Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis

Tomas Olsson[1], Lisa F. Barcellos[2] and Lars Alfredsson[3]

Abstract | Genetic predisposition to multiple sclerosis (MS) only explains a fraction of the disease risk; lifestyle and environmental factors are key contributors to the risk of MS. Importantly, these nongenetic factors can influence pathogenetic pathways, and some of them can be modified. Besides established MS-associated risk factors — high latitude, female sex, smoking, low vitamin D levels caused by insufficient sun exposure and/or dietary intake, and Epstein–Barr virus (EBV) infection — strong evidence now supports obesity during adolescence as a factor increasing MS risk. Organic solvents and shift work have also been reported to confer increased risk of the disease, whereas factors such as use of nicotine or alcohol, cytomegalovirus infection and a high coffee consumption are associated with a reduced risk. Certain factors — smoking, EBV infection and obesity — interact with HLA risk genes, pointing at a pathogenetic pathway involving adaptive immunity. All of the described risk factors for MS can influence adaptive and/or innate immunity, which is thought to be the main pathway modulated by MS risk alleles. Unlike genetic risk factors, many environmental and lifestyle factors can be modified, with potential for prevention, particularly for people at the greatest risk, such as relatives of individuals with MS. Here, we review recent data on environmental and lifestyle factors, with a focus on gene–environment interactions.

Multiple sclerosis (MS) is a demyelinating disease that mainly affects young adults and is characterized by repeated waves of inflammatory cells that enter the CNS. This process is often subclinical, but can also affect nerve tracts to produce signs and symptoms that are noted as clinical relapses. During the years preceding the diagnosis of MS, health care utilization[1] and chronic fatigue[2] are increased, and individuals are less likely to reproduce than the general population[3,4]; moreover, MRI already reveals several lesions in many individuals at the clinical onset of MS. These findings support the existence of subclinical MS, a phase that can last for several years. Thus, lifestyle and environmental factors that predispose an individual to MS might act years before clinical onset, complicating epidemiological studies that aim to identify risk factors for the disease.

As MS progresses, untreated relapses and neurological disabilities accumulate; eventually, most individuals with the disease enter a progressive phase (FIG. 1), and ~10% of people diagnosed with MS have a progressive disease course from the onset. Most current treatments have potential to postpone, but not stop, the deteriorating clinical course. Modern therapies act broadly on the immune system with potential for serious adverse events. Thus, effective treatments of MS remains an unmet medical need, and it is critical to obtain detailed understanding on mechanisms that allow MS to occur, preferably via studies that combine genetic risk factors with lifestyle and environmental factors. Besides providing mechanistic clues to help with development of more effective therapy, knowledge of environmental factors might aid in prevention of the disease.

Since previous extensive reviews on associations between MS risk and Epstein–Barr virus infection, vitamin D levels or sun exposure, and smoking[5,6] were published, the field of MS epidemiology has expanded. It is, therefore, timely to review the evidence again, and incorporate further developments, such as interactions of environmental and lifestyle factors with MS risk genes, and certain other risk factors that have recently been identified as potential contributors to MS risk. Here, we chose to discuss distinct lifestyle and environmental factors, focusing on those that potentially interact with established MS risk genes, starting with the most established risk factors.

[1]*Neuroimmunology Unit, Center for Molecular Medicine, L8:04, Karolinska University Hospital (Solna), 17176 Stockholm, Sweden*
[2]*Genetic Epidemiology and Genomics Laboratory, California Institute for Quantitative Biosciences (QB3), Office: 308D Stanley Hall, University of California, Berkeley, CA 94720–3220*
[3]*Institute of Environmental Medicine, Karolinska Institutet, Box 210, 171 77 Stockholm, Sweden*

Correspondence to T.O.
tomas.olsson@ki.se

doi:10.1038/nrneurol.2016.187
Published online 9 Dec 2016

Key points

- Epstein–Barr virus (EBV) infection, smoking, low vitamin D and lack of sun exposure are well established factors associated with risk of multiple sclerosis (MS); recently, adolescent obesity has been added to this list
- Less established factors include exposure to organic solvents and night shift work, which associate with increased risk, whereas oral tobacco use, cytomegalovirus infection, alcohol use and coffee consumption associate with decreased risk
- Some of these factors should be considered in primary prevention
- Most lifestyle and environmental factors seem to have the greatest effect during a particular time window — adolescence
- Certain factors, such as EBV infection, smoking and adolescent obesity interact with human leukocyte antigen MS risk genes, with substantial risk increases in individuals who carry genes that predispose them to MS
- The interaction with these immune response genes provides strong evidence that these lifestyle and environmental factors act on adaptive immunity, leading to autoimmune attack on the nervous system

Genetic data are important for characterizing pathogenetic mechanisms, and for elucidating the complex picture of disease initiation in the context of lifestyle and environmental factors. Our discussion has a particular focus on the HLA class I and II genes, which are the genetic risk factors that are most strongly associated with MS. The field of human genetics and complex disease studies required many years to reach consensus on study design and statistical power necessary to claim that a reported association is causal, and many candidate gene approaches utilizing small case–control comparisons have later been refuted[7]. Similar problems affect epidemiological studies of lifestyle and environmental factors. In this Review, we have concentrated on factors revealed in adequately powered studies, particularly those that have been replicated.

General remarks on risk factors in MS

MS is a complex disease, and besides genetic variants, lifestyle and environmental factors can be important contributors to disease risk. A combined analysis of both prominent genetic and environmental risk factors showed that a major fraction of MS risk could be explained by currently known risk factors[8]. The lifestyle and environmental factors that increase the risk of MS include exposure to tobacco smoke and organic solvents, Epstein–Barr virus (EBV) infection, adolescent obesity, lack of sun exposure or low levels of vitamin D, and working night shifts. Factors potentially associated with reduced risk include use of oral tobacco, high coffee consumption, alcohol consumption, and serological evidence of cytomegalovirus (CMV) infection (TABLE 1).

As a cautionary note, evaluation of results from observational epidemiological studies must take into consideration different potential sources of systematic errors, as observed estimates of association can be affected by biases which will influence interpretation of results. These biases can include reverse causation (the disease itself is causing the association), confounding factors that are not considered or inadequately addressed (such as residual confounding) in analytical models, differential misclassification of lifestyle and environmental factors (for example, as a result of recall bias), and selection bias.

Evidence for nongenetic factors

Previous studies of the heritability of MS have estimated that a sibling of an individual with MS has an almost 17-fold increased risk of the disease[9]. However, recent population-based studies have estimated a siblings' relative risk to be increased by 7-fold, indicating a much lower importance of genetic predisposition, and underscoring the role of environmental influences[10,11].

A prominent argument for the influence of environmental factors on the risk of MS is the latitude gradient[12]. Interestingly, migration studies show that the risk of MS depends on the age at which an individual migrates: the risk of MS for those who migrate from a low-risk country to a high-risk country before adolescence is similar to that of those who are born and reside in the high-risk country[13–16]. Another argument is that the risk of MS reduces with increasing numbers of younger siblings, possibly owing to greater exposure to common infections at young age, which has a potentially protective action and could interact with the MS-associated risk variants in the HLA complex[2,17,18].

Changing patterns of MS incidence in certain regions are not fully accounted for by changes in known MS risk factors[19]. Most countries have experienced a distinct increase of MS among women in the past few decades that has been too fast to be explained by changes in genetic composition, and is therefore likely to be explained by changes in lifestyle and environmental factors affecting women, including increased smoking and obesity, and changes in reproductive behaviour[20–23].

The immune repertoire — the spectrum of T cell antigen specificities — is likely to be important for MS, and is largely driven by nonheritable environmental factors[24]. Inflammatory comorbidities, which are common in MS, do not seem to be caused by sharing of genetic risk variants, but can be attributed to lifestyle or environmental factors. For example, we have used the Swedish multigeneration registry to investigate the diagnoses of parents of patients with MS. Although patients with MS often have other inflammatory comorbid conditions, the presence of these conditions (apart from MS) is not increased within the parent generation[25]. Indeed, only a minor fraction of non-HLA risk gene loci are also risk genes for other inflammatory diseases, and effects of single variants can regulate different diseases in opposite directions[26].

Evidence for genetic factors

HLA-associated genetic variants. Our knowledge of MS genetics has evolved dramatically recently in the past decade. Genes within the HLA complex are the strongest genetic risk factors for MS. The HLA class II and I genes are particularly relevant modifiers of disease risk: variants of class II genes encode products that present antigens to $CD4^+$ T lymphocytes, and class I products present antigens to $CD8^+$ lymphocytes. In MS, the class II variant *HLA-DRB1*15:01* has a striking

Latitude gradient
A gradual decrease in incidence and prevalence of MS from north to south in the northern hemisphere, and in the opposite direction in the southern hemisphere.

HLA complex
A region on human chromosome 6 containing ~200 genes, most of which have functions in the immune system; of these, class II genes encode molecules that bind and present peptide antigens to $CD4^+$ T_H cells, and class I genes encode molecules that present peptide antigens to $CD8^+$ cytotoxic T cells.

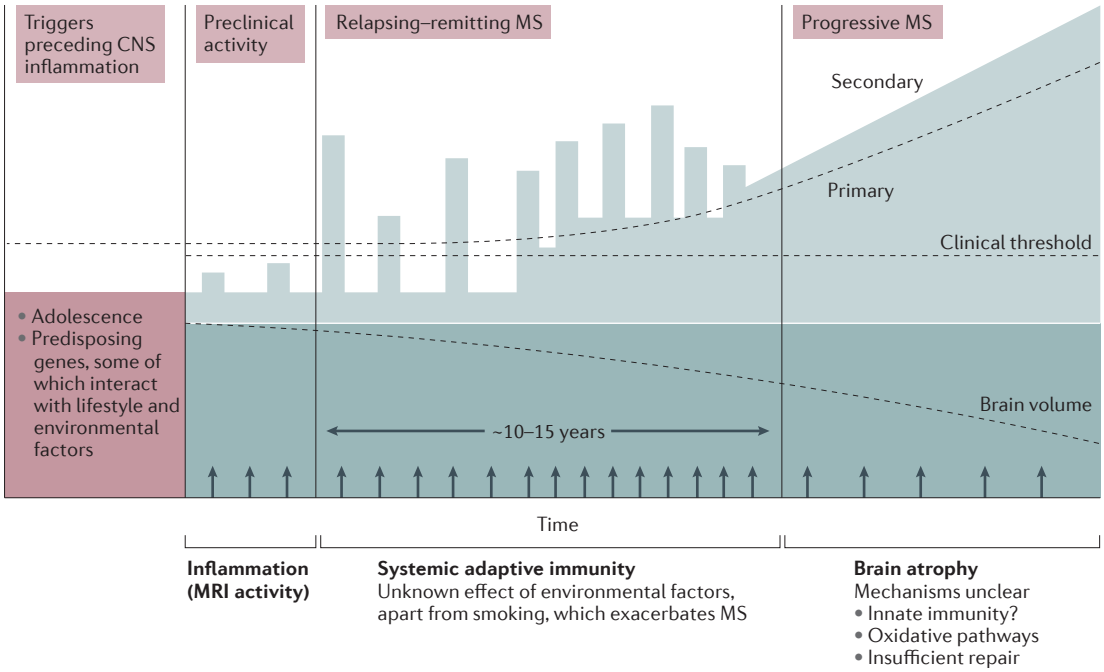

Figure 1 | **Evolution of multiple sclerosis.** Several factors that predispose to multiple sclerosis (MS) are at play during the subclinical phase of the disease, before clinically recognizable symptoms are present. The light blue area represents progression of MS, and bars denote events that cause inflammatory damage to the CNS, some of which do not reach the clinical threshold. Arrows denote episodes of new inflammation in the CNS, recorded by MRI, and are much more frequent than clinical relapses.

association with an increased risk of MS (odds ratio (OR) ~3), whereas the class I variant *HLA-A*02* is associated with protection from the disease (OR ~0.6). The absence of *HLA-A*02* and the presence of *DRB1*15:01* has a combined OR of ~5 (REFS 26–29).

Other genetic variants. Genome-wide association studies (GWAS) have detected ~110 non-HLA single nucleotide polymorphisms (SNPs), each of which confers a modest influence on MS risk; ongoing large GWAS are expected to identify more such SNPs. Nearly all of the known MS-associated SNPs are located close to genes and regulate either adaptive or innate immunity, providing further evidence that MS is primarily an immune-mediated disease. Rather than being traditional monogenic disease genetic variants, these SNPs are common variants that occur in the healthy population, and are probably selected for because they have a survival benefit, such as increased protection against infections.

Gene–environment interactions

When assessing interactions between causal factors, we have used departure from additivity of effects as a criterion in our research[30,31], which, if present, indicates action of the two factors on the same biological pathway. In simple terms, interaction is present when the risk of developing disease among those exposed to both (causal) factors is higher than the risk expected on the basis of the sum of the absolute effects attributed to each factor individually (FIG. 2).

Genome-wide association studies (GWAS)
Single-nucleotide polymorphisms (SNPs) are identified throughout the genome, usually several hundreds of thousands of SNPs, in very large case–control cohorts, allowing identification of associations between diseases and discrete genome loci.

Smoking and use of oral tobacco

Smoking

Smoking was initially suggested as a risk factor for MS by a pooled analysis of several small studies that showed an OR of ~1.5 (REFS 32,33); this result was later verified in a large case–control study[34]. Smoking and MS risk have a dose–response relationship: cumulative smoking is associated with an increase in the risk[34,35]. Elevated levels of cotinine in the serum or plasma (≥10 ng/ml), reflecting smoking, from patients before they developed MS, were associated with a similar pattern in risk increase[36]. Passive exposure to smoking has also been associated with increased risk for MS, suggesting that even minor lung irritation is important[37]. If the association between smoking and MS is caused by nonspecific lung irritation, one might even consider factors like air pollution as a trigger of CNS neuroinflammation (FIG. 3). In fact, this hypothesis has recently received some support[38], although larger replication studies are required for definitive confirmation. In addition, some evidence supports an association between organic solvent exposure and MS, possibly mediated by nonspecific lung irritation[39].

Interestingly, smoking is not only associated with an increased risk of MS, but also with the risk of developing neutralizing antibodies against biologics used in treatment of MS, including natalizumab[40] and IFN-β[41]. In addition, other inflammatory diseases, for example inflammatory myositis with Jo-1 antibodies[42] and rheumatoid arthritis[43], are associated with smoking. Thus, lung irritation could trigger immune responses to biologics and a series of organ-specific inflammatory diseases.

Table 1 | **Established and possible lifestyle and environmental risk factors for MS**

Factor	OR	HLA gene interaction	Combined OR (nongenetic factor + HLA allele)	Effect during adolescence	Immune system implied	Level of evidence
Smoking	~1.6	Yes	14	No	Yes	+++
EBV infection (seropositivity)	~3.6	Yes	~15	Yes	Yes	+++
Vitamin D level <50 nM	~1.4	No	NA	Probably	Yes	+++
Adolescent obesity (BMI >27 at age 20 years)	~2	Yes	~15	Yes	Yes	+++
CMV infection (seropositivity)	0.7	No	NA	Unknown	Yes	++
Night work	~1.7	No	NA	Yes	Yes	++
Low sun exposure	~2	No	NA	Probably	Yes	++
Infectious mononucleosis	~2	Yes	7	Yes	Yes	++
Passive smoking	~1.3	Yes	6	No	Yes	+
Organic solvent exposure	~1.5	Unknown	Unknown	Unknown	Unknown	+
Oral tobacco/nicotine	0.5	No	NA	Unknown	Yes	+
Alcohol	~0.6	No	NA	Unknown	Yes	+
Coffee	~0.7	No	NA	Unknown	Yes	+

Level of evidence for a role of a particular lifestyle or environmental factors in MS is not easy to define. Large prospective studies are, with few exceptions, rare in MS. CMV, cytomegalovirus; EBV, Epstein–Barr virus; HLA, human leukocyte antigen; MS, multiple sclerosis; NA, not applicable; OR, odds ratio; +++, high level of evidence: drawn from large prospective studies or if a case–control observation is supported by Mendelian randomization studies; ++, Case–control observations, if replicated and/or supported by independent methods; +, Non-replicated observations (included to enable further observations).

Oral tobacco

The use of oral tobacco (snuff) is very common in Sweden, making it possible to distinguish between the role of specific tobacco constituents and smoke in lung irritation. Indeed, the use of oral tobacco has a dose-dependent association with a decreased risk of MS[34,44] (assuming this association does not reflect unmeasured confounding or other biases). Nicotine stands out as a key candidate for such possible protection given that nicotine affects the α7 subunit of the acetylcholine receptor present on immune cells, thereby dampening receptor activity[45]. This observation strengthens the notion that lung inflammation itself drives the increase in risk increase, despite the apparent protective effects of nicotine.

Mechanisms and interactions with genes

Smoking provokes lung inflammation and promotes proinflammatory pathways[46]. If CNS auto-antigenic cells are present in the lung, such cells might be activated to attack the CNS; indeed, some experimental evidence supports this hypothesis: in the experimental autoimmune encephalomyelitis (EAE) rodent model of MS, encephalitogenic cells acquire a migratory phenotype in the lung, which allows them to attack the CNS[47].

Experimental autoimmune encephalomyelitis
A model disease induced in experimental animals, commonly mice or rats, by immunizing the animal with CNS components that induce an autoimmune attack against the CNS that mimics many aspects of MS; can also be induced by transfer of CNS autoreactive T cells.

Smoking interacts with HLA variants. Smoking displays a considerable interaction with MS-associated HLA risk alleles. As mentioned earlier, in the Scandinavian population, having the class II *HLA-DRB1*15:01* MS risk allele confers an OR of ~3, and lack of *HLA-A*02* confers an OR of ~1.8, resulting in a combined OR of ~5 among non-smokers; however, in smokers, the combined OR is much higher, at ~14 (REF. 48) (FIG. 2). Such a gene–environment interaction has been replicated in studies of passive smoke exposure[49]. In addition, a striking interaction between smoking and a common variant of a non-HLA gene, *NAT1* (which encodes an enzyme involved in metabolism of smoke products) has been demonstrated[50]. Thus, the effect of smoking on the risk of MS depends on the individual's HLA genotype and possibly other aspects of genetic composition. This interaction indicates a causal role of smoking in MS because, although the apparent influence of reported or actual smoking could be subject to reverse causation, recall bias or any other bias, HLA genes are unlikely to regulate smoking behaviour (FIG. 3).

The interaction of smoking with MS HLA risk alleles can shed light on disease mechanisms in MS. Smoking is also associated with rheumatoid arthritis, with interaction involving another HLA class II variant (*HLA-DRB1*04*)[43]; a similar interaction is seen between smoking, *DRB1*03* and risk of polymyositis[42]. *HLA-DRB1**04 and DRB1**03* code for antigen-presenting molecules that differ in their peptide-binding preferences. Hypothetically, T cell recognition of different sets of peptides as a result of different peptide-binding abilities of the HLA molecules could be the basis for activating autoimmune $CD4^+$ T cells directed against different organs that eventually lead to MS, rheumatoid arthritis or myositis. Furthermore, smoking is known to induce enzymes in the lung, causing post-translational modifications of peptides, such as citrinullation[43]; T cells that recognize such peptides might not have undergone

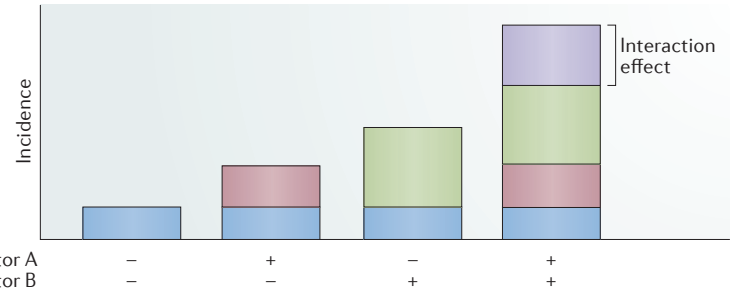

Figure 2 | **Principles of additive interactions between risk factors.** Interaction between two risk factors (A and B), is revealed when the risk of a disease is higher in the double-exposed individuals than expected on the basis of the sum of the absolute effects from each factor in the absence of the other.

clonal deletion in the thymus, resulting in autoimmune T cells in the peripheral compartment as a further step in inducing autoimmunity.

Interpretation of the protective association of the class I allele *HLA-A*02*, which encodes molecules presenting antigens to $CD8^+$ T cells, in MS is even more challenging. A role for $CD8^+$ T cells is supported by histochemical studies of brain lesions from patients with MS; in these lesions, $CD8^+$ cells dominate over $CD4^+$ cells. Although $CD8^+$ T cells can be cytotoxic, they also convey suppressor functions through production of molecules such as TGFβ[51–53]. In fact, a class I allele specific suppression mediated by $CD8^+$ cells and TGFβ has been demonstrated in EAE[54–56]. Further research on $CD8^+$ cells in relation to allelic influences is strongly warranted.

Prospects for prevention and prognostication. The smoking data also have practical implications. On the basis of Swedish data, 20.4% of all MS cases in Sweden are estimated to be attributable to smoke exposure. Among individuals who carry the genetic risk factor *HLA-DRB1*15:01* and lack the protective *HLA-A*02*, 41% of the MS cases were attributable to smoking[57]. From a public health perspective, the impact of smoking and passive smoking on MS risk is thus considerable, and preventive measures to reduce tobacco smoke exposure are therefore essential. Such preventive measures would also apply to organic solvent exposure. In particular, individuals with a family history of MS should be informed about the impact of smoking on the risk of MS, and the importance of preventing their children from being exposed to passive smoke.

Besides the risk of developing MS, history of smoking is associated with a worse prognosis in MS[58], and smoking is associated with an aggravated disease course[59–67]. In addition, continued smoking is associated with increased risk of developing neutralizing antibodies against biologics used for the treatment of MS[40,41], meaning that patients with MS should be advised to quit smoking.

Epstein-Barr virus and cytomegalovirus

Epstein-Barr virus

Many infectious agents have been proposed to have a role in MS, but one of the most interesting candidate viruses is EBV. Extensive literature supports the hypothesis that EBV infection increases the risk of MS, but as with many other factors linked to MS, it is difficult to establish a causal relationship between EBV and the disease. The collective circumstantial evidence is compelling: according to a recent meta-analysis[68], people who have had clinically overt infectious mononucleosis have a >2-fold increased risk of developing MS. Supporting an association between EBV and MS, individuals with MS have much higher levels of antibodies against EBV nuclear antigen 1 (EBNA1) and a particular fragment of it (amino acids 385–420)[69,70]. A most striking observation in a nested case–control study was that primarily all EBNA1-negative individuals had serologically converted to being EBNA1 antibody positive before MS onset[71]. Also, there seems to be a critical time window for EBV infection: only infection during adolescence or later, and not in childhood, is relevant to the risk of MS[72,73].

As potential further evidence for causality of EBV in MS, the genetic risk for elevated anti-EBNA1 titres is positively correlated with the development of MS[74]. In a pattern similar to that seen with smoking, infectious mononucleosis and increased EBNA1 antibody titres interact with HLA MS risk genetic variants[70], and infectious mononucleosis interacts with *HLA DRB1*15:01* (REF. 75), to increase the risk of MS. As the HLA risk alleles encode molecules that regulate T cell adaptive immunity, the interaction with measures of EBV infection may indicate common pathogenetic pathways in triggering MS. Similar commonality applies to the recently observed interaction between infectious mononucleosis during adolescence and MS risk, as well as obesity and MS risk (discussed below): the combination of these two risk factors results in an OR of ~14 (REF. 76).

Interestingly, EBV-related RNA has been detected in CNS lymphoid infiltrates in one study[77], although this finding has been only partially replicated in other independent studies[78]. EBV resides latent in B cells, and anti-CD20 treatments that deplete these cells have been very successful in MS[79], which has provided some support for the EBV hypothesis. Numerous different mechanisms for a role of EBV in MS could be considered, such as molecular mimicry.

Still, all these observations do not fully confirm a role for EBV in the triggering of MS. Research to confirm the EBV hypothesis has been hampered by the lack of suitable experimental models. Moreover, EBV is also very common in individuals without MS or other neuroinflammatory diseases; rather than reflecting a causal role of EBV infection, the increased antibody titres to EBNA1 could be the consequence of poor elimination of the virus owing to insufficient cell-mediated immunity against the pathogen, perhaps due to the immune–genetic setup, in people with MS. In analogy to observations in carriers of JC virus, where high antibody titres reflect poor control of viral replication that is regulated by the sets of immune genes, in particular class II genes[80], the increased humoral antibody reactivity against EBV in MS could be a attributed to MS-predisposing genes or the disease itself.

Mass vaccination against EBV, with the goal of avoiding MS later in life, has been proposed; however, the causal role of EBV is still not completely established,

Molecular mimicry
A phenomenon in which parts of a microbial agent have a molecular structure similar to a host molecule, thereby eliciting an immune response that is autoreactive against the host.

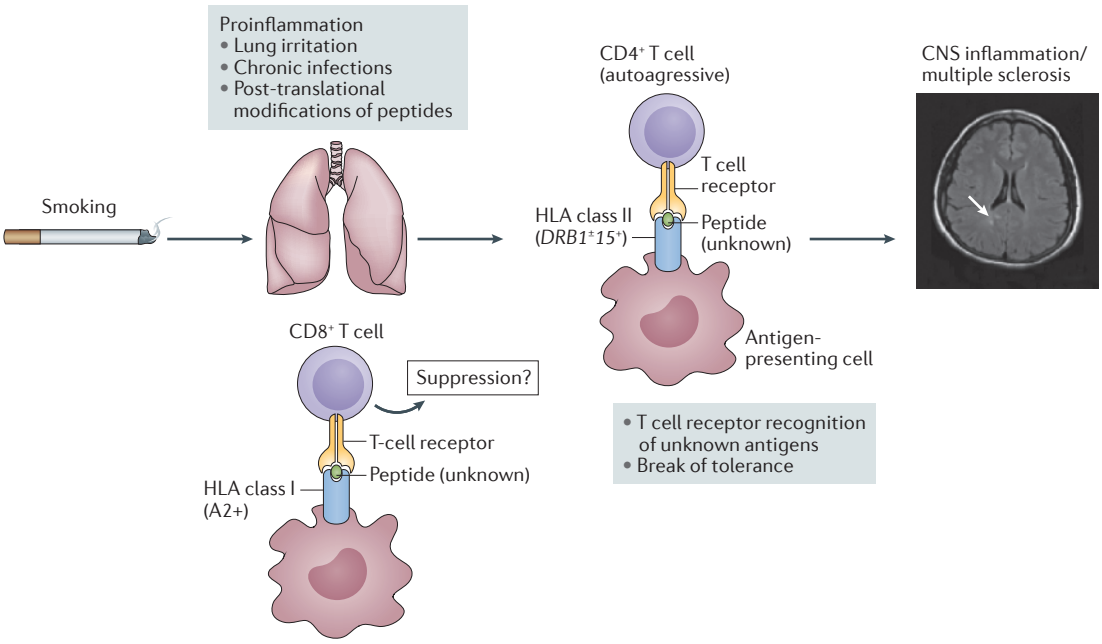

Figure 3 | **Hypothetical mechanisms of smoking-associated processes that contribute to risk of multiple sclerosis.** Smoking promotes activation of proinflammatory pathways, and alters post-translational modification of peptides. Both events can lead to activation of resident CD4⁺ CNS-antigen-specific autoimmune T cells through recognition peptides presented by *HLA-DRB1*15:01* molecules, a hypothesis supported by the fact that smoking increases the risk of rheumatoid arthritis in conjunction with *HLA-DRB1*04*. The less well understood protective role of class I *HLA-A*02* might involve CD8⁺ cells.

and even if EBV is critically involved in the development of MS, a modestly effective vaccination could postpone the most common occurrence of EBV infection from childhood to adolescence, which seems to be a sensitive period for lifestyle and environmental factors in MS, and therefore could have a negative influence on the prevention of MS. The role of EBV in MS needs to be clarified before practical issues related to MS care or prevention can be pursued and addressed.

Cytomegalovirus

CMV belongs to the same family of herpes viruses as EBV. CMV is a common virus, and CMV seroprevalence increases almost linearly with age[81]. Infection by CMV is mostly asymptomatic. Several underpowered studies have failed to identify any association between seropositivity and MS risk, though a negative association was first described in paediatric MS[73,82,83]. We have replicated this finding in an adult MS case–control cohort, and in a meta-analysis of previously published underpowered studies; together, these findings indicate a history of CMV infection (defined by seropositivity) to reduce the risk of MS with an OR of ~0.7 (REF. 84). In line with these findings, a recent study showed a lower rate of conversion to MS after a first neuroinflammatory attack among CMV-seropositive individuals than among those who were negative for CMV, with a hazard ratio of 0.42 (REF. 73). Some small cohort studies have reported no apparent difference in CMV serology between patients with MS and controls[85–87], but in view of the modestly lower ORs recorded in the large case–control cohorts [85], the size of these cohorts, primarily studying the role of EBV, are insufficient to allow detection of associations. As a cautionary note, lifestyle changes in the preclinical phase of MS cannot be completely excluded as a cause of the observed difference in CMV serology in incident MS cases, though this scenario seems unlikely.

The reasons for the potential protective effects of CMV remain unclear and speculative. CMV infection leads to an intense immune response in which a large part of the T cell repertoire becomes occupied as they develop CMV specificities, with profound developmental influences on the immune system[88]. At this point in time, the CMV observations have no practical implications in MS care or prevention.

Sun exposure and vitamin D levels

Epidemiological observations of a latitude-dependent variation in MS incidence and prevalence[12,20] — although confounded by the increased prevalence of the MS-predisposing *HLA DRB1*15:01* allele enriched in the Northern gradients — have provoked a large number of studies investigating sun exposure and vitamin D in the risk of MS. We refer the reader to other extensive reviews on the topic[6,89].

Ultraviolet radiation

Conversion of vitamin D to its active metabolite is dependent on ultraviolet radiation (UVR), making it very difficult to distinguish between the effects of UVR and vitamin D, or determine whether they are mutually non-exclusive. Both UVR and vitamin D have been shown to be important factors in protecting against MS, as recently and extensively reviewed[6]. Increased UVR

exposure is related to a decreased risk of MS[12,90,91] and, even after correcting for vitamin D levels, UVR exposure habits are associated with the risk of MS[91]; however, this finding should be interpreted with caution, as vitamin D levels were measured at diagnosis and not during the preclinical phase of MS.

The physiological basis of a potential protective effect of UVR is not completely understood. In the EAE rodent model, UVR exposure protects against neuroinflammation independently of vitamin D[92]. Moreover, UVR exposure reduces peripheral inflammation experimentally in mice[93] with an increased T regulatory (T_{REG}) cell activation, with effects on antigen-presenting dendritic cells[94,95], potentially by promoting production of *cis*-uronic acid[96].

Vitamin D

In a pioneering study, Munger *et al.*[97] demonstrated that increasing vitamin D levels, in particular before the age of 20 years, is associated with a decreased risk of MS later in life, and later data on supplementation and sun exposure have supported the role of vitamin D in reducing the risk of MS[98,99]. In addition, a diet rich in fatty fish that contains vitamin D also reduces MS risk in individuals with low sunlight exposure[100]. Recently, high vitamin D levels were shown to correlate with decreased axonal damage, as assessed by cerebrospinal fluid (CSF) neurofilament light chain levels in people with MS[101].

To assess the effect of maternal vitamin D levels during pregnancy and the risk of MS in offspring, we obtained blood samples that had been collected from babies at birth and stored for diagnosis of phenylketonuria. We observed no difference between patients with MS and population-based non-MS controls, though it should be noted that the confidence intervals in this study were quite large[102]. This finding is in line with an EAE animal model study which demonstrated that disease incidence and course is only affected by vitamin D levels in adolescent rats, but not during pregnancy or in adult rats[103]. However, these findings are not undisputed: in another study, low vitamin D levels in mothers, sampled during the first trimester, resulted in a twofold increase in the risk of MS in the offspring[104]. Of many possible explanations for these results, the different timing of sampling, and/or a potential 'inherited' difference in sun exposure behaviours are two likely reasons. Interestingly, epidemiological studies of sun exposure in Australia have provided evidence for an association between low exposure in mothers during the first trimester and risk of MS in the offspring[105]. In any case, it seems that vitamin D and/ or sun exposure in adolescence is an important modulator of future MS risk, and that vitamin supplementation during this period might mitigate MS risk to some extent.

Involvement of genetic variants. The role of vitamin D in MS is also supported by findings from genetic studies. For example, polymorphisms close to a central vitamin D metabolism enzyme gene *CYP27B1* are associated with increased risk of MS[28,106]. Moreover, two case–control studies have demonstrated that genes regulating vitamin D levels have a notable effect on the risk of MS[107,108]. Given that the distribution of such genetic variants is random, it represents a form of blinded clinical trial, or Mendelian randomization[107,108]. Interestingly, on the basis of *in vitro* studies, vitamin D was proposed as the first example of a gene–environment interaction involving the strongest genetic risk factor for MS, *HLA DRB1*15:01* (REF. 109), but this finding has not been reproduced in case–control studies[91].

Prospects for prevention and treatment. Vitamin D supplementation is non-toxic up to high levels (no upper limit has been defined), and at the moment it seems reasonable to conduct large clinical trials of vitamin D supplementation in individuals at risk of MS, for example those who have close relatives with the disease. Given that vitamin D seems to reduce risk of not only MS but also a number of other diseases, we consider vitamin D supplementation at relatively high doses recommendable for all adolescents. Making recommendations for sun exposure is more difficult because excessive exposure to sun increases the risk of developing skin cancer. However, a modest exposure to sunlight seems fair to recommend, and sun exposure within current cancer prevention guidelines should be safe.

It is not known whether vitamin D and/or sun exposure might also have a therapeutic effect on established MS. Despite many studies in which vitamin D has been added to conventional therapy, this important question has not been resolved. However, high vitamin D levels are associated with decreased axonal injury, as measured by CSF neurofilament light chain[101], and during an IFN-β trial, levels of vitamin D negatively correlated with MRI-evidenced disease activity and disease progression[110,111].

Although the jury is still out regarding therapy in individuals with established MS, many patients with MS know about the relationship between vitamin D and MS risk, and use supplementation anyhow, especially during winter time. In the absence of clinical guidelines based on evidence from randomized controlled trials, a wide range of dosages is used. It is essential to ensure that supplementation does not preclude use of specific MS treatment.

Early-life obesity and BMI

Growing evidence from studies in the past 7 years strongly supports a role for obesity in the risk of MS. Large cohort studies have associated obesity during adolescence with future risk of MS in females[112,113]. Our study replicated this finding with an OR of ~2 in both males and females, whereas a high adult BMI at diagnosis had no influence[114]. This observation withstands adjustment in models for other lifestyle and environmental factors such as smoking, sun exposure habits, and EBV status[115]. The association is strongest for a BMI >27, although being more modestly overweight is also associated with increased risk of MS. Obesity is also associated with increased risk of paediatric-onset MS[113,116]. According to our observation, adolescence seems to be the critical period in which weight affects

Mendelian randomization
A method to determine causal effects of modifiable factors that takes advantage of the fact that gene variants for certain traits are independently segregated and randomly assigned at meiosis, thereby minimizing bias such as confounding.

the risk of MS in adulthood, as a high BMI at 10 years of age was not associated with future disease risk[117]. Largely similar observations in Norwegian and Italian populations were published just 1 year ago[118], again supporting the idea of a critical ontological time window during which a risk factor has the most marked effect.

Moreover, Mendelian randomization studies show that genetic determinants for high BMI are associated with increased MS risk. These studies bypass many potential confounders and bias related to reverse causality, and provide strong support for a causal role of obesity in MS[119,120]. Although the association between obesity and MS risk is well established, whether high BMI influences disease course is not known. As with smoking and EBV, BMI interacts with HLA genetic variants: individuals with a high BMI who carry *DRB1*15:01* and do not have the protective *HLA-A*02* have a ~14-fold increased risk of MS[114], providing further support for the hypothesis that risk factors affect common biological pathways involving inflammation and adaptive immunity, as well as support for a causal role of obesity.

The detailed mechanistic pathways through which obesity is involved in MS pathogenesis are far from clear. We propose that at least three pathways are involved; these pathways are mutually non-exclusive and partly overlap with each other. First, obesity is characterized by a low-grade inflammation in which increased levels of proinflammatory mediators are produced in the fat tissue[121,122]; indeed, promotion of T_H1-promoting immune responses, which involve production of cytokines (including IFN-γ and tumour necrosis factor α) and decreased function of T_{REG} cells, have been described in obesity[123]. Second, obesity is associated with increased levels of leptin, a mediator connected to proinflammatory processes[123,124]. Third, obesity also leads to decreased bioavailability of vitamin D, which further promotes proinflammatory processes[125]. Any of these potential mechanisms can enhance the activation of adaptive autoreactive immune cells, which can trigger bouts of neuroinflammatory activity. The facts that HLA genes encode the antigen-presenting molecules necessary for activation of T cells and interact with obesity to confer MS risk further support the notion that obesity predisposes to MS via promoting adaptive immunity that is relevant to the neuroinflammation in MS.

The relevance of obesity in relation to a putative immune attack on the CNS is also strongly supported by the observed interaction between EBV or infectious mononucleosis and BMI: a history of either EBV infection or obesity alone increases the risk of MS twofold, but a combination of both factors increases the risk 14-fold[76] independent of the contribution of the *HLA-DRB1*15:01* class II risk allele. The reasons for this interaction remain speculative. Obesity might compromise the immune response against EBV, given that obesity is associated with reduced immune defence against infections in general[126,127]. Another explanation could be that a combination of the obesity-associated proinflammatory milieu and the currently undefined effects of EBV that predispose to MS would together exacerbate the risk of neuroinflammation. We argue that the mere fact that interaction between two MS risk factors, EBV and obesity, is associated with MS, support an argument for a causal role for both of them in triggering the onset of MS.

The data on obesity and MS could have a direct bearing on prevention of the disease, especially for individuals at high risk of MS, such as children of individuals with MS, or other close relatives. The data discussed above are also of interest in relation to the global obesity epidemic and could be one important factor in the increase in incidence of MS among women.

Shift work

Shift work has been linked to an increased risk of developing autoimmune thyroid disorders[128]. We have explored the association of shift work with MS in two different studies. In the first, shift work during adolescence (before the age of 20 years) increased the risk of MS ~1.7-fold[129]. In a recent replication study, we also found significant associations beyond the age of 20 years, although these associations were not as strong as those observed in the first study[130]. In mouse experiments, living in complete darkness disturbed diurnal rhythms, affected the melatonin oscillations, and aggravated collagen-induced arthritis, which, like MS, is an organ-specific inflammatory disease[131]. A study published in 2015 investigated the role of melatonin, which inhibited the differentiation of T cells into pathogenic T_H17 cells *in vitro* via several molecular mechanisms. In the EAE model, administering melatonin ameliorated the neuroinflammatory disease[132]. However, seemingly by contrast, epiphysioectomy ameliorates experimental arthritis[133]. Despite the contrasting findings, melatonin dysregulation could provide a molecular rationale for associations of shift work with MS. Avoiding night shift work, particularly in individuals at high risk of MS, is another modifiable lifestyle factor that could help prevent the disease.

Alcohol and coffee

Alcohol

Studies assessing the role of alcohol and coffee consumption in MS have yielded inconsistent results. In general, prospective risk factor studies are preferable because they are not prone to recall bias and reverse causation; however, they also usually have the weakness of observing only a low number of incident cases. In one prospective study, no impact of caffeine or alcohol on MS risk was found[134]. Our large case–control studies suggested a dose-dependent, inverse association between MS and alcohol use, with ORs in the range of 0.7–0.8 (REF. 135). A dose-dependent inverse association has also been described between alcohol use and rheumatoid arthritis[136]. The data do not provide any arguments for individuals at risk of MS to refrain from alcohol.

Coffee

Coffee consumption and MS risk was recently investigated in two independent population-based case–control cohorts[137]. In individuals who reported a high coffee consumption (>900 ml of coffee daily), risk of MS

Epiphysioectomy
Surgical removal of the epiphysis (also known as the pineal gland), the main source of melatonin.

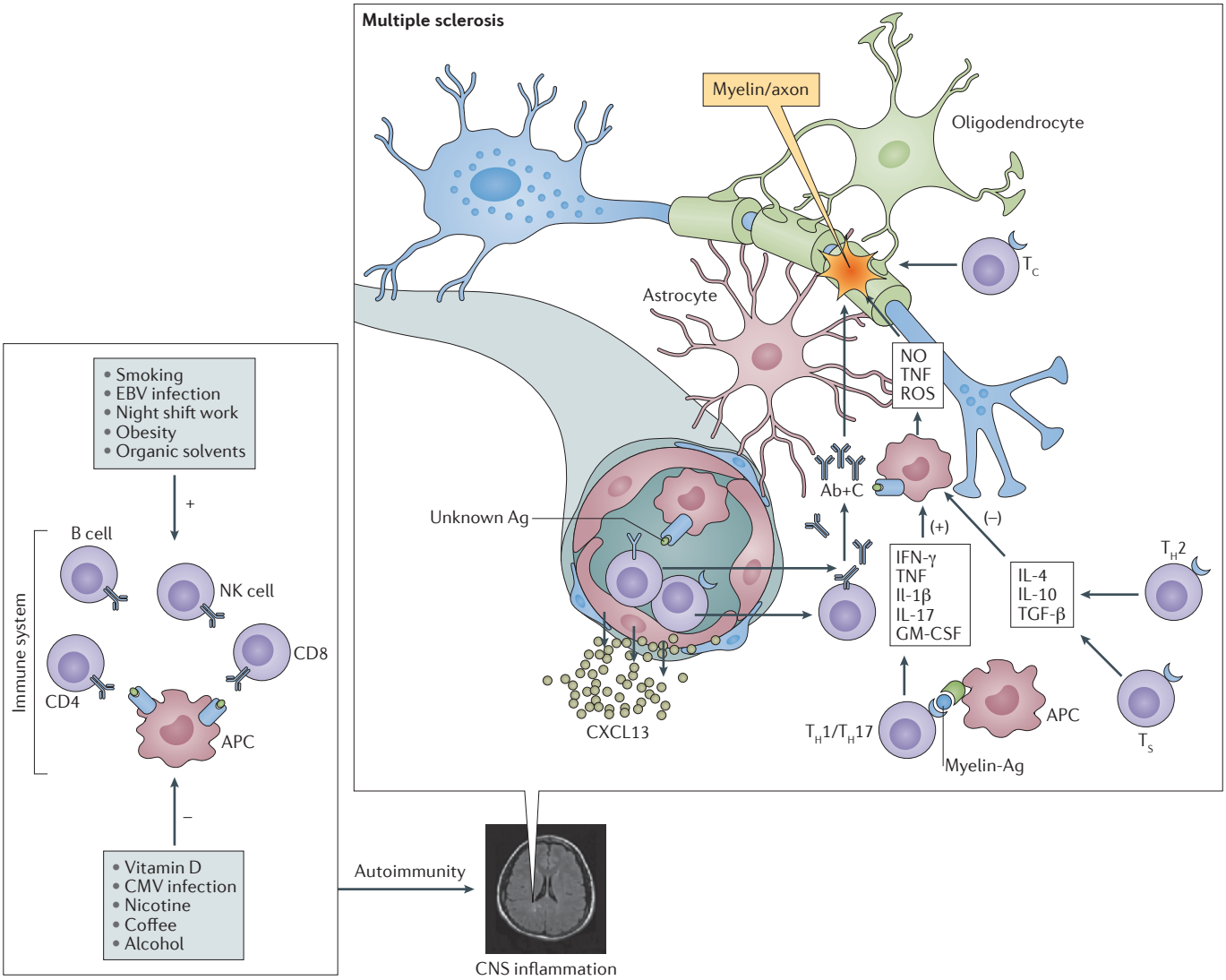

Figure 4 | **Lifestyle and environmental factors affect the immune system to trigger and/or perpetuate multiple sclerosis.** Depicted here are lifestyle and environmental factors that can affect immune system function, which can lead to neuroinflammatory attack on the CNS and eventually lead to multiple sclerosis. APC, antigen-presenting cells; Ab, antibody; C, complement; CMV, cytomegalovirus; EBV, Epstein–Barr virus; GM-CSF, granulocyte monocyte colony stimulating factor; myelin-Ag, myelin antigens; NO, nitric oxide; ROS, reactive oxygen species; T_C, T cytotoxic cell; TGF-β, transforming growth factor β; T_H, T helper cell; TNF, tumour necrosis factor; T_S, T suppressor cell.

was reduced by ~30%[137]. In animal models of MS, caffeine decreases the risk of developing neuroinflammation, and has neuroprotective[138] and anti-inflammatory properties[139]. Currently, the available data are too weak to support any recommendations regarding coffee and risk of MS.

Dietary salt and microbiota

Salt

3 years ago, salt came up as a potential trigger of MS. *In vitro* experiments showed that high salt conditions activated serum/glucocorticoid-regulated kinase 1, promoting T-cell differentiation into pathogenic T_H17 cells, and mice who consumed a diet very high in salt developed a more severe course of EAE[140,141]. However, the amount of dietary salt intake in these experiments would corresponded to >500 g of salt daily in humans, as pointed out by Gareth Pryce in a comment on the original article[141] in PubMed. Human prospective or case–control studies of salt intake and diets in general are notoriously difficult to design; however, in a small study in an Argentinian population, individuals with MS who had high salt intake had markedly more relapses and MRI-evidenced disease activity than did those with a low salt consumption[142]. Further studies are required to establish whether salt intake at everyday levels indeed affects MS risk or disease course before any broad public recommendations can be made.

Microbiota

In transgenic mice that express T-cell receptors for a neuroantigen, T cells can become activated and cause MS-like neuroinflammation. Such transgenic mice that

are free from bacteria, however, did not develop neuroinflammation, suggesting that bacteria in the gut takes part in triggering activation of adaptive immune cells to attack the CNS[143]. Furthermore, spontaneous EAE developed differently in such mice when kept at different animal facilities, suggesting that different strains of bacteria differ in their propensity to cause neuroinflammation[143]. Together, these findings support hypotheses that the type and/or distribution of gut microbiota could affect the risk and/or course of MS. Human data to support the influence of microbiota are largely lacking, and such effects are not easy to study given the enormous number of different bacterial species in the gut, many which are unknown, and difficulty in linking a specific flora to the triggering and perpetuation of neuroinflammation in MS, as any agent found in patients with MS can be argued to be an epiphenomenon and/or secondary to disease. However, with the striking animal data and technical developments that are facilitating studies of the microbiome, it is highly likely that we will see many studies on this topic during the years ahead. Partly related to this are the observations that parasitic infections can suppress symptoms of MS, perhaps through induction of T_{REG} cells and dampening of proinflammatory cytokine production[144].

Epigenetics

In the years to come, epigenetic studies will have an important role in providing a link between genetics and lifestyle and environmental factors. Different cell types, including those in the immune system and the CNS, exhibit distinct patterns of modifications to their genome, such as DNA methylation and post-translational histone modifications. The modifications constitute epigenetic mechanisms that orchestrate activity of the genome in response to external signals and lead to unique transcriptional profiles and cellular phenotypes. Variation in DNA methylation patterns is in part explained by the genotype[145,146], and partly by environmental exposures such as smoking[147]. Epigenetic mechanisms might, therefore, mediate the effect of genetic variation and environmental exposures, alone or in combination, to convey altered cellular phenotype in diseases such as MS. The first studies investigating global and genome-wide changes in epigenetic modifications have revealed some differences between patients with MS and controls[148–154]. However, much larger studies are necessary to investigate dynamic epigenome changes in patients with MS at a cellular level and in relation to the underlying genetic sequence and environmental exposures. Such studies can improve our understanding of different pathogenetic mechanisms on the molecular level and provide new therapeutic options based on epigenome editing.

Conclusions

An increasing number of lifestyle and environmental factors have been defined that can trigger and exacerbate MS (FIG. 4). Interaction studies that assess the combined influence of genetic and environmental factors have contributed to the understanding of MS; factors that interact with genetic risk factors for MS, mainly HLA variants, are likely to share aetiological pathways underlying the disease, and argue for their action on the immune system. This applies to smoking, EBV infection and adolescent obesity. The mechanistic understanding is still in its infancy, though the influence of the vast majority of lifestyle and environmental factors defined so far can be traced to their effects on the immune system, as can the genetic predisposing factors, which strongly supports the argument that the peripheral immune system has a primary role in driving MS. Some of the factors identified so far have important population-level implications for the risk of developing MS. In any case, knowledge about protective and disease-triggering factors can increasingly be incorporated into practical health care and perhaps prevention, particularly for individuals who have a family history of MS and are therefore at increased risk of the disease.

1. Marrie, R. A., Yu, N., Wei, Y., Elliott, L. & Blanchard, J. High rates of physician services utilization at least five years before multiple sclerosis diagnosis. *Mult. Scler.* **19**, 1113–1119 (2013).
2. Hughes, A. M. *et al.* Early-life hygiene-related factors affect risk of central nervous system demyelination and asthma differentially. *Clin. Exp. Immunol.* **172**, 466–474 (2013).
3. Hedstrom, A. K., Hillert, J., Olsson, T. & Alfredsson, L. Reverse causality behind the association between reproductive history and MS. *Mult. Scler.* **20**, 406–411 (2014).
4. Nielsen, N. M. *et al.* Reproductive history and risk of multiple sclerosis. *Epidemiology* **22**, 546–552 (2011).
5. Ascherio, A., Munger, K. L. & Lunemann, J. D. The initiation and prevention of multiple sclerosis. *Nat. Rev. Neurol.* **8**, 602–612 (2012).
6. Lucas, R. M., Byrne, S. N., Correale, J., Ilschner, S. & Hart, P. H. Ultraviolet radiation, vitamin D and multiple sclerosis. *Neurodegener. Dis. Manag.* **5**, 413–424 (2015).
7. Sawcer, S. The complex genetics of multiple sclerosis: pitfalls and prospects. *Brain* **131**, 3118–3131 (2008).
8. van der Mei, I. *et al.* Population attributable fractions and joint effects of key risk factors for multiple sclerosis. *Mult. Scler.* **22**, 461–469 (2016).
9. O'Gorman, C., Lin, R., Stankovich, J. & Broadley, S. A. Modelling genetic susceptibility to multiple sclerosis with family data. *Neuroepidemiology* **40**, 1–12 (2013).
10. Westerlind, H. *et al.* Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden. *Brain* **137**, 770–778 (2014).
11. Westerlind, H. *et al.* Identity-by-descent mapping in a Scandinavian multiple sclerosis cohort. *Eur. J. Hum. Genet.* **23**, 688–692 (2015).
12. Simpson, S. *et al.* Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J. Neurol. Neurosurg. Psychiatry* **82**, 1132–1141 (2011).
13. Gale, C. R. & Martyn, C. N. Migrant studies in multiple sclerosis. *Prog. Neurobiol.* **47**, 425–448 (1995).
14. Berg-Hansen, P. *et al.* Prevalence of multiple sclerosis among immigrants in Norway. *Mult. Scler.* **21**, 695–702 (2015).
15. Ahlgren, C., Oden, A. & Lycke, J. A nationwide survey of the prevalence of multiple sclerosis in immigrant populations of Sweden. *Mult. Scler.* **18**, 1099–1107 (2012).
16. Ahlgren, C., Lycke, J., Oden, A. & Andersen, O. High risk of MS in Iranian immigrants in Gothenburg, Sweden. *Mult. Scler.* **16**, 1079–1082 (2010).
17. Montgomery, S. M., Lambe, M., Olsson, T. & Ekbom, A. Parental age, family size, and risk of multiple sclerosis. *Epidemiology* **15**, 717–723 (2004).
18. van der Mei, I. A. *et al.* Human leukocyte antigen-DR15, low infant sibling exposure and multiple sclerosis: gene-environment interaction. *Ann. Neurol.* **67**, 261–265 (2010).
19. Barnett, M. H., McLeod, J. G., Hammond, S. R. & Kurtzke, J. F. Migration and multiple sclerosis in immigrants from United Kingdom and Ireland to Australia: a reassessment. III: risk of multiple sclerosis in UKI immigrants and Australian-born in Hobart, Tasmania. *J. Neurol.* **263**, 792–798 (2016).
20. Koch-Henriksen, N. & Sorensen, P. S. The changing demographic pattern of multiple sclerosis epidemiology. *Lancet Neurol.* **9**, 520–532 (2010).
21. Westerlind, H. *et al.* New data identify an increasing sex ratio of multiple sclerosis in Sweden. *Mult. Scler.* **20**, 1578–1583 (2014).
22. Ponsonby, A. L. *et al.* Offspring number, pregnancy, and risk of a first clinical demyelinating event: the AusImmune Study. *Neurology* **78**, 867–874 (2012).
23. Magyari, M., Koch-Henriksen, N., Pfleger, C. C. & Sorensen, P. S. Reproduction and the risk of multiple sclerosis. *Mult. Scler.* **19**, 1604–1609 (2013).
24. Brodin, P. *et al.* Variation in the human immune system is largely driven by non-heritable influences. *Cell* **160**, 37–47 (2015).
25. Roshanisefat, H., Bahmanyar, S., Hillert, J., Olsson, T. & Montgomery, S. Shared genetic factors may not explain the raised risk of comorbid inflammatory diseases in multiple sclerosis. *Mult. Scler.* **18**, 1430–1436 (2012).
26. Sawcer, S. *et al.* Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* **476**, 214–219 (2011).

27. Brynedal, B. *et al. HLA-A* confers an *HLA-DRB1* independent influence on the risk of multiple sclerosis. *PLoS ONE* **2**, e664 (2007).
28. Beecham, A. H. *et al.* Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* **45**, 1353–1360 (2013).
29. Moutsianas, L. *et al.* Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat. Genet.* **47**, 1107–1113 (2015).
30. Rothman, K. J., Greenland, S. & Walker, A. M. Concepts of interaction. *Am. J. Epidemiol.* **112**, 467–470 (1980).
31. Rothman, K. J., Greenland, S. & Lash, T. L. (eds) in *Modern Epidemiology*. 3rd edn Ch. 3 pp. 71–83 (Lippincott Williams & Wilkins, 2008).
32. Hawkes, C. H. Smoking is a risk factor for multiple sclerosis: a metanalysis. *Mult. Scler.* **13**, 610–615 (2007).
33. Handel, A. E. *et al.* Smoking and multiple sclerosis: an updated meta-analysis. *PLoS ONE* **6**, e16149 (2011).
34. Hedstrom, A. K., Baarnhielm, M., Olsson, T. & Alfredsson, L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology* **73**, 696–701 (2009).
35. Ghadirian, P., Dadgostar, B., Azani, R. & Maisonneuve, P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis. *Can. J. Public Health* **92**, 281–285 (2001).
36. Salzer, J. *et al.* Smoking as a risk factor for multiple sclerosis. *Mult. Scler.* **19**, 1022–1027 (2013).
37. Hedstrom, A. K., Baarnhielm, M., Olsson, T. & Alfredsson, L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. *Mult. Scler.* **17**, 788–793 (2011).
38. Heydarpour, P. *et al.* Potential impact of air pollution on multiple sclerosis in Tehran, Iran. *Neuroepidemiology* **43**, 233–238 (2014).
39. Barragan-Martinez, C. *et al.* Organic solvents as risk factor for autoimmune diseases: a systematic review and meta-analysis. *PLoS ONE* **7**, e51506 (2012).
40. Hedstrom, A. *et al.* Smokers run increased risk of developing anti-natalizumab antibodies. *Mult. Scler.* **20**, 1081–1085 (2013).
41. Hedstrom, A. K. *et al.* Smoking and risk of treatment-induced neutralizing antibodies to interferon β-1a. *Mult. Scler.* **20**, 445–450 (2014).
42. Chinoy, H. *et al.* Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. *Ann. Rheum. Dis.* **71**, 961–965 (2012).
43. Klareskog, L., Catrina, A. I. & Paget, S. Rheumatoid arthritis. *Lancet* **373**, 659–672 (2009).
44. Hedstrom, A. K., Hillert, J., Olsson, T. & Alfredsson, L. Nicotine might have a protective effect in the etiology of multiple sclerosis. *Mult. Scler.* **19**, 1009–1013 (2013).
45. Nizri, E. *et al.* Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of T_H1 and T_H17 responses. *J. Immunol.* **183**, 6681–6688 (2009).
46. Shan, M. *et al.* Lung myeloid dendritic cells coordinately induce T_H1 and T_H17 responses in human emphysema. *Sci. Transl. Med.* **1**, 4ra10 (2009).
47. Odoardi, F. *et al.* T cells become licensed in the lung to enter the central nervous system. *Nature* **488**, 675–679 (2012).
48. Hedstrom, A. K. *et al.* Smoking and two human leukocyte antigen genes interact to increase the risk for multiple sclerosis. *Brain* **134**, 653–664 (2011).
49. Hedstrom, A. K. *et al.* Interaction between passive smoking and two HLA genes with regard to multiple sclerosis risk. *Int. J. Epidemiol.* **43**, 1791–1798 (2014).
50. Briggs, F. B. *et al.* Smoking and risk of multiple sclerosis: evidence of modification by *NAT1* variants. *Epidemiology* **25**, 605–614 (2014).
51. Friese, M. A. & Fugger, L. Autoreactive $CD8^+$ T cells in multiple sclerosis: a new target for therapy? *Brain* **128**, 1747–1763 (2005).
52. Friese, M. A. *et al.* Opposing effects of HLA class I molecules in tuning autoreactive $CD8^+$ T cells in multiple sclerosis. *Nat. Med.* **14**, 1227–1235 (2008).
53. Dendrou, C. A., Fugger, L. & Friese, M. A. Immunopathology of multiple sclerosis. *Nat. Rev. Immunol.* **15**, 545–558 (2015).
54. Mustafa, M. *et al.* The major histocompatibility complex influences myelin basic protein 63-88-induced T cell cytokine profile and experimental autoimmune encephalomyelitis. *Eur. J. Immunol.* **23**, 3089–3095 (1993).
55. Mustafa, M. *et al.* Protective influences on experimental autoimmune encephalomyelitis by MHC class I and class II alleles. *J. Immunol.* **153**, 3337–3344 (1994).
56. Issazadeh, S., Kjellen, P., Olsson, T., Mustafa, M. & Holmdahl, R. Major histocompatibility complex-controlled protective influences on experimental autoimmune encephalomyelitis are peptide specific. *Eur. J. Immunol.* **27**, 1584–1587 (1997).
57. Hedstrom, A. K., Olsson, T. & Alfredsson, L. Smoking is a major preventable risk factor for multiple sclerosis. *Mult. Scler.* **22**, 1021–1026 (2016).
58. Sundstrom, P. & Nystrom, L. Smoking worsens the prognosis in multiple sclerosis. *Mult. Scler.* **14**, 1031–1035 (2008).
59. Correale, J. & Farez, M. F. Smoking worsens multiple sclerosis prognosis: two different pathways are involved. *J. Neuroimmunol.* **281**, 23–34 (2015).
60. Di Pauli, F. *et al.* Smoking is a risk factor for early conversion to clinically definite multiple sclerosis. *Mult. Scler.* **14**, 1026–1030 (2008).
61. Healy, B. C. *et al.* Smoking and disease progression in multiple sclerosis. *Arch. Neurol.* **66**, 858–864 (2009).
62. Hernan, M. A. *et al.* Cigarette smoking and the progression of multiple sclerosis. *Brain* **128**, 1461–1465 (2005).
63. Pittas, F. *et al.* Smoking is associated with progressive disease course and increased progression in clinical disability in a prospective cohort of people with multiple sclerosis. *J. Neurol.* **256**, 577–585 (2009).
64. Manouchehrinia, A. *et al.* Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. *Brain* **136**, 2298–2304 (2013).
65. Zivadinov, R. *et al.* Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. *Neurology* **73**, 504–510 (2009).
66. Manouchehrinia, A., Weston, M., Tench, C. R., Britton, J. & Constantinescu, C. S. Tobacco smoking and excess mortality in multiple sclerosis: a cohort study. *J. Neurol. Neurosurg. Psychiatry* **85**, 1091–1095 (2014).
67. Ramanujam, R. *et al.* Effect of smoking cessation on multiple sclerosis prognosis. *JAMA Neurol.* **72**, 1117–1123 (2015).
68. Handel, A. E. *et al.* An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS ONE* **5**, e12496 (2010).
69. Sundstrom, P., Nystrom, M., Ruuth, K. & Lundgren, E. Antibodies to specific EBNA-1 domains and *HLA DRB1*1501* interact as risk factors for multiple sclerosis. *J. Neuroimmunol.* **215**, 102–107 (2009).
70. Sundqvist, E. *et al.* Epstein-Barr virus and multiple sclerosis: interaction with HLA. *Genes Immun.* **13**, 14–20 (2012).
71. Levin, L. I., Munger, K. L., O'Reilly, E. J., Falk, K. I. & Ascherio, A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. *Ann. Neurol.* **67**, 824–830 (2010).
72. Ascherio, A. & Munger, K. L. EBV and autoimmunity. *Curr. Top. Microbiol. Immunol.* **390**, 365–385 (2015).
73. Makhani, N. *et al.* Viral exposures and MS outcome in a prospective cohort of children with acquired demyelination. *Mult. Scler.* **22**, 385–388 (2016).
74. Zhou, Y. *et al.* Genetic loci for Epstein-Barr virus nuclear antigen-1 are associated with risk of multiple sclerosis. *Mult. Scler.* (2016).
75. Nielsen, T. R. *et al.* Effects of infectious mononucleosis and *HLA-DRB1*15* in multiple sclerosis. *Mult. Scler.* **15**, 431–436 (2009).
76. Hedstrom, A. K., Lima Bomfim, I., Hillert, J., Olsson, T. & Alfredsson, L. Obesity interacts with infectious mononucleosis in risk of multiple sclerosis. *Eur. J. Neurol.* **22**, 578–e538 (2015).
77. Serafini, B. *et al.* Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. *J. Exp. Med.* **204**, 2899–2912 (2007).
78. Lassmann, H., Niedobitek, G., Aloisi, F. & Middeldorp, J. M. Epstein–Barr virus in the multiple sclerosis brain: a controversial issue—report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. *Brain* **134**, 2772–2786 (2011).
79. Hauser, S. L. *et al.* B-Cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.* **358**, 676–688 (2008).
80. Sundqvist, E. *et al.* JC polyomavirus infection is strongly controlled by human leucocyte antigen class II variants. *PLoS Pathog.* **10**, e1004084 (2014).
81. Staras, S. A. *et al.* Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. *Clin. Infect. Dis.* **43**, 1143–1151 (2006).
82. Waubant, E. *et al.* Common viruses associated with lower pediatric multiple sclerosis risk. *Neurology* **76**, 1989–1995 (2011).
83. Waubant, E. *et al.* Antibody response to common viruses and human leukocyte antigen-DRB1 in pediatric multiple sclerosis. *Mult. Scler.* **19**, 891–895 (2013).
84. Sundqvist, E. *et al.* Cytomegalovirus seropositivity is negatively associated with multiple sclerosis. *Mult. Scler.* **20**, 165–173 (2014).
85. Ascherio, A. *et al.* Epstein–Barr virus antibodies and risk of multiple sclerosis: a prospective study. *JAMA* **286**, 3083–3088 (2001).
86. Levin, L. I. *et al.* Temporal relationship between elevation of Epstein–Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. *JAMA* **293**, 2496–2500 (2005).
87. DeLorenze, G. N. *et al.* Epstein–Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. *Arch. Neurol.* **63**, 839–844 (2006).
88. Chidrawar, S. *et al.* Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. *Clin. Exp. Immunol.* **155**, 423–432 (2009).
89. Munger, K. L. *et al.* Molecular mechanism underlying the impact of vitamin D on disease activity of MS. *Ann. Clin. Transl. Neurol.* **1**, 605–617 (2014).
90. Kampman, M. T., Wilsgaard, T. & Mellgren, S. I. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. *J. Neurol.* **254**, 471–477 (2007).
91. Baarnhielm, M. *et al.* Sunlight is associated with decreased multiple sclerosis risk: no interaction with human leukocyte antigen-DRB1*15. *Eur. J. Neurol.* **19**, 955–962 (2012).
92. Becklund, B. R., Severson, K. S., Vang, S. V. & DeLuca, H. F. UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 6418–6423 (2010).
93. Rana, S., Rogers, L. J. & Halliday, G. M. Systemic low-dose UVB inhibits CD8 T cells and skin inflammation by alternative and novel mechanisms. *Am. J. Pathol.* **178**, 2783–2791 (2011).
94. Breuer, J. *et al.* Ultraviolet B light attenuates the systemic immune response in central nervous system autoimmunity. *Ann. Neurol.* **75**, 739–758 (2014).
95. Navid, F. *et al.* The aryl hydrocarbon receptor is involved in UVR-induced immunosuppression. *J. Invest. Dermatol.* **133**, 2763–2770 (2013).
96. Correale, J. & Farez, M. F. Modulation of multiple sclerosis by sunlight exposure: role of *cis*-urocanic acid. *J. Neuroimmunol.* **261**, 134–140 (2013).
97. Munger, K. L., Levin, L. I., Hollis, B. W., Howard, N. S. & Ascherio, A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* **296**, 2832–2838 (2006).
98. Cortese, M. *et al.* Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: the EnvIMS study. *Mult. Scler.* **21**, 1856–1864 (2015).
99. Bjornevik, K. *et al.* Sun exposure and multiple sclerosis risk in Norway and Italy: the EnvIMS study. *Mult. Scler.* **20**, 1042–1049 (2014).
100. Baarnhielm, M., Olsson, T. & Alfredsson, L. Fatty fish intake is associated with decreased occurrence of multiple sclerosis. *Mult. Scler.* **20**, 726–732 (2014).
101. Sandberg, L. *et al.* Vitamin D and axonal injury in multiple sclerosis. *Mult. Scler.* **22**, 1027–1031 (2015).
102. Ueda, P. *et al.* Neonatal vitamin D status and risk of multiple sclerosis. *Ann. Neurol.* **76**, 338–346 (2014).
103. Adzemovic, M. Z., Zeitelhofer, M., Hochmeister, S., Gustafsson, S. A. & Jagodic, M. Efficacy of vitamin D in treating multiple sclerosis-like neuroinflammation depends on developmental stage. *Exp. Neurol.* **249**, 39–48 (2013).
104. Munger, K. L. *et al.* Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish maternity cohort. *JAMA Neurol.* **73**, 515–519 (2016).
105. Staples, J., Ponsonby, A. L. & Lim, L. Low maternal exposure to ultraviolet radiation in pregnancy, month of birth, and risk of multiple sclerosis in offspring: longitudinal analysis. *BMJ* **340**, c1640 (2010).
106. Sundqvist, E. *et al.* Confirmation of association between multiple sclerosis and CYP27B1. *Eur. J. Hum. Genet.* **18**, 1349–1352 (2010).
107. Rhead, B. *et al.* Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. *Neurol. Genet.* **2**, e97 (2016).

108. Mokry, L. E. *et al.* Vitamin D and risk of multiple sclerosis: a Mendelian randomization study. *PLoS Med.* **12**, e1001866 (2015).
109. Handunnetthi, L., Ramagopalan, S. V. & Ebers, G. C. Multiple sclerosis, vitamin D, and *HLA-DRB1*15*. *Neurology* **74**, 1905–1910 (2010).
110. Ascherio, A. *et al.* Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurol.* **71**, 306–314 (2014).
111. Fitzgerald, K. C. *et al.* Association of vitamin D levels with multiple sclerosis activity and progression in patients receiving interferon Beta-1b. *JAMA Neurol.* **72**, 1458–1465 (2015).
112. Munger, K. L., Chitnis, T. & Ascherio, A. Body size and risk of MS in two cohorts of US women. *Neurology* **73**, 1543–1550 (2009).
113. Munger, K. L. *et al.* Childhood body mass index and multiple sclerosis risk: a long-term cohort study. *Mult. Scler.* **19**, 1323–1329 (2013).
114. Hedstrom, A. K. *et al.* Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis. *Neurology* **82**, 865–872 (2014).
115. Gianfrancesco, M. A. *et al.* Obesity during childhood and adolescence increases susceptibility to multiple sclerosis after accounting for established genetic and environmental risk factors. *Obes. Res. Clin. Pract.* **8**, e435–447 (2014).
116. Langer-Gould, A., Brara, S. M., Beaber, B. E. & Koebnick, C. Childhood obesity and risk of pediatric multiple sclerosis and clinically isolated syndrome. *Neurology* **80**, 548–552 (2013).
117. Hedstrom, A. K., Olsson, T. & Alfredsson, L. Body mass index during adolescence, rather than childhood, is critical in determining MS risk. *Mult. Scler.* **22**, 878–883 (2016).
118. Wesnes, K. *et al.* Body size and the risk of multiple sclerosis in Norway and Italy: the EnvIMS study. *Mult. Scler.* **21**, 388–395 (2015).
119. Mokry, L. E. *et al.* Obesity and multiple sclerosis: a Mendelian randomization study. *PLoS Med.* **13**, e1002053 (2016).
120. Gianfrancesco, M. A. *et al.* Genetic variants associated with body mass index demonstrate a causal effect on multiple sclerosis susceptibility. *Am. J. Epidemiol.* In press http://dx.doi.org/10.1093/aje/kww120 (2016).
121. Lumeng, C. N., Bodzin, J. L. & Saltiel, A. R. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* **117**, 175–184 (2007).
122. Procaccini, C., Pucino, V., Mantzoros, C. S. & Matarese, G. Leptin in autoimmune diseases. *Metabolism* **64**, 92–104 (2015).
123. Matarese, G. *et al.* Leptin increase in multiple sclerosis associates with reduced number of $CD4^+CD25^+$ regulatory T cells. *Proc. Natl Acad. Sci. USA.* **102**, 5150–5155 (2005).
124. Matarese, G., Carrieri, P. B., Montella, S., De Rosa, V. & La Cava, A. Leptin as a metabolic link to multiple sclerosis. *Nat. Rev. Neurol.* **6**, 455–461 (2010).
125. Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z. & Holick, M. F. Decreased bioavailability of vitamin D in obesity. *Am. J. Clin. Nutr.* **72**, 690–693 (2000).
126. Karlsson, E. A. & Beck, M. A. The burden of obesity on infectious disease. *Exp. Biol. Med. (Maywood)* **235**, 1412–1424 (2010).
127. Paich, H. A. *et al.* Overweight and obese adult humans have a defective cellular immune response to pandemic H1N1 influenza A virus. *Obesity (Silver Spring)* **21**, 2377–2386 (2013).
128. Magrini, A. *et al.* Shift work and autoimmune thyroid disorders. *Int. J. Immunopathol. Pharmacol.* **19**, 31–36 (2006).
129. Hedstrom, A. K., Akerstedt, T., Hillert, J., Olsson, T. & Alfredsson, L. Shift work at young age is associated with increased risk for multiple sclerosis. *Ann. Neurol.* **70**, 733–741 (2011).
130. Hedstrom, A., Akerstedt, T., Olsson, T. & Alfredsson, L. Shift work influences multiple sclerosis risk. *Mult. Scler.* **21**, 1195–1199 (2015).
131. Hansson, I., Holmdahl, R. & Mattsson, R. Constant darkness enhances autoimmunity to type II collagen and exaggerates development of collagen-induced arthritis in DBA/1 mice. *J. Neuroimmunol.* **27**, 79–84 (1990).
132. Farez, M. F. *et al.* Melatonin contributes to the seasonality of multiple sclerosis relapses. *Cell* **162**, 1338–1352 (2015).
133. Hansson, I., Holmdahl, R. & Mattsson, R. Pinealectomy ameliorates collagen II-induced arthritis in mice. *Clin. Exp. Immunol.* **92**, 432–436 (1993).
134. Massa, J., O'Reilly, E. J., Munger, K. L. & Ascherio, A. Caffeine and alcohol intakes have no association with risk of multiple sclerosis. *Mult. Scler.* **19**, 53–58 (2013).
135. Hedstrom, A. K., Hillert, J., Olsson, T. & Alfredsson, L. Alcohol as a modifiable lifestyle factor affecting multiple sclerosis risk. *JAMA Neurol.* **71**, 300–305 (2014).
136. Kallberg, H. *et al.* Alcohol consumption is associated with decreased risk of rheumatoid arthritis: results from two Scandinavian case-control studies. *Ann. Rheum. Dis.* **68**, 222–227 (2009).
137. Hedstrom, A. K. *et al.* High consumption of coffee is associated with decreased multiple sclerosis risk; results from two independent studies. *J. Neurol. Neurosurg. Psychiatry* **87**, 454–460 (2016).
138. Chen, G. Q. *et al.* Chronic caffeine treatment attenuates experimental autoimmune encephalomyelitis induced by guinea pig spinal cord homogenates in Wistar rats. *Brain Res.* **1309**, 116–125 (2010).
139. Horrigan, L. A., Kelly, J. P. & Connor, T. J. Immunomodulatory effects of caffeine: friend or foe? *Pharmacol. Ther.* **111**, 877–892 (2006).
140. Kleinewietfeld, M. *et al.* Sodium chloride drives autoimmune disease by the induction of pathogenic T_H17 cells. *Nature* **496**, 518–522 (2013).
141. Wu, C. *et al.* Induction of pathogenic T_H17 cells by inducible salt-sensing kinase SGK1. *Nature* **496**, 513–517 (2013).
142. Farez, M. F., Fiol, M. P., Gaitan, M. I., Quintana, F. J. & Correale, J. Sodium intake is associated with increased disease activity in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **86**, 26–31 (2015).
143. Berer, K. *et al.* Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* **479**, 538–541 (2011).
144. Correale, J. & Farez, M. F. The impact of environmental infections (parasites) on MS activity. *Mult. Scler.* **17**, 1162–1169 (2011).
145. Zhang, D. *et al.* Genetic control of individual differences in gene-specific methylation in human brain. *Am. J. Hum. Genet.* **86**, 411–419 (2010).
146. Liu, Y. *et al.* GeMes, clusters of DNA methylation under genetic control, can inform genetic and epigenetic analysis of disease. *Am. J. Hum. Genet.* **94**, 485–495 (2014).
147. Gao, X., Jia, M., Zhang, Y., Breitling, L. P. & Brenner, H. DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. *Clin. Epigenetics* **7**, 113 (2015).
148. Mastronardi, F. G. *et al.* Increased citrullination of histone H3 in multiple sclerosis brain and animal models of demyelination: a role for tumor necrosis factor-induced peptidylarginine deiminase 4 translocation. *J. Neurosci.* **26**, 11387–11396 (2006).
149. Baranzini, S. E. *et al.* Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature* **464**, 1351–1356 (2010).
150. Pedre, X. *et al.* Changed histone acetylation patterns in normal-appearing white matter and early multiple sclerosis lesions. *J. Neurosci.* **31**, 3435–3445 (2011).
151. Graves, M. C. *et al.* Methylation differences at the HLA-DRB1 locus in $CD4^+$ T-Cells are associated with multiple sclerosis. *Mult. Scler.* **20**, 1033–1041 (2014).
152. Huynh, J. L. *et al.* Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. *Nat. Neurosci.* **17**, 121–130 (2014).
153. Bos, S. D. *et al.* Genome-wide DNA methylation profiles indicate $CD8^+$ T cell hypermethylation in multiple sclerosis. *PLoS ONE* **10**, e0117403 (2015).
154. Maltby, V. E. *et al.* Genome-wide DNA methylation profiling of $CD8^+$ T cells shows a distinct epigenetic signature to $CD4^+$ T cells in multiple sclerosis patients. *Clin. Epigenetics* **7**, 118 (2015).

Acknowledgements
We thank Mohsen Khademi for preparing the schematic figures and Maja Jagodic for input on the epigenetics section. The original studies by T.O. cited in the text have been supported by the Swedish Research Council, the Knut and Alice Wallenberg foundation, the AFA foundation, the Swedish Brain Foundation, Margareta af Ugglas Foundation and the EUfp7 Neurinox 2012–278611. L.A. has received grants for multiple sclerosis research from the Swedish Research Council, the Swedish Research Council for Health, Working Life and Welfare and the Swedish Brain Foundation.

Author contributions
All authors researched data for article, and provided substantial contribution to discussion of content, writing, reviewing and editing of the manuscript.

Competing interests statement
T.O. has received honoraria for lectures and/or advisory boards as well as unrestricted multiple sclerosis research grants from Allmiral, Astrazeneca, Biogen, Genzyme, Merck and Novartis. L.A. has received lecture honoraria from Biogen and Teva.

Review criteria
We searched PubMed for full-length papers on "multiple sclerosis" and all lifestyle and environmental factors discussed in this Review in January 2012–May 2016. This generated 1,502 published papers, from which we omitted reviews, treatment and comorbidity studies and pure genetics articles. We evaluated each of the remaining 108 studies; the ones considered relevant for this Review were included in discussion. Relevant articles published before 2012 were included if they were referred to in the selected papers. Additional studies and reviews were included if found relevant for the topic by the authors.