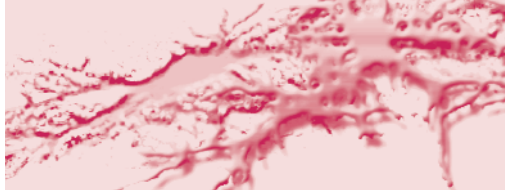

Environmental risk factors in multiple sclerosis aetiology

Ruth Ann Marrie

The epidemiology of multiple sclerosis (MS) has been intensively studied. It is conceptualised as a complex disease in which genetic and environmental factors act together to cause disease. There are temporal and geographic variations in disease risk, and risk of disease may be affected by migration between regions of differing risk. Numerous potential causal factors including infection, immunisations, physical and emotional stressors, climate, diet, and occupational exposures have been studied using various observational study designs. Thus far, no single environmental exposure has been consistently identified as a causal factor in MS, but sufficient data have accumulated that causal pathways should be postulated and tested. This review will focus on the environmental epidemiology of MS.

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An extensive body of literature addresses the epidemiology of multiple sclerosis (MS). MS is a demyelinating disease of the CNS and a leading non-traumatic cause of disability in young adults. All diagnostic criteria require evidence of dissemination of neurological dysfunction in space and time. Early diagnostic criteria, such as those of Schumacher and colleagues,[1] depended entirely on history and physical examination. Later diagnostic criteria also made use of paraclinical data, including MRI and CSF examination.[2] The most recently developed diagnostic criteria are the McDonald criteria,[3] which allow earlier diagnosis in some patients than previous criteria.[4] Use of different diagnostic criteria has implications for comparability of incidence and prevalence rates between studies, as discussed below.

It is important to distinguish MS from its variants or other demyelinating disorders.[5] Clinically isolated syndromes are episodes of acute or subacute demyelination that involve the spinal cord, brainstem, or optic nerves, and which may or may not portend the subsequent development of MS.[3] Acute disseminated encephalomyelitis is a monophasic syndrome of abrupt onset that may be associated with a depressed level of consciousness, seizures, multifocal lesions, and extensive lesions on MRI.[5] Neuromyelitis optica (Devic's disease) is a monophasic or relapsing-remitting inflammatory demyelinating disorder characterised by temporally linked optic neuritis and transverse myelitis with differing underlying pathology from that of classical MS. Other variants include Marburg disease and Balos concentric sclerosis. Existence of variants makes case definition a critical aspect of any study of MS epidemiology.

Pathophysiology

MS is thought to be a cell-mediated autoimmune disease of the CNS.[6] Evidence to support this concept of autoimmunity includes (1) predominance of women affected, similar to autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus;[7] (2) transient amelioration of disease activity during pregnancy, a relatively immunosuppressed state;[8] (3) association with other autoimmune diseases both in affected individuals and their family members;[9] (4) association with HLA type;[10] (5) similarity to experimental autoimmune encephalomyelitis, an autoimmune animal model of MS;[6] and (6) presence of autoantibodies to myelin antigens in serum and CSF.[6]

Despite our knowledge of the autoimmune nature of MS, the impact of immunomodulatory therapies on disease course is modest. Axonal transection occurs early in disease in spatial relation to focal brain inflammation.[11,12] It may also occur in regions of the brain where there is no apparent inflammation. Later in the disease course, progressive axonal degeneration occurs as a consequence of chronic demyelination. These observations suggest that MS is also a neurodegenerative disorder.[13] The picture is further complicated by clinical and pathological evidence of disease heterogeneity.[14]

A complex disease

MS is conceptualised as a complex disease, in which several environmental factors act together in a genetically susceptible individual to cause disease. Family members of affected individuals have a greater risk of disease than the general population.[15,16] Half-siblings of affected persons have roughly half the risk of full siblings of developing MS, and adopted siblings have no greater risk than the general population.[16] This indicates that genetic factors do contribute to an individual's risk of MS. Although monozygotic twins have a greater concordance (~30%) than dizygotic twins (~5%), concordance is less than 100%, indicating that genetics alone can not explain development of disease.[17] A recent review has discussed the genetic epidemiology of MS in detail.[10] My review will focus on environmental epidemiology.

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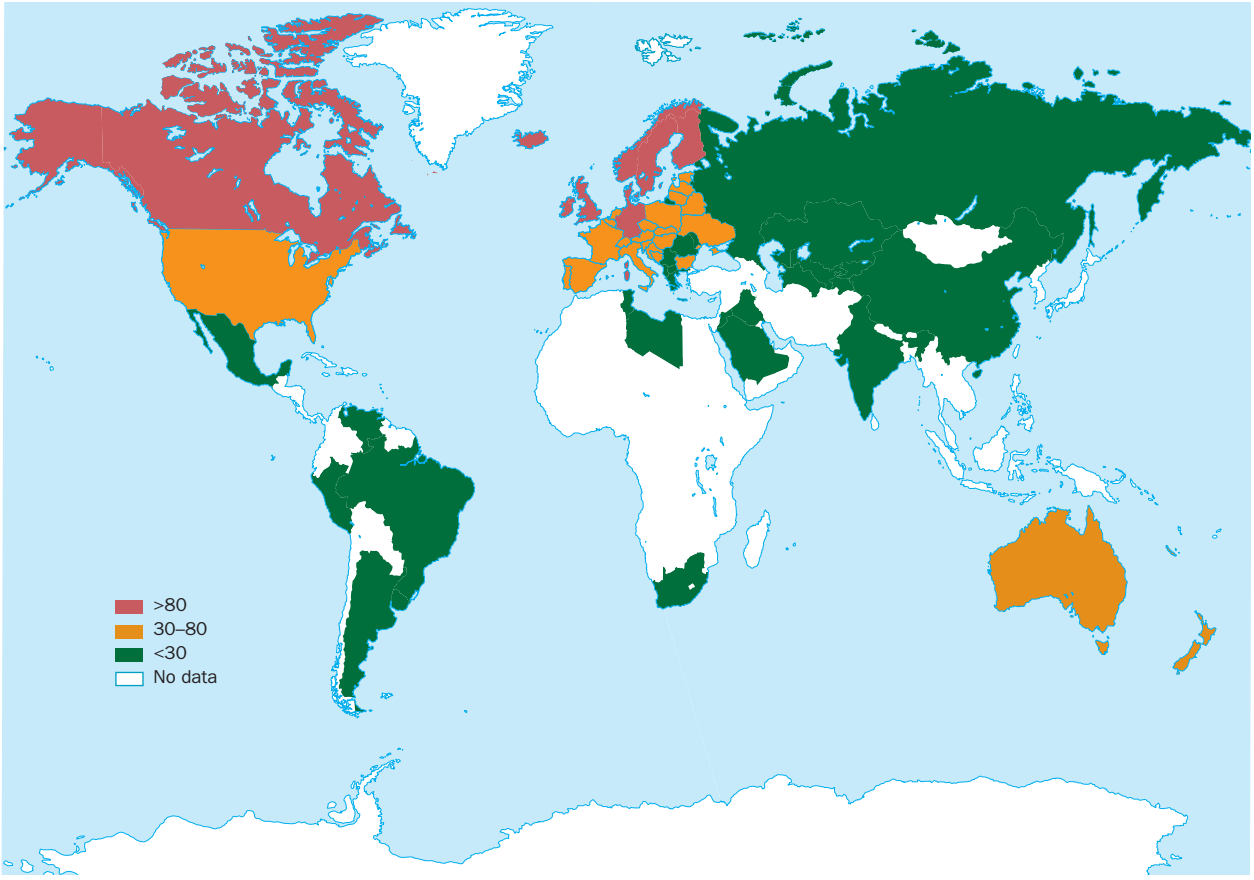

Worldwide prevalence of multiple sclerosis per 100 000 population.

Descriptive epidemiology

The geographic and temporal variations in the incidence and prevalence of MS have been intensively studied. However, it is important to note that several difficulties may arise when comparing incidence and prevalence studies from different areas or time intervals: (1) the populations studied may vary with respect to their size, age distribution, and ethnicity; (2) there may be differences between studies in the completeness of case ascertainment (affected by access to medical care, availability of diagnostic procedures such as MRI, public awareness of MS, number of neurologists, resources available to investigators); and (3) there may be differences in the diagnostic criteria used and variability in their application.

Geographic variation

Differences in the risk of MS by region have been reported. The disease tends to be rare in tropical areas but common in temperate areas, although there are some exceptions.[18] Kurtzke[19] has collated existing surveys and defined bands of MS prevalence. High prevalence (>30 per 100 000) areas include northern Europe, the northern USA and Canada, southern Australia, and New Zealand. Medium prevalence (5–30 per 100 000) areas include southern Europe, the southern USA, and northern Australia. Low prevalence (<5 per 100 000) areas include Asia and South America (figure).

Repetition of prevalence surveys in previously studied areas suggests that this characterisation of the distribution of risk may be exaggerated. Some differences in prevalence between regions may be accounted for by differences in methods and diagnostic criteria used.[20] Zivadinov and colleagues[21] reported an analysis of population-based incidence and prevalence studies of MS from 1980 to 1998. On the basis of mean crude prevalence rates, they found a latitude gradient that increased from south to north. The magnitude of the gradient decreased when these rates were standardised by age or sex to a common population. The gradient disappeared when similar adjustments were applied to the crude incidence rates. However, most of the studies analysed were conducted within latitudes of 40–60°.

Variations in prevalence are apparent over very small geographic distances,[22,23] partly reflecting geographic variations in ethnicity. Even in areas where disease is common, some groups are at lower risk, including the Samis, Turkmen, North and South Amerindians, Canadian Hutterites, Africans, and New Zealand Maoris.[18] Studies of US Army veterans identified a north–south gradient of decreasing risk that reflects the distribution of Scandinavian ancestry throughout the USA.[24]

Temporal variation

Temporal changes in incidence have been reported in several geographic areas.[22,25,26] Alterations in ascertainment or diagnostic criteria potentially explain some of these changes.

In Sardinia, where the population is genetically homogeneous and stable, repeated surveys showed increasing disease incidence. The timescale of these changes tends to suggest changes in environmental factors.[26] Although it is probable that some reported changes in incidence reflect changes in ascertainment, it is also likely that there are real changes in incidence in some areas.

Migration studies

Immigrants provide an opportunity to study the effects of changes in physical, social, and cultural environments on disease risk. Many studies have assessed the impact of migration from one country to another, as well as within a country.[27] However, migration studies suffer from many potential problems.[27] Migrants tend not to be representative of their region of origin, typically being younger, healthier, and of higher socioeconomic status. Data may be inadequate to assess the risk of disease in the country of origin, and differences in health care may lead to differences in the likelihood of being diagnosed (unequal ascertainment). Failure to standardise rates by age from different regions may lead to apparent rather than actual differences in risk.

Dean and colleagues[28] reported that immigrants from areas of low risk to the UK (an area of high risk) retained the low risk of their area of origin. Migrants within the USA showed similar findings, and migrants from areas of high risk to low risk had a risk intermediate to their areas of origin and destination.[29] Children of immigrants to the UK had risks of MS similar to those of other UK-born children, that is a higher risk than their parents.[30] Such rapid changes in risk over the course of a single generation implicate environmental factors in MS aetiology.

Alter and colleagues[31] assessed the impact of age at migration to Israel on the risk of developing MS. European immigrants migrating at older ages (>15 years) retained a high risk, whereas those migrating before age 15 years had a lower risk than expected. Many of the individuals under age 15 years at the time of migration were young at the time of the study, and could still be at risk of developing MS. Detels and coworkers[32] reported that age at migration did not affect the risk of MS in persons migrating from one, low risk, part of the USA to another. In persons migrating from an area of high risk to an area of low risk, the risk of MS was lower in those who migrated at earlier ages. Risk reduction was seen even in those aged over 15 years at the time of migration, although it was less than the risk reduction in those aged less than 15 years at migration. An Australian study did not show an association between age at migration and disease risk, which suggests that environmental factors affecting disease risk may still operate after adolescence.[33]

Despite potential problems with migration studies, the results are consistent.[27] People migrating from an area where MS is common to an area where it is less common experience a decrease in disease rates to a level intermediate to the places of origin and destination, whereas people who migrate from areas of low risk to areas of higher risk tend to retain the lower risk of their area of origin. Data on age at migration suggest the risk of disease is established largely in the first two decades of life, although a strict cut-off point (eg, age 15 years) cannot be established.

Are clusters and epidemics real?

A cluster is an excess of new cases in relation to time or space, or both, and may indicate either a true biological event or a random increase in incidence.[34] Randomness of events does not imply uniformity, and thus detection of temporal or spatial clustering may or may not have causal significance. Study of apparent MS clusters sometimes shows no evidence of a true cluster, and these investigations tend to be better at generating hypotheses about exogenous risk factors than testing them.[35,36]

Kurtzke and colleagues[37] reported an apparent MS epidemic in the Faroe Islands after the British occupation during the 1940s. Their data are often cited to support an environmental cause of MS. Poser and colleagues[38,39] discussed at length the limitations of Kurtzke and coworkers' studies,[37] including assumptions that the ability to diagnose MS reliably remained constant over time, the date of clinical onset was predictably related to date of acquisition of disease, the date of clinical onset was accurately defined for all MS patients, and that no patient had MS beginning before the 1940s. Given these assumptions, and the sensitivity of the original analyses to misclassification of even a single patient,[38] it is doubtful that this truly represents an epidemic.

Analytic epidemiology

Associations between putative environmental risk factors and disease are assessed using observational studies. Ecological studies look for associations at the population or group level. However, they are subject to the ecological fallacy if the association observed between variables at the group level is then applied to individuals.[40] In a case-control study, individuals with and without disease are compared with respect to risk factors (exposures) of interest.[41] This design is efficient for the study of rare diseases such as MS, but is subject to several types of bias. It can also be difficult to establish temporality, that is, whether the exposure of interest preceded disease onset. A prospective cohort study starts with a group of exposed and unexposed disease-free individuals who are then followed over time to see whether they develop the outcome of interest.[42] Although ideal for establishing temporality, this design can be costly and time-consuming for rare diseases. A comparison of observational study designs and the features required to limit bias in MS studies is shown in table 1. Two variations on these designs deserve special mention. In a retrospective (historical) cohort study, a cohort is assembled using existing records and is followed up to the present to identify cohort members who develop the outcome of interest.[42] School health or occupational records are examples of potential data sources. This design is less costly and time consuming than a prospective cohort study, but the investigator must rely on data collected for a purpose other than the hypothesis under study. In a nested case-control study, all cases are identified within a well-defined cohort. Controls are then selected from the same cohort. This design combines the reduced selection bias and correct temporality

Table 1. Comparison of observational study designs[40–42]

Characteristics	Case-control	Cohort	Ecological
Participants	Diseased cases Non-diseased controls	Exposed persons Unexposed persons	Population-level data
Measure of association	Odds ratio	Risk ratio or hazard ratio	Correlation coefficient
Advantages	Efficient for study of rare disease May be done quickly Less costly than cohort studies Can examine several exposures at once	Correct temporality Less risk of bias (selection, recall)	Less costly than other designs Easy to conduct External validity Dose-response assessment included
Disadvantages	Difficult to establish temporality More susceptible to bias (selection, ascertainment) Difficult to select appropriate controls Incomplete control of confounding	Costly Potential loss to follow-up Time-consuming Inefficient for rare disease Fewer hypotheses can be tested at once	Ecological fallacy (cannot be used to establish association in individuals) Temporality not established Difficult to control for confounding Better for hypothesis generation than testing
Desired features	Use of incident cases Clearly described case definition Controls selected from a study base defined a priori Exposure ascertainment methods with interviewers blinded to subject status and hypotheses Exposure ascertainment described and standardised for cases and controls Confirmatory source for recalled data Inclusion of an aetiologically relevant time period Statistical analysis specified and appropriate	Clearly defined cohort Exposure of interest well-defined Clearly described case definition Statistical analysis estimates risk or hazard ratio	Use of completely assessed incidence/prevalence material with subclassification by childhood residence Disease rates across differing geographic regions determined with uniform methodology Adjustment for confounding Selection of an appropriate timeframe between exposure and disease Calculation of correlation coefficient with confidence interval

between exposure and outcome of a cohort design with the efficiency of a case-control design.

Such observational studies are used to show associations, but association and causation are not equivalent. Guidelines exist for judging causality in observational studies.[43] Of these the most important is the establishment of temporality. The induction period is the time between the action of any causal factor and disease initiation.[44] The latent period is the time between disease initiation and detection of clinical onset. In MS the causes are unknown, and thus we cannot distinguish between the induction and latent periods. The empirical induction period incorporates both the induction period and the latent period, and is likely to be long in MS.[44] This makes it particularly difficult to establish temporality. These issues should be considered when evaluating the studies discussed below.

Environmental risk factors

Potential risk factors that are commonly studied include infection, vaccinations, stress, occupation, climate, and diet. The roles of diet and sex hormones in the aetiology of MS are discussed elsewhere.[45] The reason they are not discussed here is because the data are of insufficient quantity and quality to adequately assess whether diet and sex hormones play a causal role in MS.

Infection

Infection is often touted as a putative causal agent, particularly childhood viral infection. This is partly because migration studies have been interpreted to indicate the importance of an early environmental exposure. Despite many claims that a transmissible MS agent, presence of a virus, viral antigen, or viral genome have been identified in the brains (or other tissues) of MS patients, these have not been reproducibly shown.[46–49] Many serological studies have searched for indirect evidence of a viral cause, with various results. These studies have tended to show an increased prevalence of antibodies of several viruses in the sera and CSF of MS patients, often with higher titres than in healthy controls.[50,51] These differences were less apparent when patients were compared with siblings,[52] individuals of the same HLA type, or with individuals suffering from neurological or non-neurological inflammatory diseases.[53] The interpretation of all of these findings is unclear. Seroepidemiological studies document evidence of previous infection but cannot establish when an infection occurred, its severity, or even whether it was clinically symptomatic or asymptomatic. These studies are therefore more appropriate for generating than for testing aetiological hypotheses.

Measles, mumps, rubella, and varicella are common childhood infections and all have been considered potential causal agents.[54] Investigations of associations between childhood infections and MS are reported in several case-control studies and a few historical cohort studies. At least 30 case-control studies have investigated the association between measles and MS, with mixed results.[55–83] If we consider only case-control studies with the strongest study designs, there is no evidence that measles infection occurs

more frequently among MS patients.[59,63,79,84] Similarly, results are mixed for the association between rubella and MS.[55–63,65,66,70,71,73,74,76,77,80,82,84,85] Positive findings tended to occur in those studies with weaker study designs,[65,76,77] and, in particular, those in which exposure ascertainment was unblinded. Studies of mumps and varicella consistently show no association between the frequency of these infections and MS.[55–68,70–72,74–80,82,84,86,87]

Epstein-Barr virus (EBV) is an infectious agent of particular interest. Acute infection leads to lifelong, latent infection of B lymphocytes that affects 90% of the population by adulthood.[88] Infection is often mild in early childhood, whereas infection later in life may present as infectious mononucleosis. Several seroepidemiological studies of EBV infection and MS have been published, one in paediatric MS patients.[53,60,89–100] Most studies found increased EBV antibody seroprevalence or serum titres in MS patients than controls, although some had methodological problems.[101] Four case-control studies and two well-designed historical cohort studies found an increased risk of MS in individuals with a history of infectious mononucleosis,[57,102–107] whereas several case-control studies did not.[59,63,71,74,76,80,84] Current evidence is insufficient to show that EBV infection increases the subsequent risk of MS, although this remains biologically plausible.

The latest infectious candidates

Among the infectious candidates that have been recently proposed are human herpesvirus type 6, retroviruses, and *Chlamydia pneumoniae*. The role of viruses in MS aetiology is the subject of an upcoming review in this journal, and therefore the subsequent discussion will focus solely on *C pneumoniae*. In 1999, Sriram and colleagues[108] reported that 97% of MS patients had a positive PCR result for *C pneumoniae* in CSF compared with 18% of controls. However, subsequent studies have produced conflicting results. Layh-Schmitt and colleagues[109] detected *C pneumoniae* in CSF of MS patients, but at a lower frequency. Munger and colleagues[110] did a nested case-control study with data from the Nurses Health Studies' cohorts and found an increased risk of MS in persons with positive *C pneumoniae* serology, but in most cases blood was collected after MS onset. Numazaki and colleagues[111] had negative results of *C pneumoniae* in CSF of children with MS. Two groups were unable to detect *C pneumoniae* in brain tissue.[46,112] In a study in that masked identical CSF samples from MS patients and controls that were sent to four different laboratories, only Sriram's group had positive PCR results.[113] All of these studies were done after MS onset, so it cannot be established whether infection preceded disease. At present, there is no standardised, uniformly accepted technique for DNA extraction and PCR for *C pneumoniae*, and currently available assays are technically difficult and may give different results.[114] Thus, evidence for an association between MS and *C pneumoniae* is weak.

Age at infection

The data on the importance of age at infection for specific infectious diseases have been inconsistent, with significant differences occurring most often in studies with weaker designs for exposure ascertainment (table 2). A few studies have investigated the occurrence of any childhood infection at different ages among cases and controls, rather than focusing on a specific illness.[56,62,70,84,115] A well-designed case-control study by the Italian MS Study Group, which used incident cases and trained interviewers (who were blind to case or control status), found that MS patients were more likely to report at least one childhood illness (including measles, mumps, varicella, rubella) after age 6 years (odds ratio 1·52; 95% CI 1·05–2·20).[84] Although methodologically weaker, other studies have reported similar results.[56,62,70,115]

Observational studies have not identified a single infectious agent as a causal factor in MS. It is possible that any one of several agents could produce the same result under the appropriate circumstances (ie, genetically susceptible host, critical time of exposure). There is a suggestion that MS patients tend to have had at least one childhood infection at later ages. That the frequency of MS among first-degree adopted relatives of affected patients is no greater than that expected in the general population strongly suggests that MS is not a transmissible disease.[15]

Vaccinations

Vaccinations have also been considered as causal factors. A series of case reports in France raised particular concern about demyelinating events developing after hepatitis B vaccination.[116] Ascherio and colleauges[117] did a nested case-control study with data from the Nurses Health Studies in which 192 women with MS were matched to 645 controls. The odds ratio of MS associated with hepatitis B vaccination occurring any time before disease onset was 0·9 (95% CI 0·5–1·6). With vaccination in the 2 years before disease onset the odds ratio was 0·7 (95% CI 0·3–1·8).[117] Other case-control studies similarly found no evidence of an association.[116,118] Case-control and cohort studies are consistent in showing no association between other childhood vaccinations (measles, mumps, rubella) and MS.[55,59,64,68,87,119]

Occupational exposures and toxins

Several studies have assessed the association between occupational exposures and MS, with most focusing on exposure to organic solvents.[59,76,80,120–130] A prevalence survey in Florence, Italy, found a prevalence ratio of 4·9 (95% CI 1·6–14·9) among employees in the shoe and leather industry compared with both the general and employed populations.[126] In this study, exposure assessment was based on occupational status.

The various results of case-control and historical cohort studies on the association between MS and exposure to organic solvents are summarised in table 3, along with their methodological issues.[59,76,80,121–125,127–130] Most case-control studies used prevalent cases, and relied on self-report for exposure assessment, and thus potentially suffer from survivorship and recall biases. Only three studies discussed an induction period between exposure and disease onset, or defined the duration of exposure necessary for individuals to be categorised as "exposed".[59,125,127] However, two of these studies included possible MS cases.[125,127] It is not clear that

Table 2. Case-control studies: age at acquisition of infection and multiple sclerosis (MS)

First author (year)	Number of participants	Results	Potential sources of bias
Alter (1976)[66]	30 cases 30 controls	Cases: more had one illness at age 5–9 years (p<0·01)	Prevalent cases Blinding not reported
Berr (1989)[58]	63 cases 63 controls	Cases: tendency to have rubella, varicella at later ages (p>0·05)	Prevalent cases Unblinded
Casetta (1994)[59]	104 cases 150 controls	Cases: measles (OR 2·1; 95% CI 1·25–3·68), rubella (OR 2·7; 95% CI 1·03–7·53) more frequent before age 5 years; no difference for mumps, rubella, herpes zoster, infectious mononucleosis	Prevalent cases Unblinded
Compston (1986)[60]	177 cases 164 controls	Cases: DR2+ cases: mumps later (p<0·01); measles later (p<0·05); rubella later (p<0·02); no difference for varicella	Included clinically isolated syndromes Some prevalent cases Unblinded
Gronning (1993)[62]	155 cases 200 controls	Cases: higher mean age at measles (p=0·056)	Prevalent cases Unblinded
Bachmann (1998)[67]	666 cases Swiss general population as controls	Cases: measles more often at 5–9 years (p<0·05); mumps, rubella, varicella more often at 5–14 years (p<0·05); curves for acquisition of childhood diseases shifted to older age	Prevalent cases Unblinded Case and control data ascertained differently
Haile (1982)[70]	72 cases 72 controls	Cases: more measles, mumps, varicella, rubella, or at least one of those infections at age 5–9 years (OR 1·18; 95% CI 0·53–2·60)	Prevalent cases Unblinded
Panelius (1973)[73]	229 cases 391 controls	Cases: trend to later age of measles infection (p<0·05)	Prevalent cases Blinding not reported
Poskanzer (1980)[74]	77 cases 154 controls	Cases: no difference in age of infection with measles, rubella, mumps, varicella, infectious mononucleosis; tended to lower mean age for measles	Prevalent cases Blinding not reported
Sullivan (1984)[77]	88 cases 88 controls	Cases: measles at later age (p=0·02); sporadic cases reported mumps at later age (p=0·01); no differences for rubella, varicella	Prevalent cases
Riikonen (1989)[78]	28 cases 184 controls	Cases: measles, parotitis later (p<0·0001); no difference for varicella, rubella	Prevalent cases Blinding not reported
Zilber (1996)[79]	93 cases 94 controls	Cases: fewer reported rubella after age 15 years; one or more childhood infections after age 6 years (OR 1·52; 95% CI 1·05–2·20)	Prevalent cases
Zorzon (2003)[80]	140 cases 140 controls	No difference in age at infection for varicella, measles, mumps, rubella, infectious mononucleosis	Prevalent cases Possibly inappropriate controls
Italian MS Study Group (1989)[84]	318 cases 1975 controls	Cases: at least one childhood infection after age 6 (OR 1·52; 95% CI 1·05–2·20)	
Hays (1992)[86]	63 cases 63 controls	Cases: mumps occurred at later age; increased risk if no mumps prior to age 7 years (OR 1·9)	Prevalent cases Unblinded
Martyn (1993)[104]	225 cases 164 controls	In seropositive subjects with history of infectious mononucleosis before age 17 years (OR 7·9; 95% CI 1·7–37·9)	Included clinically isolated syndromes Prevalent cases
Hernan (2001)[107]	31 cases 1416 controls	Cases: increased risk of MS if mumps after age 15 years (OR 2·3; 95% CI 1·2–4·3) or measles after age 15 years (OR 2·8; 95% CI 0·8–9·1)	Prevalent cases
Lauer (1994)[115]	150 cases 150 controls	Cases: higher age at acquisition of at least one common childhood infection (OR 2·12, p<0·01)	Prevalent cases Unblinded

OR=odds ratio.

exposure assessments really took account of an aetiologically relevant time period. Adjustment for confounding due to sex or socioeconomic status was often lacking, and some studies were based on very small numbers. The cohort studies have fewer methodological issues, but still give divergent results.[120,121,123,124] A meta-analysis of the case-control studies produced a pooled relative risk estimate of 1·7 (95% CI 1·1–2·4).[131] Given the inconsistency and methodological issues of these studies, an association between organic solvent exposure and MS cannot be excluded.

Physical environment (sunlight)

The observed geographic variation in MS risk prompted study of climatic factors as causal agents. Two ecological studies showed strong inverse correlations (r=–0·87 and –0·88) between levels of ultraviolet radiation and the frequency of MS.[132,133] Several case-control studies looked at the association between sunlight exposure and risk of MS, with mixed results.[87,134–140] Three studies found no association,[87,137,138] whereas two found that individuals with MS reported more sun exposure before disease onset.[136,140]

Table 3. Case-control and cohort studies: organic solvents and multiple sclerosis (MS)

First author (year)	Number of participants	Results	Potential sources of bias
Case-control studies			
Casetta (1994)[59]	104 cases 150 controls	OR 4·0 (95% CI 1·2–11·1)	Prevalent cases No blinding of interviewers
Hopkins (1991)[64]	16 cases 61 controls	No difference	Prevalent cases Small sample size
Souberbielle (1990)[76]	230 cases 230 controls	More hairdressers had MS (p<0·05)	Interviewer: unblinded investigator
Zorzon (2003)[80]	140 cases 140 controls	OR 0·8 (95% CI 0·5–1·4)	Prevalent cases Blinding not reported
Koch-Henriksen (1989)[122]	187 cases 187 controls	OR 2·0 (95% CI 0·8–4·7)	Prevalent cases Unstandardised data collection Interviewer unblinded
Flodin (1988)[125]	83 cases 467 controls	OR 1·9 (95% CI 0·9–3·7)	Included possible MS cases
Landtblom (1993)[127]	91 cases 348 controls	OR 2·8 (95% CI 1·3–5·5)	Included possible MS cases
Gronning (1993)[128]	139 cases 161 controls	OR 1·55 (95% CI 0·83–2·90)	Prevalent cases Hospital controls
Nelson (1994)[129]	20 cases 856 controls	OR 2·0 (95% CI 0·6–6·9)	Small numbers
Juntunen (1989)[130]	21 cases 21 co-twins	OR 0·40 (p>0·1)	Small numbers
Cohort studies			
Stenager (2003)[120]	2558 nurse anaesthetists	Expected 1·54 cases Observed 0 cases	
Flodin (2003)[121]	2083 nurse anaesthetists 10 affected	SIR 2·9 (95% CI 1·3–5·3)	Small number of MS cases Possible underascertainment Rough estimates of expected cases
Riise (2002)[123]	11 542 painters 46 213 food or construction workers	RR 2·0 (95% CI 0·9–4·5)	No validation of MS diagnoses No measurement of exposure
Mortensen (1998)[124]	124 766 exposed 87 502 unexposed	Expected 90–94 cases Observed 87 cases	No measurement of exposure

OR=odds ratio; RR=risk ratio; SIR=standardised incidence ratio

Recent studies have been more consistent in their findings.[132,134,135,139] Freedman and colleagues[139] reported mortality from MS was negatively associated with residential and occupational exposure to sunlight. In Australia, individuals who reported high levels of sun exposure between the ages of 6 and 15 years were less likely to have MS (OR 0·31; 95% CI 0·16–0·69).[134] Greater levels of actinic skin damage, as measured using silicone casts of the hand, were also associated with decreased risk (OR 0·32; 95% CI 0·11–0·88). This study enrolled prevalent cases and it is not possible to exclude changes in sun-related behaviour after symptom onset. Goldacre and colleagues[135] hypothesised that if solar radiation is protective against MS, then individuals with MS should be less likely to develop skin cancer than the general population. They reported that MS was associated with a lower risk of skin cancer (rate ratio 0·49; 95% CI 0·24–0·91).

Biological mechanisms have been postulated to explain these findings. Ultraviolet light may have immunosuppressive effects, and it increases production of vitamin D in the skin.[141] Vitamin D has beneficial effects on autoimmune encephalomyelitis, and affects T-cell function.[141] Munger and colleagues[142] reported that nurses with higher levels of vitamin D intake were at lower risk for MS.[142] However, it is difficult to separate differences in vitamin D intake from differences in the rest of their dietary intake. Fukazawa and colleagues[143] reported an association between vitamin D receptor polymorphisms and MS in the Japanese population, whereas Steckley and coworkers[144] reported no association in a large Canadian study. It is conceivable that interactions between genetic factors that regulate the effects of vitamin D, and environmental exposure to sunlight, may explain some of the geographic variability in MS risk, but additional work is needed.

Stress

Physical and emotional stressors continue to be studied as potential MS risk factors. Important issues to be considered are the definitions of stress used, and the period of exposure thought to be relevant. A review excluded any association other than a small effect between cranial trauma and MS onset.[145] Evidence for the role of emotional stress in MS aetiology was weak, but left open the possibility that emotional stressors could be causal factors. Particular

Search strategy and selection criteria

Data for this review were identified by searches of MEDLINE (1965–2004) with the search terms "multiple sclerosis" and "case-control", "cohort", "ecologic", "cluster", "infection", "measles", "mumps", "rubella", "varicella", "infectious mononucleosis", "EBV", "Chlamydia", "vaccination", "hepatitis B", "organic solvents", "trauma", "stress", "occupation", "sunlight", "vitamin D", "risk factors", "virus", "dietary", "fat", "nutrition", "hormones", and "oral contraceptive pill". Bibliographies of all articles retrieved were searched for additional articles not indexed in MEDLINE, and references were also identified from searches of the author's files. Only papers published in English were reviewed. Studies were categorised by subject, and then subcategorised by methodology into groups of seroepidemiological, case-control, cohort, ecological, and descriptive studies. Although this review was not designed as a formal systematic review, methodological rigour of identified studies was informally assessed with published guidelines for the epidemiological study of MS.[40–42] The final reference list was chosen on the basis of originality and relevance to the topics covered in this review.

problems were potential biases in recall and measurement of exposure. Warren and colleagues[146] reported that more MS patients had unwanted stress in the 2 years before disease onset than controls. Grant and colleagues[147] reported that patients with MS had more severely threatening life events than controls in the 6 months before disease onset. More recently, a Danish cohort study found that death of a child was subsequently associated with a greater hazard of MS (hazard ratio 1·56; 95% CI 1·05–2·31).[148] This was a well-designed study that avoided many of the measurement and selection biases of previous case-control studies. However, it cannot be excluded that death of a child is simply a marker rather than being the causal factor, as such an event may be associated with changes in behaviour or the environment.

Conclusion

Studying the role of environmental risk factors in the aetiology of MS is difficult for several reasons. Any given environmental agent may be only one of many factors capable of causing MS in a genetically susceptible individual, and these factors might be neither necessary or sufficient causes. The interaction of the components of cause may vary from study to study, producing inconsistent results.[149] Exposure to putative agents is likely to be highly prevalent among individuals with and without MS, which means that large sample sizes are required for the identification of an effect. MS is a rare disease, probably with a long latent period between exposure and symptom onset, which makes it more difficult to verify whether exposure preceded disease.

Tremendous effort has been expended in studying the potential causal factors for MS. Thus far, the focus has been on testing the effects of single risk factors without testing a causal pathway. Enough biological and epidemiological data have accumulated that it is now reasonable to postulate causal pathways (eg, vitamin D receptor polymorphism, decreased sunlight exposure, and infection) and to systematically test these pathways. These pathways may or may not include the genetic template as the first step.

Given the large sample sizes and resources required for such studies, large multinational collaborative efforts will be needed with careful attention paid to study design (table 1). Greater consideration should be given to the use of existing sources of data collected for other purposes, as already seen in several historical cohort studies. Combinations of population-based birth cohorts may be a particularly useful resource. Although this will be challenging, it is the next step towards solving the MS puzzle.

Conflict of interest

I have no conflicts of interest.

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