

## Meeting Review

# Environmental modifiable risk factors for multiple sclerosis: Report from the 2016 ECTRIMS focused workshop

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**Abstract:** Multiple sclerosis (MS) is an inflammatory and neurodegenerative demyelinating disease of the central nervous system (CNS), most likely autoimmune in origin, usually beginning in early adulthood. The aetiology of the disease is not well understood; it is viewed currently as a multifactorial disease which results from complex interactions between genetic predisposition and environmental factors, of which a few are potentially modifiable. Improving our understanding of these factors can lead to new and more effective approaches to patient counselling and, possibly, prevention and management of the disease. The 2016 focused workshop of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) addressed the topic of environmental, modifiable risk factors for MS, gathering experts from around the world, to collate experimental and clinical research into environmental factors that have been associated with the disease onset and, in a few cases, disease activity and progression. A number of factors, including infections, vitamin D deficiency, diet and lifestyle factors, stress and comorbidities, were discussed. The meeting provided a forum to analyse available evidence, to identify inconsistencies and gaps in current knowledge and to suggest avenues for future research.

**Keywords:** Multiple sclerosis, environmental risk factors, infections, vitamin D, lifestyle, comorbidities

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## Introduction

Multiple sclerosis (MS) is an inflammatory and neurodegenerative demyelinating disease of the central nervous system (CNS) with onset usually in young adulthood; it is likely autoimmune in origin.<sup>1</sup> Prevalence and incidence rates vary with geography and ethnicity, with prevalence figures ranging from 2 per 100,000 in Japan to greater than 100 per 100,000 in Northern Europe and North America. Women are affected two to three times more frequently than men,<sup>2</sup> and there is evidence that this disparity is increasing in some, but not all, geographical areas.<sup>3</sup> MS is usually diagnosed during the most active and productive period of life in the majority of individuals, with disability eventually developing in a sizeable proportion of patients. There is therefore a compelling need to understand whether modifiable factors may alter disease risk, as well as disease activity and progression.

The aetiology of MS is most certainly heterogeneous: multiple factors, both genetic and environmental,

determine disease risk and these risk factors interact with one another in a complex manner.<sup>4</sup> The strongest contributor to genetic susceptibility is the major histocompatibility complex (MHC). The HLA-DRB1\*1501-DRB5\*0101 haplotype, in particular, shows a strong association with MS risk in Caucasians.<sup>5</sup> Many other susceptibility gene variants have now been identified, although their effects are generally minor.<sup>6,7</sup> The genetics of the HLA region, however, is complex with potential epistatic interactions between DR haplotypes and heterogeneity depending on ethnicity.<sup>8</sup> Notwithstanding this, for a substantial number of people who are genetically susceptible to MS, it does appear that non-genetic, potentially modifiable factors play a role in whether they develop the disease or not. In addition, some modifiable factors have been identified which are associated with disease activity and progression.<sup>9</sup> Together, this suggests that interventions could be developed to prevent some proportion of MS cases and/or alleviate the severity of the disease course.

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ECTRIMS held its fourth focused workshop on environmental, modifiable risk factors for MS on 3–4 March 2016 in Florence, Italy. The purpose of this report is to summarise the highlights from this meeting and their implications for patient care and to identify avenues for future research that may generate more effective approaches to prevention and management of MS.

### Gene–environment interactions

More than 110 loci have been associated with MS onset in large, multicentre, genome-wide association studies, although the strongest association remains with the HLA-DRB1\*1501-DRB5\*0101 haplotype.<sup>6,7</sup> Epidemiological studies have aimed to identify causal associations between gene–environment interactions or environment–environment interactions and MS risk.<sup>4</sup>

Among various research initiatives, the Swedish MS Registry has launched the development of the Epidemiology in Multiple Sclerosis (EIMS) and Genes and Environment in Multiple Sclerosis (GEMS) projects.<sup>10</sup> Lifestyle questionnaires and extensive genotyping of subjects included in these cohort studies have enabled identification of environmental and lifestyle MS risk factors and their possible interaction. The studies have shown interactions between MS HLA risk genes and smoking (both active and passive), Epstein–Barr virus (EBV) serology, infectious mononucleosis (IM), adolescent obesity and organic solvents. It is noteworthy that in the registry dataset 60% of all cases were associated with an interaction between EBV serology and HLA, with an additive effect.

In general, whether MS risk factors act in sequence and depend on each other or whether they act independently and in an additive or multiplicative fashion is poorly understood. The fact that no factor has yet been shown to be present in all patients with MS, with the possible exception of the EBV in adult-onset MS, suggests that causal pathways are likely to differ between individuals and would support an additive effect.<sup>11</sup>

### Infections and vaccines

Research on infectious causes of MS is based on clinical and pathological observations or experiments in animal models.<sup>12</sup> Another approach involves the assessment of the extent to which an infectious agent could be implicated in the epidemiology of MS.<sup>13</sup> In particular, the potential relationships between MS epidemiology and infections have been analysed in

the context of the ‘hygiene’ and ‘old friends’ hypotheses (see below). A common aspect to both is that a widespread microbe, rather than a rare pathogen, may be involved in MS. Overall, epidemiological data provide some support to the ‘hygiene’ hypothesis, but with the additional proviso for a key role of EBV in determining MS risk.

A correlation between ‘hygiene’, as defined by reduced early life exposure to infectious agents, and MS risk has long been noted. It has been suggested that better hygiene or lower exposure to infectious agents in childhood can favour the development of a Th1 pro-inflammatory cellular immune response, whereas exposure to multiple infections in childhood could deviate the immune response towards a regulatory T-cell phenotype.<sup>14</sup>

The ‘hygiene hypothesis’ may explain some epidemiological findings, including a lower incidence of MS in developing countries (with poorer hygiene) as compared to countries with higher socioeconomic standards, and an increased MS prevalence associated with improving education and socioeconomic status (SES) in some early investigations,<sup>14</sup> although more recently the inconsistency of data surrounding SES and MS risk has been recognized.<sup>15</sup> This hypothesis is also corroborated by the observation of an increased risk of MS with a late age at infection with EBV and manifestation as IM.<sup>16</sup> However, the hypothesis does not completely fit with epidemiological MS data, since those subjects who are seronegative for EBV infection have a very low MS risk status. This represents a ‘paradox’: if the hygiene hypothesis was valid for MS, these individuals would be expected to be at high MS risk because the lack of infection from EBV (an almost ubiquitous pathogen) is a marker of a highly hygienic environmental status.<sup>14</sup> This paradox could be solved if EBV itself is a contributory cause of MS – that is, the adverse effect of good hygiene on MS risk becomes manifest only after EBV infection.

### EBV

The identification of the paradox described above, and the demonstration that EBV-seronegative individuals are virtually immune to MS until the time they are infected with EBV but are at high MS risk after EBV infection,<sup>17</sup> provides convincing evidence that EBV stands out as the most important infectious agent that may explain many of the key features of MS epidemiology.

Furthermore, EBV and IM (which is usually caused by primary EBV infection during adolescence) have

been linked to MS onset in several epidemiological studies, both retrospective and prospective in design, and in meta-analyses and basic research. A number of EBV-associated autoimmune disorders and malignancies may develop after an extended period of latency following primary infection. In particular, a history of IM is associated with a two- to threefold increased risk of developing MS. Moreover, an immune response to the CD4<sup>+</sup> T-cell antigen EBNA1 is prominently elevated in MS subjects, and titres of anti-EBNA1 antibodies appear to increase the risk of the disease in a dose-dependent manner.<sup>14</sup>

However, the link between EBV and MS by itself cannot explain the decline in risk among migrants from high to low MS prevalence areas.<sup>13,18</sup> This decline may suggest that either EBV strains in low-risk areas have less propensity to cause MS or that other infectious or non-infectious factors modify the host response to EBV or contribute to determine MS risk.<sup>14</sup>

Proposed mechanisms of action for the association of EBV with MS include molecular mimicry, bystander damage to the CNS by EBV-specific T cells, induction of an immune response to EBNA1 antigen, chronic infection of B cells and an indirect mechanism due to reactivation of human endogenous retroviruses (HERV), such as HERV-W.<sup>14</sup>

In conclusion, while overall evidence strongly supports a role for EBV in MS aetiology, the exact mechanism by which EBV infection can predispose to MS remains elusive. Further studies are strongly advocated to elucidate the nature of this association. In addition, further work should investigate whether EBV infection can also drive subsequent disease activity or progression in those with established MS.<sup>9</sup>

#### *Implications for prevention and patient care*

Based on the above evidence on the role of EBV in MS, there could be relevant implications for therapeutic/preventive strategies. Antiviral therapy, based on nucleoside analogues, has been studied principally in EBV-related lymphoproliferative disorders, but it is generally ineffective, probably because the virus is in a latent phase of its cycle. Research into the development of prophylactic and therapeutic EBV vaccines has started. EBV vaccination, initially targeting IM, is under investigation, whereas induction of a viral neutralisation antibody response preventing primary infection may be appropriate for the prevention of primary EBV infection in the general population. An alternative strategy to induce a

cell-mediated immunity to control latent-infected cells in persistently infected individuals may have a role in the prevention of EBV-related malignancies and autoimmune disorders.<sup>19</sup>

Due to the capability of the virus to establish latent infection in B cells,<sup>14</sup> in those who already have MS, potential therapeutic strategies include B-cell-depleting therapies and anti-retroviral drugs. Rituximab, ocrelizumab and ofatumumab are monoclonal antibodies targeting CD20<sup>+</sup> B cells which have shown disease-ameliorating effects in clinical trials.<sup>20</sup> The role of HERVs in MS pathogenesis is under investigation, and agents such as raltegravir, a retroviral integrase inhibitor, have been tested in clinical trials for the treatment of MS.<sup>21,22</sup>

The Bacillus Calmette–Guérin (BCG) vaccine is under study as a possible option for an ‘intermediate’ preventive strategy, based on vaccination of those who are at high risk of developing MS. In a randomised double-blind trial on subjects with clinically isolated syndrome (CIS), BCG vaccine, as compared to placebo, was associated with significantly reduced development of gadolinium-enhancing lesions at magnetic resonance imaging (MRI) for a 6-month period before starting immunomodulating therapy.<sup>23</sup> BCG vaccine may act as a tumour necrosis factor (TNF)-inducer, and preclinical studies have shown that it may have a neuroprotective effect.<sup>23</sup> A study with BCG vaccine in subjects with radiologically isolated syndromes (RIS) is in preparation (Marco Salvetti, MD, University of Rome, personal communication).

#### *Parasites*

The ‘old friend’ hypothesis suggests that early exposure to large numbers of parasites could protect against development of MS in later life.<sup>24</sup> Indeed, the risk of MS shows an inverse correlation with helminth infections, and it is possible that helminths – which have been parasitic throughout human evolution – act as immune regulators in people naturally infected with them. Helminths can establish a life-long equilibrium with the host, favouring the release of anti-inflammatory cytokines and expansion of regulatory T and B cells. There are some positive results with the use of helminths as a potential treatment in animal models of MS. A few preliminary clinical studies with helminth therapy have been conducted in MS patients.<sup>24</sup> A small controlled trial of *Trichuris suis* ova failed to show any benefit.<sup>25</sup> Future research should focus on the host–parasite interactions resulting from specific molecules isolated from parasites,

perhaps providing a more targeted approach and minimising adverse effects related to helminth therapy.

## Vitamin D deficiency

### *Vitamin D and MS risk*

A substantial evidence base now exists to support an association between vitamin D deficiency and low levels of its metabolite 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) in the onset and development of MS.<sup>26</sup> Experimental studies have shown that vitamin D has potent immunomodulatory activity.<sup>26</sup> It may have an anti-inflammatory role in the secondary lymphoid organs and the CNS, and it may also have neuro- and myelin-protective and/or myelin-regenerative effects in the CNS.<sup>27</sup> Genetic effects may also contribute to MS risk as genes associated with vitamin D metabolism have been identified in an MS genome association study. In addition, transcription of the HLA-DRB1\*1501 locus is regulated by vitamin D.<sup>28</sup>

The bulk of evidence for this association is derived from extensive epidemiological research based on different study designs. Collectively, the results support the importance of vitamin D sufficiency in adolescence and adulthood, up to the time of MS onset. Furthermore, they also suggest the importance of vitamin D in childhood or even in utero.<sup>14</sup> Recently, the role of maternal vitamin D deficiency during pregnancy has been documented in a nested case-control study by the Finnish Maternity Cohort,<sup>29</sup> where insufficient maternal vitamin D levels during pregnancy were associated with an almost twofold increased risk of MS in the offspring.

The causality of the relationship between vitamin D and MS risk is further supported by the results of Mendelian randomisation studies. These results provide compelling evidence that vitamin D deficiency is an independent risk factor for MS, although they do not exclude a contribution of other factors. Some uncertainty remains about whether there is a critical timing of exposure to low 25(OH)D levels and what absolute level of 25(OH)D is associated with increased MS risk.<sup>30,31</sup>

### *Vitamin D and MS disease activity or progression*

Vitamin D deficiency has also been associated with increased disease activity or progression in subjects with already established disease. The findings of a few small therapeutic trials using vitamin D in MS patients have been inconsistent,<sup>32–37</sup> and none of these studies was powered sufficiently to observe a

treatment effect. Large randomised interventional clinical trials in the United States, Europe and Australasia are currently evaluating vitamin D supplementation to decrease the risk of relapses (Table 1). These studies should also help to define appropriate methods of dosing to obtain optimal serum vitamin D levels that may reduce clinical MS activity.

Finally, observational studies based on the datasets from Phase III trials of interferon beta-1b support an influence of vitamin D on the disease course. In the betaferon/betaseron in newly emerging MS for initial treatment (BENEFIT) trial, 25(OH)D levels in the first year of the study were studied in association with clinical and MRI outcomes after 5 years, which helped minimise the possibility of reverse causation.<sup>42</sup> The study results showed that higher serum 25(OH)D levels early in the course of MS robustly predicted a lower degree of clinical and MS activity, MRI lesion load, brain atrophy and clinical progression over the 5 years of follow-up. In the large cohort from the betaferon/betaseron efficacy yielding outcomes of a new dose in MS patients (BEYOND) trial in relapsing-remitting multiple sclerosis (RRMS), sub-group analyses based on the patient vitamin D status were possible.<sup>43</sup> There were profound geographical differences in patient vitamin D status; the analyses showed a decreased risk of accumulating new active lesions at MRI in subjects with higher levels of 25(OH)D and suggested as ‘best guess’ an optimal level of 25(OH)D corresponding to 100 nmol/L. In this regard, further investigations are needed to identify definitively optimal levels of vitamin D and to determine whether these results apply to different races or ethnicities, to patients with the secondary or primary progressive course of MS and to patients receiving drugs other than interferon beta-1b.

### *Implications for prevention and patient care*

Questions remain as to whether the MS incidence or ongoing disease activity and progression can be reduced by vitamin D supplementation. Available data suggest that MS risk is reduced at an average serum 25(OH)D level above 100 nmol/L, currently achieved by only one-fifth of the at-risk population in the United States, and in a smaller proportion in most at-risk European populations.<sup>14</sup> This level of vitamin D could be reached by taking 2000–4000 IU/day of vitamin – a dose that is well above current recommendations, but has not been associated with major adverse outcomes to date.<sup>14</sup>

Due to the above uncertainties, many experts believe that more conclusive experimental evidence should

**Table 1.** Ongoing randomised clinical trials of vitamin D in MS patients.

Trial name	SOLAR <sup>38</sup>	VIDAMS <sup>39</sup>	VITADEM	EVIDIMS <sup>40</sup>	PrevANZ <sup>41</sup>	D-Lay-MS
Location	Europe	USA	Spain	Germany	Australasia	France
Participants	230 RRMS	172 RRMS	100 RRMS	80 RRMS or CIS	240 CIS	316 CIS
Vitamin D3 intervention	7000 IU/day 4 weeks up to 14,000 IU/day 92 weeks vs placebo (add on to IFNB-1a)	5000 vs 600 IU/ day (add on to galtiramer acetate)	4000 IU/day vs placebo	20,400 IU/every other day vs 400 IU/every other day (add on to IFNB-1b)	1000 vs 5000 vs 10,000 IU/ day vs placebo	100,000 IU/ every 14 days vs placebo
Duration	96 weeks	96 weeks	Not known	18 months	48 weeks	2 years
Primary outcomes	Mean number CUA lesions at week 48; proportion relapse free at the end of study	Percentage of participants having relapse by EOS	Percentage of participants having relapse by EOS	Number of new T2 lesions	Conversion to CDMS/MDMS	Conversion to CDMS/MDMS
Secondary outcomes	Annualised relapse rate, EDSS change at predefined time points	Annualised relapse rate, EDSS change, number of new active lesions, lesion volume	EDSS change, number of new active lesions, number of new T2 hyperintense lesions	Annualised relapse rate, percentage of relapse free at EOS, EDSS and MSFC, change at predefined time points		Number of relapses, number of new lesions, new active lesions, EDSS change, EQ5D
End of study	2015	2016	Not known	2016	Not known	2017

MS: multiple sclerosis; RRMS: relapsing remitting multiple sclerosis; CIS: clinically isolated syndrome; IFNB: interferon beta; CUA: combined unique activity; EOS: end of study; CDMS: clinically definite multiple sclerosis; MDMS: McDonald multiple sclerosis; EDSS: expanded disability status scale; MSFC: multiple sclerosis functional composite; EQ5D: European quality of life 5 dimensions.

be awaited before implementing nationwide universal vitamin D supplementation programmes aimed at reducing the risk of new cases of MS. However, for many individuals, including those living in areas where vitamin D deficiency is highly prevalent and those at high MS risk due to their family history, in addition to CIS and MS patients, the use of vitamin D supplements may represent a reasonable choice, considering, on the one hand, the strength and quality of evidence available and, on the other hand, the low risk of adverse events using the vitamin D dose suggested above.

### Lifestyle risk factors

#### Cigarette smoking

**Cigarette smoking and MS risk.** There is extensive and robust evidence that cigarette smoking, both active and passive, is a risk factor for MS onset. Smoking increases the risk of MS by approximately 50% in a number of epidemiological studies,<sup>44</sup> and there appears to be a consistent dose–response relationship with cumulative smoking dose.<sup>45,46</sup> Elevated levels of cotinine (a surrogate marker for nicotine) were detected in blood samples taken before the development of MS, supporting epidemiological evidence for the increased

risk of MS in individuals who smoke.<sup>46</sup> Differential changes over time in smoking behaviour between men (with decreasing numbers of smokers) and women (with a relatively constant number of smokers) over the past several decades could, in part, explain the increase in the female:male ratio in MS incidence noted in a few regions.<sup>14,47</sup> The critical period for exposure to cigarette smoke is unclear, with some studies detecting no effect of age,<sup>45</sup> and others observing increased susceptibility to MS with exposure during adolescence or young adulthood.<sup>46</sup>

**Cigarette smoking and MS disease activity or progression.** There is also some evidence of negative effects of smoking on disease course and progression. Smoking has been associated with a faster transition from onset of symptoms to a definitive MS diagnosis,<sup>48</sup> a more rapid shift to the secondary progressive phase of the disease, and progression in disability.<sup>49,50</sup>

Numerous mechanisms have been proposed to explain the adverse effects of smoking on MS risk, including effects on demyelination, disruption of the blood–brain barrier, immunological effects and increased nitric oxide and nitric oxide metabolites, but all remain speculative. The observation that tobacco

smoking, but not Swedish tobacco snuff, was associated with an increased MS risk suggests that combustion and/or inhalation are required to increase the risk of MS. Moreover, the increased risk of MS associated with organic solvents<sup>51</sup> was found to be higher in never smokers, suggesting lung irritation may play a crucial role,<sup>52</sup> which is also supported by studies in animal models.<sup>53</sup>

*Implications for prevention and patient care.* Based on the above data, from a public health perspective, the impact of smoking and passive smoking on both MS onset and subsequent progression appears considerable. Promoting smoking cessation may be one of the most straightforward interventions available to reduce the incidence of MS.

Individuals with a history of MS in the family should be informed regarding the impact of smoking on the risk of developing MS, and the importance of preventing their children from being exposed to passive smoke.

Given the potential prognostic implications, patients with MS should be advised to stop smoking, to lessen risks for comorbidities and to avoid possible aggravating MS-related disability.

#### *Body mass index*

*Body mass index and MS risk.* In epidemiological studies, overweight/obesity is associated with a two- to threefold increased risk of MS.<sup>54–59</sup> An interaction with other risk factors, including vitamin D status and genetic predisposition, has also been documented. Data from the Swedish EIMS study have shown that individuals whose body mass index (BMI) exceeded 27 kg/m<sup>2</sup> at age 20 had a twofold increased risk of developing MS in comparison with subjects with normal body weight.<sup>57</sup> In a further Swedish study using EIMS and GEMS data, striking associations were observed between BMI status and HLA genotype with regard to MS risk. Obese individuals with the MS-associated HLA genotype (presence of the HLA-DRB1\*1501 allele and absence of the HLA-A\*02 allele) had a 16-fold greater probability of developing MS compared to non-obese individuals without this genetic risk factor.<sup>58</sup> Other population-based studies have shown that the risk of MS is increased in obese children (aged 7–13 years)<sup>60</sup> and adolescents (aged 12–18 years),<sup>61</sup> with the effects being most pronounced in girls. As for the possible mechanism of action, it is speculated that a chronic, low-grade inflammatory state can develop as a result of the part played

by adipocytes in several metabolic pathways. High levels of leptin, an adipokine secreted by adipose tissue, have been associated with reduced regulatory T-cell activity and increased disease severity in animal models.<sup>62,63</sup>

*BMI and MS disease activity or progression.* There is also some evidence of a negative impact of obesity on disease-related outcomes in those with MS. A higher BMI has been associated with a poorer response to interferon beta-1b in MS patients.<sup>64</sup> Moreover, obesity has been associated with increased T1 lesion volume at MRI,<sup>65</sup> higher disability levels in a cohort of MS subjects who were obese compared with those of normal weight<sup>66</sup> and in patients at MS diagnosis.<sup>67</sup>

*Implications for prevention and patient care.* Clearly, educational interventions should not take BMI as the only indicator of health status and should focus more broadly on lifestyle. Adolescents and young adults at high risk of developing MS should be counselled on the need to modify their lifestyle to mitigate their risk. Patients with MS should be encouraged to modify their lifestyle in order to reduce the negative impact of obesity on disease-related outcomes and the risk of associated comorbidities.

#### **Other risk factors**

##### *Salt*

There is some experimental information on the implications of dietary salt on the pathogenesis of CNS autoimmunity. Studies using animal models of MS have shown that high salt diet promotes pathogenic T-cell responses and aggravates autoimmunity, suggesting that high dietary salt intake may promote CNS autoimmunity and increase the risk of MS.<sup>68</sup> These findings have not as yet been demonstrated in clinical studies, but it would seem prudent for those at high risk of developing MS, and perhaps those already with MS, to moderate salt intake as a lifestyle choice.

##### *Alcohol and caffeine*

The Swedish EIMS and GEMS studies have assessed the possible association between alcohol and caffeine consumption and the risk of developing MS. A dose-dependent inverse association between alcohol consumption and MS risk was statistically significant in both men and women. In addition, the detrimental effects of smoking were more pronounced among non-drinkers.<sup>69</sup>

Studies in animal models of MS have shown that caffeine may decrease the risk of developing the disease, possibly because of its neuroprotective properties and its role in the suppression of the production of proinflammatory cytokines.<sup>70</sup> Data from population-based studies showed that the risk of MS was substantially reduced in individuals reporting a high coffee consumption.<sup>71</sup> However, in two large cohorts of women participating in the Nurse Health Study, the association between MS risk and alcohol or caffeine intake was not confirmed.<sup>72</sup> Therefore, due to limited research and inconsistent findings, caution is needed in interpreting these results and their significance warrants further investigation.

### *Stress*

It has been suggested that psychological stress may play a role in the risk of MS, but the evidence in the field is limited and interpretation is difficult due to methodological issues and complexities in the definition and quantification of stress. A number of nationwide studies have been performed in Denmark. In the first study, parents exposed to the loss of a child had an increased risk of MS which was significant only when follow-up was at least 8 years.<sup>73</sup> In the second study, there was little evidence for a causal association between major stressful life events, such as divorce or the loss of a child or a spouse, and subsequent MS risk.<sup>74</sup> A third study focused on the associations between stressful life events occurring in childhood (parental divorce, parental death, death of a sibling) and subsequent risk of MS reported only weak or modest associations.<sup>75</sup>

As for the potential impact of stress on disease activity, severe stress associated with war increased the frequency of MS relapses.<sup>76</sup> A meta-analysis of 14 studies showed that non-traumatic stressful life events were associated with an increased risk of disease exacerbation. However, the association between the stressors and MS exacerbation is complex and no firm conclusions can be drawn because measurement of effects was heterogeneous between studies.<sup>77</sup> It has been postulated that the control of immune function by the major stress systems is impaired in MS and the endogenous inhibiting systems may become inefficient against inflammation. This hypothesis should be tested in prospective epidemiological studies that employ endocrine, immunological and MRI activity markers.<sup>78</sup>

### **Comorbidities**

Interest in MS-related comorbidities is increasing. Most studies have focused on the presence or impact

of comorbidities in patients with MS. Comorbidities have been associated with delays in diagnosis and disability progression.<sup>67</sup> Modifiable comorbidities arise directly or indirectly from the primary disease (e.g. osteoporosis due to reduced mobility) and their management impacts overall patient outcomes. The interaction between MS and comorbidities is complex. For example, comorbidities may affect decisions on initiation of disease-modifying drugs (DMDs)<sup>79</sup> and DMDs may increase the risk of certain comorbidities or they may be contraindicated if these comorbidities are already present.<sup>80</sup> Moreover, common cancers may be diagnosed less frequently in MS patients because of MS-related immunological changes, the adoption of a healthier lifestyle following an MS diagnosis or a delay in cancer detection.<sup>67</sup>

Gaining a better understanding of MS comorbidities may provide insight into the underlying disease processes and promote the health and wellbeing of MS patients.

### *Implications for patient care*

Comorbidities in MS are finally being given the profile and importance their potential impact warrants. MS neurologists are advised to help prevent comorbidities and to systematically pursue early diagnosis and optimal treatment of modifiable comorbidities, which may produce substantial improvements in patient quality of life.<sup>80</sup>

### **The gut microbiota–brain axis**

There is growing evidence implicating gut microbiota in the starting phase of several autoimmune diseases. As for MS, the stimuli triggering the autoimmune response have been commonly attributed to environmental factors, in particular microbial infections. However, using the relapsing–remitting mouse model of spontaneously developing experimental autoimmune encephalomyelitis, it has been shown that the commensal gut flora – in the absence of pathogenic agents – is essential in triggering immune processes, leading to a relapsing–remitting autoimmune disease driven by myelin-specific CD4<sup>+</sup> T cells.<sup>81,82</sup>

Further research is now ongoing to determine the composition of the intestinal microbiota associated with an increased susceptibility to MS,<sup>83,84</sup> in addition to disease outcomes in those already with MS,<sup>85</sup> and this research may provide a conceptual basis for exploring new, less invasive treatment strategies.

**Table 2.** Achievements and challenges: key points.

The aetiology of MS is multifactorial with both genetic and non-genetic determinants contributing to the risk of the disease

Four environmental factors – EBV infection, low levels of vitamin D, cigarette smoking and increased BMI – are notable for robust evidence supporting their association with the disease

Primary infection with EBV and a history of IM double an individual's risk of MS, with elevated antibody titres to EBV nuclear antigen being observed before the disease onset

Longitudinal studies of vitamin D supplementation and pre-onset serum levels of 25(OH)D support a protective role of vitamin D on MS risk

Cigarette smoking (both active and passive) increases the risk of MS by two to three times

Increased BMI is associated with two- to threefold increased risk of MS and female adolescents are at higher risk

Each of the above factors may interact in a complex manner with other genetic and non-genetic determinants

Our understanding of the mechanisms by which EBV infection, vitamin D, cigarette smoking and increased BMI influence the risk of MS are limited or mainly speculative and further research is required

There is some – albeit not definitive – evidence that vitamin D and cigarette smoking may also influence the subsequent MS disease course (relapses and/or progression)

MS: multiple sclerosis; EBV: Epstein–Barr virus; BMI: body mass index; IM: infectious mononucleosis; 25(OH)D: 25-hydroxyvitamin D<sub>3</sub>.

## Achievements, gaps in knowledge and future perspectives

### *Achievements*

Over the past decades much progress has been made in the identification of genetic and environmental, modifiable risk factors for the onset of MS, including the role of EBV, vitamin D levels, tobacco smoking and BMI (Table 2). These successes have resulted in neurologists being able to provide some guidance on ways to reduce risk in patients' relatives, CIS and RIS subjects and to MS patients regarding lifestyle modification to potentially alter their clinical course.

The literature provides estimates that assist in determining the specific proportion of cases of MS that could be prevented through prevention of the above four major environmental risk factors.<sup>86</sup> In particular, considering preventive interventions to occur sequentially, and under specific assumptions related to the increased risk associated with each risk factor and the prevalence of exposure in the population, it has been calculated that smoking avoidance would produce 8%, vitamin D supplementation 44%, obesity prevention 15% and IM prevention 12% reduction. Together, curtailing these risk factors may prevent over 60% of MS cases. An EBV vaccine or other intervention that modulates EBV infection or the immune response to EBV could potentially prevent around 90% of MS cases.

### *Gaps in knowledge and future perspective*

Most of the existing data concerning established risk factors for MS are derived from RRMS and are

naturally dominated by women, so future investigations are warranted in the primary and secondary progressive forms of MS, and in men, that look for modifiable factors to decrease disease risk and possibly ameliorate the subsequent disease course.

Moreover, our understanding of the pathogenic mechanisms underlying the identified risk factors is limited or mainly speculative. An in-depth elucidation of how these factors interfere with the development of MS, or with disease outcomes in those with MS, could lead to new approaches to prevention and treatment.

Future research should also focus on novel, emerging risk factors for which evidence is limited or conflicting to date. To achieve these goals, further efforts should necessarily reinforce the multidisciplinary approach, combining basic and epidemiological research. In this context, there may be an increasing role for 'omic technologies' in elucidating pathogenic mechanisms due to their abilities to give a snapshot of the activity of cells, tissue and organisms at individual points in time.<sup>14</sup> Future genetic studies may reveal mechanisms that can be modulated by developing novel therapeutic strategies. Epidemiological studies of gene–environment interactions can confirm causal associations, and epidemiological methodology can provide additional evidence of associations suggested by genetic studies. Meta-analyses are powerful ways of combining data and highlighting consistent patterns among studies. It is possible that traditional epidemiological methods may soon become more efficient by the use of social media and mobile technologies in tracking individuals included in epidemiological surveys.<sup>87</sup>



Furthermore, a major gap in knowledge is understanding interactions between different genetic and environmental risk factors. Clarifying these interactions and modelling all known risk factors could enable robust ‘risk stratification’ and, possibly, the evaluation of an ‘individualised’ risk estimate, in order to identify those individuals who may benefit most from preventive strategies.

Some preliminary attempts at combining risk factors have been undertaken.<sup>11</sup> The most comprehensive study to date involved the combination of 16 genetic risk loci, sex, and EBV titres into a prediction model.<sup>88</sup> However, the model fell short of being clinically useful, and more detailed studies of these and additional risk factors in large cohorts are needed to better understand their predictive power in combination.

Finally, there is preliminary work on environmental factors, vitamin D, smoking and obesity in particular, as potential modifiers of the disease course and response to DMDs,<sup>89</sup> a field that warrants further research.

Overall, although changes in environmental and nutritional factors would surely not eradicate MS completely, they may account for a large number of cases and further progress in this field could have a dramatic impact on the occurrence of the disease. Some of these factors may also impact the subsequent disease course in those who develop MS and hence inform the management of the disease.

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## Appendix 1

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