligodendrocytes themselves and, to a somewhat lesser extent, to the nerve cells and axons (Weinshenker et al., 1989; Runmarker and Andersen, 1993; Liguori et al., 2000; Confavreux et al., 2003; Pittock et al., 2004; Confavreux and Vukusic, 2006; Compston et al, 2006; Kremenchutsky et al., 2006; Tremlett et al., 2006). Immune mechanisms are definitely involved in MS pathogenesis, either primarily or secondarily, and, in fact, most authorities favor primary autoimmunity as the pathogenic basis for this disease (Compston et al., 2006). Evidence of both acute and chronic inflammation is typically found in the cerebrospinal fluid (CSF) of individuals, especially during acute clinical attacks (Compston et al., 2006). This evidence includes the findings of an elevation in total CSF protein concentration, an increased number of mononuclear cells in the CSF, and an increase in the gamma-globulin (IgG) fraction in the CSF, as reflected by the presence of unique oligoclonal CSF IgG bands on gel electrophoresis and/or an increase in the so-called CSF IgG index (Compston et al., 2006; Housley et al., 2015).

In the northern parts of North America and Europe, where the disease is most common, the prevalence is between 0.1 and 0.2% of the population and the incidence is approximately 5–6 per 100 000 population per year (Wynn et al., 1990). There is, however, considerable variation in the prevalence of MS in different countries around the world (Fig. 11.1). Women are affected two to three times more often than men. Typically, the disease becomes clinically apparent between the ages of 20 and 40 years (mean age at onset: ~28 years), although it can begin as early as the first or second year of life (Ruggieri et al., 1999; Gadoth, 2003; Lee and Chitnis, 2016) or as late as the seventh decade of life (Confavreux et al., 2003; Confavreux and Vukusic, 2006). In addition, it is now recognized that the onset of the disease generally precedes the first clinical manifestation, often by many years, if not decades (Okuda et al., 2009, 2014). Indeed, some individuals with pathologically verified MS may be discovered incidentally at autopsy. In fact, based on several large autopsy series performed before the era of magnetic resonance imaging (MRI), the prevalence of such "asymptomatic" MS was reported to be approximately 0.1% (Vost et al., 1964; Georgi, 1966; Gilbert and Sadler, 1983; Engell, 1989). If so, this suggests that, over their entire lifetime, as many as half of pathologically proven MS cases will never experience sufficient clinical symptoms to bring them to medical attention. The experience in the post-MRI era seems to confirm these earlier autopsy observations. Thus, with the ever-increasing availability of MRI, the appearance of radiographic disease in persons who have never had neurologic symptoms is now a common finding (e.g., Okuda et al., 2009, 2014). Nevertheless, after 5 years, approximately a third

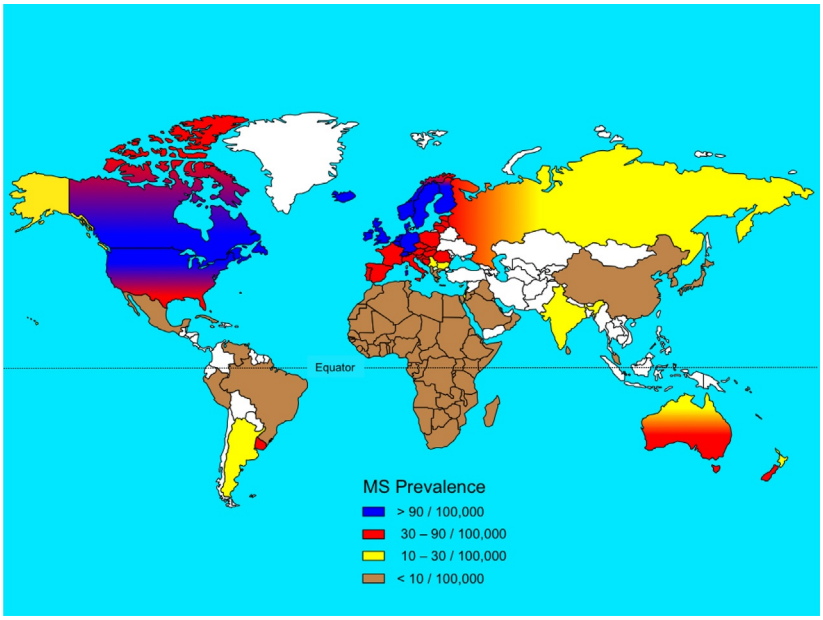

Fig. 11.1. Global distribution of multiple sclerosis (MS) prevalence. Dotted black line indicates the Equator. Data taken from reviews of worldwide epidemiology of MS (Rosati, 2001; Compston et al., 2006).

of these so-called radiographically isolated syndrome patients will have experienced clinical evidence of MS disease activity (Okuda et al., 2009, 2014).

THE PATHOLOGY OF MS

Pathologically, MS is characterized by multifocal patches of demyelination and gliosis (plaques) within the CNS white matter. Indeed, within these plaques there is evidence of white-matter tissue injury of differing ages both to the myelin sheaths (surrounding the axons) and to the oligodendrocytes themselves (Bar-Or et al., 1999; Conlon et al., 1999; Compston et al., 2006; Hauser and Oksenberg, 2006). In addition, there is evidence of axonal injury – especially within active MS lesions; and gray-matter demyelination – especially later in the disease course (Ferguson et al., 1997; Trapp et al., 1998; Peterson et al., 2001; Bø et al., 2003; Lucchinetti et al., 2011). Within acute lesions, presumably guided by cellular adhesion molecules and proinflammatory cytokines, autoreactive immune cells cross the blood–brain barrier (BBB) and enter into the CNS – these invading cells include cluster of differentiation (CD)4+, thymic-derived lymphocytes (T cells), CD20+ bone marrow-derived lymphocytes (B cells), CD8+ cytotoxic lymphocytes, and CD68+ macrophages (Bar-Or et al., 1999; Conlon et al., 1999; Compston et al., 2006; Hauser and Oksenberg, 2006). These activated cells are thought to contribute to the CNS tissue damage that occurs in acute MS lesions.

On histopathologic examination, lesions that are characterized on MRI as having only T2 hyperintensity (i.e., T2-only lesions) are much more likely to demonstrate myelin preservation compared to lesions that, on MRI, are characterized by T2 hyperintensity in addition to persistent T1 hypointensity and a reduced magnetization transfer ratio (MTR: i.e.,T2/T1/MTR lesions) – in fact, only 20–45% of T2-only lesions are associated with demyelination on pathologic examination compared to 80–83% of the T2/T1/MTR lesions (Fisher et al., 2002; Moll et al., 2009). Nevertheless, regardless of the state of myelin preservation, most of the T2-only MRI lesions still contain activated microglia and evidence of BBB breakdown and, thus, would show up as gadolinium-enhanced (Gd+) lesions on routine imaging.

Even in the so-called normal-appearing white matter, 30% of the regions examined histopathologically were still found to contain activated microglia (Fisher et al., 2002; Moll et al., 2009). It is unknown whether activated microglia within myelinated T2-only lesions are causing new damage. Thus, it is possible that these microglial cells are simply responding to a cytokine release associated with either the breakdown of the BBB, or Wallerian degeneration, or both. Indeed, it may well be that the microglia may actually be involved in removing debris from lesions and possibly even promoting repair, whereas the macrophages may be cells responsible for the actual tissue damage (Yamasaki et al., 2014). Regardless, it is clear that MS lesions demonstrate a considerable histopathologic diversity – ranging from chronic gliotic demyelinated scars, to highly inflamed demyelinating lesions, to less inflamed regions in which the myelin seems to be completely preserved.

The endothelial cells in the CNS are not fenestrated and have extraordinarily tight junctions between them. For many years, these tight junctions were thought to be primarily responsible for creating and maintaining the BBB. Nevertheless, it is now understood that this barrier is actually produced by a complex interaction between the vascular system and the CNS, which, collectively, is referred to as the neurovascular unit (Holman et al., 2011; Engelhardt et al., 2014; Muoio et al., 2014). This unit includes the endothelial cells, the extracellular matrix, the basement membrane, and also the cells surrounding the endothelial cells, notably the pericytes and the astrocytes. Together, this unit works both to provide mutual trophic support and to make the CNS entry by hydrophilic molecules (either by active transport or by diffusion) and entry by transcytosis extremely selective. A focal breakdown of the BBB can be caused by any one of a variety of CNS insults, including inflammation, toxic exposure, ischemia, trauma, and neoplasia. In MS, the breakdown of the BBB is thought to represent both a critical step in the development of a new MS lesion and the pathogenic basis of an acute MS attack. Nevertheless, whether this BBB breakdown is the initial event in lesion formation is not entirely clear (e.g., Filippi et al., 1998; Goodkin et al., 1998). Thus, using the MTR, focal changes in the relative concentrations of free and bound water can be detected in regions of otherwise normal-appearing CNS white matter that, months later, are destined to become Gd+lesions seen on MRI. Presumably, these MTR changes reflect biochemical alterations, which are the initial events in lesion formation. It is nonetheless possible that these early events represent a selective breakdown in the BBB, which is not detectable by conventional MRI and, in this view, the more general breakdown of the BBB, which is reflected by Gd+lesions, would be a secondary phenomenon.

THE CLINICAL COURSE OF MS

The symptoms of MS depend, to a large extent, upon the location of plaques within the CNS and commonly include visual impairment, weakness, muscle spasms, sensory disturbances (including pain and paresthesias), incoordination, gait abnormalities, bladder dysfunction, and double vision (Compston et al., 2006). Three

different disease courses of MS have been defined (Lublin et al., 2014), although it is unclear whether these different courses reflect a variable clinical expression of a single disease or whether these represent different diseases with different pathophysiologic underpinnings. The first, and by far the most common course, is relapsing/remitting MS (RRMS). About 85–90% of MS cases begin with this course. RRMS is characterized by self-limited "attacks" of neurologic dysfunction, (variously referred to as "attacks," "relapses," "bouts," "exacerbations," or "flares"). These attacks develop acutely, evolve over hours to days, last days to months, and are followed by a recovery of neurologic function (sometimes incomplete). Between these episodes, the patient is neurologically stable. On MRI, these patients often have new lesions on T2-weighted images (new T2 lesions) or they may have Gd+lesions, which reflect a focal breakdown of the BBB due to the new inflammatory activity, or they may have both (Compston et al., 2006).

The second defined clinical course is secondary progressive MS (SPMS), which always begins as typical RRMS but, at some point in time, it changes course such that the acute inflammatory episodes become less frequent and the patient begins to experience an insidious and progressive decline in neurologic function, independent of acute attacks. On MRI, the evidence of acute inflammation (new T2 lesions and Gd+lesions) becomes less conspicuous and the occurrence of brain atrophy becomes more prominent. SPMS ultimately develops in the majority of RRMS patients and causes most of the disability experienced by patients. For example, longitudinal population-based studies have estimated that 50% of RRMS patients will require the use of a cane to assist with their ambulation after 15–30 years of disease (Weinshenker et al., 1989; Runmarker and Andersen, 1993; Liguori et al., 2000; Confavreux et al., 2003; Pittock et al., 2004; Confavreux and Vukusic, 2006; Kremenchutsky et al., 2006; Tremlett et al., 2006; Scalfari et al., 2010). Nevertheless, predicting the clinical course for any individual patient is difficult, and, as noted earlier, many patients with pathologic MS are discovered only incidentally at autopsy (Vost et al., 1964; Georgi, 1966; Gilbert and Sadler, 1983; Engell, 1989). Men, patients with high initial attack rates, patients with early involvement of motor or cerebellar pathways, patients with moderate disability after 5 years of illness, and patients with a large MRI disease burden are more likely to become disabled compared to patients without these risk factors (Weinshenker et al., 1989; Runmarker and Andersen, 1993; Confavreux et al., 2003; Scalfari et al., 2010). However, even among patients who have experienced little disability in the first 10 years of their illness, significant disability can still develop subsequently (Hawkins and McDonnell, 1999) and, for this reason, the diagnosis of so-called "benign MS" can only be made in retrospect. Moreover, any definition of "benign" MS must include a consideration of the patient's cognitive function, which can be affected by MS, independently from a person's physical abilities (Correale et al., 2012).

The third clinical type, primary progressive MS (PPMS), accounts for only about 10% of cases. In PPMS, the onset of illness is insidious and, from the beginning, the patient experiences a steady decline in neurologic function without acute clinical attacks (although these may develop subsequently). Similarly to SPMS, these patients have less evidence of active inflammation on MRI compared to patients with RRMS. Also, PPMS patients have a more equal sex ratio, a later age of onset, and a worse prognosis for ultimate disability compared to patients with RRMS. In fact, within the first decade of the illness, over 50% of PPMS patients will require a cane to assist with ambulation (Weinshenker et al., 1989; Runmarker and Andersen, 1993; Liguori et al., 2000; Confavreux et al., 2003; Pittock et al., 2004; Confavreux and Vukusic, 2006; Kremenchutsky et al., 2006; Tremlett et al., 2006; Scalfari et al., 2010).

It is possible that the poor long-term prognosis for untreated MS (which is often assumed) may be overestimated. For example, in patients with attacks of idiopathic optic neuritis (ON), a condition closely associated with MS and having similar genetic determinants, the conversion rate to clinically definite MS (CDMS) may be as low as 40% after 40 years (Rodriguez et al., 1994). Even in the long-term follow-up of the patient cohort who participated in the ON treatment trial (Optic Neuritis Study Group, 2008), the conversion rate to CDMS was reported to be only 50%. Because this cohort is known to include individuals who already had MS at baseline (Goodin, 1999), this 50% number undoubtedly overestimates the true risk of MS following an episode of isolated ON. Thus, for some patients with clinically isolated syndromes such as ON, it seems that benign forms of demyelinating disease may be much more common than is currently believed by many authors. Nevertheless, in patients presenting with a clinically isolated syndrome, certain laboratory or imaging features such as characteristic MRI abnormalities, evidence of inflammation in the CSF (i.e., either the presence of oligoclonal IgG bands or an elevation in the IgG index), or abnormalities on evoked potential testing significantly increase the likelihood of that individual developing MS in the future (Sharief and Thompson, 1991; O'Riordan et al., 1998; Gronseth and Ashman, 2000). For example, over 60% of patients with a monosymptomatic clinically isolated syndrome will have MRI abnormalities consistent with MS and, of these, more than 80% will develop CDMS within the next 20 years

(Fisniku et al., 2008). By contrast, in the absence of such MRI abnormalities, the 20-year risk of developing CDMS is only about 20% (Fisniku et al., 2008). The spinal MRI may have special prognostic significance. Indeed, one study reported that the odds ratio (OR) for conversion from radiographically isolated syndrome to CDMS was more than 75 when a spinal cord lesion was present (Okuda et al., 2011).

CONSIDERATIONS REGARDING THE CAUSES OF MS AND ITS EXACERBATION

In considering the causation of MS, two possibly separate questions need to be addressed. The first is to consider what is known about those factors that may trigger an attack of the disease in an individual who already has MS. The second, and perhaps more important, question is to ask what is known about the causal factors that lead to the disease in the first place.

Factors associated with the exacerbation of MS

The timing of acute MS attacks seems to be largely (although not entirely) a chance event. If factors exist that do trigger MS attacks, these are, almost certainly, environmental events (or environment–gene interactions). They are not likely to be primarily genetic events because the genetic make-up of an individual is fixed. Indeed, certain environmental factors do seem to be consistently associated with an increased or decreased likelihood of a person experiencing an MS attack. For example, three high-quality studies have looked at the effect of pregnancy on the likelihood of MS exacerbations (Confavreux et al., 1998; Salemi et al., 2004; Vukusic et al., 2004). Each of these studies reported a significant reduction in the likelihood of a woman experiencing an attack during pregnancy (especially during the last trimester) compared to her prepregnancy risk (Confavreux et al., 1998; Salemi et al., 2004; Vukusic et al., 2004). Also, during the period 3–6 months postpartum, the risk of a women with MS experiencing an attack is increased compared to her prepregnancy state (Confavreux et al., 1998; Salemi et al., 2004; Vukusic et al., 2004). The basis for these observations is not known, but one possibility is that fluctuations in hormonal levels (possibly in estrogen), which occur during and after pregnancy, influence the likelihood of MS attacks. Nevertheless, there are many other (nonhormonal) physiologic changes that occur during pregnancy (Houtchens, 2007) and there is no basis on which to discount these factors as being responsible for the observed pregnancy-related changes in attack risk.

Another factor that seems to influence the attack risk consistently is the occurrence of nonspecific infectious syndromes (e.g., rhinorrhea, fever, cough, malaise, nausea, abdominal pain, diarrhea, and so forth). Four high-quality studies have looked at the association between these clinical syndromes (often attributed to nonspecific upper respiratory or gastrointestinal viral infections) and each has reported that there is an increased risk of an MS attack around the symptomatic period compared to other times during the year (Sibley et al., 1985; Andersen et al., 1993; Panitch, 1994; Edwards et al., 1998).

Some authors have suggested that vaccinations (e.g., to influenza or hepatitis B) can be related to the timing of MS attacks, although the available data are unconvincing (Merelli and Casoni, 2000). Other factors have also been suggested as both causal factors and as influencing the likelihood of an MS attack (e.g., trauma and psychologic stress), and these are considered below.

Factors associated with the development of MS

Before considering the different candidate factors that might be in the causal pathway leading to MS, it is important to review the implications of several of the general epidemiologic observations that have been made with regard to MS pathogenesis.

General considerations

Chronic diseases such as MS typically have complex etiologic bases (Rothman and Greenland, 1998). Both individual genetic background and the environmental events that they experience during their lives are critical to whether they will ultimately develop the disease. For example, as noted earlier, an individual from northern North America or northern Europe has a lifetime risk of developing MS of approximately 0.1–0.2% (Compston et al., 2006). The risk for individuals with an affected family member increases roughly in proportion to the genetic similarity between themselves and the proband (French Research Group on Multiple Sclerosis, 1992; Mumford et al., 1994; Ebers et al., 1995, 2004; Robertson et al., 1996; Sadovnick et al., 1996; Compston and Coles, 2002; Willer et al., 2003; Nielsen et al., 2005; Compston et al., 2006; Islam et al., 2006; Ristori et al., 2006). Siblings of an MS proband (~50% similarity) have a 20–30-fold increased risk compared to the general population. By contrast, monozygotic twins (~100% genetic similarity) have more than 200 times the general population risk.

Despite this strong genetic predisposition, however, it is clear that genetics is not the only factor. If it were, the proband-wise concordance rate for monozygotic twins – an estimate of the lifetime risk of MS for an individual with a monozygotic twin who has MS (Witte et al., 1999) – would be much closer to 100% than to the

20–30% reported in these northern populations (Mumford et al., 1994; Ebers et al., 1995; Willer et al., 2003). In southern populations, where the proband-wise concordance rate for monozygotic twins is approximately half that in the north (French Research Group on Multiple Sclerosis, 1992; Islam et al., 2006; Ristori et al., 2006), this conclusion is even more evident. Consequently, it is clear that, in addition to any genetic contributions, there must be environmental and/or epigenetic factors that contribute in an important ways to MS pathogenesis.

When considering the environmental events that might relate to MS pathogenesis, although anticipated on theoretic grounds (Goodin, 2016), it is noteworthy that the observed microenvironmental contributions to MS risk seem to be minimal. Thus, studies in conjugal couples, brothers and sisters of different birth order, adopted individuals, and in siblings and half-siblings raised together or apart have generally indicated that MS risk is unaffected by these microenvironmental influences (Sadovnick et al., 1996, 2005; Ebers et al., 2000, 2004; Bager et al., 2006; Dyment et al., 2006). If so, then the relevant environmental events in MS pathogenesis must be acting at a population level. Moreover, if, in addition to a genetic predisposition, one or more population-level environmental events is necessary for MS to develop, then it is only natural to enquire as to how many such events there are and whether these events need to occur at any particular time or in any particular order. Several published epidemiologic findings bear on these issues.

Environmental factors near birth

The first finding is the presence of the so-called "maternal effect" in MS (Ebers et al., 2004). Epidemiologic support for such "maternal effect" is provided by three independent observations. The first is that half-siblings (i.e., siblings who share one, but not both, biologic parents), who are concordant for MS, are twice as likely to share the mother as they are to share the father (Sadovnick et al., 1996; Ebers et al., 2004). Such a circumstance suggests that MS susceptibility is transmitted from mother to child through some mechanism other than the passage of nuclear genes. An environmental exposure, occurring either in the intrauterine period or soon thereafter, is one possibility. Once the child is born and becomes independent of the mother, however, such a maternal effect would be not be expected from an environmental event.

This maternal effect, however, need not be environmental. It could equally be the result of mitochondrial inheritance, from genetic imprinting favoring expression of certain maternal genes, or from other epigenetic factors (Bartolomei and Tilghman, 1997). With respect to these other possibilities, however, there has been an interesting discussion in the literature about the possible existence of a so-called "Carter effect" in MS (Kantarci et al., 2006; Herrera et al., 2007). This hypothetic effect occurs because men (thought to be less susceptible to MS than women) are presumed to have more "potent" susceptibility genes when they actually develop the disease. In such a circumstance, one would anticipate paternal transmission of MS to be more common when the father's side is "genetically loaded" compared to maternal transmission when the mother's side is similarly "loaded." One report found weak evidence ($p = 0.032$) for such a "Carter effect" (Kantarci et al., 2006), whereas another (larger) study did not (Herrera et al., 2007). Importantly, however, neither study provided evidence for the excessive maternal transmission expected if mitochondrial genes, genetic imprinting, or epigenetic factors were the basis of the "maternal effect" in MS (Ebers et al., 2004). By contrast, any potentially responsible environmental factor would not be expected to produce a maternal effect in these studies because the intrauterine and early postnatal environments are the same irrespective of which parent transmits the MS risk.

The second observation is that the MS concordance rate for fraternal twins seems to be greater than that for full siblings. For example, in a large cohort from Canada (Willer et al., 2003), the concordance rate for MS in full-siblings was 2.9%, with a standard error (SE) of 0.6%, compared to a concordance rate in dizygotic twins of 5.4%. Although few studies directly compare these rates, other large studies (Robertson et al., 1996; Islam et al., 2006), including a review (Compston and Coles, 2002), generally support the same conclusion, although, in a population-based study from Sweden, dizygotic twins and siblings seemed to have a similar risk (Hansen et al., 2005a, b). Such a disparity in recurrence risk between siblings and dizygotic twins (if it exists) cannot be attributed to mitochondrial inheritance, genetic imprinting, or epigenetic factors because, on average, these factors should be similar for both siblings and fraternal twins sharing the same biologic parents. Rather, this discrepancy must be due to environmental events occurring during the shared intrauterine or in the early post-natal period.

The third observation relates to the possible month-of-birth effect for MS, which has now been reported in studies from Canada, northern Europe, and Australia (Templer et al., 1992; Willer et al., 2005; Sadovnick et al., 2007; Staples et al., 2010; Torkildsen et al., 2014). Thus, combining patients from the northern hemisphere (Canada, Denmark, and Sweden), significantly more MS patients were reportedly born in May and fewer were born in November, compared to other months of the year (Willer et al., 2005). Another study found more

RRMS patients born in May than November (Sadovnick et al., 2007). Finally, in 67 Canadian patients, born in the southern hemisphere, this month-of-birth effect seemed reversed (Willer et al., 2005). Recently, this reversal has been fully documented in Australia, where MS risk peaks for babies born in November/December and has its nadir for children born in May/June (Staples et al., 2010).

There has been, however, some controversy regarding the possibility that this month-of-birth effect may be due to an analytic bias (e.g., Fiddes et al., 2013, 2014; Torkildsen et al., 2014). Nevertheless, if these month-of-birth observations cannot be explained away as simple artifacts (Torkildsen et al., 2014), such an effect would provide unequivocal evidence for an early environmental event involved in MS pathogenesis that is time-locked to birth. As the interval between the birth and any environmental event increases, the coupling between birth and the event will inevitably become less precise and, as a result, the observed birth signal will become increasingly less distinct. The fact that this signal remains so clear (Willer et al., 2005; Staples et al., 2010) would then indicate that the responsible environmental event underlying this effect must occur very near to the birth itself. In addition, the timing of this environmental event is periodic and it is coupled to the solar cycle (Willer et al., 2005; Staples et al., 2010). Perhaps importantly, mothers of May babies in the northern hemisphere (or November babies in the southern hemisphere) spend much of their pregnancy during the winter months (with less sun exposure) compared to mothers who are pregnant over the summer months. This *circa annum* periodicity to MS susceptibility might be due to variations in vitamin D levels from differences in maternal sun exposure while the child is *in utero* (Chaudhuri, 2005; Willer et al., 2005). Seasonal infections might also produce such periodicity although, because intrauterine infections of the child are uncommon, any such association would probably need to be secondary.

Environmental factors during adolescence

A second environmental factor is suggested by observations in people who migrate from one geographic region to another with differing MS risks (Dean and Kurtzke, 1971; Alter et al., 1978; Elian et al., 1990; Kahana et al., 1994; Cabre et al., 2005; Compston et al., 2006). For example, when individuals move (prior to their adolescent years) from an area of high MS prevalence to an area of low prevalence (or vice versa), their MS risk becomes similar to that of the region to which they moved. By contrast, when they make the same move after adolescence, their MS risk remains similar to that of the region from which they moved. Moreover, the children of immigrants from low-MS-prevalence areas who are born in a high-MS-prevalence area have an MS risk similar to their birth country rather than their country of ethnic origin (Elian et al., 1990). These observations (if correct) indicate that there is some environmental event, which is involved in MS pathogenesis, that occurs some time between birth and adolescence.

Environmental factors during adult life

Third, the initial clinical symptoms in MS are generally delayed considerably (often by decades) following the period when the maternal factor and the migratory factor take place. It is possible that these early environmental events, by themselves, are sufficient to cause MS although, in that case, the long delay between these events and MS onset seems somewhat difficult to rationalize. Consequently, it seems likely that a subsequent environmental event (or events) is responsible for the timing of symptom onset.

ENVIRONMENTAL FACTORS

Many potential environmental triggers, including trauma, stress, vaccinations, obesity, tobacco, typhoid, smallpox, Epstein–Barr virus (EBV), human herpesvirus (HHV)-6, chickenpox, *Chlamydia*, other infections, vitamin deficiencies, low sunlight, cosmic rays, occupational hazards, living with domesticated animals, dietary habits, and toxic exposures, have been postulated to be linked to MS pathogenesis (Compston et al., 2006). Of these, EBV infection, vitamin D deficiency, tobacco, and obesity have attracted the greatest current interest for their potential role in MS pathogenesis. Nonetheless, several of these other factors continue to have strong proponents and no single factor has been proven conclusively to be related. Neither has any factor been completely excluded, although many of the proposed associations lack credible scientific evidence, biologic plausibility, or both.

Epstein–Barr virus

EBV is a double-stranded linear DNA virus of the herpes family. It is a very common infection of humans, with over 90% of the population becoming infected (Ascherio et al., 2001; Goldacre et al., 2004; Sundström et al., 2004; Ponsonby et al., 2005; Thacker et al., 2006; Ascherio and Munger, 2007, 2016; Farrell, 2007; Nielsen et al., 2007; Serafini et al., 2007). Following birth, as the antibody protection provided by the mother subsides, infants become susceptible to EBV infection. In many parts of the world the initial EBV infection occurs during early childhood and is either asymptomatic or produces nonspecific symptoms

indistinguishable from many other childhood illnesses. However, if the initial infection is delayed until adolescence or young adulthood (which often happens in highly developed regions such as North America and Europe), the syndrome of infectious mononucleosis (glandular fever) develops in 35–50% of cases. The viral infection seems to specifically target the epithelial cells of the oropharynx and the B cells.

Once a cell is infected, the viral genome becomes circularized and persists within the cell as an episome. Latent rather than lytic infection ultimately predominates (probably due to immune responses by the host). Latently infected B lymphocytes proliferate and are directed to specific sites (e.g., the bone marrow) where the virus persists indefinitely, periodically becoming reactivated, resulting in further cell lysis and producing fresh viral particles.

During either the late incubation period or early in the acute illness, antibodies to antigens associated with the process of viral replication, such as the viral capsid antigen (VCA) and the diffuse and restricted early antigens (EA), are found in the serum (Henle et al., 1987). Antibodies to VCA are initially of the IgM class. However, this response lasts only 1–2 months, after which time the anti-VCA response shifts to the IgG class. These antibodies persist for the lifetime of the individual. Antibodies to EA are also of the IgG class but usually drop to undetectable levels after 3–6 months. Thus, the EA antibodies are generally taken as a sign of active infection, although, in approximately 20–30% of patients, these antibody titers may persist for years. Moreover, these antibodies can be found in patients with chronic active infections or with secondary complications such as nasopharyngeal carcinoma or Burkitt's lymphoma. The EBV nuclear antigens (EBNA 1–5) are expressed in latently infected B cells, and antibodies to these antigens typically appear 3–6 weeks following the initial infection. These too persist for the lifetime of the individual. In addition to any possible role in MS pathogenesis, EBV has been implicated in the pathogenesis both of certain malignancies (e.g., EBV-positive Hodgkin lymphoma, nasopharyngeal carcinoma, and Burkitt's lymphoma) and of several autoimmune diseases, such as rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus (Vaughan, 1995).

Despite the fact that EBV infection has been consistently linked to MS, especially when it causes symptomatic mononucleosis infection (Ascherio et al., 2001; Goldacre et al., 2004; Sundström et al., 2004; Ponsonby et al., 2005; Thacker et al., 2006; Ascherio and Munger, 2007, 2016; Farrell, 2007; Nielsen et al., 2007; Serafini et al., 2007), it is notable that fewer than one in 900 individuals with an EBV infection and only a small fraction of patients with mononucleosis will ever develop MS. Nevertheless, the evidence that EBV plays some role in MS pathogenesis seems quite compelling (Ascherio et al., 2001; Goldacre et al., 2004; Levin et al., 2005; Ponsonby et al., 2005; Thacker et al., 2006; Ascherio and Munger, 2007, 2016; Farrell, 2007; Nielsen et al., 2007; Serafini et al., 2007). Thus, even though EBV infection occurs in over 90% of the non-MS population (Sumaya et al., 1980, 1985; Bray et al., 1983; Larsen et al., 1985; Shirodaria et al., 1987; Munch et al., 1998; Myhr et al., 1998; Wagner et al., 2000; Ascherio et al., 2001; Haahr et al., 2004; Sundström et al., 2004; Ponsonby et al., 2005), the evidence for prior EBV infection in adult-onset MS is essentially 100% and is significantly more likely in cases than in controls (Table 11.1). Even in those rare MS patients who test negatively for prior exposure to EBV, this finding could easily be a false-negative result because, in every such case, the antibody response was not measured to the entire set of EBV antigens (Table 11.1). Also, the prior nature of the EBV infection is supported both by the presence of IgG (not IgM) antibodies to EBV antigens and by the unequivocal evidence (when it has been assessed) of infection years prior to the onset of clinical symptoms (Ascherio et al., 2001; Goldacre et al., 2004; Sundström et al., 2004; Ponsonby et al., 2005; Thacker et al., 2006; Ascherio and Munger, 2007, 2016; Farrell, 2007; Nielsen et al., 2007). It should be noted that, here, the term "prior" is being used to mean "prior to the symptomatic onset of MS." As discussed earlier, disease onset frequently (perhaps generally) occurs several years before symptom onset. Nevertheless, such a strong association between EBV and MS is very difficult to ignore.

Moreover, this high prevalence of EBV antibodies in adult-onset MS does not seem to be the consequence of either false-negative tests within the general population or false-positive tests in MS patients. Certainly, as shown in Table 11.2, the near-100% prevalence cannot be due to a general hyperimmune state in MS patients because their antibody responses to other common pathogens (e.g., mumps, measles, chickenpox, cytomegalovirus, herpes simplex) are not similarly increased (Bray et al., 1983; Ascherio et al., 2001; Haahr et al., 2004; Sundström et al., 2004; Ponsonby et al., 2005). Interestingly, one report found evidence of EBV infection in a substantial proportion of those B lymphocytes infiltrating the CNS in 21 of 22 MS cases examined at postmortem (Serafini et al., 2007). This isolated report, however, has not been replicated. Finally, the increased risk of MS, either with delayed exposure to EBV (Ascherio et al., 2001; Haahr et al., 2004; Ponsonby et al., 2005; Thacker et al., 2006) or following symptomatic mononucleosis (Goldacre et al., 2004; Sundström et al., 2004; Thacker et al., 2006; Nielsen et al., 2007), strongly

Table 11.1

Prevalence of antibodies to Epstein–Barr virus (EBV) in the sera of patients and controls

Study	EBV+ multiple sclerosis cases (%)	EBV+ controls (%)	*p*-value
Sumaya et al., 1980‡	155/157 (98.7%)	76/81 (93.8%)	0.05
Bray et al., 1983‡	309/313 (98.7%)	363/406 (89.4%)	0.0001
Larsen et al., 1985‡	93/93 (100%)	78/93 (83.9%)	0.0001
Sumaya et al., 1985*	104/104 (100%)	23/26 (88.5%)	0.007
Shirodaria et al., 1987‡‡	26/26 (100%)	24/26 (92.3%)	–
Munch et al., 1998†	137/138 (99.3%)	124/138 (89.9%)	0.0004
Myhr et al., 1998*	144/144 (100%)	162/170 (95.3%)	0.008
Wagner et al., 2000†	107/107 (100%)	153/163 (93.9%)	0.01
Ascherio et al., 2001††	143/144 (99.3%)	269/287 (93.7%)	0.008
Sundström et al., 2004	234/234 (100%)	693/702 (98.7%)	NS
Haahr et al., 2004†	153/153 (100%)	50/53 (94.3%)	0.05
Ponsonby et al., 2005‡‡	136/136 (100%)	252/261 (96.6%)	0.05
Total	1741/1749 (99.5%)	2267/2406 (94.2%)	$p < 10–23$

*Study measured antibodies against the Epstein–Barr nuclear antigens (EBNA), the viral capsid antigen (VCA), and the early antigens (EA).
†Study measured antibodies only against EBNA and EA.
‡Study measured antibodies only against VCA.
††Study measured antibodies only against EBNA and VCA. One person was antibody-negative to each antigen, but it is unclear from the text whether this was the same person. The review by Haahr et al. (2004) suggests it was not.
‡‡Study measured antibodies only against EBNA and VCA.
NS, not significant.

suggests that the association between MS and this particular pathogen is genuine. Taken at face value, the near-100% association with a "prior" EBV infection would seem to indicate that EBV is a necessary (but not a sufficient) condition for adult MS to develop and, therefore (if this is correct), that EBV must be a part of the causal pathway leading to MS.

Even in childhood-onset MS, high-quality evidence indicates that MS is associated with a prior EBV exposure (Alotaibi et al., 2004; Pohl et al., 2006; Krone et al., 2008; Makhani et al., 2016). However, despite such an unequivocal association, the actual prevalence of prior EBV infection reported in children with MS has varied considerably. Thus, in an early study from Canada, 83% of MS cases had evidence of prior EBV infection (Alotaibi et al., 2004). A more recent Canadian study also made a very similar observation (Makhani et al., 2016). Thus, in this second study, 23.5% of 247 patients with acquired demyelinating syndrome (ADS) were diagnosed as MS and, of these MS cases, 84.5% had evidence of a "prior" EBV infection. This rate was markedly different from the rate of prior EBV exposure (44.4%) found in patients with monophasic forms of ADS (Makhani et al., 2016). Both of these observations suggest that, unlike adult-onset MS, a sizable proportion of patients with childhood MS do not have a previous exposure to EBV. By contrast, a large German study, which included even younger children than those studied in Canada, found that virtually all (98.6%) of childhood MS cases have evidence of a prior EBV infection (Pohl et al., 2006). In fact, these authors argue that, based on the distribution of the antibody concentrations, their two EBV-negative MS patients were actually the expected number of "false negatives" (Krone et al., 2008).

It is unclear how to account for these differences between reports – either those between geographic regions in children or those between adults and children in general. Nevertheless, if the observations out of Canada are correct (as seems likely), it must be the case that some children can develop MS independently from any EBV exposure. Perhaps some children develop different demyelinating diseases, with a different pathogeneses, from typical adult MS or perhaps children can develop the disease through different pathways, which are unavailable to adults. Alternatively, as noted above, the disease onset frequently precedes, often by many years, the symptom onset (Okuda et al., 2009, 2014) so that, perhaps, the disease onset actually begins very much earlier than is currently suspected and that the EBV association is due to the disease making EBV infection both more likely to occur in general and more likely to occur at an earlier age. This latter explanation, however, would not easily account either for the migration data (Dean and Kurtzke, 1971; Alter et al., 1978; Elian et al., 1990; Kahana et al., 1994; Cabre et al., 2005) or for the apparent association of MS with mononucleosis – the manifestations of a late EBV infection (Goldacre et al., 2004; Thacker et al., 2006; Farrell,

Table 11.2

Prevalence of antibodies to different viruses in the sera of patients with multiple sclerosis (MS) and controls[†]

Virus	Virus+ MS cases (%)	Virus+ controls (%)	*p*-value	OR
Adults with MS				
EBV[‡]	1741/1749 (99.5%)	2267/2406 (94.2%)	$p < 10^{-23}$	13.3
HSV*	507/666 (76.1%)	997/1287 (77.4%)	NS	0.93
HSV1*	109/138 (79.0%)	159/205 (77.6%)	NS	1.09
HSV2*	40/138 (29.0%)	29/205 (14.1%)	$p < 0.01$	2.5
VZV*	516/637 (81.0%)	1004/1179 (85.2%)	NS	0.74
CMV*	312/693 (45.0%)	447/854 (52.3%)	NS	0.74
Measles*	661/749 (88.3%)	1309/1609 (81.4%)	NS	1.72
Mumps*	224/386 (58.0%)	305/524 (58.2%)	NS	0.99
Rubella*	226/235 (96.2%)	299/307 (97.4%)	NS	0.67
Chlamydia pneumoniae[‡‡]	85/129 (65.9%)	160/258 (62.0%)	NS	1.18
Children with MS				
EBV**	145/147 (98.6%)	106/147 (72.1%)	$p < 0.001$	27.3
HSV-1/2	68/133 (51.0%)	(?)/152 (46.7%)	NS	1.19
HSV-2**	65/149 (43.5%)	(?)/152 (36.2%)	$p < 0.0001$	1.36
VZV**	88/99 (88.4%)	147/152 (96.7%)	$p < 0.0001$	0.26
Measles**	70/82 (85.6%)	122/152 (90.1%)	$p < 0.0001$	0.65
Mumps**	74/90 (82.0%)	(?)/152 (73.0%)	NS	1.68
Rubella**	101/122 (82.5%)	123/152 (80.9%)	NS	1.11
HHV-6**	126/132 (95.6%)	144/152 (94.7%)	NS	1.22
Adenovirus**	8/127 (6.3%)	20/152 (13.2%)	NS	0.44
Influenza A**	24/127 (18.9%)	15/152 (9.9%)	$p < 0.05$	2.12
Parainfluenza 2**	27/123 (22.0%)	30/152 (19.7%)	$p < 0.05$	1.15

*Data from Ascherio and Munger (2007).
[†]OR, odds ratio; EBV, Epstein–Barr virus; HSV, herpes simplex virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; HHV, human herpesvirus; NS, not significant.
[‡]Data from Table 11.1.
**Data from Krone et al. (2008). This is data from children with MS. A (?) appears when the numbers provided don't make sense completely. Data on EBV are partly derived from Pohl et al. (2006).
[‡‡]Data from Munger et al. (2004a).

2007). Consequently, some variant of the first set of explanations seems more likely.

Presumably, if EBV is in the (or a) causal pathway to MS, it cannot be the factor responsible for the "maternal effect" in MS because EBV infection typically occurs neither *in utero* nor during the early postpartum period. If EBV infection is permissive in the manner suggested then, like the second environmental factor in MS pathogenesis, it probably acts during adolescence (when both late infection and mononucleosis occur) or thereafter and, consequently, it would be a much better candidate for the second (or a later) environmental event. Nevertheless, although the timing of the EBV infection and the second environmental event seems to occur at similar times, the migration data are difficult to explain on the basis of a late infection by a single strain of EBV. Thus, individuals from low-prevalence regions for MS (presumably) would have already acquired EBV early so that moving after early childhood to a high-prevalence region should not matter. Similarly, why children from a high-prevalence region (who presumably have not yet acquired EBV) should adopt a low prevalence when they move is equally unclear. Nevertheless, it is known that there are at least two different strains of EBV (Zimber et al., 1986; Young et al., 1987) with different geographic distributions and, perhaps, a better understanding of the complexity of EBV biology might help to rationalize the migration data.

Regardless of these complexities, however, on the basis of abundant and very consistent high-quality evidence, it seems clear that EBV infection plays some role in MS pathogenesis.

Other infectious agents

Over the years, many other common infectious agents have been suggested as possible causes of MS. Certainly, the evidence is consistent that MS attacks occur more

frequently than expected around the time that patients are experiencing nonspecific infectious syndromes (Sibley et al., 1985; Andersen et al., 1993; Panitch, 1994; Edwards et al., 1998). Nevertheless, as causal agents involved in MS pathogenesis, other than for EBV, the evidence is considerably less compelling (Munger et al., 2004a; Pohl et al., 2006; Ascherio and Munger, 2007, 2016; Krone et al., 2008). For example, considering the OR for the seroprevalence of antibodies against many common pathogens in both MS and non-MS populations reveals that, with the notable exception of EBV (in both adults and children with MS), there is not much evidence of an association between specific agents and the likelihood of MS (Table 11.2). Similarly, even for recently proposed agents such as HHV-6 and *Chlamydia pneumoniae*, the findings are inconclusive (Moses and Sriram, 2001; Kaufman et al., 2002) and the seroprevalence data are unimpressive (Table 11.2).

The fact that the seroprevalence data do not particularly suggest a role for infectious agents other than EBV, however, does not exclude the possibility that these other agents might be involved in MS pathogenesis. For example, in the pediatric population (Krone et al., 2008), it is of interest that, although IgG antibodies against *C. pneumoniae* were no different between patients and controls (Table 11.2), there was a highly significant increase in the presence of IgM antibodies (presumably indicating a more recent infection) in patients. However, for unclear reasons, this excess of IgM antibodies was not replicated in an adult series of MS cases (Munger et al., 2004a). Also, the reported excess occurrence of *C. pneumoniae* DNA in the CSF of MS patients (Bagos et al., 2006) and reports of an excess of *C. pneumoniae*-specific immune complexes in the serum of MS patients, especially early in the disease the course (Parratt et al., 2008), if consistently confirmed, will require explanation.

Similarly, in the case of HHV-6 infection, where the prevalence of infection in the general population is high, the seroprevalence data could be misleading. Thus, if the critical factor was either the timing of the HHV-6 infection or a penetration of the CNS by the organism, which occurs randomly in infected individuals, then the seroprevalence data may well be nonsuggestive despite the organism playing a critical causal role. Consequently, it is hard to exclude any of these infections as being potential contributors to MS pathogenesis. Moreover, as noted above, there are at least three environmental factors involved in the cause of MS and, at the moment, there are no leading candidates for the third environmental event.

Vitamin D deficiency

The production of active vitamin D by mammals *in vivo* requires the two-step conversion of 7-dehydro-cholesterol into vitamin D_3 (Holick, 1998; Hayes et al., 2003; Nagpal et al., 2005; Lips, 2006). The first step – the synthesis of previtamin D_3 – is catalyzed by the exposure of 7-dehydro-cholesterol in the skin to ultraviolet B (UVB) radiation (wavelength = 280–320 nm). The second step – a rearrangement of the internal double-bond structure of the previtamin D_3 molecule – forms vitamin D_3. Vitamin D_3 is then hydroxylated, first to 25(OH) D_3 by 25-hydroxylase (primarily in the liver) and second by 1-α hydroxylase in the tissues (in the kidneys and many others) to form $1,25(OH)_2D_3$ (active vitamin D). The dietary intake of vitamin D_3 can circumvent the UVB-dependent part of this pathway and can allow an individual to have normal vitamin D_3 serum levels even in the absence of adequate UVB radiation. Vitamin D, however, is found in only a few natural dietary sources. Sufficient quantities are only present in a few animals (Gillie, 2006) such as oily fishes and reindeer, which derive it or its precursors from their diet (fish from phytoplanctonic algae in the sea and reindeer from lichen on the tundra). Interestingly, two human populations with a notably low MS risk (Sinclair, 1977; Koch-Henderson, 1995; Grønlie et al., 2000; Gillie, 2006) are the Inuit or Eskimos (who consume large quantities of oily fish) and the Sami or Lapps (who eat reindeer meat regularly). In both instances, the principal source of vitamin D for the population comes from their respective diets (Gillie, 2006). Other human populations, by contrast, require sufficient exposure of the skin to UVB radiation in order to maintain adequate vitamin D_3 serum levels throughout the year.

Furthermore, it should be noted that the grouping of vitamin D together with the other vitamins is probably inappropriate. Thus, typically, vitamins are organic molecules, which are involved in specific chemical reactions, and which humans (during the course of their evolution) have lost the ability to synthesize. Presumably, humans were able to do this because these compounds (in general) are widely available in our foods and, consequently, most of our diets provide a sufficient supply, even without specific supplementation. By contrast, for truly essential compounds such as cholesterol (critical for the function of every cell), humans have preserved the ability to synthesize them, even when they are abundant in our diet. Clearly, vitamin D also falls into this second category. Humans have the ability to synthesize it, it is not prevalent in our diet, and it does not participate in any specific biochemical reaction. Rather, vitamin D acts (together with its receptor and the retinoid X receptor) as a transcription factor that controls the expression of thousands of nuclear genes throughout the body and it has become increasingly apparent that its deficiency is associated with a number of medical conditions (Tavera-Mendoza and White, 2007). Moreover, it

seems very likely that, for diverse human populations living in temperate (and more extreme) regions of the earth, the occurrence of lighter skin tones represents a convergent evolutionary adaptation to the need for adequate vitamin D in these areas (Jablonski and Chaplin, 2000, 2002).

In this context, it is interesting that, as latitude increases (both north and south of the equator), the angle at which the arriving sunlight strikes the earth's atmosphere becomes more oblique. Such a circumstance causes the light to travel for a longer path through the atmosphere before reaching the earth's surface, thereby reducing the amount of UVB radiation available to human populations for vitamin D synthesis. In central and north-east Africa (where *Homo sapiens* evolved) there is plenty of UVB radiation for vitamin D_3 synthesis throughout the year (Jablonski and Chaplin, 2000, 2002; Kimlin et al., 2007). By contrast, once human populations left Africa and began to inhabit the temperate (and even more extreme) regions of the earth, UVB exposure became inadequate for vitamin D_3 synthesis during some (or many) months of the year. For example, it has been estimated that the level of UVB radiation at the US–Canadian border during most months of the year (Fig. 11.2) is insufficient to produce an adequate amount of vitamin D_3 (Adams, 1989; Jablonski and Chaplin, 2000, 2002; Kimlin et al., 2007). Moreover, as noted by several authors (Adams, 1989; Jablonski and Chaplin, 2000, 2002; Rosati, 2001; Compston et al., 2006) and consistent with a putative role for vitamin D deficiency in MS pathogenesis, worldwide distribution maps of reduced UVB availability (Fig. 11.2) are strikingly similar to comparable maps of MS prevalence (Fig. 11.1).

Vitamin D acts by binding to its receptor (VDR), which is located on the surface of cells throughout the body. This receptor–ligand complex is then internalized to form a heterodimer together with the retinoid X receptor, a complex which is then translocated to the cell nucleus, where it binds to a short sequence of DNA bases (the vitamin D response element or VDRE) located in the promoter region of several (many) nuclear genes (Nagpal et al., 2005; Tavera-Mendoza and White, 2007). Interestingly, the VDRE has recently been identified in the promoter region adjacent to the human leukocyte antigen (HLA) DRB1*1501 allele (Ramagopalan et al., 2009). This observation is notable because, as discussed below, this genomic region (and this allele in particular) has been consistently linked to MS pathogenesis for decades (Compston et al., 2006).

The roles of vitamin D in calcium homeostasis and the maintenance of bone health have been widely known for years (Holick, 1998; Lips, 2006). Its role in other cellular processes, including a variety of immune functions, such as cell proliferation, differentiation, and immunomodulation, as well as its antineoplastic actions, has been less widely recognized (Cantorna, 2000; Deluca and

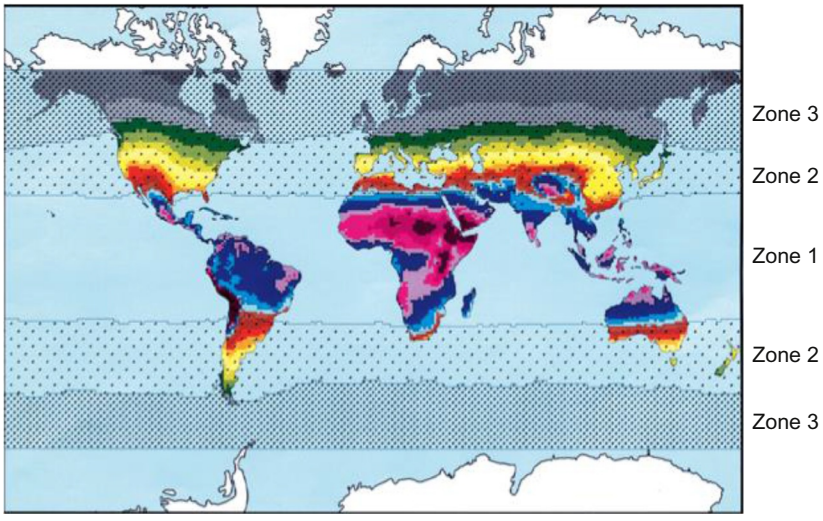

Fig. 11.2. Global distribution of ultraviolet B (UVB) radiation expressed as the number of months estimated to be insufficient for adequate vitamin D synthesis. In zone 2 (where there is a variable insufficiency), an increasing number of insufficient months is indicated by the coloration changing from reds to oranges to yellows to greens. The darkest green indicates insufficient UVB for 11/12 months. The amount of UVB radiation also varies in zones 1 and 3, but is either always adequate (zone 1) or always inadequate (zone 3). (Reproduced from Jablonski and Chaplin, 2000, with permission.)

Cantorna, 2001; Griffin et al., 2001; Hayes et al., 2003; Nagpal et al., 2005; Tavera-Mendoza and White, 2007). Nevertheless, the VDR is expressed on cells throughout the body, including activated T and B cells and on macrophages (Cantorna, 2000; Deluca and Cantorna, 2001; Nagpal et al., 2005). Vitamin D has also been implicated in the maturation of dendritic cell and in the modulation of antigen-specific immune responses *in vivo* (Cantorna, 2000; Deluca and Cantorna, 2001; Griffin et al., 2001). Human decidual cells synthesize active vitamin D during gestation (especially in early pregnancy), suggesting that vitamin D may play a role in the regulation of both acquired and innate immune responses at the fetal–maternal interface (Evans et al., 2006). Finally, vitamin D deficiency seems to play a role in the pathogenesis of several autoimmune diseases, such as insulin-dependent diabetes mellitus, rheumatoid arthritis, experimental autoimmune encephalomyelitis, and inflammatory bowel disease (Cantorna, 2000; Deluca and Cantorna, 2001; Cantorna and Mahon, 2004). Because autoimmune diseases, in general, are more common in women, it is possible that there exist gender-specific differences in the physiologic responses to vitamin D and, indeed, there is some evidence for this proposition (Suarez et al., 1998; Spach and Hayes, 2005). Notably, in one study (Spach and Hayes, 2005) vitamin D supplementation seemed to confer protection against experimental autoimmune encephalomyelitis only to surgically naïve female mice but not to male mice or to ovariectomized females.

With this background, there have been several notable studies, which have explored, more directly, the possible relationship of vitamin D to MS. For example, one group of investigators (Van der Mei et al., 2003) retrospectively interviewed 136 Tasmanian MS cases (through a recruitment campaign that included informational meetings, physician referrals, and posted fliers) as well as 272 sex- and birth year-matched controls recruited from the same source population (using the roll of registered electors). Participants filled in calendars (using a validated questionnaire) and were interviewed about the amount of time they spent in the sun during weekends and holidays during summer for each year of their life. They were also asked about measures taken to protect against the sun and the use of vitamin D supplementation. Higher sun exposure during childhood and early adolescence (6–15 years), as documented by calendar, was associated with a decreased risk of MS ($p < 0.01$). No attempt was made in this study to measure sun exposure in the 0–5-year age range.

Another study (Munger et al., 2004b) reported results from two prospectively established cohorts of nurses, the first established in 1976 (consisting of 121 700 nurses aged 30–55 years) and the second established in 1989 (consisting of 116 671 nurses aged 25–42 years). Both cohorts filled out validated semiquantitative food frequency questionnaires on several occasions. Total vitamin D intake (dietary plus supplemental) was estimated and persons were ranked according to their quintile of vitamin D consumption. Estimates of MS cases per person-year were calculated for each quintile of these cohorts, both collectively and separately. In this study, total vitamin D intake at baseline was found to be inversely associated with risk of MS. Thus, the age-adjusted pooled relative risk (RR) of MS comparing the highest with the lowest quintile of vitamin D consumption was 0.67 ($p = 0.03$). When dietary and supplemental vitamin D were examined separately, the effect seemed to be largely related to the amount of supplemental vitamin D intake.

In another report (Munger et al., 2006), the investigators retrospectively studied 315 MS cases who had previously served in the US armed services. Cases were identified from all active duty personnel (using medical records from the Physical Evaluation Boards' database) and participants were required to have at least one stored serum sample from entry into the military available for analysis. Controls (two per case) were randomly selected from the same population for each case and were matched to the cases on the bases of age, sex, race/ethnicity, dates of sample collection, and branch of military service. Using regression analysis, among whites (but not blacks), there was a significant 41% decrease in MS risk for every 50 nmol/L increase in 25-(OH)-vitamin D ($p = 0.04$), Comparing the top quintile of 25-(OH)-vitamin D level to the bottom quintile yielded an RR of 0.38 ($p = 0.006$).

As a result of these considerations, vitamin D deficiency would seem to be a good candidate for the "maternal" factor in MS pathogenesis. Not only is vitamin D (like this maternal factor) coupled to the solar cycle in temperate regions (Willer et al., 2003), it is known to be involved in immune system maturation (Cantorna, 2000; Deluca and Cantorna, 2001; Griffin et al., 2001; Hayes et al., 2003; Nagpal et al., 2005), its deficiency has been associated with other autoimmune disorders (Cantorna, 2000; Deluca and Cantorna, 2001; Cantorna and Mahon, 2004), its worldwide distribution mirrors that of reduced UVB radiation (Figs 11.1 and 11.2), extreme northern populations with high dietary intake of vitamin D (e.g., the Inuit and Sami people) have a very low prevalence of MS (Sinclair, 1977; Koch-Henderson, 1995; Grønlie et al., 2000; Gillie, 2006), and there are known interactions between the physiologic effects of vitamin D and gender in some mammals (Suarez et al., 1998; Spach and Hayes, 2005). This last aspect of vitamin D physiology might provide insight to the gender specificity of MS and, perhaps, also, to

the basis of the increase in MS incidence among women (Hernán et al., 1999; Koch-Henriksen, 1999; Celius and Vandvik, 2001; Barnett et al., 2003; Sarasoja et al., 2004; Orton et al., 2006).

However, regardless of its possible connection to the maternal factor in MS, if vitamin D deficiency acts at all, it could also act during childhood, during adolescence, later in life, or even at multiple different times. In fact, as noted above, the available direct data supporting a role of vitamin D in MS pathogenesis actually suggest that there may be an effect during childhood or adolescence (Van der Mei et al., 2003; Munger et al., 2004b, 2006). This uncertainty about when vitamin D deficiency might play a critical role has important implications for the design of any clinical trial meant to test the so-called "vitamin D hypothesis." Thus, if, as has been suggested previously (Ascherio and Munger, 2007), a clinical trial is designed to enrich the study cohort for individuals at high risk of developing MS by including only first-degree relatives of MS probands, then the study will be a failure if the critical time for the environmental exposure occurs *in utero*, during the early postnatal period, or even during childhood. Thus, in such a circumstance, by the time that MS probands are identified, most of their brothers and sisters will have already passed their window of therapeutic opportunity. Obviously, similar concerns also apply to any therapeutic trial targeting any other potentially early-acting environmental events (e.g., EBV exposure).

Physical trauma

The idea that physical trauma or psychologic stress might play a role in either the causation or exacerbation of MS dates back to the late 19th century and to the earliest descriptions of MS. Since that time there has been an ongoing debate about these possible relationships (Goodin et al., 1999). Nevertheless, actual experimental evidence from well-controlled clinical studies has generally been missing. For example, it was not until 1952 that the first controlled study of the effects of trauma on MS was reported (see Goodin et al., 1999). The authors of this study interviewed 250 MS patients and 250 controls and reported that 36 (14.4%) MS patients had a history of trauma within the 3 months prior to the clinical onset of their MS (which could have taken place years before the interview). By contrast, only 13 control subjects (5.2%) reported a history of trauma in the 3 months prior to the time of the interview ($p < 0.01$). However, because the MS patients were interviewed about remote events, whereas controls were interviewed about recent events, there is likely a difference in recall between the groups and, thus, this study is probably biased. Moreover, the study definition of trauma included events (e.g., peripheral injuries and dental procedures) which are not considered to be biologically plausible antecedents of MS even by the most ardent advocates for an association between MS and trauma (see Goodin et al., 1999). Most importantly, however, every one of the other six controlled trials (of varying levels of quality) have not found any evidence to support a relationship between MS and trauma (Goodin et al., 1999). The most definitive of these studies was a population-based study (Goldacre et al., 2006), which compared the incidence of MS in a cohort of 110 993 patients admitted to hospital following a head injury to the same incidence in a reference cohort of 534 600 admitted to hospital for other reasons. These authors found no significant difference in MS risk between cohorts at any time interval after head injury or with any length of hospital stay. This study effectively excludes any clinically important relationship between the clinical onset of MS and head trauma. In another, more recent study, Spitzer et al. (2012) administered the Childhood Trauma Questionnaire to 234 adult MS cases and 885 control adults from the general population. After adjusting for sociodemographic factors and current depression, these authors found that adult MS patients reported significantly more emotional abuse, sexual abuse, and emotional neglect during childhood compared to adult controls. Notably, however, the MS patients did not report more physical abuse, suggesting that physical trauma during childhood is not associated with MS.

Some authors have suggested that, perhaps, the relationship is most evident following cervical rather than head trauma although, even here, the data (such as they exist) are quite unconvincing. For example, the first study to suggest this relationship was a case series of 16 MS patients who also had cervical spondylosis (Brain and Wilkinson, 1957). In this study, the authors provided pathologic evidence from just two autopsied patients in whom the worst cord demyelination was not at the level of maximum disk disease. Remarkably, despite this apparently negative finding, and without marshalling either other evidence or reasoned argument, these authors concluded that MS lesions within the spinal cord were "associated with the presence of the spondylotic bars" (Brain and Wilkinson, 1957). In a subsequent uncontrolled case study of the distribution of MS lesions within the cervical spinal cord in 18 patients with MS (Oppenheimer, 1978), the author reported on three autopsied patients with severe degenerative changes in their cervical spine and noted that his findings were in conflict with the spondylotic bar "hypothesis" proposed earlier (Oppenheimer, 1978). In fact this author specifically concluded that this hypothesis "loses its force" because the lesions of MS "do not appear to be related to points of compression by spondylotic bars" (Oppenheimer, 1978).

Another uncontrolled case series of 39 MS patients (Chaudhuri and Behan, 2001) examined the relationship between whiplash injury and MS attacks and concluded that such injuries were related to the occurrence of MS symptoms. Nevertheless, there are several serious concerns regarding this study and these conclusions. Thus, the cervical trauma was quite mild, as evidenced by the fact that no patient suffered cervical vertebral fracture, dislocation, or spinal cord compression. Moreover, the nature and severity of the injury were judged retrospectively over a period ranging from 1 to 10 years, a circumstance leading to a high probability of recall bias. In addition, it is unclear on what basis the authors concluded that certain clinical syndromes (e.g., ON, oscillopsia, and internuclear ophthalmoplegia) were precipitated by minor cervical trauma. Most tellingly, however, the population of patients was compiled from a series of patients who were referred for medicolegal consultation specifically because they were known to have a history of both MS and antecedent cervical trauma. Such a "study" cannot provide any useful information about a possible causal relationship between cervical trauma and MS. Indeed, based on all of these considerations, it seems clear that none of these studies provide any substantive evidence that either cervical whiplash injury or trauma from cervical spondylosis can cause (or exacerbate) MS.

Psychologic stress

A possible association between psychologic stress and MS is more difficult to assess. Part of this difficulty is related to the lack of any consistent, agreed-upon measure or definition of stress in the literature. Part, also, is related to the fact that the relationship between psychologic stress and MS (if any) is likely to be different for different types and different severities of life stress. Indeed, the number of potential variations is quite large. Thus, life stress may be acute and severe but self-limited, it may be chronic and mild but long-lasting, or it can be any combination of these attributes. It can range in severity from only a minor disturbance of a person's life to a life-threatening, psychologically traumatic event. Finally, it seems almost certain that the nature and severity of stress produced by similar life events (e.g., marriage, loss of job, emotional abuse, a financial reversal, or the death of a spouse) will vary considerably both between individuals and for individuals at different times of their life. All of these factors combine to make this a particularly difficult area of scientific inquiry.

In a review of this topic, the American Academy of Neurology concluded that a relationship between MS and psychologic stress was, at best, only possible (Goodin et al., 1999). Since then, several further investigations have been undertaken. In 1999, a study of the relationship between stress and MS exacerbation in 61 patients was published (Kroencke and Denney, 1999). Patients retrospectively filled out questionnaires for the Hassles scale, the Uplifts scale, and Ways of Coping scale regarding the previous 6 months. Patients were classified as being in remission, in exacerbation, or in the chronic phase of the illness at the time they filled out the questionnaires. These authors reported that the exacerbation subgroup experienced more hassles and used more passive-avoidant or aggressive coping compared to the chronic subgroup ($p = 0.02$ and $p = 0.05$ respectively) but not compared to the remission subgroup. There are several problems with this study. The retrospective assessment of hassles and uplifts introduces the possibility of recall bias, which can substantially contaminate studies of this type (Teschke et al., 2000; Kip et al., 2001; McIntosh et al., 2002; Ebers, 2004). Also, the absence of a difference between the exacerbation and remission subgroups is hard to rationalize if the apparent effect of stress on MS exacerbations is genuine. Lastly, the failure to statistically adjust for the numerous between-group comparisons made in this study, together with the marginal significance of the reported observations, makes it quite likely that some, if not all, of these observations are actually type I errors.

A prospective study explored the relationship between life stress and new lesion formation on brain MRI in 36 patients (Mohr et al., 2003). Life stress was measured using the Holmes-Rahe Social Readjustment Rating Scale (SRRS), the Hassles Scale, and the Profile of Mood States. This study reported that the number of new MRI lesions was increased after a lag of 8 weeks ($p < 0.001$) following an increase in "conflicts and disruption in routine" subscale of the SRRS (e.g., family or job conflict, changes in routine). No such increase in MRI activity was seen following "major negative" life events (e.g., death of a family member), "positive stress" (e.g., outstanding personal achievement), or daily hassles. Nor did the authors find a similar increase in MRI activity after lags of 0, 4, or 12 weeks. Although the use of MRI is an important methodologic improvement compared to earlier studies, it is noteworthy that: (1) the 8-week lag was not an *a priori* prediction; (2) no adjustment was made to the statistical significance of this *ad hoc* observation despite the large number of between-group comparisons undertaken; (3) there was no evidence of a dose–response (i.e., major stressful life events did not impact on MRI activity); and (4) there was no clinical accompaniment to the reported MRI changes. All of these facts, taken together, raise substantial concern about the reliability of the reported findings.

In a 2003 study of the relationship between stress and MS (Buljevac et al., 2003), 110 eligible patients were identified, 37 of whom refused to participate because

of the intense follow-up required. Another 13 dropped out during the course of the study. Thus, only 60 patients (55%) completed the trial. Weekly diaries were collected which catalogued stressful events ranging from "stress related to a holiday" to "death of close family member." The authors excluded 48/505 (9.5%) events, which were thought to be "caused by multiple sclerosis itself." These authors found that patients with stress in the preceding 4 weeks were more likely to experience a first or second exacerbation compared to patients who were stress-free ($p = 0.01$–0.02). Although no analysis of the relationship of stress severity to exacerbation is provided in the text, there was no difference between the effect of a single stressful event compared to the effect of multiple such events in the prior 4 weeks. This study has several sources of potential bias. First, despite the fact that patient diaries were completed on Sunday of each week, there is still a substantial probability of recall bias. For example, if an MS exacerbation began prior to the Sunday survey, it seems likely that patients, aware of the research hypothesis being tested, would report more stress in the prior week than if they had been symptom-free during the same interval. Indeed, a substantial impact of such recall bias has already been well documented in a prospective study of similar design (Kip et al., 2001). Moreover, the large number of dropouts and excluded events may have biased the findings. As a result of these considerations, this study represents only weak evidence in favor of an association. Moreover, the 4-week (or shorter) interval between the stress and its apparent clinical effect is inconsistent with the 8-week lag between the stress and its measurable MRI effect reported by others (Mohr et al., 2003).

Also in 2003, the results of another study examining the relationship between stress and MS exacerbations were reported (Ackerman et al., 2003). This study included 50 patients (all women) who were followed for a period of up to 1 year. Subjects completed weekly questionnaires regarding life events, which may or may not have occurred during the previous week. These events were rated on a four-point scale as either severe (levels 1 or 2) or nonsevere (levels 3 or 4). As in the earlier study (Buljevac et al., 2003), events "potentially related to MS disease activity (e.g., losing a job following an MS attack) were excluded from analysis." The number of excluded events is not clear from the text but, in a preliminary report of the findings in the first 23 subjects (Ackerman et al., 2002), 63% of the severe events and 26% of the total events were excluded on this basis (Ackerman, 2004; Goodin, 2004). In this study the apparent risk of MS exacerbation in the 6 weeks following level 1–3 life events was greater than following level 4 events ($p < 0.05$). The authors found no difference in risk between severe and nonsevere events. Interestingly, no comparison seems to have been made between patients with and without any events. Also, these authors found that the density of life events (i.e., number of events/week) was "positively correlated with the proportion of weeks ill with MS-exacerbations" ($p < 0.05$). This trial also has a substantial risk of recall bias for the same reasons discussed in connection with the earlier study (Buljevac et al., 2003). Moreover, bias may have been introduced by the exclusion of such a large proportion of life events and the failure to compare the findings in patients with and without any life events in the preceding 6 weeks (Ackerman, 2004; Goodin, 2004). As a result this study represents only weak evidence of an association. Most notably, however, the results from this trial seem inconsistent with earlier reports. Thus, the finding that both severe and nonsevere events are associated with MS exacerbations is at odds with one of the reports cited earlier (Mohr et al., 2003), and the finding that event density is associated with MS attacks is at odds with another (Buljevac et al., 2003).

In 2004, the results of a nationwide Danish cohort study were reported (Li et al., 2004). This study retrospectively examined the subsequent risk of MS in parents who had lost a child between 1980 and 1996 compared to parents who had not sustained such a loss. These authors found that the hazard ratio for the development of MS was significantly greater ($p < 0.05$) in bereaved parents. However, this effect was significant only in the subgroup of patients followed for more than 8 years but not in those followed for less time. The major difficulty with this study, therefore, is its apparent marked inconsistency (compared to earlier reports) with respect to the time lag between the occurrence of stress and the onset of disease (Goodin et al., 1999). Moreover, even though not directly analogous to a possible effect of stress on MS exacerbations, a lag of more than 8 years between the stressor and its clinical impact seems quite unexpected based on the reports discussed earlier (Ackerman et al., 2002, 2003; Buljevac et al., 2003; Mohr et al., 2003).

In 2008, Golan and colleagues reported what appeared to be markedly increased occurrence of MS exacerbations requiring steroids in 156 RRMS patients residing in northern Israel during the 33-day Israel–Hezbollah war in 2006. Although there is no question that this war was extremely stressful for the civilian populations of both northern Israel and southern Lebanon and although chance is a very unlikely explanation for the reported findings, the nature of the relationship is still very difficult to decide. Without objective MRI evidence, it is hard to be certain that the observed "neurologic events" actually represented new areas of focal CNS inflammation, even though these attacks were "confirmed" by a neurologist and steroid treatment

recommended. It is well described (Compston et al., 2006) that external heat, internal fever, and exercise are capable of causing the re-emergence of previously recovered symptoms without any with new inflammation. These episodes are termed "pseudo-attacks" and, obviously, the comparable mechanisms might conceivably produce symptoms under conditions of extreme stress.

Moreover, this study did not find any time lag between the stress and the exacerbation (Golan et al., 2008). Because the stress in this study was far more severe than that reported in the earlier study (Mohr et al., 2003), it is possible that this difference altered the reported association. If so, however, then it is noteworthy that, during the first Persian Gulf War of 1991, Israeli MS patients experienced fewer MS exacerbations (compared to baseline) both during and after the stress of the Scud missile attacks (Nisipeanu and Korczyn, 1993). Unfortunately, such confusing (and conflicting) results are typical of the literature in this area of research.

Finally, as discussed earlier, in a recent study regarding traumatic events during childhood (Spitzer et al., 2012), the authors found that, even after making adjustments for sociodemographic factors and current depression, adult MS patients still reported significantly more emotional abuse, sexual abuse, and emotional neglect during their childhood compared to adult controls from the same population.

In summary, therefore, although there is some evidence for an association between antecedent psychologic stress and the onset or exacerbation of MS, even the recent data on this subject are inconclusive. At present, there are important conflicts between reports, both with respect to the effect of different degrees of stress and about the timing of these relationships, which prevent any strong conclusions from being drawn. Therefore, a relationship between stress and either MS onset or MS exacerbation remains, at best, only a possibility.

Tobacco

The first case-control study suggesting a possible association between the occurrence of MS and tobacco smoking was published in the mid-1960s (Antonovsky et al., 1965). Subsequent to this initial report, there have now been several similar case-control studies of this relationship (Villard-Macintosh and Vessey, 1993; Thorogood and Hannaford, 1998; Hernán et al., 2001, 2005; Riise et al., 2003; Sundström et al., 2008; Da Silva et al., 2009; Rodríguez Regal et al., 2009) and, even though some individual studies have not achieved statistical significance, each demonstrates an excess of MS cases in smokers compared to nonsmokers. Consequently, in aggregate, this association seems reasonably well established. The basis for this relationship, however, is more difficult to understand. Smoking has been linked to other autoimmune diseases such as systemic lupus erythematosus, Crohn's disease, rheumatoid arthritis, and thyroid disease (Harel-Meir et al., 2007) and, perhaps, the association is due to a direct or indirect physiologic effect of nicotine on the CNS (e.g., immunomodulatory changes or altered vascular permeability). However, it might also be due to an interaction with certain MS-susceptibility genes. In fact, an interesting report in rheumatoid arthritis has provided evidence of an interaction between smoking behavior and the presence of one or more of the HLA DRB1 susceptibility alleles, which are associated with MS (Padyukov et al., 2004). Thus, the risk of rheumatoid arthritis was substantially greater in smokers who carried one or more of these susceptibility alleles compared not only to nonsmokers who also carried these alleles but also to smokers who did not carry them (Padyukov et al., 2004).

In addition, smoking might be associated with disease progression in MS patients, although these reports have been somewhat inconsistent and, therefore, the evidence provided by them is inconclusive. Interestingly, one report claimed that passive exposure to tobacco smoke during childhood was associated with a greater risk of subsequently developing MS (Mikaeloff et al., 2007). Nevertheless, a very detailed subsequent report failed to confirm this finding (Montgomery et al., 2008).

Obesity

In 2009, the cohorts of women who took part in the Nurses' Health Study ($n = 121\ 700$) and the Nurses' Health Study II ($n = 116\ 671$) were analyzed and, after 40 years, 593 MS cases were identified (Munger et al., 2009). In this study the body mass index (BMI) of the participants was estimated at ages 5, 10, 18, and 20 years. These authors reported that patients at age 18 with a BMI of ≥ 30 had a greater than twofold increase in the RR of MS compared to individuals with a BMI of $18.5 - 23$. A similar risk was found for individuals who were obese at age 20 but not for obesity at ages 5, 10, and during adulthood, suggesting that adolescence was the principal period of risk. A study from Sweden also found a similar relationship (Hedström et al., 2012). Thus, in this study, individuals (men and women) with a BMI of >27 at age 20 years were at twice the risk of MS compared to their nonobese controls. A study out of Denmark (Munger et al., 2013) reported that, among school-aged children (7–13 years), girls in the 95th percentile for BMI had an RR of MS ($RR = 1.61$), which was significantly greater than for nonobese individuals. By contrast, the risk in obese boys seemed to be attenuated compared to girls (Munger et al., 2013). A similar result was found in a study utilizing the large Kaiser database in California

(Gianfrancesco et al., 2014), where girls who were obese at ages 10 and 20 had a greater MS risk than their non-obese counterparts. Finally, in a study from Norway and Italy, the authors found that in Norway (but not Italy) obesity in the period of childhood through young adulthood (in both sexes) increased the likelihood of MS subsequently (Wesnes et al., 2015). Thus, in sum, there seems to be quite consistent evidence that obesity during childhood and young adulthood is a risk factor for MS. Whether this effect is confined to (or most conspicuous in) females is unclear, but possible. Also, the biologic basis for this apparent association is unknown, although it has been suggested that it might be due to the induction of a chronic inflammatory state (with elevated levels of proinflammatory cytokines and with lower circulating serum levels of vitamin D) in obese individuals (Munger et al., 2009; Wesnes et al., 2015).

Dietary factors

More than 60 years ago, Swank (1950) put forth the hypothesis that variations in the frequency of MS seen worldwide could be accounted for by variations in the amount (and possibly the nature) of fat consumption. For example, in North America and Europe, which have high levels (>100 g/day) of per capita fat consumption, there is a considerably greater prevalence of MS compared to Asia, Latin America, and Africa, where this a low level (<60 g/day) of per capita fat consumption (Swank, 1950; Swank et al., 1952). Also, Swank thought that some of the variations in MS incidence both during and after World War II could be explained by variations in fat consumption during that period. And lastly, he noted the greater incidence of MS in northern compared to southern Switzerland and speculated that this was related to differences in the fat content of the diets between these two regions.

In a second paper (Swank and Grimsgaard, 1988), the group studied the occurrence of MS in different regions of Norway. These authors reported a fourfold increase in MS prevalence in the inland (farming) regions compared to coastal regions and related this to a lower fat consumption in the coastal areas where residents depended upon fishing for their livelihoods. The differences in consumption of butter and animal fat between regions, however, were most conspicuous when the authors compared the contemporary fat consumption in the inland areas to fat consumption in the coastal areas determined from an earlier study of diet. When they made the same comparison using simultaneously acquired dietary information, the difference in fat consumption between regions was not as apparent and, thus, their conclusions regarding dietary fats are of uncertain validity. Moreover, these authors, themselves, noted that their observations could not exclude the possibility that fat consumption was correlated with other factors having the same geographic distribution. Also, it is possible that a difference in the incidence of MS might relate to dietary factors other than fats (e.g., the consumption of vitamin D from fish in the coastal regions).

Nevertheless, Swank continued to follow a cohort of 156 patients over the period from 1949 to 1984 (Swank and Dugan, 1990; Swank, 1997). This cohort was derived from a larger group of 264 MS patients seen at the Montreal Neurological Institute between December 1948 and April 1954, 108 of whom declined to participate in the prospective study (41% initial dropout rate). All patients were encouraged to participate in the dietary recommendations. Only 8 patients were lost to follow-up and, of these, 4 were subsequently relocated. Initially, only the total fat intake was restricted, resulting in a drop in daily fat consumption from 125 to 20–30 g/day. Beginning in 1951, however, the recommended diet was modified. Butter fats and hydrogenated oils were eliminated. The consumption of cod liver oil (5 g/day) and vegetable oil (10–40 g/day) was added. In addition, the consumption of animal fats was limited to 15 g/day. During the follow-up interval, commercially processed foods and pen-fattened beef were also restricted because of the increased fat content of these products. Protein intake was limited to 60–90 g/day. This protein was mostly in the form of fish, seafood, and the white meat from chicken and turkey (with the skins removed), although skim milk, lean meats, an occasional egg, vegetables, cereals, and nuts were also allowed. Carbohydrates were consumed as necessary for energy. Not everyone in the cohort elected to follow the diet strictly, and patients were, therefore, divided into three groups based on their total fat consumption ($\leq$20 g/day, 20–30 g/day, and >30 g/day).

These authors report significant differences in outcome based upon the degree of patient adherence to the diet ($p < 0.001$). Thus, the "good dieters" ($\leq$20 g/day) had a 31% mortality (mostly due to MS) and, after 34 years of follow-up, had an increase in disability of approximately one point on the Expanded Disability Status Scale (EDSS). By contrast, the mortality in the "poor dieters" (the other two groups combined) was 80% (also mostly due to MS) and their disability increased by five to six points on the EDSS scale at the completion of the study (Swank and Dugan, 1990; Swank, 1997). Although these results suggest that total fat consumption might influence the course of MS, there are important limitations to the study design that prevent any strong conclusions from being drawn from its results. Thus, it is even unclear which aspect of the diet might have been responsible for the apparently beneficial effect. For example, the consumption of cod liver oil (very high in vitamin D) was also recommended in addition to the restriction of total fat consumption, so that

there is no way to determine which, if either, of these two elements to the diet was important. The same ambiguity is present for each individual aspect of the recommended diet and, importantly, we are not provided with enough information in the published papers to sort out these potential relationships. Moreover, because the study was not randomized, there are concerns that the two groups may have differed in some important respects other than their daily fat consumption. For example, there is evidence that disability levels (possibly from self-selection) were maldistributed between the "good" and "poor" dieters at entry into the study (Swank, 1997), and it is possible that this maldistribution has biased the trial results in favor of the "good dieters." Thus, 32% (23/72) of the "good dieters" who entered the study had minimal disability (neurologic grade of 1) on examination. By contrast, significantly fewer (8% or 6/72) of the "poor dieters" has such a low level of disability at study entry ($z = 3.59$; $p < 0.001$).

Two other small clinical studies (Alter et al., 1974; Fitzgerald et al., 1987) also suggest that dietary factors may play a role in the causation or exacerbation of the illness. Thus, the short-term trial of Fitzgerald et al. (1987), studying a diet low in fat and high in polyunsaturated oils in 83 MS patients, found that those patients who complied with their recommended diet had fewer clinical attacks and less neurologic disability compared to patients who did not comply with the diet. A study by Alter et al. (1974) examined the covariance of MS prevalence and diet in 22 countries with good information on both parameters. For inclusion, the countries had to have both good medical facilities and fairly standardized case-finding methods. Dietary information was derived from information supplied by the United Nations. These authors found that there were significant linear correlations ($p < 0.01$) between the consumption of either animal fat (expressed as the total calories) or fats and oils and the prevalence of MS in the different countries. In a case-control study from Croatia (Sepcic et al., 1993), the ORs for the consumption of full-fat milk (OR = 21.7) and of potatoes with lard and fresh or smoked meat (OR = 20.7) were significantly greater in MS patients compared to controls. In another case-control study in Canada, the authors reported that there was a significantly increased risk of MS in persons who consumed more animal fats and a significantly lower risk in persons consuming more vegetable protein and dietary fiber (Ghadirian et al., 1998).

In summary, although far from conclusive, there is some indication in the literature that dietary factors may possibly be involved (at least partially) in either the causation or the exacerbation of MS. Such a conclusion, however, does not distinguish the dietary hypothesis from the potential role that vitamin D might play in MS pathogenesis. Nevertheless, because of the established (non-MS) health benefits for the so-called "Mediterranean" diet (Estruch et al., 2013) and because of the similarity of this diet to that proposed by Swank, it is probably appropriate to recommend the Mediterranean diet to patients who express an interest in dietary approaches to the management of their disease.

Other factors

As noted earlier, there have been a wide variety of other factors suggested as having some role in either the cause or exacerbation of MS. In general, however, these reports have generally been inconsistent and inconclusive and often the mechanisms by which these factors are proposed to operate in MS pathogenesis lack biologic plausibility.

CHANGING ENVIRONMENTAL EXPOSURES

MS epidemiology has changed in important ways over the past several decades. Thus, the incidence (prevalence) of MS is increasing, especially in women (Hernán et al., 1999; Koch-Henriksen, 1999; Freedman et al., 2000; Celius and Vandvik, 2001; Barnett et al., 2003; Ranzato et al., 2003; Sundström et al., 2003; Sarasoja et al., 2004; Orton et al., 2006). As a consequence of this, the gender ratio has been altered (Orton et al., 2006) and a switch in the latitude gradient for MS incidence has been reported (Hernán et al., 1999). Because MS genetics seems unlikely to have shifted in so short an interval, these observations presumably relate to a change in the environmental determinants of MS. Although many widespread environmental changes are known to be taking place (e.g., increasing atmospheric concentrations of CO_2, CH_4, and other pollutants; increasing global temperatures; a depletion of stratospheric ozone; a greater dietary consumption of *trans* fats and processed foods), one recent change (potentially relevant to the possible role of vitamin D deficiency) is that people are increasingly encouraged to avoid prolonged sun exposure and to use sunblock to prevent skin cancer (Emmons and Colditz, 1999). Nevertheless, sunblock with sun-protective factor (SPF) 15 blocks approximately 94% of the incoming UVB radiation and higher SPF levels block even more (Emmons and Colditz, 1999). As a result, any widespread use of sunblock and/or sun avoidance will exacerbate any population deficiency of vitamin D synthesis and, presumably, will increase the likelihood of diseases related to vitamin D deficiency. By contrast, the pattern of EBV infection seems to have changed little over this interval (Müller et al., 2005).

In summary, the current epidemiologic evidence seems to support the existence of three (or more) environmental events which contribute to MS pathogenesis. The first event occurs near birth, the second occurs during childhood, and the third occurs long after the first two have already taken place. At present, the two best candidate factors identified are vitamin D deficiency and EBV infection. Indeed, these two factors seem particularly well suited to the first two environmental events in MS pathogenesis. However, because there are at least three distinct events involved in MS pathogenesis, there is no need to choose between vitamin D and EBV. They could, easily, both be involved. Nevertheless, even if these two environmental events are implicated as part of a pathway to adult MS, this does not guarantee that they are on the same or the only pathway. Indeed, assuming that each factor is part of some causal pathway, there are several possible ways these two environmental events might interact to produce MS (Goodin, 2009). No pathway can be excluded entirely, although, if prior EBV infection is a necessary condition for MS, then this implies that these two factors act sequentially and are part of a causal cascade of environmental events, which leads to adult MS (Goodin, 2009).

GENETIC FACTORS

As noted earlier, the risk of developing MS for individuals who have an affected family member increases in rough proportion to the amount of shared genetic information between themselves and the proband (Mumford et al., 1994; Ebers et al., 1995, 2004; Robertson et al., 1996; Sadovnick et al., 1996; Compston and Coles, 2002; Willer et al., 2003; Nielsen et al., 2005; Compston et al., 2006; Islam et al., 2006). Thus, for example, siblings of an MS proband (50% genetic similarity) have a 20–30-fold increased risk compared to the general population, whereas monozygotic twins (100% genetic similarity) have a risk more than 200 times that in the general population (e.g., Mumford et al., 1994; Willer et al., 2003; Hansen et al., 2005a,b; Islam et al., 2006; Ristori et al., 2006; Kuusisto et al., 2008). These observations, by themselves, unequivocally implicate genetic factors in the pathogenesis of MS.

General considerations

The field of the genetic epidemiology of MS has been under particularly active investigation for the past two decades and several large genomewide association studies (GWAS) have now been completed and have identified almost 200 MS-susceptibility genes, in addition to the long-established linkage to the HLA class II region on chromosome 6, which is thought to be due to an association with the DRB1*1501 allele (Baranzini et al., 2009; De Jager et al., 2009; International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium, 2011; International Multiple Sclerosis Genetics Consortium, 2014). However, such GWAS investigations focus their attention upon the analysis of approximately 500 000 single-SNPs (single-nucleotide polymorphisms: located in scattered regions throughout the genome) and these analyses identify those single-SNPs, which are significantly associated with the disease. Although, rarely, an SNP identified in this manner may actually represent the genetic alteration responsible for the disease association, in most cases these SNPs exists on haplotypes within a particular genomic region and merely "tag" an allele of some nearby gene, which is the actual basis of the observed disease relationship. In fact, most of these single-SNPs are located in either intronic or intergenic regions of the genome (e.g., Fig. 11.3). This type of association has been referred to as a "synthetic" association

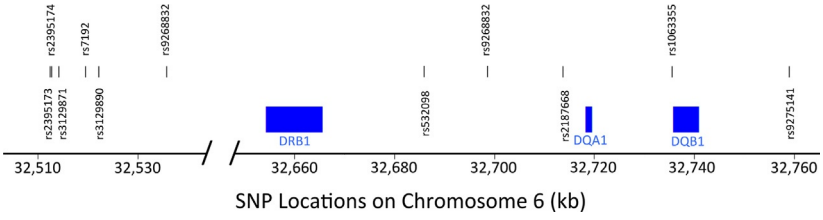

Fig. 11.3. Location of the 11 single-nucleotide polymorphisms (SNPs) in the SNP haplotype surrounding the DRB1 gene on chromosome 6, which has the most significant disease association (see text). The two single-SNPs in this particular SNP set, which have the greatest disease association, are: rs2395173 ($\rho = 0.63$) and rs3129871 ($\rho = 0.59$). Both are located on the extreme left-hand edge of the SNP haplotype, far from the DRB1 locus. The blue bars represent the location of the start and stop points for the DRB1, DQA1, and DQB1 genes. Table 11.3 lists some of the SNP haplotypes (composed of these 11 SNPs) found in this genomic region. The locations of other human leukocyte antigen (HLA) genes discussed in the text (not depicted) are as follows:

HLA A: 30 018–30 021 kb
HLA C: 31 345–31 348 kb
HLA B: 31 430–31 433 kb

(Adapted from Khankhanian et al., 2015.)

(Dikson et al., 2010). However, because a single-SNP can tag more than one haplotype, these synthetic associations require a very large number of patients to uncover, their SNPs typically have a greater allelic frequency than the underlying susceptibility allele, these SNP associations are generally weak and underestimate the strength of the true genetic association (Dikson et al., 2010; Goodin and Khankhanian, 2014), and typically, the GWAS analysis using single-SNPs leaves a large residual "gap" of unexplained heritability (Gourraud et al., 2012; Hofker et al., 2014). As a consequence, these SNP associations only identify genomic regions of interest and do not really help to identify the actual basis for the association (e.g., Fig. 11.3). By contrast, using these SNPs to create SNP haplotypes in the different genetic regions can markedly reduce the heritability gap compared with single-SNP methods and make it clear that these associations identify genetic regions rather than particular genes (Khankhanian et al., 2015).

In fact, these haplotype methods can be used to fine-map the genetic associations within regions of interest previously identified by a single-SNP GWAS (Goodin and Khankhanian, 2014; Khankhanian et al., 2015). Thus, multi-SNP haplotypes detected disease associations in 32 of the 110 regions that were at least 1000-fold more significant than those detected by single-SNPs (Khankhanian et al., 2015). By contrast, single-SNPs were never similarly more significant than the multi-SNP haplotypes in detecting disease associations. Moreover, the nature of the disease associations identified was altered, even within a previously defined genomic region, and the amount of the heritability of MS explained by the observed associations was markedly improved using multi-SNP haplotype methods compared to the use of only single-SNPs (Khankhanian et al., 2015).

In addition, the use of these SNP haplotype methods can help to clarify some of the underlying genetic relationships of MS. For example, Khankhanian and coworkers (2015) reported that, in the class II region of chromosome 6, the most disease-associated haplotype was an 11-SNP haplotype consisting of SNPs (rs2395173_A; rs2395174_C; rs3129871_A; rs7192_A; rs3129890_G; rs9268832_A; rs532098_A; rs17533090_A; rs2187668_A; rs1063355_A; and rs9275141_C), which includes 246 kb of DNA surrounding the DRB1 gene (Fig. 11.3). The final letter in each SNP name designates the minor allele nucleotide (in the control population) at that SNP location. This minor allele is coded as equal to "1" in the haplotype, whereas the major allele is coded as "0" (Table 11.3). Several of the 20 major haplotypes in this region are listed in Table 11.3. These 20 haplotypes account for over 95% of all haplotypes identified and were found at similar frequencies (Table 11.3) in both the EPIC and WTCCC datasets (Baranzini et al., 2009; International Multiple Sclerosis Genetics Consortium, 2014; Khankhanian et al., 2015). The EPIC dataset consisted of 882 controls and 975 MS cases; the WTCCC dataset consisted of 18 872 controls and 11 376 MS cases. Each SNP haplotype has been named arbitrarily according to its order of identification in the EPIC dataset (Goodin and Khankhanian, 2014), using the SNP set presented above and in Figure 11.3. There was a notable consistency between the EPIC and WTCCC control populations with regard to the haplotype identity and frequency, both of which consisted, largely, of persons having a northern European ancestry (Table 11.3). Moreover, each of the SNP haplotypes in this region was very specific with regard to its HLA haplotype association (Fig. 11.4) and each of these SNP haplotypes (as well as the others not listed) had the same HLA association in both datasets (Khankhanian et al., 2015).

In addition, as expected from previous observations in Caucasian populations (e.g., Ahmad et al., 2003; Wennerström et al., 2013; Zúñiga et al., 2013), several of the haplotypes in this region were extremely extended. Nevertheless, the frequency of such extended haplotypes in this dataset seems somewhat surprising (Fig. 11.5). Thus, for example, considering only extended

Table 11.3

Single-nucleotide polymorphism (SNP) haplotypes in the class II region of chromosome 6*

		Haplotype frequency	
Name	SNP haplotype	EPIC	WTCCC
a1	10110100010	0.11	0.12
a2	00000000100	0.02	0.02
a3	00000010001	0.21	0.19
a4	00000000001	0.13	0.11
a5	10100010001	0.08	0.09
a6	01011100100	0.09	0.10
a8	10110100011	0.00	0.00
a9	01000001010	0.11	0.11
a11	00000010010	0.03	0.02
a27	10100100011	0.00	0.00
a36	10100100010	0.00	0.00

*The "name" is arbitrary and indicates the order of haplotype identification in the EPIC dataset (Goodin and Khankhanian, 2014). The SNP haplotype represents the haplotypes identified using the set of 11 SNPs, shown in Figure 11.1. The number "0" indicates the presence of the major allele (in the control population) at the particular SNP location. By contrast, the number "1" indicates the presence of the minor allele (in the control population) at this particular location. Only 11 selected SNP haplotypes are listed. Haplotype frequencies found in two independent datasets (EPIC and WTCCC) are shown (Goodin and Khankhanian, 2014; Khankhanian et al., 2015).

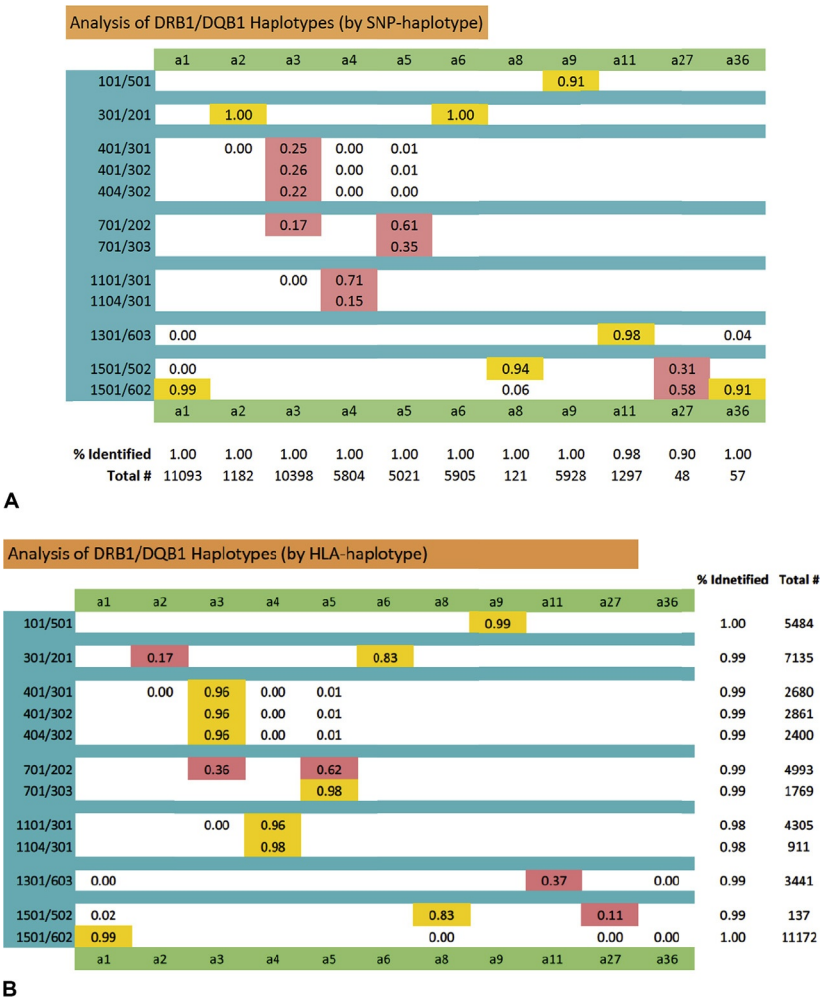

Fig. 11.4. The human leukocyte antigen (HLA) haplotype–single-nucleotide polymorphism (SNP)haplotype associations – both by SNP haplotype (**A**) and also by HLA-haplotype (**B**) – for the selected SNP haplotypes presented in Table 11.3. Haplotypes not presented also had very specific haplotype–haplotype associations (Khankhanian et al., 2015).

haplotypes that are composed of a specific SNP haplotype, together with a specific combination of alleles at each of three HLA class I loci (A, B, and C) and two HLA class II loci (DRB1 and DQB1) – i.e., haplotypes which span a genomic region of more than 2.5 mb of DNA – there are more than 4 billion possible unique combinations of these SNP haplotypes together with these five HLA alleles. Despite this large number of possibilities, however, there were only 10 076 unique combinations represented within the set of 59 882 haplotypes of the WTCCC population. Moreover, of this total of 59 882 haplotypes, 22% were accounted for by only 10 unique combinations, 30% were accounted for by 25 combinations, and 71% were accounted for by 810 combinations (Table 11.4). Furthermore, for each of the top 25 haplotypes, the association of the class I component with the class II component of the haplotype was markedly greater than chance ($p << 10^{-300}$). By contrast, 6014 (60%) of the combinations were unique (i.e., had only a single representative) in the WTCCC dataset. Similarly, 7410 (74%) of the combinations had two or fewer representatives but accounted for only 8806 (15%) of the total number of combinations in the WTCCC dataset.

Of the 14 extended haplotypes in Caucasians described by Ahmad and coworkers (2003), six were also in the top 25 in the WTCCC dataset (Table 11.4). Another two haplotypes in these top 25 were extremely similar to those described previously (Table 11.4); both of the haplotypes actually reported were very rare in the WTCCC – one was not present and the other had only two representations. Similarly, of the 17 extended haplotypes for the A, B, and DRB1 loci in Caucasians described by Wennerström and coworkers (2013), nine were in the top 25 in the WTCCC dataset (Table 11.4). And finally, in the small Caucasian population from

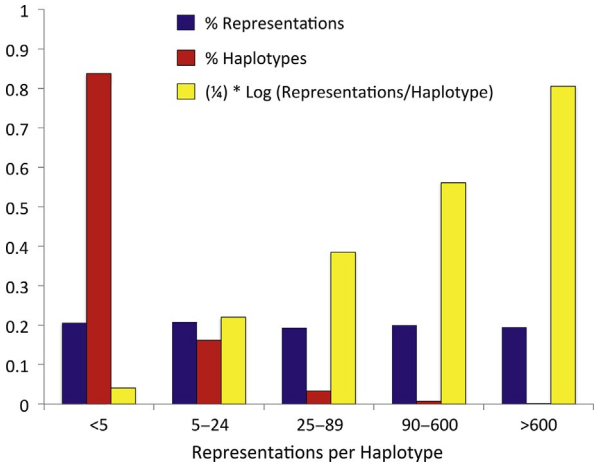

Fig. 11.5. Plots of the number of different haplotypes and the number of representations for each haplotype in the WTCCC dataset. Groups have been divided into quintiles based on the total number of haplotypes represented in the WTCCC data (blue). Also plotted are the number of haplotypes per group (red) and one-quarter of the logarithm (base 10) of the ratio of representations to the number of haplotypes represented (yellow).

the report by Zúñiga and coworkers (2013), three of the six haplotypes were in the top 25 (Table 11.4). Of the remaining 17 described haplotypes, all but the three from Zúñiga and coworkers (2013) had at least some representation and 11 of them had 10 or more representations in the WTCCC dataset.

The reason for these differences between the Caucasians studied is not known, although it seems likely to be a consequence of differences (possibly subtle) between populations. For example, a recent study from Nigeria also reported a high prevalence of extended HLA haplotypes (Testi et al., 2015). Thus, in this study, 14 extended 5-locus West African haplotypes (A-C-B-DRB1-DQB1) accounted for 16% of the haplotypes present in their population. Also, in an Amerindian population, a high prevalence of 5-locus extended HLA haplotypes was, again, reported (Zúñiga et al., 2013). Thus, in their mixed population (which included Amerindians, Caucasians, Africans, and persons of undetermined ancestry), 16 Amerindian haplotypes accounted for 13% of the haplotypes present. Presumably, if the different groups had been segregated in this report (Zúñiga et al., 2013), this percentage would be much higher in the Amerindian population. Interestingly, however, there was essentially no overlap between any of these different populations. Common haplotypes in West Africa and Amerindians were completely distinct from each other (Zúñiga et al., 2013; Testi et al., 2015). Moreover, in the WTCCC dataset, only a single individual carried a single copy of the most common West African haplotype. The remainder of the WTCCC was completely distinct from the common haplotypes in both the Amerindian and West African populations.

Regardless of the explanation for these differences, however, the fact remains that the large majority of haplotypes in the WTCCC dataset are represented multiple (≥ 3) times and that a high prevalence of extended HLA haplotypes also characterizes other populations. These observations suggest that, while different haplotype combinations frequently do arise from one generation to the next, most of these combinations are under very strong negative selection pressure. Such a

Table 11.4

Most common (top) 25 extended haplotypes in the WTCCC

Haplotype (A_C_B_DRB1_DQB1_SNP)†	Frequency	%††
01:01_07:01_08:01_03:01_02:01_a6*§	3782	0.063
03:01_07:02_07:02_15:01_06:02_a1*§	2961	0.049
02:01_07:02_07:02_15:01_06:02_a1¶§	1465	0.024
03:01_04:01_35:01_01:01_05:01_a9§	1086	0.018
02:01_05:01_44:02_04:01_03:01_a3*§	906	0.015
29:02_16:01_44:03_07:01_02:02_a5**¶	730	0.012
24:02_07:02_07:02_15:01_06:02_a1	728	0.012
02:01_03:04_15:01_04:01_03:02_a3**§	556	0.009
01:01_06:02_57:01_07:01_03:03_a5*	554	0.009
11:01_04:01_35:01_01:01_05:01_a9*	539	0.009
25:01_12:03_18:01_15:01_06:02_a1	440	0.007
02:01_03:04_40:01_13:02_06:04_a19§	416	0.007
01:01_07:02_07:02_15:01_06:02_a1	405	0.007
02:01_07:01_08:01_03:01_02:01_a6	397	0.007
02:01_06:02_13:02_07:01_02:02_a3§	361	0.006
01:01_07:01_08:01_15:01_06:02_a1	320	0.005
23:01_04:01_44:03_07:01_02:02_a5	312	0.005
02:01_06:02_57:01_07:01_03:03_a5	293	0.005
02:01_05:01_44:02_15:01_06:02_a1	289	0.005
30:01_06:02_13:02_07:01_02:02_a3*	285	0.005
31:01_03:04_40:01_04:04_03:02_a3	253	0.004
11:01_07:02_07:02_15:01_06:02_a1§	229	0.004
30:02_05:01_18:01_03:01_02:01_a2¶	212	0.004
02:01_01:02_27:05_01:01_05:01_a9	211	0.004
03:01_08:02_14:02_13:02_06:09_a25	201	0.003
Total	17931	0.299

†Extended haplotypes including the human leukocyte antigen (HLA) loci (A, C, B, DRB1, and DQB1), together with a particular SNP haplotype (see Table 11.3).

††Percentage (%) of the 59 882 haplotypes in the WTCCC dataset represented by each extended haplotype.

*Extended haplotypes (in Caucasians) described previously by Ahmad et al. (2003) without inclusion of the SNP haplotypes.

**Very similar haplotypes to those described by Ahmad et al. (2003), except that A_29:01 is replaced by A_29:02; and C_03:03 is replaced by C_03:04.

¶Extended haplotypes (in Caucasians) described by Zúñiga et al. (2013), again without the SNP haplotypes.

§Extended haplotypes (for only the A, B, and DRB1 loci) from Finnish (and other) populations, described by Wennerström et al. (2013).

circumstance further suggests that natural selection is operating upon specific combinations of alleles that "work" together (whatever this means) rather than on the specific alleles within each combination (e.g., Ahmad et al., 2003). If so, this also raises the possibility that any observed disease association in this genomic region similarly relates to the combination of alleles rather than to a particular allele – a consideration which has obvious relevance to the discussions that follow.

DRB1*1501 – DQB1*0602 HLA haplotype

This two-locus HLA haplotype is strongly associated with the (a1) SNP haplotype (Table 11.3; Fig. 11.4). It also occurs in specific association with several other SNP haplotypes, including (a27), (a34), and (a36). For example, among the 52 individuals who carried the (a36) SNP haplotype, 48 also carried the DRB1*1501 – DQB1*0602 HLA haplotype but did not carry an (a1) SNP haplotype. Of these persons, 38 (79%) were controls. Consequently, the OR for those rare individuals who carry a single-copy of the (a36) DRB1*1501 – DQB1*0602 haplotype but not the (a1) SNP haplotype (compared to those who do not carry either the (a36) SNP haplotype or the DRB1*1501 – DQB1*0602 HLA haplotype) was significantly different from the disease association found for carriers of a single copy of the (a1) DRB1*1501/DQB1*0602 haplotype (Fig. 11.6).

Similarly, 14/19 (74%) of the carriers of the (a27) SNP haplotype, who also carry the DRB1*1501/DQB1*0602 haplotype but not the (a1) SNP haplotype, were controls. Thus, those non-(a1) individuals who carry a single-copy of the (a27) DRB1*1501 – DQB1*0602 haplotype (compared to those who do not carry either the (a27) SNP-haplotype or the DRB1*1501 – DQB1*0602 HLA haplotype) also had a significantly different disease association from that found for single-copy carriers of the (a1) DRB1*1501 – DQB1*0602 haplotype (Fig. 11.4). Indeed, considering all non-(a1) DRB1*1501 – DQB1*0602 carriers, the OR for disease association was both not significantly different from unity and was significantly different lower than the OR in (a1) DRB1*1501 – DQB1*0602 carriers (Fig. 11.6).

By contrast, those rare individuals who carry the (a1) SNP haplotype but not the DRB1*1501/DQB1*0602 HLA haplotype, have a disease risk (compared to noncarriers of either haplotype) which does not differ significantly from the risk in single-copy carriers of the (a1) DRB1*1501 – DQB1*0602 haplotype (Fig. 11.6). Also, the disease risk in single-copy carriers of the (a1) DRB1*1501 – DQB1*0602 haplotype is unaffected by having a second copy of the DRB1*1501 allele (Fig. 11.6).

As anticipated from earlier publications (e.g., Ahmad et al., 2003; Wennerström et al., 2013; Zúñiga et al., 2013), the (a1) SNP haplotype resides on one of several very long extended haplotypes, which stretch over a genomic distance of more than 2.5 mb of DNA (Table 11.4). Many of these haplotypes share the motif of C*0702 – B*0702, in addition to having the DRB1*1501 – DQB1*0602 class II haplotype (Table 11.4). Nevertheless, dividing the (a1) carriers into those who have this motif and those who do not, there was no difference between groups with regard to their association with disease risk (Khankhanian et al., 2015).

Taken together, these observations suggest that the apparent association of MS with the DRB1*1501 – DQB1*0602 HLA haplotype is not due to either of these HLA alleles but, rather, is due to something else about extended (a1) SNP haplotype. If so, then the apparent association of these two HLA alleles and MS, which has been observed for decades (Baranzini et al., 2009; De Jager et al., 2009; International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium, 2011; International Multiple Sclerosis Genetics Consortium, 2014), is presumably an artifact that is caused by the extremely tight coupling between the (a1) SNP haplotype and this particular extended HLA haplotype (Figs 11.4 and 11.6).

DRB1*0301 – DQB1*0201 HLA haplotype

This particular HLA haplotype is very tightly coupled to both the (a2) and the (a6) SNP haplotypes (Fig. 11.4). These two SNP haplotypes, however, clearly have different disease associations from each other. In the case of (a6), heterozygotes carry no risk and the pattern seems to be recessive (Fig. 11.6). By contrast, in the case of (a2), the heterozygotes do carry a significant risk and the pattern suggests either a dominant or a dose-dependent effect (Fig. 11.6).

Similar to the (a1) SNP haplotype, both the (a2) and the (a6) SNP haplotypes reside on very long extended haplotypes (Khankhanian et al., 2015). Thus, in the case of (a6), this extended haplotype includes the A*0101, C*0701, and B*0801 alleles, whereas, in the case of (a2), the extended haplotype includes the A*3002, C*0501, and B*1801 alleles (only a single case was homozygous). If only these extended haplotypes are considered, the patterns of disease association (noted above) are exaggerated (Fig. 11.6). This, again, suggests that the disease association is not due to either the DRB1*0301 – DQB1*0201 HLA haplotype or to one of these two HLA alleles individually. Rather, it seems that the disease risk must be conferred by something else about the (a2) and (a6) SNP haplotypes.

In addition, there are several general points about the genetics of MS that deserve mention, as they are material to the interactions that take place between genetic

Relationships of HLA Haplotypes with the (a1)-SNP-haplotype

SNP Haplotypes	HLA Haplotype {DRB1*1501 / DQB1*0602}	OR (1 v. 0)	OR (2 v. 0)
All		3.00 (CI = 2.85 - 3.16)	6.38 (CI = 5.59 - 7.28)
a1		3.04 (CI = 2.88 - 3.20)	6.52 (CI = 5.70 - 7.45)
a27, non-a1		0.91 (CI = 0.33 - 2.52)	na
a36, non-a1		0.67 (CI = 0.33 - 1.34)	na
non-a1		1.42 (CI = 0.98 - 2.06)	na
	*2 Copies: {DRB1*1501 / DQB1*0602}*		
a1		2.77 (CI = 1.22 - 6.29)	6.52 (CI = 5.70 - 7.45)
	*No Copies: {DRB1*1501 / DQB1*0602}*		
a1		1.97 (CI = 1.15 - 3.36)	na
	*1 Copy: {DRB1*1501 / * }*		
All		2.99 (CI = 2.84 - 3.15)	
a1		3.03 (CI = 2.87 - 3.19)	6.54 (CI = 5.72 - 7.47)
non-a1		1.52 (CI = 1.18 - 1.95)	
	*2 Copies: {DRB1*1501 / * }*		
a1		2.76 (CI = 1.59 - 4.82)	6.52 (CI = 5.76 - 7.52)

Relationships of HLA Haplotypes with the (a2) and (a6) SNP-haplotypes

SNP Haplotypes	HLA Haplotype DRB1*0301 / DQB1*0201	OR (1 v. 0)	OR (2 v. 0)
All		0.98 (CI = 0.92 -1.04)	1.67 (CI = 1.41 -1.97)
a2		1.31 (CI = 1.16 -1.48)	1.91 (CI = 0.93 -3.91)
a6		0.94 (CI = 0.89 -1.01)	1.65 (CI = 1.35 -2.02)
a2 + a1		4.61 (CI = 3.44 -6.16)	na
a6 + a1		3.89 (CI = 3.45 -4.89)	na
a2, non-a1		2.10 (CI = 1.84 -2.41)	3.64 (CI = 1.78 -7.47)
a6, non-a1		1.08 (CI = 1.00 -1.16)	2.60 (CI = 2.13 -3.19)
non-a2, non-a6		0.75 (CI = 0.40 -1.42)	na
	HLA-hyplotype including *A*3002; C*0501; B*1801*		
a2		1.73 (CI = 1.33 -2.33)	na
non-a2		0.67 (CI = 0.21 -2.13)	na
	HLA-hyplotype including *A*0101; C*0701; B*0801*		
a6		0.98 (CI = 0.91 -1.06)	2.11 (CI = 1.55 -2.89)
non-a6		0.71 (CI = 0.36 -1.41)	na

Fig. 11.6. Relationships between and the a1, a2, and a6 single-nucleotide polymorphism (SNP) haplotypes and multiple sclerosis (MS) in conjunction with different combinations of human leukocyte antigen (HLA) haplotypes. The odds ratios (OR) are given for comparing cases and controls carrying either one or two copies of the risk SNP haplotype to cases and controls carrying zero copies. In both circumstances, the disease association varied markedly, depending upon which SNP haplotype carried the HLA haplotype. Such an observation indicates that the observed disease associations were not due to these specific HLA alleles but, rather, to something else about these SNP haplotypes (see text for a discussion). CI, confidence interval.

susceptibility and environmental exposure. First, it seems that most MS cases occur in genetically susceptible individuals who experience the necessary environmental exposures (Goodin, 2016). MS very rarely develops (if at all) in the remainder of the population (i.e., in nonsusceptible individuals). Second, the prevalence of such susceptibility is, at most, 8.5% in the general population, and likely to be much less (Goodin, 2016). By contrast, the environmental factors must occur at a largely population-wide exposure level (Sadovnick et al., 1996, 2005; Ebers et al., 2000, 2004; Bager et al., 2006; Dyment et al., 2006; Goodin, 2016).

Consequently, a person's genetic make-up is, by far, the most important component of that risk. Thus, it seems that more than 92.5% of the individuals (and likely a lot more) in these different populations are genetically incapable of getting MS, regardless of what environmental events they experience during their lives (Goodin, 2016). Moreover, because the prevalence of genetic susceptibility seems to be so similar in the different regions

of Europe and North America (Goodin, 2009), it must be a difference in the environmental events which underlies the observed variations in MS epidemiology between these geographic regions. In addition, the mechanisms underlying genetic susceptibility to MS are likely to be quite complex. Thus, despite the fact that the (a1) SNP haplotype (which usually carries the DRB1*1501 allele) has the largest and most consistent association with MS susceptibility of any genetic marker (Baranzini et al., 2009; De Jager et al., 2009; International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium, 2011; International Multiple Sclerosis Genetics Consortium, 2014), the probability of being in the genetically susceptible subset is still quite small, even when this haplotype is present (Goodin, 2016). Also, 45–50% of individuals with MS lack this haplotype entirely (International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium, 2011; International Multiple Sclerosis Genetics Consortium, 2014). And, finally, the likelihood of developing MS in "genetically" susceptible individuals is approximately the same regardless of whether the susceptible genotype includes or does not include this haplotype (Goodin, 2016). As a consequence, based on all of these considerations, it seems that this particular haplotype may not be as uniquely important for MS-susceptibility as is currently believed by many.

Genetic and environmental interactions

There are important interactions between the genetic make-up of individuals and their environment, which are indicated by the observation that the proband-wise monozygotic twin concordance rate for women (0.34) is significantly larger ($p < 0.001$) than the same rate (0.065) for men (Willer et al., 2003). The consequence of this observation (if correct) is that men are 60% more likely to be genetically susceptible to getting MS than are women (Goodin, 2016). Also, a direct consequence of this difference, together with the observation that the greater current prevalence of MS is greater in women than men, is that women must be more likely to experience the necessary environmental events, more likely to get MS in response to these events, or both (Goodin, 2016). These relationships can be derived mathematically from the available epidemiologic data (Goodin, 2016) and are illustrated in Figure 11.7. Considering some of the potential environmental factors involved in MS pathogenesis, it might be that women use more sunblock or sun avoidance than men and, therefore, they experience greater vitamin D deficiency. Or it might be women have better hygiene as children than men and, thus, acquire EBV infections later in life. Alternatively,

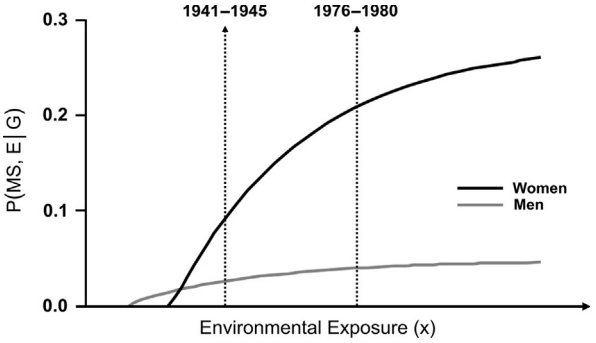

Fig. 11.7. Response curves for developing multiple sclerosis (MS), derived from a simple mathematic model (Goodin, 2009, 2016), in men and women to an increasing likelihood of a "sufficient" environmental exposure (E). Proportionate (but not necessarily equal or constant) hazards have been assumed for the two genders. The conditional probability of developing MS in a genetically susceptible individual (G) is shown on the *y*-axis. The transformed exposure level (x) is shown on the *x*-axis (Goodin, 2009, 2016). One environmental unit is defined (arbitrarily) as the change in the environmental exposure level (whatever this represents and however many factors this involves), which has taken place in Canada between the time periods of 1941–1945 and 1976–1980. Response curves are derived solely based on published epidemiologic observations (Willer et al., 2003; Orton et al., 2006). The response curve for men begins at a smaller value of x than women, but their response is almost at its plateau by 1941–1945. By contrast, women are nowhere near their (much higher) plateau in 1941–1945 and have a much steeper rise in incidence as a response to environmental changes during the interval of observation compared to men. (Adapted from Goodin, 2009, 2016.)

it could be that there are gender-specific differences in vitamin D metabolism, such that men and women experience a deficiency at different levels of absolute exposure (Suarez et al., 1998; Spach and Hayes, 2005). Alternatively, it could be that women have a greater likelihood of actually developing MS once the necessary environmental and genetic events have come together or it could be that some combination of these factors contributes to the observed gender-specific differences.

Importantly, however, not all of the current epidemiologic observations are consistent with this formulation derived from the Canadian data. Thus, a US study also looked at the monozygotic twin concordance rates in 95 men and 323 women (Islam et al., 2006). These authors identified 55 twin pairs concordant for MS (11 men and 45 women), none of whom were "doubly ascertained" (Islam et al., 2006). They estimated that their sampling methods had identified "approximately 27% of the North American twin cases prevalent at any time during the [study] period". In contrast to the Canadian data, however, these authors reported that the pair-wise concordance rate in female twin pairs of

13.9% (45/323) was not significantly different from the rate of 11.6% (11/95) in male twin pairs (Islam et al., 2006). Thus, unlike the Canadian data, these results would imply that women are substantially more likely to be genetically susceptible to MS than men (Goodin, 2016). Although such a conclusion might superficially seem to fit well with certain preconceived notions about MS susceptibility, the actual findings of this particular study are suspect for two reasons. First, the finding that the twin concordance rate in men and women is approximately equal indicates that the penetrance (i.e., the probability of actually developing MS for a susceptible individual) is similar for men and women (Goodin, 2016). Such a conclusion is hard to rationalize with the repeated observation that the prevalence of MS is increasing around the world and that this increase is predominantly or exclusively found in women (Hernán et al., 1999; Koch-Henriksen, 1999; Celius and Vandvik, 2001; Barnett et al., 2003; Sarasoja et al., 2004; Orton et al., 2006). Second, using their sampling methods, the authors found an extremely low rate of double ascertainment (i.e., zero). Low rates of double ascertainment are recognized as an indicator of a biased method of sampling (Witte et al., 1999) and, in this study, the probability of not doubly ascertaining a single twin pair is extremely unlikely by chance alone ($p < 10^{-6}$). Consequently, the sample used in this study is unquestionably biased and, therefore, the data out of Canada (where the authors report 54% double ascertainment) are likely to provide more reliable estimates (Willer et al., 2003).

Also, the data out of Canada are notable for the marked and continuous change in the gender ratio that has taken place over the 35-year interval of the study (Orton et al., 2006). This observation, together with the difference in twin concordance rate between men and women, has important implications with respect to the interaction between the gender of the individual and the environmental factors involved in MS pathogenesis (Goodin, 2016). From the gender ratio change alone, it is evident that there must have been a greater than 32% increase in the prevalence of MS in Canada over the 35-year period (Goodin, 2016). Although better diagnostic imaging and laboratory methods could contribute to such an increase, the fact that both the minimum increase in MS prevalence and the change in gender ratio are direct reflections of a disproportionate increase in MS prevalence among women (Goodin, 2016) suggests that better diagnostic techniques are an unlikely explanation. Moreover, the change in gender ratio also indicates that men begin to get MS at lower levels of environmental exposure than women (i.e., men have a lower threshold of exposure for getting the disease compared to women). Importantly, the parameter estimates for each of these effects (i.e., the increased prevalence of MS in the population and the lower threshold in men) are quite stable using any of the observed data points during the 35-year period to estimate them (Goodin, 2009).

From the observed difference in twin concordance rate between men and women, together with the two conclusions regarding disease prevalence and threshold, and assuming a proportionate hazard in men and women, the response curves for men and women to changes in their environmental exposure can be derived (Goodin, 2016). From these response curves it is apparent that, although men are more likely to be genetically susceptible than women and that they begin to get the disease at lower levels of environmental exposure, women are far more responsive the environmental changes (whatever these are), which have been taking place and that they approach a substantially higher response plateau for their probability of getting the disease (Goodin, 2016).

In addition, assuming environmental exposure levels have been increasing for longer than just the period of this study, the fact that men have a lower threshold than women suggests the possibility that, at some time in the past, MS may have been more prevalent in men than in women. Viewed in this context, it is quite interesting that a 1922 survey of MS cases in Europe and the USA (Wechsler, 1922) found that approximately 58% of cases (in each region) were men.

SUMMARY

Based on known epidemiology, it seems clear that the causal pathway leading to adult MS involves both multiple genetic susceptibility factors and, at least, three necessary (and sequential) environmental events or factors. If this is indeed the case, then, potentially, the disruption or modification of any one of these environmental factors might prevent the disease from ever occurring, regardless of an individual's genetic make-up. Of the promising candidate factors considered, the easiest to modify would be vitamin D deficiency, because supplemental vitamin D_3 is both widely available and very inexpensive. Nevertheless, any test of such a therapeutic strategy (or another similar approach directed at other factors) will require large numbers of persons to be followed for long time periods (>30 years). Randomized, placebo-controlled designs in this context are not feasible, so that testing such a notion will require the use of nonrandomized, open-label studies and applying statistical methods for bias mitigation (e.g., Rosenbaum and Rubin, 1983; Trojano et al., 2007; Goodin et al., 2011). The only requirement would be to monitor prospectively the vitamin D intake of all participating persons and, ideally, monitor their blood levels as well. Also, data could be simultaneously collected about a

variety of other candidate factors, such as EBV infection, other viral exposures, birth location, ethnicity, migration history, smoking, the occurrence of traumas, and so forth. Because such a study would require persons (both adults and their children) to be followed prospectively, realistically, it is only feasible in places that have universal healthcare (and, thus, have large population-based centralized medical records) or in communities where the population is nonmobile and where complete ascertainment and longitudinal follow-up can be accomplished. Nevertheless, such a design would be cost-effective (only information easily available needs to be captured), it could be accomplished without much difficulty (including everyone who wants to participate), and it poses no ethical dilemmas (each person, together with their physician, is free to choose what they feel is best for themselves). It also seems like extremely important information to begin to collect. Certainly, such a longitudinal observational study carries minimal risk, is inexpensive, and, potentially, could provide inestimable benefits to future patients.

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