Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses

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Summary

Background The cause of multiple sclerosis is believed to involve environmental exposure and genetic susceptibility. We aimed to summarise the environmental risk factors that have been studied in relation to onset of multiple sclerosis, assess whether there is evidence for diverse biases in this literature, and identify risk factors without evidence of biases.

Methods We searched PubMed from inception to Nov 22, 2014, to identify systematic reviews and meta-analyses of observational studies that examined associations between environmental factors and multiple sclerosis. For each meta-analysis we estimated the summary effect size by use of random-effects and fixed-effects models, the 95% CI, and the 95% prediction interval. We estimated the between-study heterogeneity expressed by I^2 (defined as large for $I^2 \geq 50\%$), evidence of small-study effects (ie, large studies had significantly more conservative results than smaller studies), and evidence of excess significance bias (ie, more studies than expected with significant results).

Findings Overall, 44 unique meta-analyses including 416 primary studies of different risk factors and multiple sclerosis were examined, covering a wide range of risk factors: vaccinations, comorbid diseases, surgeries, traumatic events and accidents, exposure to environmental agents, and biochemical, infectious, and musculoskeletal biomarkers. 23 of 44 meta-analyses had results that were significant at p values less than 0·05 and 11 at p values less than 0·001 under the random-effects model. Only three of the 11 significant meta-analyses (p<0·001) included more than 1000 cases, had 95% prediction intervals excluding the null value, and were not suggestive of large heterogeneity (I^2<50%), small-study effects (p for Egger's test >0·10), or excess significance (p>0·05). These were IgG seropositivity to Epstein-Barr virus nuclear antigen (EBNA) (random effects odds ratio [OR] 4·46, 95% CI 3·26–6·09; p for effect size=$1 \cdot 5 \times 10^{-19}$; I^2=43%), infectious mononucleosis (2·17, 1·97–2·39; p=$3 \cdot 1 \times 10^{-50}$; I^2=0%), and smoking (1·52, 1·39–1·66; p=$1 \cdot 7 \times 10^{-18}$; I^2=0%).

Interpretation Many studies on environmental factors associated with multiple sclerosis have caveats casting doubts on their validity. Data from more and better-designed studies are needed to establish robust evidence. A biomarker of Epstein-Barr virus (anti-EBNA IgG seropositivity), infectious mononucleosis, and smoking showed the strongest consistent evidence of an association.

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Introduction

Multiple sclerosis is the most common demyelinating disease in high-income countries[1] and, according to a report by the Multiple Sclerosis International Federation,[2] the global median prevalence of multiple sclerosis has increased from 30 per 100 000 in 2008 to 33 per 100 000 in 2013. Prevalence varies considerably between countries[3,4] and is highest in North America (140 per 100 000) and Europe (108 per 100 000) and lowest in sub-Saharan Africa (2·1 per 100 000) and east Asia (2·2 per 100 000).[2] The cause of multiple sclerosis is multifactorial; both genetic and environmental factors contribute to disease risk. In particular, several environmental risk factors, such as Epstein-Barr virus infection,[5] smoking,[6] and latitude,[7] have been proposed; however, the causes of multiple sclerosis are still largely unknown and there are at present no well-established risk factors to assist disease prevention.[8]

Numerous meta-analyses and systematic reviews for environmental risk factors associated with multiple sclerosis have been published. However, to our knowledge, there has been no effort to summarise the evidence from these meta-analyses and their associated limitations, such as the presence of diverse biases. We did the first umbrella review of the evidence across existing systematic reviews and meta-analyses of observational studies, to provide an overview of the range and validity of the reported associations of diverse environmental risk factors with multiple sclerosis. We summarise the risk factors that have been associated with multiple sclerosis in meta-analyses, assess whether there is evidence for diverse biases in these meta-analyses, and finally assess which of the previously studied associations that have been synthesised in meta-analyses have robust evidence.

Methods

Search strategy and eligibility criteria

We did an umbrella review (a systematic collection and assessment of multiple systematic reviews and meta-analyses done on a specific research topic).[9] We systematically searched PubMed from inception to Nov 22, 2014 to identify systematic reviews and meta-

analyses of observational studies examining associations between environmental (non-genetic) factors and multiple sclerosis. The search strategy used the keywords "multiple sclerosis" AND ("systematic review" OR "meta-analysis"). The full text of potentially eligible articles was scrutinised independently by two investigators (LB and VB). We excluded meta-analyses that investigated the association between genetic markers and risk of multiple sclerosis because these factors have been examined elsewhere. When a study included meta-analyses of both genetic and environmental risk factors, we only extracted information on environmental factors. Additionally, meta-analyses with an outcome related to relapse or remission of multiple sclerosis or severity of symptoms were excluded. We also excluded meta-analyses that examined multiple sclerosis as a risk factor for other medical conditions, and systematic reviews of ecological studies with no individual data. We did not apply any language restrictions in the selection of eligible studies. When more than one meta-analysis on the same research question was eligible, the meta-analysis with the largest number of component studies with data on individual studies' effect sizes was retained for the main analysis.

For more on the **association between genetic markers and risk of multiple sclerosis** see http://msgene.org/

Data extraction

Data extraction was done independently by two investigators (LB and VB) and, in the case of discrepancies, the final decsion was that of a third investigator (IT). From each eligible article, we recorded the first author, journal, year of publication, examined risk factors, and number of studies included. If a quantitative synthesis was done, we also extracted the study-specific relative risk estimates (risk ratio, odds ratio [OR], hazard ratio, or incident risk ratio) together with the corresponding CI and the number of cases and controls in each study for each risk factor. Whenever the studies used several control groups, we used the data from the healthy controls as the control group. For studies with no quantitative synthesis, we recorded a statement summarising the authors' main interpretations of their findings and the reason why a quantitative synthesis was not attempted.

Statistical analysis

For each meta-analysis, we estimated the summary effect size and its 95% CI with both fixed-effects and random-effects models.[10,11] We also estimated the 95% prediction interval, which further accounts for between-study heterogeneity and evaluates the uncertainty for the effect that would be expected in a new study addressing that same association.[12,13] For the largest study of each meta-analysis, we estimated the SD of the effect size and examined whether the SD was less than 0·10. In a study with an SD of less than 0·10, the difference between the effect estimate and the upper or lower 95% CI is less than 0·20 (ie, this uncertainty is less than what is considered a small effect size).

In the case of meta-analyses with continuous data, the effect estimate was transformed to an OR with an established formula.[14] Between-study heterogeneity was assessed by the I^2 metric.[15] I^2 ranges between 0% and 100% and is the ratio of between-study variance over the sum of the within-study and between-study variances.[16] Values exceeding 50% or 75% are usually judged to represent large or very large heterogeneity, respectively. The 95% CI of I^2 estimates can be wide when there are few studies.[17]

We assessed whether there was evidence for small-study effects (ie, whether smaller studies tend to give substantially larger estimates of effect size compared with larger studies)[18] with the regression asymmetry test proposed by Egger and colleagues.[19] A p value less than 0·10 with more conservative effect in larger studies than in random-effects meta-analysis was judged to be evidence for small-study effects.

We applied the excess statistical significance test, which assesses whether the observed number of studies with nominally significant results (positive studies, $p<0·05$) is larger than their expected number.[20] This test assesses whether the number of positive studies among those in a meta-analysis is too large based on the power that these studies have to detect plausible effects at α of 0·05. The expected number of studies with significant results is calculated in each meta-analysis by the sum of the statistical power estimates for each component study. The true effect size for any meta-analysis is not known. We estimated the power of each component study using the effect size of the largest study (smallest SE) in a meta-analysis.[21] The power of each study was calculated with an algorithm using a non-central t distribution.[22] Excess statistical significance for single meta-analyses was claimed at p less than 0·10 (one-sided $p<0·05$, with observed values greater than expected values as previously proposed[20]). The observed versus expected comparison was done separately for each meta-analysis, and it was also extended to groups including many meta-analyses after summing the observed and expected values from each meta-analysis.

In six meta-analyses presented in three articles,[23–25] data on effect sizes or sample size for individual studies were not available. For five meta-analyses presented in two articles,[23,24] the estimation of fixed-effects summary effect size was not done because of inadequate data. For one meta-analysis,[24] the I^2 and the Egger's test could not be estimated because the study-specific relative risk estimates for component studies were not reported. In one other meta-analysis,[25] the total number of cases was not reported. All six meta-analyses presented in three articles[23–25] were not included in the excess significance bias analysis because the data needed for power calculations were not reported.

Finally, we identified associations that had the strongest validity and were not suggestive of bias. Specifically, we noted which associations met the following criteria: had

significance according to fixed-effects and random-effects at p less than 0·05 and at p less than 0·001;[26,27] were based on greater than 1000 cases; had between-study heterogeneity that was not large (I^2 <50%); had 95% prediction interval that excluded the null value; and had no evidence of small-study effects and excess significance.

The statistical analysis and the power calculations were done with STATA version 12.0.

Role of the funding source

There was no funding source for this study. All authors had full access to all the study data. The corresponding author had final responsibility for the decision to submit for publication.

Results

Overall, 609 articles were searched and 20 articles were deemed eligible (figure). 17 of the 20 eligible articles had a quantitative synthesis providing a summary estimate. 13 of 33 articles screened at full text were excluded because a larger meta-analysis that examined the same risk factors and population was found. These articles pertained to chronic cerebrospinal venous insufficiency,[28,29] Epstein-Barr virus infection[30] and seronegativity,[31] hepatitis B vaccination,[32,33] immunisations,[34] infectious mononucleosis,[35] tetanus vaccination,[36] organic solvents,[37] smoking,[38] serum homocysteine,[39] and traumatic injury.[40] Three of these excluded articles were systematic reviews without a quantitative synthesis;[32–34] one article[40] did not present adequate data to do our analyses so we included an older meta-analysis[41] with better reporting. For the remaining nine meta-analyses,[28–31,35–39] when we compared the summary effects with the effects of the more recent respective meta-analysis, agreement existed on the direction and presence of nominal statistical significance of the association. The difference in magnitude of the effect size varied from 4% to 70%, but this variability could be attributed to the larger number of datasets included in the more up-to-date meta-analyses.

The three systematic reviews of observational studies without further quantitative synthesis examined stress[42] (five studies, 503 patients with multiple sclerosis), socioeconomic status[43] (21 studies, 7632 patients with multiple sclerosis), and serum prolactin[44] (23 studies, 1199 patients with multiple sclerosis) as risk factors for the onset of multiple sclerosis. In these systematic reviews the authors stated in their conclusions that there is evidence for associations between risk for multiple sclerosis and stress, high socioeconomic status, and elevated serum prolactin on the basis of descriptive presentation of the results of individual studies without quantitative synthesis. The authors stated that they did not attempt quantitative synthesis owing to the large heterogeneity in systematic reviews for stress and for socioeconomic status, but did not present any reasons for not doing quantitative synthesis in the systematic review for serum prolactin.

The 17 remaining articles correspond to 44 unique meta-analyses, including 416 primary observational studies in total. The median number of studies per meta-analysis was eight (IQR 6–12) and the median number of cases was 933 (IQR 471·5–2045·5). The 44 meta-analyses covered a wide range of risk factors (table 1). 20 (46%) of 44 meta-analyses studied associations between infections or vaccinations and multiple sclerosis. Seven (16%) of 44 meta-analyses examined biomarkers of Epstein-Barr virus or infectious mononucleosis. Additionally, other meta-analyses examined associations of biochemical biomarkers (n=5), musculoskeletal biomarkers (n=3), comorbid diseases (n=5), surgeries, traumatic events, or accidents (n=8), and exposure to toxic environmental agents (n=3). The number of cases was greater than 1000 in 21 meta-analyses. All eligible meta-analyses used summary-level data from published literature and none of them had access to individual participant data.

23 (52%) of 44 meta-analyses reported effects that were significant at p values less than 0·05. 11 (25%) were significant at p values less than 0·001 under the random-effects model (table 1): IgG seropositivity for Epstein-Barr virus nuclear antigen (EBNA),[50] IgG seronegativity for Epstein-Barr virus,[50] IgG seropositivity to Epstein-Barr

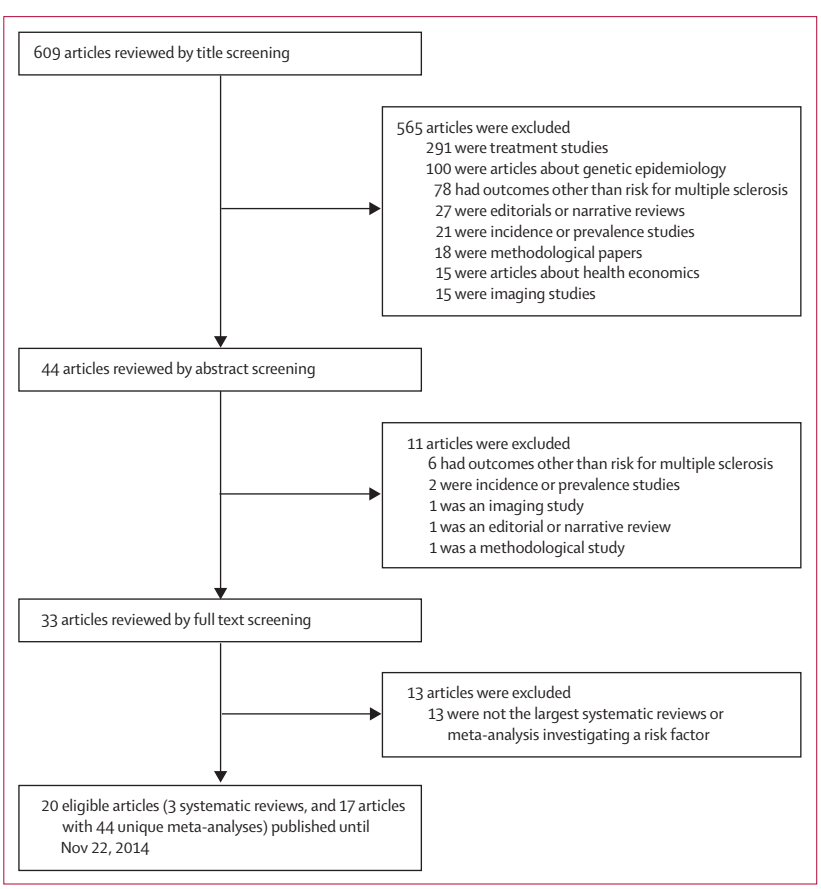

Figure: **Flow chart of literature search**

	Risk factor	Number of primary studies	Total number of cases/controls	Random-effects summary effect size (95% CI)	95% PI	p random	p fixed	Largest study		I^2	Egger test p value
								Effect size (95% CI)	SE		
Biochemical biomarkers											
Duan et al[45]*	Serum vitamin D	11	1007/829	0·44 (0·24–0·78)	0·05–3·70	0·005	$3·0×10^{-5}$	1·22 (0·90–1·66)	0·16	89%	0·05†
Liu et al[25]*	Serum uric acid	8	NA	0·28 (0·14–0·57)	0·02–3·29	$4·1×10^{-4}$	$1·0×10^{-22}$	0·27 (0·16–0·46)	0·27	87%	0·91
Zhu et al[46]*	Serum vitamin B_{12}	8	377/353	0·64 (0·44–0·93)	0·26–1·60	0·02	0·002	0·75 (0·41–1·37)	0·29	38%	0·99
Zhu et al[46]*	Serum folate	6	324/314	0·85 (0·61–1·17)	0·46–1·55	0·33	0·33	1·08 (0·59–1·97)	0·28	12%	0·01†
Zhu et al[46]*	Serum homocysteine	8	639/430	4·57 (1·40–14·89)	0·06–338	0·01	$1·6×10^{-8}$	0·34 (0·23–0·51)	0·20	96%	0·01
Musculoskeletal biomarkers											
Huang et al[47]*	BMD in femoral neck	10	518/902	0·36 (0·21–0·61)	0·06–2·26	$1·33×10^{-4}$	$9·1×10^{-15}$	0·68 (0·43–1·08)	0·23	81%	0·20
Huang et al[47]*	BMD in lumbar spine	11	560/930	0·34 (0·24–0·50)	0·11–1·12	$1·07×10^{-8}$	$2·0×10^{-13}$	0·52 (0·33–0·81)	0·23	67%	0·05†
Huang et al[47]*	BMD in hip	8	457/842	0·33 (0·18–0·60)	0·04–2·65	$2·95×10^{-4}$	$2·2×10^{-16}$	0·55 (0·35–0·86)	0·23	86%	0·22
Exposure to toxic environmental agents											
Aminzadeh and Etminan[48]	Dental amalgam	4	321/20 656	1·24 (0·96–1·62)	0·41–3·79	0·11	0·38	2·05 (1·19–3·53)	0·28	77%	0·02
Barragan-Martinez et al[49]	Organic solvents	15	1811/1 140 990	1·54 (1·03–2·29)	0·37–6·39	0·03	0·12	0·68 (0·40–1·15)	0·27	77%	0·06†
Handel et al[6]	Smoking	14	3052/457 619	1·52 (1·39–1·66)	1·37–1·68	$1·7×10^{-18}$	$4·7×10^{-19}$	1·50 (1·30–1·80)	0·08	0%	0·50
Infections or vaccinations											
Almohmeed et al[50]	Anti-EA IgG seropositivity	14	1354/1521	1·40 (0·93–2·10)	0·36–5·47	0·11	$8·9×10^{-5}$	1·35 (0·79–2·29)	0·27	70%	0·51
Almohmeed et al[50]	Anti-EBNA IgG seropositivity	30	3511/3797	4·46 (3·26–6·09)	1·46–13·62	$1·5×10^{-19}$	$5·0×10^{-35}$	9·04 (3·57–22·86)	0·47	43%	0·06
Almohmeed et al[50]	Anti-EBV IgG seronegativity	7	933/1.427	0·13 (0·05–0·32)	0·01–1·39	$2·0×10^{-5}$	$1·1×10^{-9}$	0·05 (0·003–0·87)	1·45	52%	0·05
Almohmeed et al[50]	Anti-VCA IgG seropositivity	24	2949/3376	4·52 (2·85–7·15)	0·87–23·42	$3·4×10^{-10}$	$2·8×10^{-23}$	9·15 (3·25–25·78)	0·53	58%	0·24
Bagos et al[23]‡	*Chlamydia pneumoniae* DNA in CSF	19	822/688	3·22 (1·20–8·59)	NA	0·02	NA	10·27 (2·34–45·02)	0·75	88%	0·46
Bagos et al[23]‡	Ig for *Chlamydia pneumoniae* in serum	12	740/950	1·07 (0·75–1·53)	NA	0·72	NA	1·70 (1·10–2·70)	0·23	91%	0·36
Bagos et al[23]‡	Ig for *Chlamydia pneumoniae* in CSF	6	264/261	3·82 (0·72–20·37)	NA	0·12	NA	1·28 (0·60–2·74)	0·39	83%	0·32
Bagos et al[23]‡	Intrathecal production of Ig for *Chlamydia pneumoniae*	6	313/315	3·84 (1·32–11·21)	NA	0·01	NA	1·54 (0·65–3·66)	0·44	55%	0·15
Farez and Correale[51]	BCG vaccination	6	536/751	0·96 (0·69–1·34)	0·60–1·54	0·82	0·82	1·00 (0·40–2·60)	0·48	0%	0·01†
Farez and Correale[51]	Diphtheria vaccination	3	237/387	0·60 (0·40–0·91)	0·04–8·57	0·02	0·02	0·80 (0·37–1·74)	0·39	0%	0·88
Farez and Correale[51]	Hepatitis B vaccination	6	15 241/12 339	1·00 (0·74–1·37)	0·45–2·26	0·99	0·10	0·92 (0·84–1·01)	0·05	48%	0·36
Farez and Correale[51]	Influenza vaccination	4	14 997/10 128	0·97 (0·77–1·23)	0·42–2·23	0·81	0·77	1·02 (0·96–1·09)	0·03	37%	0·84
Farez and Correale[51]	MMR vaccination	3	568/1880	1·02 (0·64–1·62)	0·05–20·58	0·94	0·94	0·90 (0·40–1·80)	0·38	0%	0·33
Farez and Correale[51]	Poliomyelitis vaccination	7	570/725	0·87 (0·61–1·26)	0·39–1·98	0·46	0·59	0·80 (0·07–2·80)	0·94	29%	0·13
Farez and Correale[51]	Tetanus vaccination	8	929/3203	0·71 (0·57–0·88)	0·47–1·07	0·002	$1·2×10^{-4}$	0·60 (0·40–0·80)	0·18	17%	0·15
Farez and Correale[51]	Typhoid fever vaccination	4	288/467	1·05 (0·72–1·53)	0·37–3·01	0·81	0·79	1·02 (0·50–2·06)	0·36	14%	0·92
Handel et al[5]	Infectious mononucleosis	18	19 519/16 136	2·17 (1·97–2·39)	1·96–2·41	$3·1×10^{-50}$	$3·1×10^{-50}$	2·06 (1·71–2·48)	0·09	0%	0·68
Santiago et al[52]	EBV DNA in CSF and brain tissue	7	211/251	1·57 (0·66–3·73)	0·23–10·94	0·31	$3·6×10^{-4}$	2·80 (0·99–2·49)	0·24	36%	0·53

(Table 1 continues on next page)

	Risk factor	Number of primary studies	Total number of cases/controls	Random-effects summary effect size (95% CI)	95% PI	p random	p fixed	Largest study		I^2	Egger test p value
								Effect size (95% CI)	SE		
(Continued from previous page)											
Santiago et al[52]	EBV DNA in mononuclear cells and serum	6	392/337	1·84 (1·02–3·30)	0·39–8·63	0·04	0·001	3·10 (1·81–5·38)	0·28	49%	0·79
Sundqvist et al[24]‡	Cytomegalovirus infection	11	2030/2192	0·77 (0·67–0·87)	NA	$1·0\times10^{-6}$	NA	0·73 (0·58–0·92)	0·12	NA	NA
Surgeries, traumatic events, or accidents											
Lunny et al[53]	Tonsillectomy at age ≤20 years	12	4414/4422	1·32 (1·09–1·61)	0·80–2·18	0·005	$5·2\times10^{-10}$	1·35 (1·22–1·51)	0·05	44%	0·72
Lunny et al[53]	Tonsillectomy at age >20 years	9	1801/1618	1·19 (0·94–1·50)	0·69–2·05	0·15	0·12	1·02 (0·77–1·34)	0·14	32%	0·29
Lunny et al[53]	Appendectomy at age ≤20 years	7	21218/205124	1·17 (1·02–1·34)	0·99–1·38	0·02	0·02	1·11 (0·94–1·31)	0·08	0%	0·13
Lunny et al[53]	Appendectomy at age >20 years	5	21360/205021	1·26 (0·92–1·72)	0·52–3·07	0·15	0·38	1·02 (0·93–1·11)	0·05	46%	0·09†
Lunny et al[53]	Adenoidectomy at age ≤20 years	3	458/636	1·07 (0·68–1·68)	0·02–73·45	0·78	0·96	0·85 (0·54–1·34)	0·23	35%	0·15
Lunny et al[53]	Other surgeries at age ≤20 years§	4	485/1202	0·80 (0·50–1·28)	0·12–5·18	0·36	0·14	1·00 (0·75–1·33)	0·15	63%	0·90
Lunny et al[53]	Other surgeries at age >20 years§	15	1099/1371	1·19 (0·86–1·65)	0·40–3·57	0·30	0·25	1·00 (0·75–1·33)	0·15	67%	0·46
Warren et al[41]	Traumatic injury	12	1486/1479	1·41 (1·03–1·92)	0·60–3·29	0·03	0·01	1·29 (0·69–2·40)	0·32	42%	0·29
Comorbid diseases											
Monteiro et al[54]	Allergic disease	8	1834/255374	0·91 (0·68–1·23)	0·37–2·28	0·54	0·80	1·45 (1·13–1·87)	0·13	72%	0·18
Monteiro et al[54]	Allergic rhinitis	6	2061/262707	0·82 (0·59–1·12)	0·30–2·18	0·23	$2·0\times10^{-4}$	1·32 (0·78–2·24)	0·27	70%	0·56
Monteiro et al[54]	Asthma	8	2726/263562	0·83 (0·47–1·44)	0·12–5·63	0·53	0·23	2·39 (1·79–3·18)	0·15	89%	0·17
Monteiro et al[54]	Eczema	4	1435/255629	0·93 (0·71–1·22)	0·45–1·95	0·61	0·70	0·76 (0·45–1·29)	0·27	13%	0·18
Tsivgoulis et al[55]	Chronic cerebrospinal venous insufficiency	19	1250/899	8·45 (3·47–20·56)	0·33–217	$3·5\times10^{-6}$	$1·2\times10^{-21}$	4·10 (2·66–6·30)	0·22	80%	0·23

The effect sizes are expressed as odds ratios (ORs). For infectious, biochemical, and musculoskeletal biomarkers, the level of comparison is high values versus low values, whereas for the remaining risk factors the level of comparison is exposed versus not exposed. BMD=bone mineral density. PI=prediction interval. p random=p value for random-effects meta-analysis. p fixed=p value for fixed-effects meta-analysis. EA=early antigen. EBNA=Epstein-Barr virus nuclear antigen. EBV=Epstein-Barr virus. VCA=viral-capsid antigen. NA=not available. MMR=measles, mumps, and rubella. *Random-effects summary effect size estimated from standardised mean differnce transformed to OR. †Both criteria for existence of small-study effects fulfilled (p value for Egger's test <0·10, and largest study with a smaller [more conservative] effect size than random-effects summary effect size). ‡Meta-analyses did not provide adequate data to estimate the random-effects summary effect size, and we report the random-effects summary effect size as presented by the authors of the original meta-analyses. §In these analyses, the component studies did not define the type of surgery.

Table 1: **Characteristics, quantitative synthesis, and bias assessment of the 44 eligible meta-analyses of environmental risk factors for multiple sclerosis**

virus viral-capsid antigen (VCA),[50] bone mineral density in femoral neck, lumbar spine, and hip,[47] chronic cerebrospinal venous insufficiency,[55] cytomegalovirus infection,[24] infectious mononucleosis,[5] serum uric acid,[25] and smoking.[6] In only three meta-analyses—those of anti-EBNA IgG seropositivity, infectious mononucleosis, and smoking—the 95% prediction interval rule for random-effects model did not include the null. The remaining meta-analyses of risk factors had prediction intervals that included the null value, showing that, although on average some risk factors are associated with multiple sclerosis, this might not always be the case in specific settings (tables 1, 2).

The results of the largest study were more conservative than the summary result in 19 (43%) meta-analyses. However, the largest study was typically not very large or substantially different in weight from other studies. In seven meta-analyses, the SD of the largest study was less than 0·10 in a log OR scale (it was <0·20 in 16 meta-analyses).

21 (48%) meta-analyses had large heterogeneity (I^2≥50%) and 12 (27%) meta-analyses had very large heterogeneity (I^2>75%). The meta-analyses with very large heterogeneity examined asthma, dental amalgam, bone mineral density in femoral neck and hip, *Chlamydia pneumoniae* DNA in CSF, chronic cerebrospinal venous insufficiency, immunoglobulins for *C pneumoniae* in serum, immunoglobulins for *C pneumoniae* in CSF, organic solvents, serum homcysteine, serum vitamin D, and serum uric acid.

Evidence for small-study effects was noted in ten (23%) meta-analyses by use of the Egger's test. In six of these ten meta-analyses (serum vitamin D, BCG vaccination, serum folate, anti-EBV IgG seronegativity, bone mineral density in lumbar spine, and organic solvents) the largest individual study had a more

	Sample size (number of cases)	Significance threshold reached (under the random-effects model)	95% prediction interval rule	Estimate of heterogeneity*	Small-study effects or excess significance bias	Random-effects summary effect size (95% CI)
Associations supported by convincing evidence						
Anti-EBNA IgG seropositivity[50]	>1000	<0·001	Excluding the null value	Not large	Neither	4·46 (3·26–6·09)
Infectious mononucleosis[5]	>1000	<0·001	Excluding the null value	Not large	Neither	2·17 (1·97–2·39)
Smoking[6]	>1000	<0·001	Excluding the null value	Not large	Neither	1·52 (1·39–1·66)
Associations supported by suggestive evidence						
Appendectomy at age ≤20 years[53]	>1000	<0·05 but >0·001	Including the null value	Not large	Neither	1·17 (1·02–1·34)
Diphtheria vaccination[51]	<500	<0·05 but >0·001	Including the null value	Not large	Neither	0·60 (0·40–0·91)
EBV DNA in mononuclear cells and serum[52]	<500	<0·05 but >0·001	Including the null value	Not large	Neither	1·84 (1·02–3·30)
Serum vitamin B_{12}[46]	<500	<0·05 but >0·001	Including the null value	Not large	Neither	0·64 (0·44–0·93)
Tetanus vaccination[51]	>500 but <1000	<0·05 but >0·001	Including the null value	Not large	Neither	0·71 (0·57–0·88)
Tonsillectomy at age ≤20 years[53]	>1000	<0·05 but >0·001	Including the null value	Not large	Neither	1·32 (1·09–1·61)
Traumatic injury[41]	>1000	<0·05 but >0·001	Including the null value	Not large	Neither	1·41 (1·03–1·92)
Associations supported by weak evidence						
Anti-EBV IgG seronegativity[50]	>500 but <1000	<0·001	Including the null value	Large	Neither	0·13 (0·05–0·32)
Anti-VCA IgG seropositivity[50]	>1000	<0·001	Including the null value	Large	Neither	4·52 (2·85 to 7·15)
BMD in femoral neck[47]	>500 but <1000	<0·001	Including the null value	Very large	Excess significance bias	0·36 (0·21–0·61)
BMD in lumbar spine[47]	>500 but <1000	<0·001	Including the null value	Large	Small-study effects	0·34 (0·24–0·50)
BMD in hip[47]	<500	<0·001	Including the null value	Very large	Neither	0·33 (0·18–0·60)
Chlamydia pneumoniae DNA in CSF[23]	>500 but <1000	<0·05 but >0·001	NA	Very large	NA†	3·22 (1·20–8·59)
Chronic cerebrospinal venous insufficiency[55]	>1000	<0·001	Including the null value	Very large	Neither	8·45 (3·47–20·56)
Intrathecal production of Ig for *Chlamydia pneumoniae*[23]	<500	<0·05 but >0·001	NA	Large	NA†	3·84 (1·32–11·21)
Organic solvents[49]	>1000	<0·05 but >0·001	Including the null value	Very large	Small-study effects	1·54 (1·03–2·29)
Serum vitamin D[45]	>1000	<0·05 but >0·001	Including the null value	Very large	Small-study effects	0·44 (0·24–0·78)
Serum homocysteine[46]	>500 but <1000	<0·05 but >0·001	Including the null value	Very large	Neither	4·57 (1·40–14·89)
Serum uric acid[25]	NA	<0·001	Including the null value	Very large	Neither	0·28 (0·14–0·57)
Associations not adequately assessed owing to absence of data						
Cytomegalovirus infection[24]	>1000	<0·001	NA	NA	NA	0·77 (0·67–0·87)
Non-significant associations						
Anti-EA IgG seropositivity[50]	>1000	>0·05	Including the null value	Large	Neither	1·40 (0·93–2·10)
Appendectomy at age >20 years[53]	>1000	>0·05	Including the null value	Not large	Small-study effects	1·26 (0·92–1·72)

(Table 2 continues on next page)

	Sample size (number of cases)	Significance threshold reached (under the random-effects model)	95% prediction interval rule	Estimate of heterogeneity*	Small-study effects or excess significance bias	Random-effects summary effect size (95% CI)
(Continued from previous page)						
Adenoidectomy at age ≤20 years[53]	<500	>0·05	Including the null value	Not large	Neither	1·07 (0·68–1·68)
Allergic disease[54]	>1000	>0·05	Including the null value	Large	Neither	0·91 (0·68–1·23)
Allergic rhinitis[54]	>1000	>0·05	Including the null value	Large	Neither	0·82 (0·59–1·12)
Asthma[54]	>1000	>0·05	Including the null value	Very large	Neither	0·83 (0·47–1·44)
BCG vaccination[51]	>500 but <1000	>0·05	Including the null value	Not large	Small-study effects	0·96 (0·69–1·34)
Dental amalgam[48]	<500	>0·05	Including the null value	Very large	Neither	1·24 (0·96–1·62)
EBV DNA in CSF and brain tissue[52]	<500	>0·05	Including the null value	Not large	Neither	1·57 (0·66–3·73)
Eczema[54]	>1000	>0·05	Including the null value	Not large	Neither	0·93 (0·71–1·22)
Hepatitis B vaccination[51]	>1000	>0·05	Including the null value	Not large	Neither	1·00 (0·74–1·37)
Ig for *Chlamydia pneumoniae* in serum[23]	>500 but <1000	>0·05	NA	Very large	NA†	1·07 (0·75–1·53)
Ig for *Chlamydia pneumoniae* in CSF[23]	<500	>0·05	NA	Very large	NA†	3·82 (0·72–20·37)
Influenza vaccination[51]	>1000	>0·05	Including the null value	Not large	Neither	0·97 (0·77–1·23)
MMR vaccination[51]	>500 but <1000	>0·05	Including the null value	Not large	Neither	1·02 (0·64–1·62)
Other surgeries at age ≤20 years[53]	<500	>0·05	Including the null value	Large	Excess significance	0·80 (0·50–1·28)
Other surgeries at age >20 years[53]	>1000	>0·05	Including the null value	Large	Excess significance	1·19 (0·86–1·65)
Poliomyelitis vaccination[51]	>500 but <1000	>0·05	Including the null value	Not large	Neither	0·87 (0·61–1·26)
Serum folate[46]	<500	>0·05	Including the null value	Not large	Small-study effects	0·85 (0·61–1·17)
Tonsillectomy at age >20 years[53]	>1000	>0·05	Including the null value	Not large	Excess significance	1·19 (0·94–1·50)
Typhoid fever vaccination[51]	<500	>0·05	Including the null value	Not large	Neither	1·05 (0·72–1·53)

Convincing evidence criteria: more than 1000 cases, significant summary associations (p<0·001) per random-effects calculations, no evidence of small-study effects, no evidence for excess significance bias, prediction intervals not including the null, and not large heterogeneity (I^2≤50%). Suggestive evidence criteria: nominally significant summary associations (p<0·05) per random-effects calculations, no evidence of small-study effects, no evidence for excess significance bias, and not large heterogeneity (I^2<50%). Weak evidence criteria: all other risk factors with nominally significant summary associations (p<0·05). Non-significant associations were p>0·05. EBNA=Epstein-Barr virus nuclear antigen. EBV=Epstein-Barr virus. VCA=viral-capsid antigen. BMD=bone mineral density. EA=early antigen. NA=not available. MMR=measles, mumps, and rubella. *Heterogeneity was categorised as not large (I^2<50%), large (I^2≥50% but I^2≤75%), and very large (I^2>75%). †In the respondent risk factors, small-study effects were not present, but the relevant articles did not provide adequate data to do the excess statistical significance test.

Table 2: **Assessment across the 44 associations of environmental risk factors with multiple sclerosis**

conservative effect size than the random-effects summary effect size. Assuming that the effect size in the largest study was the true effect, four of the 38 meta-analyses (bone mineral density in femoral neck, tonsillectomy at age >20 years, other surgeries at age >20 years, and other surgeries at age ≤20 years) had a significant difference between the number of observed and expected positive studies.

Of the 44 eligible meta-analyses, ten (23%) had nominally significant summary associations (p<0·05) per random-effects calculation and had no evidence of small-study effects, had no evidence for excess significance bias, and did not have large heterogeneity (I^2<50%). These meta-analyses pertained to vaccination for diphtheria,[51] vaccination for tetanus,[51] smoking,[6] traumatic injury,[41] infectious mononucleosis,[5] anti-EBNA

IgG seropositivity,[50] Epstein-Barr virus DNA in mononuclear cells and serum,[52] elevated serum B_{12},[46] and appendectomy at age 20 years or younger,[51] and tonsillectomy at age 20 years or younger.[53] Of these only anti-EBNA IgG seropositivity, smoking, and infectious mononucleosis included more than 1000 cases in the meta-analysis, effects with p values less than 0·001, and a 95% prediction interval excluding the null value. Anti-EBNA IgG seropositivity had a summary OR of 4·46 (95% CI 3·26–6·09; $p=1·5\times10^{-19}$) with moderate heterogeneity (I^2=43%). Evidence was supported by a total of 3511 cases. Smoking had a summary OR of 1·52 (95% CI 1·39–1·66; $p=1·7\times10^{-18}$) with 3052 cases, and infectious mononucleosis had an OR of 2·17 (95% CI 1·97–2·39; $p=3·1\times10^{-50}$) with 19 519 cases; there was no observed heterogeneity (I^2=0%) with either association.

An overall assessment of significant risk factors for multiple sclerosis—using as criteria the sample size, the replication as expressed by the I^2 hetrogeneity metric, a more conservative p value than p less than 0·05 (p<0·001), the 95% prediction interval rule (ie, excluding the null value), and the presence of small-study effects—is presented in table 2.

In the meta-analysis on anti-EBNA IgG seropositivity, data quality was assessed by use of the Newcastle-Ottawa assessment scale[50] and in those on infectious mononucleosis and smoking data quality was assessed by study design, exposure assessment, and diagnostic criteria.[5,6] All three meta-analyses compared the summary estimates of studies of high versus low quality and reported no significant differences between the subgroups.

Discussion

We provide an overview and appraisal of environmental risk factors that have been associated with multiple sclerosis. Overall, 44 risk factors have been studied for an association with the disease, including infections and vaccinations, comorbid diseases, surgeries, traumatic events and accidents, exposure to toxic environmental agents, and biochemical biomarkers. Only three of these risk factors (anti-EBNA IgG seropositivity, infectious mononucleosis, and smoking) were supported by evidence with strong epidemiological credibility, as expressed by large sample size, not large heterogeneity, not suggestive of small-study effects and excess significance bias, a p value less than 0·001, and a prediction interval excluding the null. Among these studies, the summary effect sizes were relatively large for anti-EBNA IgG seropositivity and infectious mononucleosis (OR>2).

Our assessment did not show an overall excess of findings with significant results, by contrast with other fields, in which an excess of significant results is reported.[56–58] In our study, a large proportion of the examined meta-analyses had large heterogeneity and small-study effects. The applied Egger test is particularly difficult to interpret when between-study heterogeneity is large.[18] Heterogeneity might often be a manifestation of bias in some studies of a meta-analysis, but could also emerge from genuine differences across studies. Genuine heterogeneity might operate in the field of multiple sclerosis. Prevalence and incidence of multiple sclerosis has marked geographical heterogeneity, which might be shown here as differential association of the risk factors in different geographical regions.[8,59] Other reasons for heterogeneity include the mixture of cohort studies and case-control studies in some of the meta-analyses, differences in definition of multiple sclerosis (which varies over time as diagnostic criteria change and varies between geographical regions), differences in exposure assessment, differences in frequency of exposed control groups, and differential response rates among cases and controls in the primary studies. The reported associations with disease need to be interpreted with caution, in particular for the meta-analyses in which the heterogeneity is large, the number of studies is relatively small, the largest study is more conservative than the summary effect, and small-study effects are evident.

Meta-analyses examining infections and vaccinations were the largest proportion of studies and meta-analyses we identified. Many bacterial and viral agents have been associated with multiple sclerosis. However, Epstein-Barr virus was the only viral agent with consistent evidence for an association with multiple sclerosis. Infectious mononucleosis and anti-EBNA IgG seropositivity had significantly positive associations with multiple sclerosis with no signs of bias. The estimated prediction intervals of these meta-analyses were very wide but did not include the null, and show that the effect size of the association might vary substantially in different settings. In particular, the summary effect size of the anti-EBNA IgG seropositivity was large (OR>4) with wide 95% prediction intervals, suggesting that there is probably genuine heterogeneity in the association. Serum concentrations of anti-EBNA antibodies are thought to be a marker of good immune response to Epstein-Barr virus and are usually higher in individuals with a history of infectious mononucleosis. Epstein-Barr virus in turn is the main cause of infectious mononucleosis. Several theories have been suggested to explain the possible mechanism underpinning the epidemiological associations of multiple sclerosis and Epstein-Barr virus, but firm conclusions cannot be made. Moreover, the clinical implications are unclear because presently there is no licensed Epstein-Barr virus vaccine, and plans for developing a vaccine have been debated.[60] If phase 3 and 4 clinical trials are launched for promising Epstein-Barr virus vaccines, tracking the incidence of multiple sclerosis would be useful, but any effect might need many years of follow-up to establish. Another potential role of a Epstein-Barr virus biomarker could be in differential diagnosis (eg, low anti-EBNA titres in an

individual with clinically isolated syndrome might suggest a lower chance of multiple sclerosis).[61,62] Furthermore, the discovery of treatment for the latent Epstein-Barr virus infection could be another option for the prevention of multiple sclerosis. Rituximab, a monoclonal antibody with lytic effects on B cells, has been tested on multiple sclerosis patients with history of infectious mononucleosis, but the potential benefits and safety of such therapy for disease prevention should be assessed on a large scale with high quality randomised clinical trials and long-term follow-up.[63]

Smoking was the only other risk factor, apart from Epstein-Barr virus infection, that showed consistent association with multiple sclerosis without the presence of bias. The association between smoking and multiple sclerosis is positive but of modest effect. Numerous mechanisms have been proposed to explain the adverse effects of smoking on multiple sclerosis—including effects on the immune system and immunomodulatory effects, demyelination, and disruption of the blood–brain barrier—but all remain speculative.[61,64] Confounding cannot be totally excluded. Nevertheless, promotion of smoking cessation is probably one of the most straightforward and effective public health interventions and has multiple well established benefits, regardless of the extent to which it might also reduce the incidence of multiple sclerosis.[8]

Our analysis also confirms that the association between several risk factors and multiple sclerosis is null or has very small effect. These include several vaccinations (tetanus, diphtheria, influenza, BCG, mumps, measles and rubella, poliomyelitis, hepatitis B virus, and typhoid fever), biochemical factors, presence of dental amalgam, past surgeries and traumatic events (tonsillectomy, adenoidectomy, and traumatic injury), and presence of allergies, eczema, and chronic cerebrospinal venous insufficiency. The absence of association between these factors and multiple sclerosis might have important clinical implications because such risk factors have been thought to cause multiple sclerosis in populations leading to clinical actions that are unjustified and not evidence-based. For example, in early 1998, hepatitis B vaccination was suspended in France[65] after preliminary results of two case-control studies done in France and the UK reported a non-significant increase in the risk of multiple sclerosis among vaccinated as compared with unvaccinated patients.[66,67] The present study, together with compelling evidence from pharmacovigilance and pharmacoepidemiological sources, confirms no association between the vaccine and multiple sclerosis onset.[51,68,69]

Chronic cerebrospinal venous insufficiency, a chronic state of impaired venous drainage in the central nervous system, has also been thought to be associated with risk for multiple sclerosis by impairing blood drainage from the brain and upper spinal cord.[70,71] Some organisations promote treatment of chronic cerebrospinal venous insufficiency with percutaneous balloon angioplasty[72,73] to treat multiple sclerosis. Our analysis shows that, despite the fact that the summary OR is significant and has a relatively strong effect, the prediction interval is very wide and includes the null, and heterogeneity is very large. Presently, there is no clear scientific evidence to support the link between chronic cerebrospinal venous insufficiency and multiple sclerosis.

Vitamin D and sun exposure have also received attention as risk factors for multiple sclerosis in an attempt to explain the geographical trends and latitude of multiple sclerosis incidence.[7,8] Here, we show that, despite the significant association of higher serum 25(OH)D (a biomarker of vitamin D in serum) with lower multiple sclerosis incidence, the estimated prediction interval of the effect size included the null, which suggests that in some settings the effect of vitamin D on multiple sclerosis might be absent. Additionally, the evidence for vitamin D had very large heterogeneity and the presence of small-study effects, further suggesting that the evidence supporting low serum vitamin D concentrations as a risk for multiple sclerosis is weak and requires assessment in prospective studies and clinical trials.[74] Similarly, associations between homocysteine and multiple sclerosis had large heterogeneity and asymmetry. Presently, vitamin D and folate supplementation for multiple sclerosis prevention do not have a strong evidence base.

Our analysis has some caveats. First, some meta-analyses[23–25] were excluded from heterogeneity and bias tests because they did not provide adequate data to do the respective analyses. Second, both asymmetry and excess statistical significance tests offer suggestions of bias, and not definitive proof thereof. Third, effect inflation might affect even the results of the largest studies because often these studies were not necessarily very large or might have had inherent biases themselves. Thus, our estimates of the extent of excess statistical significance are probably conservative. Additionally, we did not appraise the quality of the individual component primary studies because this was beyond the scope of this umbrella review. This was the aim of the original systematic reviews and meta-analyses, which should include an assessment of study quality and whether the study should be included in the quantitative calculations. The meta-analysis for the association between cytomegalovirus infection and multiple sclerosis[24] had a p value of less than 0·001, but it could not be fully assessed because I^2 and the predictive interval could not be estimated owing to the absence of data. An association between cytomegalovirus infection and risk for multiple sclerosis could not be excluded. Also, in our analysis we assessed only associations considered by meta-analyses of observational studies. Thus, we might miss other associations with adequate evidence that have not yet been assessed through meta-analytic approaches.

Acknowledging these caveats, our assessment maps the status of evidence on 44 associations between

environmental risk factors and risk for multiple sclerosis. Only three risk factors provided credible evidence for positive associations with multiple sclerosis without the presence of substantial caveats: infectious mononucleosis, anti-EBNA IgG seropositivity, and smoking. The mechanisms of these risk factors are not well understood and the public health implications for disease prevention based on Epstein-Barr virus infection are not clear. Data from more studies and investigation of sources of heterogeneity are needed to better understand the associations between the remaining risk factors and multiple sclerosis. Our methods are likely to be generalisable to observational studies of other clinical areas beyond multiple sclerosis, because most biases described herein are not specific to multiple sclerosis but operate in observational research of other chronic diseases as well. As previously suggested for observational research and biomarker studies in general,[58,75] use of standardised definitions for outcomes and exposures, adoption of reporting guidelines (eg, Strengthening the Reporting of Observational Studies in Epidemiology [STROBE]),[76,77] and registration of hypothesis-testing observational studies[78,79] might help to improve the evidence in the future.

For more on the **STROBE guideline** see http://www.strobe-statement.org/

Contributors

VB, LB, JPAI, and IT had the original idea for the study and designed the study. LB, EE, and VB did the analysis. LB, VB, and IT wrote the first draft of the manuscript. All authors had full access to study data. All authors critically reviewed and wrote the final version. All authors approved the final version of the manuscript. IT had final responsibility for the decision to submit the paper for publication.

Declaration of interests

We declare no competing interests.

References

1 Gilden DH. Infectious causes of multiple sclerosis. *Lancet Neurol* 2005; **4:** 195–202.

2 Multiple Sclerosis International Federation. Atlas of MS 2013: mapping multiple sclerosis around the world. London: Multiple Sclerosis International Federation, 2013.

3 Kingwell E, Marriott JJ, Jette N, et al. Incidence and prevalence of multiple sclerosis in Europe: a systematic review. *BMC Neurol* 2013; **13:** 128.

4 Evans C, Beland SG, Kulaga S, et al. Incidence and prevalence of multiple sclerosis in the Americas: a systematic review. *Neuroepidemiology* 2013; **40:** 195–210.

5 Handel AE, Williamson AJ, Disanto G, Handunnetthi L, Giovannoni G, Ramagopalan SV. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS One* 2010; **5:** e12496.

6 Handel AE, Williamson AJ, Disanto G, Dobson R, Giovannoni G, Ramagopalan SV. Smoking and multiple sclerosis: an updated meta-analysis. *PLoS One* 2011; **6:** e16149.

7 Simpson S Jr, Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. *J Neurol Neurosurg Psychiatry* 2011; **82:** 1132–41.

8 Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *Lancet Neurol* 2010; **9:** 727–39.

9 Ioannidis JP. Integration of evidence from multiple meta-analyses: a primer on umbrella reviews, treatment networks and multiple treatments meta-analyses. *CMAJ* 2009; **181:** 488–93.

10 DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177–88.

11 Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997; **127:** 820–26.

12 Higgins JP, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. *J R Stat Soc Ser A* 2009; **172:** 137–59.

13 Higgins JP. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. *Int J Epidemiol* 2008; **37**: 1158–60.

14 Chinn S. A simple method for converting an odds ratio to effect size for use in meta-analysis. *Stat Med* 2000; **19:** 3127–31.

15 Cochran W. The combination of estimates from different experiments. *Biometrics* 1954; **10:** 101–29.

16 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21:** 1539–58.

17 Ioannidis JP, Patsopoulos NA, Evangelou E. Uncertainty in heterogeneity estimates in meta-analyses. *BMJ* 2007; **335:** 914–16.

18 Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ* 2011; **343**: d4002.

19 Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315:** 629–34.

20 Ioannidis JP, Trikalinos TA. An exploratory test for an excess of significant findings. *Clin Trials* 2007; **4:** 245–53.

21 Ioannidis JP. Clarifications on the application and interpretation of the test for excess significance and its extensions. *J Math Psychol* 2013; **57:** 184–87.

22 Lubin JH, Gail MH. On power and sample size for studying features of the relative odds of disease. *Am J Epidemiol* 1990; **131:** 552–66.

23 Bagos PG, Nikolopoulos G, Ioannidis A. *Chlamydia pneumoniae* infection and the risk of multiple sclerosis: a meta-analysis. *Mult Scler* 2006; **12:** 397–411.

24 Sundqvist E, Bergstrom T, Daialhosein H, et al. Cytomegalovirus seropositivity is negatively associated with multiple sclerosis. *Mult Scler* 2014; **20:** 165–73.

25 Liu B, Shen Y, Xiao K, Tang Y, Cen L, Wei J. Serum uric acid levels in patients with multiple sclerosis: a meta-analysis. *Neurol Res* 2012; **34:** 163–71.

26 Ioannidis JP, Tarone R, McLaughlin JK. The false-positive to false-negative ratio in epidemiologic studies. *Epidemiology* 2011; **22:** 450–56.

27 Johnson VE. Revised standards for statistical evidence. *Proc Natl Acad Sci USA* 2013; **110:** 19313–17.

28 Laupacis A, Lillie E, Dueck A, et al. Association between chronic cerebrospinal venous insufficiency and multiple sclerosis: a meta-analysis. *CMAJ* 2011; **183:** E1203–12.

29 Zwischenberger BA, Beasley MM, Davenport DL, Xenos ES. Meta-analysis of the correlation between chronic cerebrospinal venous insufficiency and multiple sclerosis. *Vasc Endovasc Surg* 2013; **47:** 620–24.

30 Ascherio A, Munch M. Epstein-Barr virus and multiple sclerosis. *Epidemiology* 2000; **11:** 220–24.

31 Pakpoor J, Disanto G, Gerber JE, et al. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. *Mult Scler* 2013; **19:** 162–66.

32 Demicheli V, Rivetti A, Di Pietrantonj C, Clements CJ, Jefferson T. Hepatitis B vaccination and multiple sclerosis: evidence from a systematic review. *J Viral Hepat* 2003; **10:** 343–44.

33 Martinez-Sernandez V, Figueiras A. Central nervous system demyelinating diseases and recombinant hepatitis B vaccination: a critical systematic review of scientific production. *J Neurol* 2013; **260**: 1951–59.

34 Rutschmann OT, McCrory DC, Matchar DB, Immunization Panel of the Multiple Sclerosis Council for Clinical Practice Guidelines. Immunization and MS: a summary of published evidence and recommendations. *Neurology* 2002; **59:** 18370–43.

35 Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. *Ann Neurol* 2006; **59:** 499–503.

36 Hernan MA, Alonso A, Hernandez-Diaz S. Tetanus vaccination and risk of multiple sclerosis: a systematic review. *Neurology* 2006; **67:** 212–15.

37 Landtblom AM, Flodin U, Soderfeldt B, Wolfson C, Axelson O. Organic solvents and multiple sclerosis: a synthesis of the current evidence. *Epidemiology* 1996; **7:** 429–33.

38 Hawkes CH. Smoking is a risk factor for multiple sclerosis: a metanalysis. *Mult Scler* 2007; **13:** 610–15.

39 Zhu Y, Liu HN, Zhang CD, Liang HY. [Meta-analysis on relationship between hyperhomocysteinemia and multiple sclerosis]. *Zhonghua Yi Xue Za Zhi* 2009; **89:** 3055–57.

40 Lunny CA, Fraser SN, Knopp-Sihota JA. Physical trauma and risk of multiple sclerosis: a systematic review and meta-analysis of observational studies. *J Neurol Sci* 2014; **336:** 13–23.

41 Warren SA, Olivo SA, Contreras JF, et al. Traumatic injury and multiple sclerosis: a systematic review and meta-analysis. *Can J Neurol Sci* 2013; **40:** 168–76.

42 Artemiadis AK, Anagnostouli MC, Alexopoulos EC. Stress as a risk factor for multiple sclerosis onset or relapse: a systematic review. *Neuroepidemiology* 2011; **36:** 109–20.

43 Goulden R, Ibrahim T, Wolfson C. Is high socioeconomic status a risk factor for multiple sclerosis? A systematic review. *Eur J Neurol* 2014; published online Nov 5.DOI: 10.1111/ene.12586.

44 Zhornitsky S, Yong VW, Weiss S, Metz LM. Prolactin in multiple sclerosis. *Mult Scler* 2013; **19:** 15–23.

45 Duan S, Lv Z, Fan X, et al. Vitamin D status and the risk of multiple sclerosis: a systematic review and meta-analysis. *Neurosci Lett* 2014; **570:** 108–13.

46 Zhu Y, He ZY, Liu HN. Meta-analysis of the relationship between homocysteine, vitamin B12, folate, and multiple sclerosis. *J Clin Neurosci* 2011; **18:** 933–38.

47 Huang Z, Qi Y, Du S, Chen G, Yan W. Bone mineral density levels in adults with multiple sclerosis: a meta-analysis. *Int J Neurosci* 2014; published online Dec 18. DOI:10.3109/00207454.2014.988332.

48 Aminzadeh KK, Etminan M. Dental amalgam and multiple sclerosis: a systematic review and meta-analysis. *J Public Health Dent* 2007; **67**: 64–66.

49 Barragan-Martinez C, Speck-Hernandez CA, Montoya-Ortiz G, Mantilla RD, Anaya JM, Rojas-Villarraga A. Organic solvents as risk factor for autoimmune diseases: a systematic review and meta-analysis. *PLoS One* 2012; **7:** e51506.

50 Almohmeed YH, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PLoS One* 2013; **8:** e61110.

51 Farez MF, Correale J. Immunizations and risk of multiple sclerosis: systematic review and meta-analysis. *J Neurol* 2011; **258:** 1197–206.

52 Santiago O, Gutierrez J, Sorlozano A, de Dios Luna J, Villegas E, Fernandez O. Relation between Epstein-Barr virus and multiple sclerosis: analytic study of scientific production. *Eur J Clin Microbiol Infect Dis* 2010; **29:** 857–66.

53 Lunny C, Knopp-Sihota JA, Fraser SN. Surgery and risk for multiple sclerosis: a systematic review and meta-analysis of case-control studies. *BMC Neurol* 2013; **13:** 41.

54 Monteiro L, Souza-Machado A, Menezes C, Melo A. Association between allergies and multiple sclerosis: a systematic review and meta-analysis. *Acta Neurol Scand* 2011; **123:** 1–7.

55 Tsivgoulis G, Sergentanis TN, Chan A, et al. Chronic cerebrospinal venous insufficiency and multiple sclerosis: a comprehensive meta-analysis of case-control studies. *Ther Adv Neurol Disord* 2014; **7:** 114–36.

56 Tsilidis KK, Panagiotou OA, Sena ES, et al. Evaluation of excess significance bias in animal studies of neurological diseases. *PLoS Biol* 2013; **11:** e1001609.

57 Tsilidis KK, Papatheodorou SI, Evangelou E, Ioannidis JP. Evaluation of excess statistical significance in meta-analyses of 98 biomarker associations with cancer risk. *J Natl Cancer Inst* 2012; **104:** 1867–78.

58 Tzoulaki I, Siontis KC, Evangelou E, Ioannidis JP. Bias in associations of emerging biomarkers with cardiovascular disease. *JAMA Intern Med* 2013; **173:** 664–71.

59 Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372:** 1502–17.

60 Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci Transl Med* 2011; **3:** 107fs7.

61 Ascherio A, Munger KL, Lunemann JD. The initiation and prevention of multiple sclerosis. *Nat Rev Neurol* 2012; **8:** 602–12.

62 Lunemann JD, Tintore M, Messmer B, et al. Elevated Epstein-Barr virus-encoded nuclear antigen-1 immune responses predict conversion to multiple sclerosis. *Ann Neurol* 2010; **67:** 159–69.

63 He D, Guo R, Zhang F, Zhang C, Dong S, Zhou H. Rituximab for relapsing-remitting multiple sclerosis. *Cochrane Database Syst Rev* 2013; **12:** CD009130.

64 van der Mei IA, Simpson S Jr, Stankovich J, Taylor BV. Individual and joint action of environmental factors and risk of MS. *Neurol Clin* 2011; **29:** 233–55.

65 Monteyne P, Andre FE. Is there a causal link between hepatitis B vaccination and multiple sclerosis? *Vaccine* 2000; **18:** 1994–2001.

66 Hernan MA, Jick SS, Olek MJ, Jick H. Recombinant hepatitis B vaccine and the risk of multiple sclerosis: a prospective study. *Neurology* 2004; **63:** 838–42.

67 Touze E, Fourrier A, Rue-Fenouche C, et al. Hepatitis B vaccination and first central nervous system demyelinating event: a case-control study. *Neuroepidemiology* 2002; **21:** 180–86.

68 Mikaeloff Y, Caridade G, Rossier M, Suissa S, Tardieu M. Hepatitis B vaccination and the risk of childhood-onset multiple sclerosis. *Arch Pediatr Adolesc Med* 2007; **161:** 1176–82.

69 Sadovnick AD, Scheifele DW. School-based hepatitis B vaccination programme and adolescent multiple sclerosis. *Lancet* 2000; **355:** 549–50.

70 Zamboni P. The big idea: iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *J R Soc Med* 2006; **99:** 589–93.

71 Zamboni P, Galeotti R, Menegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009; **80:** 392–99.

72 Zamboni P, Galeotti R, Menegatti E, et al. A prospective open-label study of endovascular treatment of chronic cerebrospinal venous insufficiency. *J Vasc Surg* 2009; **50:** 1348–58 e1-3.

73 Zamboni P, Bertolotto A, Boldrini P, et al. Efficacy and safety of venous angioplasty of the extracranial veins for multiple sclerosis. Brave dreams study (brain venous drainage exploited against multiple sclerosis): study protocol for a randomized controlled trial. *Trials* 2012; **13:** 183.

74 Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014; **348**: g2035.

75 Tzoulaki I, Siontis KC, Ioannidis JP. Prognostic effect size of cardiovascular biomarkers in datasets from observational studies versus randomised trials: meta-epidemiology study. *BMJ* 2011; **343:** d6829.

76 Gallo V, Egger M, McCormack V, et al. STrengthening the Reporting of OBservational studies in Epidemiology—Molecular Epidemiology (STROBE-ME): an extension of the STROBE statement. *PLoS Med* 2011; **8:** e1001117.

77 von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007; **4:** e296.

78 Dal-Re R, Ioannidis JP, Bracken MB, et al. Making prospective registration of observational research a reality. *Sci Transl Med* 2014; **6:** 224cm1.

79 Ioannidis JP. The importance of potential studies that have not existed and registration of observational data sets. *JAMA* 2012; **308:** 575–76.