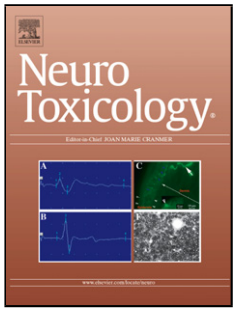

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Factors associated with onset, relapses or progression in multiple sclerosis: a systematic review

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ABSTRACT

Multiple sclerosis (MS) is a chronic disease of the central nervous system with an unidentified etiology. We systematically reviewed the literature on the possible risk factors associated with MS disease onset, relapses and progression from 1960-2012 by accessing six databases and including relevant systematic reviews, meta-analyses, case-control or cohort studies. The focus was on identifying modifiable risk factors. Fifteen systematic reviews and 169 original articles were quality assessed and integrated into a descriptive review. Best evidence, which included one or more prospective studies, suggested that lower exposure to sunlight and/or lower serum vitamin D levels were associated with an increased risk of developing MS onset and subsequent relapses, but a similar quality of evidence was lacking for disease progression. Prospective studies indicated that cigarette smoking may increase the risk of MS as well as accelerate disease progression, but whether smoking altered the risk of a relapse was largely unknown. Infections were implicated in both risk of developing MS and relapses, but data for progression were lacking. Specifically, exposure to the Epstein-Barr virus, particularly if this manifested as infectious mononucleosis during adolescence, was associated with increased MS risk. Upper respiratory tract infections were most commonly associated with an increase in relapses. Relapse rates typically dropped during pregnancy, but there was no strong evidence to suggest that pregnancy itself altered the risk of MS or affected long-term progression. Emerging research with the greatest potential to impact public health was the suggestion that obesity during adolescence may increase the risk of MS; if confirmed, this would be of major significance.

Keywords: multiple sclerosis; risk factors; systematic review; etiology; relapses; progression

1. INTRODUCTION

Multiple sclerosis (MS) is a complex neurological condition characterized by inflammation, demyelination and axonal degeneration of the central nervous system. It is considered the most common cause of neurologic disability among young adults in the West (Leary et al., 2005). Approximately 1.3 million people are affected worldwide (WHO, 2008). North America, Europe and Australasia have moderate to high rates of MS, with around 1-2 people per 1000 affected (Evans et al., 2013). MS incidence typically peaks around 30 years of age, with more women than men affected (Marrie et al., 2010). The high prevalence in conjunction with the relatively young onset age and the chronic nature of MS translates into higher societal costs than either stroke or Alzheimer's disease (Pugliatti et al., 2006).

Although many factors – including genetic and environmental – have been implicated in either triggering MS or modulating the subsequent disease course, results vary substantially between studies. Few systematic reviews consider more than one risk factor at a time, such that it is hard to establish a comprehensive understanding of the (likely) multiple risk factors involved in modifying MS risk. In addition, few risk factors have been successfully targeted or modified in order to reduce the risk of developing MS or delay disease progression (drug treatments aside). Further, the literature surrounding risk factors associated with onset or disease progression in MS has grown rapidly over the last few decades. Therefore there is a real need to understand the broad range of risk factors linked with MS and the level of evidence associated with these factors.

This systematic review of the literature aimed to integrate findings on risk factors that might influence the onset of MS or MS disease activity (relapses or progression).

2. METHODS

This review was conducted using a centralized protocol designed by the University of Ottawa, Canada's National Population Health Study of Neurological Conditions project and adapted to study MS. Key points and adaptations are summarized here:

2.1 Inclusion and exclusion criteria

To be included in this systematic review articles had to be original observational studies (case-control or cohort), systematic reviews, or meta-analyses that examined at least one risk factor associated with the onset and/or disease activity (relapses or progression) in MS. Only studies that reported a quantifiable measure of risk, involved human subjects, and were published in English or French were included.

Articles were excluded on any of the following grounds: did not specifically assess risk factors, e.g. studies which examined biomarkers for diagnostic purposes; did not analyze or report quantitative data e.g. were predominantly descriptive; the risk factor under review was a form of intervention or pharmacological treatment for the disease or part of a randomized controlled trial designed for a therapeutic intervention. An exception was made for studies occurring within a clinical trial or making secondary use of the clinical trial data and only when the exposure of interest was unrelated to the therapeutic intervention. No restriction was imposed in terms of age, sex, race, geographical residence, or source of population of participants (e.g. community, hospital, outpatient, registry or health administrative data).

Outcomes of interest were: MS onset and disease activity (relapses or progression). MS onset was typically considered as the first symptomology related to subsequent diagnosis of MS. Ideally MS diagnosis would involve internationally-recognized criteria (e.g. Schumacher, Poser, or McDonald), although studies using other methods (e.g. health administrative data or self-report) were considered. There is a large body of research describing the risk of reaching a diagnosis of MS in special subgroups of patients, e.g. those with optic neuritis or other clinically isolated syndromes. These studies were considered beyond the scope of this review and were not included.

MS disease activity is multifaceted and variable and for the purposes of this review, we considered clinical relapses (‘attacks’) and disease progression. MS relapses (acute worsening of function followed by partial or complete recovery) were considered a measure of short-term disease activity. Studies measuring longer-term disease activity, i.e. progression were also included, regardless of what measure(s) were used. The Expanded Disability Status Scale (EDSS) is a 20-point scale (ranging from 0 = normal to 10 = death due to MS, marked by 0.5 increments) and is currently the most widely used measurement (Kurtzke, 1983). It is an expansion of the Disability Status Scale (DSS), which applied the same range, without the 0.5 increments (Kurtzke, 1955). The transition from relapsing-remitting MS (RRMS) to secondary progressive MS (SPMS) is also often used as a measure of progression, typically recognized clinically as ‘progression with or without occasional relapses, minor remissions, and plateaus.’ We did not include studies that focused on death (‘all-cause mortality’) as an outcome, nor did we include studies reporting the association between our defined outcomes e.g. between relapses and progression. All risk factors (other than interventions) were considered, although our primary focus was on factors most amenable to modification, i.e. with the potential for the highest public health impact. Demographic factors, such as age and sex were not a major focus. For example, it is generally well established that age and sex influence MS risk. This information is best represented in

incidence/prevalence studies which are being comprehensively covered in a separate series of systematic reviews funded in the same cycle as this current review (Evans et al., 2013; Kingwell et al., 2013). These studies are also well-suited to examine the possible influence of latitude in relation to MS risk; therefore latitude was not covered here. Genetic risk factors associated with MS disease activity (relapses or progression) were systematically reviewed; however, genetic factors associated with MS risk were considered beyond the scope of this review. Instead, findings from a credible and comprehensive field synopsis of published genetic association studies, MSGene, were summarized (Lill et al., 2013). Finally, the vast topic on the prognosis of MS (often termed 'the natural history of MS') was not systematically re-reviewed here, rather a brief overview was provided.

2.2 Search methods for identification of study scope

Firstly, a search strategy was developed using MeSH terms in MEDLINE (OvidSP) as well as relevant keywords. Subject headings explored in OvidSP were inclusive of "multiple sclerosis", "disseminated sclerosis", "chronic progressive", "acute fulminating relapsing" and "remitting". Synonym mapping, and scope notes in MEDLINE were used to identify the appropriate subject headings. All the relevant words (e.g. sclerosis), phrases (e.g. multiple sclerosis) or a combination were used. All possible synonyms (e.g. acute vs. severe), alternate terminologies (e.g. disseminated sclerosis vs. multiple sclerosis), variant word endings (sclerosis vs. multiple) were also adopted. No alternate spellings were required. Boolean logic was used to combine concepts and drop irrelevant articles.

Secondly, the following broader MeSH terms were added to the search: "risk factor", "odds ratio", "relative risk", "risk difference", "predictor" or "prediction" or "predisposition" and "progression". Keywords adopted from other search strategies of relevant studies were added to the current search.

The search for relevant publications was carried out in two stages; the first focused on identification of systematic reviews and/or meta-analyses, the second on original observational studies. The original search was conducted in May 2011 and then updated to the end of May 2012 for original articles, and December 2012 for systematic reviews/meta-analyses. This lag-time enabled the most recent systematic reviews to be included, thereby maximizing the number of studies included, whilst balancing time and resources which prevented an update on our search for original articles to the latter date. The following databases were searched from their respective initiation (year shown in brackets): MEDLINE (1996), Cochrane Central Register of Controlled Trials (1991); EMBASE (1980); CINHAL (1982), PSCYINFO (1990), AGELINE (1982). Searches were completed via OvidSP, and EBSCO. To minimize the possibility of a missed article, references of retrieved articles were checked and experts in the field were approached to critique early drafts of the review (see acknowledgements). These resources were considered as 'additional' records. Detailed search strategies are provided in the **Supplementary Material l.**

2.3 Article collection, screening and data extraction

Articles were initially stored in Endnote®, a reference manager, and duplicates were deleted. The remaining articles were transferred to Distiller®; an online application designed to facilitate literature screening and data extraction. Articles were screened at three levels: title, abstract, and full paper.

Article titles were assessed and either excluded or promoted by two reviewers (stage 1a). Abstracts of all potentially relevant articles were then retrieved and screened in a similar manner (stage 1b). Cohen's kappa coefficient for the level of agreement for the first 100 abstracts was 0.78. Differences of opinions were resolved by consensus.

2.4 Quality assessment

Quality assessment was performed at stage 3, using a pre-validated tool to assess the methodological quality of systematic reviews (AMSTAR) (Kang et al., 2012). Observational studies were assessed using the modified Downs and Black Criteria (Wigle et al., 2009).

Findings for onset and disease activity (relapses and progression) were reported separately, with articles grouped by the main exposure (risk factor) under study using the following broad categories: environmental and lifestyle, occupational, pre-existing co-morbidities or other medical conditions, biological, genetic and miscellaneous. To avoid duplication, original articles already included in a systematic review were not reportedly separately.

3. RESULTS

3.1 Risk Factors Associated with MS Onset

The screening process for articles related to MS disease onset is outlined in **Figure 1**. Nine systematic reviews (**Table 1**) and 122 original (**Supplementary Material II**) articles were included.

3.1.1 Environmental and Lifestyle Risk Factors

Viral infections

Two systematic reviews (Thacker et al., 2006; Handel et al., 2010a) investigated the association between infectious mononucleosis and MS. Infectious mononucleosis typically occurs after adolescent exposure to the Epstein-Barr Virus (EBV, also known as human herpes virus 4 [HHV-4]). Both reviews reported positive associations with the risks ranging from 2.2 to 2.3 (Thacker et al., 2006; Handel et al., 2010a). Both reviews included a search of one database (MEDLINE), one from 1965-2005 (Thacker et al., 2006), the other 2006-2010 (Handel et al., 2010a), and included a combined total of 32 case-control studies. Heterogeneity was reported only in the more recent review (Handel et al., 2010a) and was virtually absent (I^2=0%). The risk estimates were broadly similar in a later original study which found a history of infectious mononucleosis to be associated with a greater risk of MS among 678 newly diagnosed MS cases compared to controls (OR: 1.89; 1.45 – 2.48) (Sundqvist et al., 2012).

Twelve additional papers used other methods to measure EBV exposure, of which 8 accessed samples from incident cases (prior to MS onset) (Ascherio et al., 2001; Wagner et al., 2004; Massa et al., 2007; Levin et al., 2005; Levin et al., 2010; Munger et al., 2011; DeLorenze et al., 2006; Sundström et al., 2004) and 4 accessed samples from prevalent MS cases (Sumaya et al., 1985; Villoslada et al., 2003; Santón et al., 2011; Waubant et al., 2011). In a prospective study of incident cases of MS from the USA's Nurses' Health Study, EBNA-1 and EBNA-2 levels of 18 women collected prior to disease onset were higher than their matched controls (RR for EBNA-1: 2.5; 1.0 - 6.3; RR for EBNA-2: 3.9; 1.1 - 13.7) (Ascherio et al., 2001). Using the same samples, researchers also found an increased risk (albeit not significant) associated with the presence of EBV in plasma (RR: 2.5; 0.78 – 7.8) (Wagner et al., 2004). Anti-BZLF1 antibodies [involved in EBV infection by mediating the switch between latent and lytic infection (Mauser et al., 2002)] were also associated with an increased risk (RR: 4.6; 0.84 – 25.24); however, once adjusted for EBNA-2 levels, this association disappeared (RR: 0.72; 0.06 – 8.36) (Massa et al., 2007). Three nested case-control studies accessed US Military data sources, including the USA's Department of Defense Serum Repository to access samples largely collected before clinical onset of MS (Levin et al., 2005; Levin et al., 2010; Munger et al., 2011). One study included 83 cases and 153 matched controls from Army Personnel; 26 of the 50 cases had samples collected more than 5 years prior to MS onset (Levin et al., 2005). A four-fold increase in anti-EBNA complex or EBNA-1 were both associated with a three-fold increased risk of MS (RR for EBNA complex: 3.0; 1.3 – 6.5; RR for EBNA-1: 3.0; 1.2 – 7.3) (Levin et al., 2005). A subsequent study accessed both Army and Navy personnel, including serum samples from 305 MS patients collected prior to the onset of disease and matched with 610 controls (Levin et al., 2010). Of 10 initially EBV-negative cases that went on to develop MS, 100% (10/10) converted to EBV-positive prior to their MS onset, compared to 35.7% (10/28) of controls (p = 0.0008) (Levin et al., 2010). An additional study accessed the same samples (Levin et al., 2010), although removed those already included in the first study (Levin et al., 2005), leaving 222 MS cases with samples collected prior to MS onset and 444 matched controls (Munger et al., 2011). Having average EBNA-1 antibody titer levels of $\geq$ 320 compared to <20 significantly increased the risk of developing MS (RR: 7.7; 2.6 – 23.0) (Munger et al., 2011). Forty-two members of the Kaiser Permanente Northern California, USA health plan who partook in multiphasic examinations

administered between 1965 and 1974 with serum samples collected prior to their MS onset were compared to age- and sex-matched controls (DeLorenze et al., 2006). A 4-fold increase in antibody titers to EBNA complex was associated with an increased risk of MS (RR: 2.1; 1.1-3.8) (DeLorenze et al., 2006). Serum samples from 73 incident (i.e. samples collected pre-MS symptom onset) and 161 prevalent Swedish MS cases were analyzed; high activity against EBNA-1 was associated with an increased risk of MS in both cohorts (incident OR: 4.5; 1.9-11; prevalent OR: 7.5; 3.6-1.6)) (Sundström et al., 2004).

In a comparison of 100 prevalent cases of MS with multiple control groups (healthy siblings, healthy non-blood related controls and individuals with other neurological conditions) MS patients consistently had higher antibody responses to EBV antigens (Sumaya et al., 1985). EBNA antibody titer levels were significantly increased in 49 prevalent RRMS patients compared to 50 healthy controls ($p = 0.041$) (Villoslada et al., 2003). EBV was detected more frequently in the serum of prevalent MS patients (present in 70/75, 93%) compared to healthy controls (123/186, 66%) (OR: 7.18; 2.75 – 18.67) (Santón et al., 2011). Dual infection with EBV (type 1 and type 2) was also significantly increased in the cases compared to controls (OR: 15.07; 6.36 – 35.68) (Santón et al., 2011). Elevated EBNA-1 IgG levels were also associated with a greater risk of MS in newly diagnosed Swedish MS patients compared to general population controls (OR: 1.74; 1.38 – 2.18) (Sundqvist et al., 2012). EBNA-1 seropositivity (measured on average 2 years after MS symptom onset) was associated with an increased risk of MS (OR: 3.78; 1.52 – 9.38) in 189 paediatric MS cases compared to 66 controls (Waubant et al., 2011).

One study specifically examined different strains of EBV in serum samples collected from the USA's Nurses' Health Study, but no significant differences in the proportion of mutant sequences or individual variants between 66 MS cases and unaffected controls were found ($p > 0.05$) (Simon et al., 2011). The possibility that the increased risk associated with EBV (as measured by EBNA-1) was actually mediated by genetic susceptibility was explored in a case-control study which included 148 women with MS from the Nurses' Health Study (18 of which had blood drawn before MS onset) and 296 age and sex-matched healthy controls; but no association with HLA-DRB1*1501 (presence vs. absence) was found (De Jager et al., 2008).

In summary, the results of these systematic reviews and original studies provide strong evidence to suggest that EBV infection is implicated in increasing the risk of MS.

Several studies used questionnaires to retrospectively determine the incidence of early life infections and their effect on MS risk. A European case-control study found no association between childhood infections (measles, mumps, rubella, chickenpox, herpes zoster, infectious mononucleosis, hepatitis, tuberculosis and typhoid fever) and MS (Granieri et al., 1997). Two studies reported that childhood measles infection increased the risk of MS (OR: 1.9, $p = 0.04$; OR: 2.1, 1.15-3.68, respectively) (Bansil et al., 1997; Casetta et al., 1994) while one reported a decreased risk (OR: 0.2, 0.1-0.5) (da Silva et al., 2009). Another found that MS patients had mumps at an older age

(12.3 versus 8.6 years, p<0.04) but at a lower frequency than controls (33/63 versus 60/92, p=0.08) (Hays et al., 1992). An Italian study found that the development of a rubella infection (OR: 2.7, 1.03-7.53) or whooping cough (OR: 2.2, 1.18-4.14) prior to the age of 5, was associated with an increased risk of MS (Casetta et al., 1994). A population-based study in France (Berr et al., 1989) found no significant difference between the 63 cases and their matched controls in the occurrence of various infections; however, when they analyzed serum samples they found that MS cases had significantly higher mumps antibody titers ($p < 0.02$) (Berr et al., 1989).

Again, using retrospective questionnaires completed by adults diagnosed with MS, a number of studies reported an older age at infection in MS patients more frequently than controls for the following infections: rubella and measles (Compston et al., 1986); measles, mumps, rubella, and varicella (Bachmann and Kesselring, 1998); and measles and mumps (Hernán et al., 2001). In contrast, the age at infection with measles, mumps, rubella, varicella, pertussis or scarlet fever was not associated with MS risk in a later study from Copenhagen, Denmark (Bager et al., 2004). Here, the authors used a comprehensive data linkage design to obtain information regarding childhood infections (up to age 14 years), all of which was collected largely 'real time' prior to the onset of MS, avoiding recall bias which might have compromised other studies (Bager et al., 2004).

Previous chicken pox infection, which is the clinical manifestation of varicella zoster virus (VZV), was associated with a reduced risk of early-onset MS (OR: 0.58; 0.36-0.92) (Mikaeloff et al., 2009a). Although the authors used a retrospective questionnaire design, they did verify the information against medical records for a subset of subjects (Mikaeloff et al., 2009a). Three studies investigated the association between VZV and MS risk; all found it to be increased (Kang et al., 2011; Krone et al., 2008; Rodríguez-Violante et al., 2008). Kang et al.(2011), used the Taiwan National Health Insurance Research Database and selected 315,550 new herpes zoster cases (with an ICD code for herpes zoster) along with 946,650 randomly selected controls (without an ICD code for herpes zoster). Neither cases nor controls were known to have MS. After one year of follow-up, 29 cases and 24 controls developed MS (HR: 3.96, 2.22-7.07). Krone et al. (2008) studied 152 German children with MS and matched controls to determine the association between the risk of MS and a range of infections. Although more controls had a history of VZV infection than cases (147 versus 88), the median serum VZV IgG antibody levels were significantly higher in cases (896 vs 575 units/mL, $p < 0.0001$). Further, MS cases had significantly higher levels of IgG antibodies for herpes simplex virus type 2 (median: 426 versus 89 units/mL, $p <0.0001$) (Krone et al., 2008). Finally, the association between self-reported previous VZV infection and MS risk was investigated among 126 cases and 157 unaffected controls. (Rodríguez-Violante et al., 2008). Previous VZV infection was more common among MS patients (OR: 2.72, 1.69-4.38) (Rodríguez-Violante et al., 2008).

No association was found between the onset of MS and the presence of antibodies to diphtheria or tetanus in a prospective nested case-control study conducted among members of the American military (Massa et al., 2009). Nor was an association found between exposure

to human herpes virus 6 (HHV-6) and MS risk (26.7% in clinic-based cases vs 24.2% in hospital-based controls, $p > 0.05$) based on a comparison of serum samples from 36 prevalent Jordanian MS patients and randomly selected 'healthy' hospital controls (Ahram et al., 2009), or among 46 prevalent Canadian MS patients when compared to multiple control groups ($p > 0.05$ for all) (Mayne et al., 1998). However, increased anti-HHV-6 IgM antibody levels were reported in early RRMS patients when compared to healthy controls (16%, versus 5%, $p = 0.002$) (Villoslada et al., 2003). An increased frequency of antibodies to Human Parvovirus B19 were reported in 46 prevalent Japanese MS patients compared to healthy controls (65.8% vs 40%, $p = 0.019$) (Nakashima et al., 1999) Antibodies to MS associated retrovirus (MSRV) were found in 100% (n=25) of prevalent Sardinian MS patients, compared to 12% (n=25) of healthy controls (Serra et al., 2001).

Two studies reported an increased risk of MS onset with exposure to *Chlamydia pneumoniae* (CP) (Munger et al., 2003; Krone et al., 2008) while two found no association (Munger et al., 2004; Villoslada et al., 2003). Munger et al., adopted a nested case-control study design using large cohorts for both studies (Munger et al., 2003; 2004). Among the USA's Nurses' Health Study participants, the risk of MS was associated with CP seropositivity (OR: 1.7, 1.1-2.7) (Munger et al., 2003). The same group later studied both the US Army personnel and the Kaiser Permanente Medical Care Program (KPMCP) cohorts and found no association (ORs were 1.0 and 1.5 respectively). However, serum levels of anti-CP IgG antibody were associated with an increased risk of MS in the KPMCP cohort (OR for a fourfold difference in antibody titers = 1.7; 95% CI 1.2, 2.5), but not the Army; the authors concluded that they could not rule out the role of CP in altering MS risk (Munger et al., 2004). A significantly higher prevalence of IgM antibody titers to CP were measured among children with MS compared to unaffected controls (28.9% versus 2.0%) (Krone et al., 2008). Lastly, Villoslada et al. (2003) measured IgG and IgM levels of CP antibodies and found no difference between healthy controls, and patients with RRMS or SPMS.

In summary, the influence of other infections (beyond EBV) on MS risk appears complex, with interpretation of findings complicated by the diversity of exposure measurements between studies.

Vaccination

A systematic review suggested that exposure to the tetanus vaccine was associated with a reduced risk of developing MS (OR: 0.67, 0.55-0.81) (Hernán et al., 2006). Nine case-control studies were included from a search of articles published between 1966 and September 1 2005, using Medline, LILACS, EMBASE, and Science Citation Index. The heterogeneity between studies was negligible ($I^2 = 2.5\%$, $p = 0.41$) (Hernán et al., 2006). A later systematic review also examined the effect of the tetanus vaccine, along with other vaccines on MS risk (Farez and Correale, 2011). Medline, EMBASE, and the Cochrane Register of Controlled Trials were searched from 1966, 1977, and 1961, respectively, until January 2011. No additional studies were found in the later review and a similar reduced risk of MS associated with the tetanus vaccine was reported (OR: 0.68; 0.54 – 0.84). A reduced risk of MS was also associated with the diphtheria vaccine (OR:

0.60; 0.40 – 0.90). The risk of MS was not significantly altered by exposure to the following vaccinations: hepatitis B (OR: 0.92; 0.84 – 1.004), BCG (OR: 0.96; 0.69-1.34), influenza (OR: 0.97; 0.77 – 1.23), measles-mumps-rubella (MMR) (OR: 1.02; 0.64 – 1.61), polio (OR: 0.87; 0.61 – 1.25), or typhoid fever (OR: 1.05; 0.72 – 1.53). Heterogeneity was minimal (not significant) for each of the vaccines studied (Farez and Correale, 2011). The authors were unable to determine the risk associated with vaccinations to hepatitis A, yellow fever, varicella zoster, measles, mumps, rabies, rubella, pertussis, or pneumococcal due to insufficient number of studies (< 3) or significant heterogeneity (Farez and Correale, 2011).

A single French study not included in either review examined hepatitis B vaccination in relation to paediatric MS (Mikaeloff et al., 2009). Using a population-based approach among a large cohort of children with either CNS inflammatory demyelination or confirmed MS, no altered risk of MS was found (OR: 0.74; 0.54 – 1.02); however, an increased risk of confirmed MS was associated with the Engerix B brand of vaccine (OR: 2.77; 1.23 – 6.24) (Mikaeloff et al., 2009b). A Brazilian study examined the impact of 'standard' government vaccination programs (with several vaccines considered), as reported by 81 prevalent MS cases and 81 controls (friends or neighbours). The risk of MS was increased, although the confidence interval was wide, reflected by the low number of participants (OR: 16.2; 2.3 – 115.2) (da Silva et al., 2009). A single study found an association between measles vaccination and MS onset among 140 prevalent cases compared to 131 age and sex matched controls using a retrospective structured questionnaire, however the corresponding confidence interval was wide (OR: 92.2; 12.1 – 700.2) (Zorzon et al., 2003).

Antibiotic use

Overall antibiotic use or use of antibiotics specifically active against *Chlamydia pneumoniae*, were not associated with MS risk in a nested case-control study which used the UK's expansive General Practice Research Database (Alonso et al., 2006). A later study tried to replicate these findings in a larger case-control study in Denmark, and seemingly conversely, found that penicillin use was associated with an increased risk of MS (OR: 1.21; 1.10-1.27) (Nørgaard et al., 2011). There was a trend for other antibiotics (pivmecillinam, macrolides, tetracyclines, nitrofurantoin, and sulfamethizole and/or trimethoprim) to similarly increase the risk, leading the authors to infer that perhaps an underlying infection might trigger MS rather than antibiotic use itself (Nørgaard et al., 2011).

Amalgam fillings

A systematic review found a slight, though not-significant increased risk of MS subsequent to amalgam restorations compared to those with non-amalgam fillings (OR: 1.24; 0.96 – 1.61), (Aminzadeh et al., 2007). This review included a search of two databases (Embase and MEDLINE) from 1966-2006 and found 3 case-control studies and one cohort study. Significant heterogeneity among studies was found (Q statistic = 13.7, p = 0.004) (Aminzadeh et al., 2007). No additional original studies were identified by our review.

Smoking

A systematic review suggested that smoking ('ever' versus 'never') was associated with a 1.34 times elevated risk of MS (95%CI 1.17-1.54) (Hawkes, 2007). This review included a search of 8 databases (1964-2006) and incorporated 6 case-control studies. A later review also found an increased risk of MS susceptibility due to smoking (OR: 1.48, 1.35-1.63) (Handel et al., 2011) after searching one database (1960-2010) and incorporating 14 studies (6 cohort and 8 case-control). Although the authors included studies which used a variety of exposure (smoking) definitions, heterogeneity was measured and was not found to be significant (Handel et al., 2011).

Observational studies published since these systematic reviews have reported mixed results – four studies reported a significant association between smoking or smoking-related factors and the onset of MS (Sundström et al., 2008; Hedström et al., 2011a; Hedström et al., 2011b Alonso et al., 2011), while one did not (Villard-Mackintosh and Vessey, 1993). Serum cotinine levels (a nicotine metabolite) were measured in incident cases of MS prior to disease onset and compared to healthy controls in Sweden; elevated levels were associated with an increased risk of MS (OR: 2.9; 1.3 – 6.3), particularly in women (OR: 3.9; 1.3 – 12) (Sundström et al., 2008). The Swedish Epidemiological Investigation of Multiple Sclerosis (EIMS) case-control study examined previous exposure to environmental tobacco, as well as passive smoking, with these exposures collected retrospectively from patients 'shortly' after their MS diagnosis (Hedström et al., 2011a; Hedström et al., 2011b). Smokers were at an increased risk of MS compared to non-smokers (OR: 1.6; 1.3–2.0) (Hedström et al., 2011a). Possible interactions between smoking and the HLA gene variants (HLA-DRB1 and HLA-A) were examined; smokers with presence of HLA-DRB1*15 and absence of HLA-A*02 genotype had a significantly increased risk of MS (OR: 13.5; 8.1 – 22.6) [vs non-smokers absent/present for these respective HLA variants] (Hedström et al., 2011a). Further, while smokers without the genetic risk variants were still at an increased risk, this risk was not significant compared to non-smokers (OR: 1.4; 0.9 – 2.1) (Hedström et al., 2011a). Data from three case-control studies based in the USA, Australia, and Sweden were combined and the interaction(s) between smoking, anti-EBNA antibody titers, and HLA-DRB1*15 were examined (Simon et al., 2010). In patients with high anti-EBNA antibody titers the risk of MS was increased strongly in ever smokers (OR: 3.9; 2.7 – 5.7), and modestly so in never smokers (OR: 1.8; 1.4 – 2.3), with no modification by the presence of HLA-DRB1*15 (Simon et al., 2010). The effects of passive smoking in otherwise 'never smokers' (n=695 cases) were also examined (Hedström et al., 2011b). Never-smokers were at an increased risk of MS if they had been exposed to passive smoking (vs. never-smokers not exposed to passive smoking, OR: 1.3; 1.1 – 1.6) (Hedström et al., 2011b).

Smoking was associated with an increased MS risk for women (OR 6.48; 1.46 - 28.78), but not men (OR: 0.72; 0.31 - 1.68) in Iran, based on evidence from a case-control study of prevalent MS cases (n=311 women and 83 men) (Alonso et al., 2011). Though the primary exposure of interest was oral contraceptive use, a large cohort of women (n = 17, 032) were also asked to report their smoking status in a prospective study (Villard-Mackintosh and Vessey, 1993). The risk of MS was increased, but not significantly for those who smoked 0-

14 cigarettes/day compared to never smokers (RR: 1.6; 0.8 – 3.1) (Villard-Mackintosh and Vessey, 1993). In summary, the results of the prior systematic review coupled with the subsequently published original studies, provide strong evidence for an effect of smoking on MS risk.

A significant association between parental smoking at home and risk of early-onset MS was found in a French study of prevalent paediatric MS cases (OR: 2.12, 1.43 - 3.15) (Mikaeloff et al., 2007). This risk was higher in older children (over the age of 10 years at the onset of MS versus under 10 years; RR 2.49 (1.53–4.08), which the authors attributed to greater exposure to parental smoking (Mikaeloff et al., 2007). Children of parents who smoked at home were at an increased risk of MS compared to those whose parents did not smoke at home (RR: 1.24; 1.02 – 1.51) in women enrolled in the USA's Nurses' Health Study (Gardener et al., 2009a). Although this was no longer significant when restricted to the women (cases) who were non-smokers as adults (RR: 1.2; 0.90–1.5). Further, neither maternal nor paternal smoking during pregnancy was associated with a significantly altered risk of MS (RR for mothers: 0.97; 0.77 – 1.21 and for fathers 1.50; 0.99 – 2.28) (Gardener et al., 2009a). The authors warned that as the study included women (cases) diagnosed with MS both before and after the exposure information was collected, caution was due when interpreting results (Gardener et al., 2009a). A Swedish record-linkage study found no association between maternal smoking during pregnancy (collected prospectively) and risk of MS in the offspring (OR: 0.96, 0.65 - 1.44) (Montgomery et al., 2008). Differences between this (Montgomery et al., 2008) and the former study (Mikaeloff et al., 2007) were hypothesized as possibly related to the retrospective capture of smoking information combined with low response rate from controls; 498/1536 (32%) of controls were 'non-responders' vs. 35/164 (21%) of cases in the former study (Mikaeloff et al., 2007; Montgomery et al., 2008).

Stress

Women in the USA's Nurses' Health Study were questioned prospectively regarding work and home stress, and retrospectively regarding physical and sexual abuse during childhood and adolescence (Riise et al., 2011). Having been exposed to any of these stressors did not appear to increase the risk of developing MS (all OR 95% CIs crossed 1) (Riise et al., 2011). The death of a child increased the risk of MS in parents (HR: 1.56, 1.05 – 2.31) and the unexpected loss of a child (under 18 years old) increased the risk further (HR: 2.13, 1.13 – 4.03) in a population-based Danish study which involved linkage of nationwide registries, including 21,062 bereaved parents and 293,745 matched parents who did not lose a child (Li et al., 2004). Self-reported marked adversity in the 6 months preceding onset was significantly increased in 39 prevalent MS patients in the UK compared to healthy matched controls (77% versus 35%, $p < 0.001$) when semi-structured interviews were conducted a median two years after MS onset (Grant et al., 1989). Stressful life events prior to onset were more common among 91 French MS patients than age and sex-matched healthy controls (67.2% vs 47.6%, $p < 0.05$) based on a

retrospective questionnaire (Berr et al., 1989). When compared to controls with other neurological conditions or rheumatology patients, 100 MS patients reported similar emotional backgrounds and responses to potentially stressful life events; however, during the two years prior to disease onset, MS patients recalled significantly more unwanted stress than controls (RR: 3.2, $p < 0.001$) (Warren et al., 1982). Based on the results of these five studies, there appears to be an association between emotionally stressful life events and an increased risk of MS.

Oral Contraceptives

All four studies examining the impact of the oral contraceptive pill (OCP) on the risk of MS had access to OCP use collected pre-recognition of MS, virtually eliminating any recall bias. Three studies did not find a significant association (Hernán et al., 2000; Thorogood and Hannaford, 1998; Villard-Mackintosh and Vessey, 1993). Using the USA's Nurses' Health Study cohorts, the age-adjusted relative risk of MS in oral contraceptive users was 1.2 (0.9 – 1.5) (Hernán et al., 2000). The other two studies accessed large cohorts of women in the UK; neither reported an altered risk of MS. One compared OCP users for 96+ months versus non-users (RR: 0.7; 0.3 – 1.4) (Villard-Mackintosh and Vessey, 1993) and the other current users with never users (RR: 1.2; 0.7 – 2.0) (Thorogood and Hannaford, 1998). A fourth study found a modest protective effect of recent oral contraceptive use (in the previous three years; OR: 0.6; 0.4 – 1.0) based on a nested case-control study within the UK's General Practice Research Database (Alonso et al., 2005). The authors concluded that, along with other epidemiological evidence, findings suggested that OCP use may delay the onset of MS, but not necessarily prevent or reduce the risk of MS (Alonso et al., 2005).

Vitamin D and sunlight exposure

Low vitamin D intake (Munger et al., 2004), low serum 25-hydroxyvitamin D levels (Munger et al., 2006; Kragt et al., 2009; Shaygannejad et al., 2010; Soilu-Hänninen et al., 2005) and/or low sunlight exposure (Islam et al., 2007; Dalmay et al., 2010; Dwyer et al., 2008; van der Mei et al., 2003; Alonso et al., 2011; Bäärnhielm et al., 2012) were consistently associated with an increased risk of MS. Based on prospective food-frequency questionnaires, women in the USA's Nurses' Health Study with the highest quintile of vitamin D intake were at a reduced risk of MS (RR: 0.67; 0.40 – 1.12, p for trend = 0.03) (Munger et al., 2004). Intake from supplements was also protective when comparing women who took ≥ 400 IU/day to women taking no supplemental vitamin D (RR: 0.59; 0.38 – 0.91) (Munger et al., 2004). Incident cases of MS with serum samples collected prior to onset were accessed from the USA's Department of Defense Serum Repository and compared to matched healthy controls (Munger et al., 2006). For each 50 nmol/L increase in 25(OH)D levels the risk of MS was reduced (OR: 0.59; 0.36 – 0.97) (Munger et al., 2006). An Australian study found that exposure to ultraviolet (UV) radiation during the first trimester of pregnancy decreased the risk of MS in the child (Staples et al., 2010). This association was also found to exhibit a dose-dependent relationship (p for trend < 0.05) (Staples et al., 2010). Mothers (n = 35,794) of women enrolled in the

USA's Nurses' Health Study were questioned regarding their diet during pregnancy with their daughter. Women born to mothers with high vitamin D intake during pregnancy were at a reduced risk of developing MS (highest quintile of vitamin D vs lowest quintile, RR: 0.57; 0.35 – 0.91) (Mirzaei et al., 2011).

Several studies measured serum 25(OH)D after the onset of MS. Lower levels of serum 25(OH)D were found in female MS cases (not male) vs 'healthy' controls (either the patients spouse or hospital personnel) in a study from The Netherlands (Kragt et al., 2009). For every 10 nmol/L increase in serum 25(OH)D levels measured during the winter, the odds of MS in women was reduced by 19% (OR: 0.81; 0.69 – 0.95); however, serum levels were drawn on average 11 years after MS symptom onset (Kragt et al., 2009). In Iran, serum 25(OH)D levels drawn between December-February (winter) were reduced in prevalent MS cases compared to healthy controls (48 vs 62 nmol/L, $p = 0.036$) (Shaygannejad et al., 2010). All cases had to have an EDSS lower than 5 (be able to walk without aid), but their disease duration was not reported. When 25(OH)D levels were measured around the time of MS diagnosis and compared to controls in Finland, there were no significant differences when levels were measured in the winter months, but summer levels (June to September) were lower in cases ($p < 0.05$) (Soilu-Hänninen et al., 2005).

In Tasmania, Australia, higher sunlight exposure between the ages of 6 and 15 was also associated with a reduced risk of MS (OR: 0.31, 0.16 – 0.59) (van der Mei et al., 2003). Skin casts were also used to objectively measure past sun exposure; findings concurred with the main conclusion that higher sunlight exposure may lower MS risk (van der Mei et al., 2003). This reduced risk was later shown to be influenced by the melanocortin 1 receptor genotype as well as red hair/ fair skin phenotype (Dwyer et al., 2008).

One study focused on monozygotic twins discordant for MS; the twin with MS recalled lower sunlight exposure during childhood as compared to the unaffected twin which persisted after adjustment for smoking history, childhood infection, mononucleosis, diet, and age at menarche (for female twins) (Islam et al., 2007). Sun exposure information prior to the age of 15 was collected retrospectively in Cuba, Martinique, and Sicily (Dalmay et al., 2010). Higher sun exposure on weekdays or weekends was associated with a reduced risk of MS (for every one hour/day sunlight exposure on weekdays, OR: 0.90; 0.85 – 0.98; for weekends OR: 0.93; 0.87–0.99) (Dalmay et al., 2010).

Prevalent Iranian MS patients (n=394) and their matched controls were questioned regarding their daily sunlight exposure (Alonso et al., 2011). A reduced risk of MS was associated with increased daily sunlight exposure (OR per 1-hour increment: 0.62; 0.5 – 0.73) (Alonso 2011). Exposure to ultraviolet radiation (UVR) over the previous five years was ascertained by questionnaire among 1013 newly diagnosed MS cases with an average disease duration of 4.5 years (mean) and 1194 controls in Sweden (Bäärnhielm et al., 2012). Those with the lowest UVR exposure were reported as being at a significantly increased risk of MS compared to those with the highest UVR exposure (OR: 2.2; 1.5 – 3.3), although much of the UVR exposure would have occurred after MS onset (Bäärnhielm et al., 2012).

In summary, eleven studies provided evidence implicating low exposure to sunlight or low intake or levels of serum vitamin D in increasing the risk of MS, suggesting that this is an important modifiable risk factor.

Month or season of birth

The association between month of birth and the risk of MS was examined in a systematic review, which accessed Medline and PubMed until May 2012 (Torkildsen et al., 2012). Fifteen studies were found, of which 12 reported an effect of month of birth on MS risk. Studies based in the Northern Hemisphere consistently reported an excess of MS births in April and May, with nadirs in November and December. The opposite was found in the Southern Hemisphere, with a peak in November and a nadir in May (Torkildsen et al., 2012). The potential for publication bias was not addressed. A subsequent study compared the birthdates of 26, 994 English and Scottish MS patients to the general populations of these countries (Disanto et al., 2012). Peak MS births were recorded in April (OR: 1.05; 1.00 – 1.09) and May (OR: 1.08; 1.04 – 1.13), with modest nadirs in October (OR: 0.96; 0.92 – 1.00) and November (OR: 0.96; 0.91 – 1.00) (Disanto et al., 2012).

Sibling exposure, birth order, and early life exposure to other infants

A small Danish population-based study found a reduced risk of MS in children with a greater number of older siblings (relative incidence for the 3rd child compared to the 1st or 2nd: 0.28; 0.1 – 0.79; 46 MS cases were included) (Isager et al., 1980). A later and larger Danish study prospectively-collected school records with information regarding birth order which included 455 incident cases and 1801 matched peer controls; no association between birth order and MS risk was found (p for trend of increasing birth order = 0.21) (Bager et al., 2004). In a Canadian longitudinal study, 10,995 MS cases were compared to 26,336 of their siblings. A higher than expected mean birth order in sibships of size 8, 11, or 15 was found among MS cases; however, all other sibship sizes showed no influence of birth order (Sadovnick et al., 2004). The authors concluded that birth order did not have an effect on MS risk in most families (Sadovnick et al., 2004).

A population-based Tasmanian study examined birth order and infant sibling exposure (Ponsonby et al., 2005). Similar to the earlier population-based studies, they found no association between birth order and MS (OR, 1.06; 95% CI, 0.95-1.19 per increase in birth order), however the number of younger siblings (but not older siblings) was associated with MS (OR, 0.77; 95% CI, 0.67-0.90 per sibling), especially if born within 2 years of the subject, but not greater than 6 years. Overall, exposure to infant siblings in the first 6 years of life was associated with a reduced risk of MS (compared to <1 infant year, adjusted ORs were 0.57 (0.33-0.98) for 1-<3 infant years; 0.40 (0.19-0.92) for 3-<5 infant years and 0.12 (0.02-0.88) for ≥5 infant years; test for trend p = 0.002) (Ponsonby et al., 2005). A later study from the same group examined the gene-environment interaction and found a combined effect of HLA-DR15 positivity and low infant sibling exposure on the risk of MS (OR: 7.88; 3.43-18.11) which was 3.9-times greater than expected (test for interaction,

p = 0.019) (van der Mei et al., 2010). Other studies also found associations; having three or more older or younger siblings was correlated with a lower risk of MS (OR: 0.83; 0.72 – 0.96) in a case-control study of over 4000 MS patients compared to the Swedish general population (Montgomery et al., 2004). A small Israeli study reported an increased risk of MS in first born children compared to controls (35/86, (41%) of cases vs 25/81(31%) of controls) (Zilber et al., 1988).

Multiple early life exposures gathered using a standardized questionnaire were considered in a German case-control study (Conradi et al., 2011). Having 2 or more older siblings compared to no older siblings was associated with a modest decrease in MS risk (OR: 0.54; 0.30 – 1.00). A lower risk was also associated with attending a day-care centre (OR: 0.5; 0.31 – 0.80), or residing in an urban area with a population greater than 100 000 people compared to an area with a population of less than 10 000 people (OR: 0.43; 0.23 – 0.81) (Conradi et al., 2011). Frequency of receiving day-care as an infant was not found to be related to MS risk in individuals with no siblings (54/379 cases attended day-care as a child versus 14/101 spousal controls, p = 0.92) in a Canadian study which accessed data from interviews with the mothers of cases and controls (Ramagopalan et al., 2011).

Obesity and diet

Though not systematic, a review of diet in relation to MS risk chronicled 15 case-control studies published between 1953 and 1995 (Lauer, 1997). The dietary risk factors were wide-ranging and often conflicting. The author concluded that a diet rich in animal fat was most commonly correlated with an increased risk of MS, while a diet rich in vegetables and fish was related to a reduced risk of MS (Lauer, 1997).

A case-control study from Montreal, Canada indicated that high body mass index (BMI) was associated with a reduced risk of MS (OR: 0.76; 0.61 – 0.95 per 5-unit increase in BMI) (Ghadirian et al., 1998). However, BMI was measured in prevalent cases, such that findings could be interpreted as indicating that around the time of diagnosis (i.e. when BMI was measured), those with MS weighed less than expected. A subsequent study, which used data from the USA's Nurses' Health Study, found that a higher BMI ($\geq 30 kg/m^2$) measured *prior* to MS onset, was associated with an increased risk of MS, when measured during adolescence (RR: 2.25; 1.50 – 3.37 compared to BMI of 18.5 - < 21 kg/m^2) (Munger et al., 2009). In a Swedish case-control study a BMI of >=30 (classified as obese) at the age of 20, based on a retrospective questionnaire, was associated with a significantly increased risk of MS (OR: 2.2; 1.5-3.0) (Hedström et al.,2012).

No effect of vegetable or animal fat intake (Zhang et al., 2000), nor vitamins from fruits and vegetables (Zhang et al., 2001) was found when examining the USA's Nurses' Health Study cohort in which food intake was collected prior to recognition of MS (Zhang et al., 2000; Zhang et al., 2001). Higher candy and sweet consumption was associated with an increased risk of MS (OR: 1.29; 1.07 – 1.55) as was higher fat intake (OR per 33 g increase of animal fat: 2.03; 1.13 – 3.67), while higher fruit juice consumption was found to reduce the

risk (OR: 0.82; 0.74 – 0.92) as were cereals and breads (OR: 0.62; 0.40 – 0.97) among 197 newly diagnosed MS cases who were questioned regarding their previous dietary habits compared to controls in Montreal, Canada (Ghadirian et al., 1998).

Two case-control studies also employed retrospective questionnaires regarding lifestyle and dietary habits (Pekmezovic et al., 2006; da Silva et al., 2009). Coffee and alcohol consumption were associated with an increased risk of MS in a Serbian study (OR for coffee: 1.7, p = 0.047; OR for hard alcohol: 6.7, p = 0.026) (Pekmezovic et al., 2006); however, a Brazilian study found a decreased risk of MS associated with alcohol consumption (OR: 0.2; 0.1 – 0.4) (da Silva et al., 2009).

Animal ownership

Six case-control studies from 3 different continents (North America, Asia, and Europe) used retrospective questionnaires to examine animal ownership in relation to MS risk (Read et al., 1982; Bansil et al., 1997; Warren et al., 1991; Hernán et al., 2001; De Keyser and Zwanikken 1997; Cook et al., 1987). The earliest published study reported that MS cases were more likely to have owned an indoor dog than their matched controls (p = 0.006) (Cook et al., 1978). A subsequent study found no association between dog or cat exposure and the risk of MS (RR: 1.1; 0.5 – 2.5 for dogs, RR: 1.2; 0.4 – 1.5 for cats) (Read et al., 1982). Another reported an increased risk of MS associated with exposure to cats (OR: 3.03; 1.06 – 8.33) (Warren et al., 1991), while another found an increased risk associated with exposure to dogs (OR: 2.2, p = 0.04) (Bansil et al.,1997). Dog ownership from 0-5 years of age was associated with an increased risk of MS (OR: 2.7; 1.4 – 5.0) among 100 MS cases and matched controls in the Netherlands (De Keyser and Zwanikken, 1997). Among the USA'S Nurses' Health Study participants, there was a modest association with dogs (vs. women who had never owned a dog, OR: 1.5; 0.9 – 2.3), increasing slightly to 1.7 (1.0-3.0) for those exposed at >15 years of age, but no increased risk associated with prior cat ownership (OR: 1.0; 0.7–1.4), (Hernán et al., 2001). In summary, the relationship between animal exposure and MS risk is not well-understood, given these contradictory results.

3.1.2 Occupational Risk Factors

Organic solvent exposure in relation to MS risk was examined in a systematic review which accessed the MEDLARS database (1966 to 1994) and included 9 case-control studies in a meta-analyses with a pooled RR of 2.1 (95% CI: 1.6 – 2.7) (Landtblom et al., 1996). Two studies published since also observed elevated risk, although neither reached significance (Riise et al., 2002; Landtblom et al., 2006) When compared to workers not exposed to organic solvents, painters were at a higher, albeit not significant, increased risk of MS (RR: 2.0; 0.9 – 4.5) (Riise et al., 2002). The second study included nurse anaesthetists, outlined below (Landtblom et al., 2006).

One of the studies included in the systematic review above (Landtblom et al., 1996) examined other occupational exposures through a structured interview given to 104 MS cases and 150 healthy controls in Italy (Casetta et al., 1994). Working in public administration was associated with an increased risk of MS (OR: 6.1; 2.20 – 17.58), while working in agriculture was associated with a reduced risk (OR: 0.4; 0.21 – 0.95) (Casetta et al., 1994).

Nursing itself was not found to be an occupational hazard for MS risk (SIR: 0.87; 0.66 – 1.12) (Stenager et al., 2003). Although one study found a higher than expected rate of MS amongst nurses exposed to anaesthetics (SIR: 2.9; 1.3 – 5.3) (Flodin et al., 2003), another found a modest association when compared to other professions (RR: 2.2; 0.8 – 5.5) (Landtblom et al., 2006). The authors of the earlier study cautioned that their methodology and risk estimate was crude (Flodin et al., 2003).

The incidence of MS among utility workers was not significantly increased when compared to national figures (SIR: 1.35; 0.92 – 1.91) (Johansen et al., 1999). However, radiological work exposure to X-rays or ionizing radiation was associated with an increased risk of MS in a Swedish case-control study, with the respective ORs being 1.8 (95% CI 1.2-2.6) and 4.4 (95% CI 1.6-11.6) (Axelson et al., 2001).

Shift work as a risk factor for MS was examined in one study which accessed two population-based case-control cohorts – the first included newly diagnosed cases of MS, and the other, cases with long-standing MS (mean disease durations from onset to the time of the study were 3 and 17 years, respectively) (Hedström et al., 2011c). Shift work at various ages was assessed; exposure at a young age (vs. no exposure to shift work) was associated with an increased risk of MS in both cohorts (newly diagnosed cases: OR: 1.6; 1.2 -2.1, long-standing cases: OR: 1.3; 1.0 – 1.6) (Hedström et al., 2011c). Further, the risk was elevated for those who worked 3 or more years of shift work prior to age 20 (vs. no exposure to shift work); the ORs were 2.0 (1.2–3.6) and 2.1 (1.3–3.4) for the two cohorts respectively (Hedström et al., 2011).

3.1.3 Pre-existing co-morbidities or other medical conditions

A sizable case-control study found that appendicitis (specifically a perforated appendix), as identified through linkage of prospectively collected databases was not associated with an altered risk of MS (OR: 0.86; 0.70 – 1.04) (Roshanisefat et al., 2011). The incidence of Hodgkin lymphoma (HL) was measured among MS patients and their first-degree relatives using Danish population-based cancer and MS registries. No increased risk was found for MS patients; however, their first-degree relatives were at an increased risk of young-adult-onset HL (RR: 1.93; 1.01-3.71) (Hjalgrim et al., 2004).

Lower serum uric acid levels were reported in prevalent Italian MS patients (n=124) versus controls (4.1 vs 4.7 mg/dl, p = 0.001) (Sotgiu et al., 2002). To ascertain whether this was a possible cause or just a consequence of the disease, one group accessed serum samples pre-

onset using the USA's Nurses' Health Study; they did not find a strong association with MS onset (each 1mg/dL increase in uric acid levels was associated with a RR of 0.52; 0.22 - 1.20, adjusted for BMI, smoking and blood pressure) (Massa et al., 2009).

A history of allergies was not associated with MS risk in a case-control study of 163 incident MS cases nested within the UK's General Practice Research Database cohort (Alonso et al., 2006). Although prior exposure to a sedating anti-histamine (H1-antagonist) was associated with a decreased MS risk (OR 0.2, 95% CI 0.1 to 0.8), the absolute number of cases exposed was small (Alonso et al., 2006) and the authors did not have access to over-the counter purchases of anti-histamines. The same authors also comprehensively reviewed the literature on the association between allergy and MS (Alonso et al., 2006), albeit not as a formal systematic review, but their findings are included here to avoid unnecessary duplication of efforts. In summary they identified 15 case-control studies published between 1952-2002, giving a pooled OR for MS and allergy of 1.1 (0.8-1.7) (Alonso et al., 2006). Although the authors noted that all these studies used prevalent (not incident) MS cases and significant heterogeneity was found ($p<0.001$) (Alonso et al., 2006). The same authors conducted a case-control study nested within the USA's Nurses' Health study, using questionnaires mailed to women with MS and both 'healthy' and diseased (breast cancer) controls (Alonso et al., 2008). They found no association between a history of allergies and MS risk (OR: 1.0, 0.8-1.4) (Alonso et al., 2008). Two later case-control studies in Italy found allergies to be protective against MS, with ORs of 0.30 (95%CI: 0.16 – 0.57) reported for allergic respiratory diseases (Bergamaschi et al., 2009) and 0.58 (95%CI: 0.38 – 0.89) for atopic allergy (Pedotti et al., 2009); both studies asked individuals retrospectively, either by mail or telephone. Childhood cow's milk allergy was not associated with MS risk in a Canadian case-control study which included 6638 cases and 2509 spousal controls, with information gathered retrospectively via interview with the mothers of MS cases and spousal controls. Early cow's milk allergy (to age 3 years) affected 2.3% (n=111) of the female MS cases vs. 2.1% (n=16) of female controls (p=0.82) and 1.8% (n=30) of male MS cases vs. 1.4% (n=24) of male controls (p=0.30) (Ramagopalan et al., 2010).

By accessing computerized records from the USA's Kaiser Permanente Northern California medical care program, a number of autoimmune diseases were found to be more prevalent pre-MS onset as compared to controls, including: uveitis, Bell's palsy and inflammatory bowel disease, (ORs ranged from 2.7 to 3.2) (Langer-Gould et al., 2010). Although conditions such as rheumatoid arthritis, autoimmune thyroiditis, lupus, psoriasis, type I diabetes, asthma or eczema were not more common (Langer-Gould et al., 2010). Incident MS cases from the USA's Nurses' Health Study were analysed and a higher risk of MS was reported in women with migraines diagnosed prior to their MS diagnosis, vs. non-migraineurs (RR: 1.39; 1.10 – 1.77) (Kister et al., 2012).

Head injury necessitating a hospital admission, as identified through health administrative data was not associated with subsequent MS risk (also identified via hospital data, RR: 1.1; 0.88 – 1.36) (Goldacre et al., 2006). The Taiwan National Health Insurance Research Database was accessed and 72 765 patients who had been hospitalized with a traumatic brain injury were compared to 218 295 matched

controls who had not (Kang and Lin, 2012). After six years of follow-up, the risk of MS was found to be significantly increased in people who had experienced a traumatic brain injury (HR: 1.97; 1.31 – 2.93) compared to those who had not (Kang and Lin, 2012).

The following studies examined the risk of MS within specific groups of patients who already had a chronic condition. A Swedish study accessed both the national Inpatient and Cause of Death Registries and found that among patients with a diagnosis of chronic obstructive pulmonary disease (COPD), the risk of MS was increased (HR: 2.51; 2.13-2.98), being higher if the diagnosis of COPD was before age 60 (HR: 6.41; 4.54 – 9.28) (Egesten et al., 2008). Data from the UK's General Practice Research database was used to examine MS risk subsequent to a diagnosis of inflammatory bowel disease; an increased risk was observed as compared to matched controls for ulcerative colitis (incidence rate ratio (IRR) : 2.63; 1.29 –5.15), but not Crohn's disease (IRR: 2.12; 0.94–4.50) (Gupta et al., 2005). By accessing the two Danish population-based disease registers (hospital and MS-related), patients with type 1 diabetes mellitus were found to be at an increased risk of MS (RR: 3.26; 1.80 – 5.88), with those diagnosed with diabetes before the age of 15 being at a substantially increased risk (RR: 4.23; 1.90 – 9.43) (Nielsen et al., 2006). A case-control study on the association between poliomyelitis and MS risk was conducted in Copenhagen, Denmark (Nielsen et al., 2000). Of 5652 polio patients, 19 cases of MS were observed, compared to 11 expected (SIR: 1.73, 1.04-2.74). The authors noted that although the small number of events was rather limiting, the data still suggested that polio infection might increase risk of MS (Nielsen et al., 2000).

3.1.4 Biological Risk Factors

Preterm birth was not associated with an increased risk of MS based on interview-derived data from a Canadian study which compared patients to spousal controls (5.6% vs 5.2%, p = 0.41) (Ramagopalan et al., 2008a). The same study design was used to explore the influence of birth weight on MS risk. No association was found among females (mean birth weight: 7.23 lbs for cases vs 7.19 lbs for controls, p= 0.48) or males (7.56 lbs vs 7.55 lbs, p = 0.92) (Ramagopalan et al., 2008b). Mothers of subjects enrolled in the USA's Nurses' Health Study were questioned regarding experiences surrounding their daughter's birth (Gardener et al., 2009a). An increased risk of MS was associated with late initiation of prenatal care (RR: 1.6; 1.0 – 2.4), diabetes during pregnancy (RR: 10; 2.5 – 42), and maternal pre-pregnancy overweight/obesity (RR: 1.7; 1.0 – 2.7) (Gardener et al., 2009a). An Italian study reported that being breast fed for more than 7 months reduced the risk of MS (OR: 0.38; 0.19 – 0.74) among 93 MS cases and their matched controls (Pisacane et al., 1994). Among 100 women who developed MS in their child-bearing years, the risk of onset of RRMS was increased during the 8 month period following delivery when compared with the 8 months prior to delivery (9 onset relapses occurred post-delivery versus 0 during pregnancy, p = 0.04) (Runmarker and Anderson, 1995). An earlier age at menarche was associated with an increased risk of MS in Canadian women (12.4 years for cases vs 12.6 years for controls, p = 0.00017), but no association between age at puberty and MS risk was found in men (p = 0.7); information was derived from 5493 MS cases and 1759 spousal controls (Ramagopalan et al., 2009). An

earlier age at menarche was also associated with an earlier onset age among women with RRMS in the province of Newfoundland and Labrador, Canada; for every one year increase in the age of menarche, the age of first symptoms increased by 1.16 years (R2 = 0.69, P = 0.04) (Sloka et al., 2006).

3.1.5 Genetic risk factors

The MSGene database summarized a comprehensive field-synopsis of published genetic association studies performed in MS (Lill et al., 2013). The database is regularly updated, but as of December 2012, information on 789 studies, 324 meta-analysis, 809 genes, and 2,907 polymorphisms were included, from which 69 polymorphisms had high credible associations with MS. Based on the quality of the original studies, and the effect size measured, the top 5 most strongly associated genes were listed as HLA-DRB1, IL2RA, IL7R, TAGAP, and CLEC16A. For instance, from meta-analyses, the OR was 2.27 (95%CI: 2.04-2.54) for the SNP rs3135388 (HLA-DRB1*1501) (Lill et al., 2013). In addition, SNPs residing in so-called 'gene deserts' (i.e. not linked to a specific gene) were also identified, an example being gwa-16q24.1.

3.1.6 Miscellaneous Risk Factors

Less commonly studied environmental or lifestyle-related risk factors are included here. One cohort study of 88,791 women in the USA's Nurses' health Study included 210 incident cases of MS and found that being naturally left-handed increased the risk of MS (OR: 1.62; 1.04-2.53) (Gardener et al., 2009b).

No association or increased risk was reported between mobile (cell) phone use based on population-based hospital registers (standardized hospitalization ratio: 1.0; 0.9 – 1.1) (Schüz et al., 2009). Having children by multiple partners did not increase risk (RR: 1.16; 0.88 – 1.52) (Basso et al., 2004) nor did being exposed to a classmate during childhood who later developed MS (OR: 1.1; 0.7 – 1.6) (Bager et al., 2004). Blood transfusion(s) in the year prior to onset was not a risk factor for MS onset among 150 Scottish MS patients who answered a retrospective questionnaire compared to age and sex-matched neurological controls (OR: 1.0; 0.3-3.3) (Swingler, 1993).

An Israeli study found that MS cases had higher education levels than controls, were more likely to live in tents or huts versus 'proper' housing, and were more likely to drink spring or well water as opposed to tap water ($p < 0.05$ for all) based on an extensive retrospective questionnaire (Zilber and Khana, 1996).

3.2 Risk Factors Associated with MS disease activity – relapses and progression

The screening process for articles related to MS disease activity is outlined in Figure 1. Six systematic reviews (**Table 2**) and 50 original articles (**Supplementary Material III**) were included.

3.2.1 Environmental and Lifestyle Risk Factors

Infections

Nine studies examined the association between infections and relapses; six reported an increased risk of relapse during periods of infection (Tremlett et al., 2008; Correale et al., 2006; Andersen et al., 1993; Buljevac et al., 2002; Buljevac et al., 2003, Panitch, 1994); three found no influence of infections on relapse rates (Kriesel et al., 2004; Gasperini et al., 1995; Sotgiu et al., 2002); and one found parasite infections to reduce relapse rates (Correale and Farez, 2007). Two reported the longer-term effects of infections on progression-related outcomes (Sotgiu et al., 2002; Correale and Farez, 2007).

A population-based cohort was prospectively followed for a mean of 2.3 years in Southern Tasmania, Australia. A positive correlation between upper respiratory tract infections (URTIs) and the monthly relapse rate ($r = 0.39$, $p = 0.014$) was found, with URTIs explaining around 15% of the variation in relapses (Tremlett et al., 2008). The authors also observed that both prior ambient ultraviolet radiation (lagged 1.5 months) and estimated serum vitamin D levels (no lag) were inversely associated with URTI rates and that the associations between URTIs and relapses and between 25(OH)D (or prior EUV, lagged 1.5 months) were both substantially reduced after adjustment for each other, suggesting they might be acting partly on the same pathway (Tremlett et al., 2008).

Other studies examined the risk of an MS relapse during specific 'at-risk' periods (Correale et al., 2006; Panitch, 1994; Andersen et al., 1993; Buljevac et al., 2002; Buljevac et al., 2003; Kriesel et al., 2004) defined as the time interval from two weeks prior through until five weeks after an infection (unless otherwise stated) as described by Sibley et al. (1985).

A prospective study of 60 RRMS patients in Los Angeles, USA found an increased relapse rate during the 'at risk' period surrounding systemic infection compared to the 'not at risk' period (OR: 3.2; 2.26 – 4.69) (Correale et al., 2006). The annual relapse rate during the 'at-risk' period around URTI infections was also significantly higher than during the not at-risk periods (2.92 versus 1.16, $p < 0.001$) among 30 patients from Baltimore, USA (Panitch, 1994).

Sixty Swedish patients were followed for a mean of 31 months (Andersen et al., 1993). Common infections were not associated with an increased relapse rate during at-risk periods (RR: 1.14, $p = 0.199$); however, when this period was reduced to one week prior to infection

and continued for four weeks after, there was a significant increase in relapse rates compared to the remaining 'not at risk' period (RR: 1.32, p = 0.048). In addition, a positive correlation was found between the number of common infections per month and the relapse rate (p < 0.01), which the authors concluded accounted for the reduced frequency of relapses observed in the summer months (Andersen et al., 1993).

A prospective study following 73 RRMS patients in Holland found a higher risk of relapse during 'at-risk' periods surrounding URTI or gastrointestinal infections (RR: 2.1, 1.4 – 3.0) compared to non-risk periods (Buljevac et al., 2002). The same cohort was examined for Chlamydia pneumonia (CP) infection, and again there was an increased risk of relapse during periods of acute infection (RR: 3.1, 1.3-6.7) (Buljevac et al., 2003). Further, patients infected with CP had a higher annual relapse rate than uninfected patients (RR: 2.5; 1.2-4.7) (Buljevac et al., 2003).

A cohort of 16 RRMS patients experiencing URTIs was monitored for the occurrence of relapses as well as the possible causative viral pathogen (Kriesel et al., 2004). The mean relapse rate during the at-risk period was 0.26 (95% CI: 0.13 – 0.44) per month, versus 0.09 (95%CI 0.05 – 0.17) per month during the not at risk period. Relapse-associated URT infections were more likely to be due to the picornavirus compared to non-relapse associated URTIs (OR: 4.7, 1.3-17.5) (Kriesel et al., 2004).

Eighty-nine RRMS patients who presented with a new relapse at an MS clinic over a one-year period were interviewed regarding the number of infections experienced during the previous three months. Findings were compared to 89 RRMS controls who had been stable for at least 3 months. Infectious diseases were reported *less* frequently by those who had a relapse, though not significantly so (OR: 0.5, 0.2 – 1.3) (Gasperini et al., 1995).

An Italian group investigated the association between an 'MS-associated retrovirus (MSRV)' and risk of relapse and progression (Sotgiu et al., 2002). Nine MSRV-positive and 6 MSRV- negative MS patients were monitored over a mean of three years. There was a slight, though not statistically significant increased mean annual relapse rate (0.4 vs 0.2, p > 0.05) and a higher mean EDSS score at follow-up (3.2 vs 1.6, p = 0.01) in the MSRV-positive vs.-negative patients (Sotgiu et al., 2002). These 15 patients, along with 3 others were followed for 6-years in total (Sotgiu et al., 2006). Again, the MSRV-positive patients (n=10) had a higher annual relapse rate (0.5 versus 0.3, p = 0.01) and mean EDSS score (4.3 versus 2.2, p = 0.004) and compared to MSRV-negative patients (n=8) (Sotgiu et al., 2006).

A cohort of 12 RRMS patients presenting with parasitic infections in Buenos Aires, Argentina were prospectively followed for a mean of 4.6 years (Correale and Farez, 2007). When compared to 12 non-infected matched controls, parasite-infected patients showed a significantly lower total number of relapses (3 versus 56; p < 0.0001). They also showed minimal change in disability (mean EDSS = 0),

while uninfected patients' disability increased over the 4.6 years of follow-up [mean change in EDSS ≈ 2.5, derived from Figure 1b in the original publication (Correale and Farez, 2007, Figure 1b)].

In summary, the majority of studies found an increased risk of relapses or other longer term disease activity outcomes during or after infection; however, not all studies reported a significant association and parasitic infections maybe protective.

Vaccinations

Exposure to any vaccination in the previous two months was not associated with an increased risk of relapse (RR: 0.71, 0.40 – 1.26) among 643 European patients (Confavreux et al., 2001). Further, there was no increased risk associated with specific vaccines, such as tetanus, hepatitis B, or influenza vaccination (RRs were 0.75, 0.67 and 1.08 respectively; all 95% CIs contained 1) (Confavreux et al., 2001).

Vitamin D, sunlight, ultraviolet (UV) radiation

Of five publications (Simpson et al., 2010; Tremlett et al., 2008; Smolders et al., 2008; Correale et al., 2009; Soilu-Hänninen et al., 2008) examining serum vitamin D levels (25(OH)D) or UV radiation and relapse rates in four unique cohorts, four reported that low serum vitamin D levels were associated with an increased risk of relapse (Simpson et al., 2010; Tremlett et al., 2008; Correale et al., 2009; Soilu-Hänninen et al., 2008) and one examined longer-term outcomes, albeit cross-sectionally (Smolders et al., 2008). Further, one study examined the influence of breastfeeding on 25(OH)D levels and the risk of relapse in post-partum women (Langer-Gould et al., 2011).

In a prospective, population-based cohort study of 145 Southern Tasmanian RRMS patients, an inverse relationship was found between serum 25(OH) D levels and relapse rates. Adjusting for seasonal variation, the risk of relapse was reduced (HR: 0.90, 0.83 – 0.98) per 10nmol/L increase in serum 25(OH) D levels (Simpson et al., 2010). An earlier study nested within the same cohort found that prior erythemal UV radiation was negatively correlated with monthly relapse rates (lagged by 1.5 months: $r = -0.32$, $p = 0.046$), and serum 25(OH)D levels ($r= -0.31$, $p = 0.057$) (Tremlett et al., 2008).

Using a partly retrospective, partly cross-sectional study design, levels of serum 25(OH) D and 1,25(OH)2D (the biologically active metabolite) were measured for 267 MS patients in The Netherlands (Smolders et al., 2008). High 25(OH) D levels were associated with a reduced risk of relapse (OR: 0.95, 0.91 – 0.99) (Smolders et al., 2008). Both 25(OH) D and 1,25(OH)2D metabolite levels were significantly lower in patients with primary and secondary progressive MS compared to relapsing-remitting ($p = 0.002$) (Smolders et al., 2008). Lower 25(OH) D levels were also associated with higher EDSS scores, but 1,25(OH)2D levels were not (Smolders et al., 2008).

Serum 25(OH) D levels of 58 RRMS patients in remission and 34 who were experiencing a relapse were assessed in a cross-sectional study in Buenos Aires, Argentina (Correale et al., 2009). Those experiencing a relapse had significantly reduced 25(OH) D levels (38.5 ng/ml versus 47.3 ng/ml, $p < 0.00001$) (Correale et al., 2009).

Twenty-three Finnish patients enrolled in a clinical trial (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis, PRISMS) had their 25(OH)D levels sampled every 3 months as well as during a relapse over one year. Relapse-related periods were associated with reduced 25(OH)D levels compared to periods of remission (47.4 nmol/l versus 60.0 nmol/l, $p = 0.012$) (Soilu-Hänninen et al., 2008).

Twenty-eight women with MS were followed during pregnancy and at 2, 4, and 6 months post-delivery (Langer-Gould et al., 2011). Serum vitamin D levels (25(OH)D) rose in women who breastfed non-exclusively compared to women who breastfed exclusively ($p = 0.02$, adjusted for season); however, these reduced 25(OH)D levels were not associated with an increased risk of postpartum MS relapse in this small cohort of women (Langer-Gould et al., 2011).

In summary, the six studies that examined vitamin D serum levels generally found that elevated levels were beneficial while lower levels were detrimental with regards to relapses and long-term disability.

Breast-feeding

A meta-analysis examining the association between breastfeeding and relapse rates included data from 12 studies extracted from PubMed [1988-2011 (Pakpoor et al., 2012); not including the previous study outlined above (Langer-Gould et al., 2011)]. A combined total of 869 women with MS who breastfed were found to be at a significantly reduced risk of a post-partum relapse compared to 689 women with MS who did not (OR: 0.53, 0.34 – 0.82). The authors noted significant heterogeneity across studies ($p = 0.002$, $I^2 = 63\%$). A potential confounder, as identified by the authors, was the use of disease-modifying therapies prior to pregnancy (Pakpoor et al., 2012).

Seasonal variation

Based on a Medline search of articles published between 1966 and 2000, a meta-analysis integrated data from ten studies which examined the seasonal variation of MS relapses (Jin et al., 2000). The ratio of highest to lowest seasonal proportions was calculated as 1.10 (95% CI, 1.07 – 1.13) with the highest proportion of relapses occurring in spring and the lowest in winter (Jin et al., 2000).

Studies published outside of this meta-analysis in which seasonal variation in relation to relapses were examined included: one from Tasmania, Australia (also reported under '*Infections*') which found lower relapse rates in February (summer) compared to all other

months of the year (p = 0.018) (Tremlett et al., 2008). A small retrospective Japanese study (34 patients) found relapse rates to be significantly higher in the warmest (July and August) and coldest (January and February) months compared to the remaining months of the year (p <0.05) (Ogawa 2003). However, in a four year retrospective study of 249 RRMS patients in Portugal, there was no significant difference in the mean relapse rate across all months (p = 0.54) (Fonseca et al., 2009). In addition, two studies which primarily examined the association between infections and relapse rates (see '*Infections*' section) also did not observe a significant seasonal effect on relapse rates (Panitch 1994; Buljevac et al., 2002).

A retrospective examination of the association between season of birth and disease progression accessing two large cohorts from Canada and The Netherlands found no significant association between month or season of birth and time to EDSS 6 or conversion to SPMS (Koch et al., 2008).

Stress

A systematic review by Artemiadis et al. (2011) examined the association between stress and relapse rates in 12 studies (9 cohort and 3 case-control). One database (Medline) was searched and only longitudinal original articles published between 1980 and 2010 were evaluated. Of the 12 studies included, 10 reported an increased risk of relapse associated with stress; however, due to the heterogeneity in measurement of stress, the authors could not conclude a causal relationship existed.

An additional study identified an association between war-related stress and relapses (Yamout et al., 2010). Two hundred and sixteen RRMS patients who had visited the American University Hospital, Beirut (a tertiary referral centre in Lebanon) at least once between 2005 and 2008 were included. The number of relapses during the 34 day Israeli-Lebanese war and up to 1 month after the war (15 July–15 September 2006) totalled 23, which the authors concluded was greater than expected based on the number of relapses during consecutive 2-month periods in the year preceding and following the war (a mean of 8.4, SD=0.86 was reported from between Jan/2005 to Jan/2008, excluding the war period) (Yamout et al., 2010).

A bidirectional relationship between stress and disease progression was noted in a prospective study of 101 MS patients. The risk of disease progression increased during reported stressful events (OR: 1.13, p <0.0003), and the risk of reported stressful events increased when the rate of disease progression increased (OR: 2.13, p < 0.0001) (Schwartz et al., 1999).

Physical trauma

No significant association was found between physical trauma, including dental procedures, surgery, fracture, burn, sprain, head injury, abrasion, laceration, or contusion and disease activity - either relapses or disability progression (DSS), among 170 American MS patients during 8 years of prospective follow-up (Sibley et al., 1991).

Smoking

The effects of smoking ('ever versus never') on the risk of reaching secondary-progressive MS were examined through a meta-analysis which included a search of two databases (OldMedline and Medline) for studies published between 1960 and May 2010 (Handel et al., 2011). Of the four studies included, three showed a statistically significant increased risk of progression associated with smoking; however, when they were combined the 95% confidence interval included one (RR: 1.88, 0.98 – 3.61). Further, the heterogeneity between studies was highly significant ($X^2 = 13.76$, $p = 0.003$) (Handel et al., 2011). Two additional studies not included in this systematic review also explored the association between smoking and progression (Pittas et al., 2009; Zivadinov et al., 2009). One prospective study from Tasmania, Australia found a modest association between cumulative pack years of cigarette smoking and the risk of reaching SPMS (OR: 1.03; 1.00-1.05 per pack year smoked, defined as one year of smoking 20 cigarettes/day) (Pittas et al., 2009). The authors noted that it was difficult to tease out the effects of smoking on SPMS from increasing age and disease duration which also elevated the risk of conversion to SPMS (Pittas et al., 2009). However, the same authors also found an association between pack years and disability, as measured by the multiple sclerosis severity scale (MSSS) (p for trend <0.01) (Pittas et al., 2009). Although no association was found between smoking and relapse rates (HR: 0.94, 0.69 -1.26 per pack year) (Pittas et al., 2009). A retrospective/ cross-sectional study of 368 MS cases in Buffalo, USA reported an association between smoking and increased disability, as measured by the EDSS ($p<0.004$) (Zivadinov et al., 2009).

3.2.2 Co-morbidities or other medical conditions

Nearly 9000 participants in the North American Research Committee of Multiple Sclerosis Registry (NARCOMS) with and without vascular comorbidity were compared (Marrie et al., 2010). Participants that reported one or more vascular comorbidities at MS diagnosis were at an increased risk of 'early gait' disability, equivalent to an EDSS of 4.0-4.5 (HR per vascular condition: 1.51; 1.41 – 1.61) (Marrie et al., 2010).

In a retrospective study, patients with a lipid profile test recorded within +/-6 months of an EDSS score ('baseline') along with a second EDSS score at least 6 months after baseline were included (Weinstock-Guttman et al., 2011). In total, of the 492 MS patients followed for a mean of 2.2 years, a higher baseline total cholesterol was modestly associated with worsening progression, measured by the EDSS

(r=0.15, p = 0.001) and MSSS (r=0.12, p=0.008) (Weinstock-Guttman et al., 2011). Findings were similar when restricted to those not taking a statin.

Among 156 recently diagnosed MS patients in The Netherlands, 146 were successfully followed prospectively for 3 years (examined at baseline, 6 months 1 and 3 years); those with musculoskeletal system comorbidities had greater physical decline (measured by the Functional Independence Measure) compared to those without comorbid illness ($F(3,414) = 5.50$, $p=0.005$, adjusted for age and baseline disease severity). None of the other comorbidities (e.g cardiovascular, respiratory or diabetes) examined were found to influence progression ($p>0.1$ for all) (Dallmeijer et al., 2009).

A retrospective study involving 149 patients was conducted in Groningen, The Netherlands. Neither fatigue (OR: 0.87, 0.42- 1.82) nor depression (OR: 0.57, 0.29 – 1.13) were associated with the risk of reaching secondary progressive MS (Koch et al., 2008).

3.2.3 Biological Risk Factors

Menstruation

The influence of the premenstrual period on MS relapses was examined retrospectively among 56 women with MS in Groningen, The Netherlands (Zorgdrager and De Keyser, 2002). Of the 42% (n=22) of women with a relapse during the premenstrual period (defined as the 6 days preceding the onset of menses), 10 women experienced a relapse starting in the pre-menstrual period vs. the remainder (n=12) who had relapses starting both within and outside this period. The authors concluded that the proportion of premenstrual relapses was considered to be greater than would be expected by chance alone (sign test, $p = 0.006$) (Zorgdrager and De Keyser, 2002).

Pregnancy and parity

A systematic review examining the relationship between pregnancy and relapse rates included a search of the following databases (1983-2009): EMBASE/Excerpta Medica, Medline, PubMed, LILACS, SciELO and the Cochrane Database of Systematic Reviews (Finkelsztejn et al., 2011). Original data from 13 studies, including 1221 pregnancies were incorporated in a meta-analysis. Relapse rates measured across three time periods from the different studies were pooled (rates/year shown): the year prior to pregnancy [n=10 studies; 0.44 (95% CI 0.39–0.48)]; during pregnancy [n=13 studies; 0.26 (95% CI 0.19–0.32)]; and in the 3-12 months post-delivery [n=13 studies; 0.76 (95% CI 0.64–0.87)]. Compared to the year prior to pregnancy, relapse rates were lower during pregnancy ($p<0.0001$) and higher post-delivery ($p<0.0001$) (Finkelsztejn et al., 2011).

Broadly similar findings were reported in three studies not included in the systematic review. A lower risk of relapse was observed during pregnancy compared with the 8 months postpartum (p=0.004) in a cohort of 153 women (Runmarker and Anderson, 1995). A prospective study of 227 women, enrolled around the time of pregnancy reported a lower risk of relapse during the third trimester of pregnancy ($p < 0.001$), and a higher risk in the first three months post-delivery, versus the year before pregnancy ($p < 0.001$) (Vukusic et al., 2004). The annualized relapse rates were: pre-pregnancy 0.7 (95%CI: 0.6-0.8); third trimester: 0.2 (0.2-0.3); three months post-delivery: 1.2 (1.1-1.4). Despite the relative increased risk post-partum, the authors noted that the majority of women (72%) remained relapse-free in the 3 months post-delivery (Vukusic et al., 2004). Further, the relapse rate dropped after this time, returning to pre-pregnancy levels (Vukusic et al., 2004). A higher relapse rate was also observed in the first three months post-delivery when compared to the relapse rate during the third trimester ($p = 0.005$) among 35 women followed prospectively in The Netherlands (Neuteboom et al., 2012).

Fewer studies attempted to examine the potential for pregnancy to impact the MS disease course over the longer-term. Disability, as measured by the EDSS, did not change significantly between the first trimester, one year, and three years postpartum in a cohort of 15 women compared to matched nulliparous women (Worthington et al., 1994). A retrospective study found no association between parity (the number of times a woman has given birth) and the onset of secondary progression (OR: 0.93, 0.50 - 1.72) in a cohort of 227 women with RRMS in Groningen, The Netherlands (Koch et al., 2009).

However, another study reported a significantly lower risk of developing secondary progression in women who became pregnant after MS onset compared to those who did not in a retrospective analysis of 100 women in The Netherlands (Runmarker and Anderson 1995). The risk of reaching SPMS was 3.2 times higher (95%CI: 1.1-10.3) for each year of observation in the non-pregnant state vs pregnant (Runmarker and Anderson, 1995).

Twenty-nine women with MS were followed for 5-years (from an original cohort of 39 in which 4 died and 6 were lost to follow-up) (Stenager et al., 1994). The cohort as a whole progressed (as measured by the 5-year vs baseline DSS, p=0.008), including the subgroup without children or those with MS onset largely pre-child birth (n=19, p=0.005), although not for those with MS onset more than 6 months after childbirth ($p = 0.74$) (Stenager et al., 1994). The authors concluded that it was unlikely that pregnancy influenced long-term disability in this small cohort of women (Stenager et al., 1994).

In summary, most studies indicate a decreased risk of relapse during pregnancy followed by an increased risk after delivery, while long-term disability does not seem to be unduly influenced.

Ethnicity

Ethnicity was not a significant predictor of EDSS (OR: 1.61; 0.86-2.99) when African Americans were compared to non-African Americans in New York State, USA, although a modest interaction between race and disease duration (OR: 0.96; 0.93-1.00) led the authors to conclude that African Americans exhibited greater disability with increasing disease duration (Weinstock-Guttman et al., 2003). In another cohort, African Americans (n=357) reached EDSS 6 faster than Caucasian American (n=427) MS patients, (HR: 1.67, 1.29 – 2.15) (Cree, 2004). Among 65 PPMS patients in Brazil, African ancestry was also associated with a greater risk of progression (Vasconcelos et al., 2010). Patients of African descent (n=23) reached EDSS 3 faster (1 year versus 2 years, $p<0.05$; actual p value unclear as 3 different p-values were given) and EDSS 6 faster (3 years versus 5 years, $p <0.05$) than white patients (n=42) (Vasconcelos et al., 2010).

Native North Americans (n=26) in British Columbia, Canada progressed more rapidly to EDSS 6 when compared to matched, largely Caucasian controls (median time: 14.4 years versus 35.2 years, $p = 0.004$) in a retrospective study of largely prospectively collected data (Saeedi et al., 2012).

3.2.4 Genetic Risk Factors

Ten studies examined the association between genetic factors and progression. Typically each examined a different genetic component or variant; replication of most findings is needed.

Four studies examined the influence of the HLA-DRB1 allele on progression (Romero-Pinel et al., 2011; DeLuca et al., 2007; Cournu-Rebeix et al., 2008; Runmarker et al., 1994).When Canadian patients were classified as either having a benign (n= 112) or malignant disease course (n = 51), the HLA-DRB1*01 was found more frequently in the benign cases (19% vs 3.9%, $p = 0.027$). No other HLA-DRB1 allele differed significantly between the cohorts (DeLuca et al., 2007). A later study of 380 Spanish patients reported that the presence of HLA-DRB1*01 or HLA-DRB1*04 genotype reached EDSS 6 more rapidly than patients without (first quartiles were 15.3 years vs 31.0 years (for DRB1*01), $p = 0.004$, and 17.6 years vs. 26.0 years (for DRB1*04), $p=0.02$, respectively) (Romero-Pinel et al., 2011).In a cohort of 651 French patients, of the 575 with RRMS, the median time to reach EDSS 6 was shorter for carriers of the HLA-DRB1*15 allele compared to non-carriers (20 years vs 26 years, $p = 0.026$). There was no significant influence of the HLA-DRB1*15 on progression among the smaller subgroup of 76 patients with PPMS (Cournu-Rebeix et al., 2008). Progression to EDSS 6 did not vary between HLA-DRB1*15-positive and negative patients with RRMS, SPMS, or PPMS over a 25-year follow-up; however, RRMS patients with haplotypes DR17 and DQ2 converted to SPMS more rapidly ($p = 0.04$) than those without this haplotype (Runmarker et al., 1994).

Several single nucleotide polymorphism (SNP) loci were examined in relation to MS disease severity. No association was found with SNPs of the following genes: *CLEC16A, IL2RA, IL7R, RPL5, CD58, CD40* in a cohort of 1006 Australian patients using various

measures of disease severity, including the MSSS (Jensen et al., 2010); or in a cohort of 514 Dutch patients with: *CTLA-4-318, CTLA-4+49* and *CD28-13+17* when time to EDSS 6 was assessed (Vanveen et al., 2003). SNPs in the melanocortin I receptor region were associated with disease severity, specifically rs1805009 was associated with a lower MSSS score ($p = 0.003$), while rs1805006 was associated with a higher MSSS score ($p = 0.030$) among 525 Northern European Caucasians (Strange et al., 2010). The immunoglobulin heavy chain variable (IGHV) 4-39 was not associated with disease progression when comparing 146 benign MS cases with 47 malignant MS cases ($p = 0.8$) (Watson et al., 2010). The chemokine receptor 5 δ32 allele was not associated with a specific disease course (RRMS/SPMS or PPMS), nor was it associated with a faster progression, as measured by EDSS, among 439 patients from Northern Ireland (Silversides et al., 2004). The association between the interleukin-1 (IL-1) gene and disability was examined in 377 MS patients; amongst 200 individuals with a disease duration of at least 10 years, the risk of reaching EDSS of 6 or higher was reduced in those with the IL-1 receptor antagonist genotype (OR: 0.4, 0.2-0.8), and increased in those carrying the IL-1β genotype (OR: 2.2, 1.2-4.0) (Mann et al., 2002).

3.2.5 Other Clinical and Demographic Factors

While we identified only one published systematic review of studies examining the role of additional clinical and demographic factors (such as sex, age and onset symptoms) on the 'natural history of MS' (Langer-Gould et al., 2006), the sizable number of very comprehensive reviews available in this area (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011) meant that we did not systematically re-review this topic here. However, we have briefly summarized the main findings from these reviews, as well as key findings in the wider literature related to important subgroups not specifically included in Langer-Gould's systematic review, such as primary-progressive MS or paediatric MS (who represent around 10% and <5% of all MS patients, respectively).

A systematic review aimed to identify clinical and demographic factors associated with disease progression in MS (measured as EDSS or SPMS), focusing on those with a relapsing-remitting onset (Langer-Gould et al., 2006). The search strategy spanned 5 different databases from 1966 to May 2005, including 27 studies. Meta-analyses were not possible because of study heterogeneity (Langer-Gould et al., 2006). Sphincter symptoms at onset were associated with a worse prognosis (HR range: 1.1-3.1). Sex, age at onset, and onset symptoms (excluding sphincter involvement) showed only weak associations with prognosis, but the results varied widely across studies (Langer-Gould et al., 2006). In addition, as the authors noted, the cross-sectional design of many studies, enrollment of cases from specialist referral centres and lack of multivariable adjustment were major limitations (Langer-Gould et al., 2006).

Other reviews in the area which included all 'forms' of MS generally concurred with these findings, although some have concluded that onset age is associated with outcome – for instance a younger age at onset was associated with a longer time to reach disability milestones, but these milestones were reached at a younger age vs. those older at onset (Confavreux et al., 2006; Tremlett et al., 2010;

Renoux, 2011). This observation has also been reported in studies specifically focusing on time to SPMS as an outcome (Tremlett et al., 2010). Being male (versus female) is often reported as associated with a more rapid progression (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011), although when the age at which disability milestones were reached was considered, there appears to be little difference between men and women (Tremlett et al., 2010; Renoux, 2011). Being male (vs female) was implicated in a shorter time to secondary progression (Confavreux et al., 2006; Tremlett et al., 2010); however, when other factors were considered through multivariable analysis, there was little effect of sex on long-term prognosis (Renoux, 2011). The presence (versus absence) of specific onset symptoms and their association on long-term progression appear to vary between studies, although once other demographic factors are considered (e.g. sex, age), there often appears to be little independent impact of onset symptoms (Tremlett et al., 2010; Renoux, 2011). However, in one review, optic neuritis at onset was reported as being associated with a longer time to progression, while motor, cerebellar, or sphincter dysfunction at onset were associated with more rapid progression (Renoux, 2011).

Having a primary-progressive disease course was typically associated with a worse prognosis than a relapsing-remitting course (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011). However, once the disease course has been established, few predictors of progression in those with PPMS have been identified (Miller and Leary, 2007), aside from having three or more neurological systems involved at onset (Miller and Leary, 2007), older age or absence (versus presence) of sensory symptoms (Koch et al., 2009) – all of which were associated with a faster progression in a limited number of studies (Miller and Leary, 2007; Koch et al., 2009).

In a review examining prognostic factors in paediatric MS (under 18 years at MS onset), the authors reported that unlike many adults with relapsing-remitting MS, being male was associated with a better prognosis, but there was little effect of onset age on progression (which naturally has a narrower range in those with paediatric MS versus adults) (Banwell et al., 2007).

4. DISCUSSION

The extensive epidemiological evidence for the association between a wide range of risk factors and MS disease onset and disease activity (relapses and progression) with a focus on factors most amenable to modification was systematically reviewed and summarized. Best evidence indicated that lower serum 25(OH) D levels or low sunlight exposure, exposure to the Epstein-Barr virus or cigarette smoking (either passive or active) were modifiable contributors to MS susceptibility. However, a largely non-modifiable factor, variation in the HLA-DRB1 allele, remained one of the strongest risk factors. Factors most strongly associated with MS relapses were all potentially modifiable, including low serum 25(OH)D levels or low sunlight exposure, presence of common infections, and exposure to stress. Fewer epidemiological studies examining potentially modifiable factors associated with longer-term disease progression were found, the best evidence implicated cigarette smoking with poorer outcomes in MS.

4.1 Factors associated with MS onset

While one of the strongest factors associated with MS susceptibility related to presence of the HLA-DRB1*1501 polymorphism, the effect was still modest (OR: 2.27; 2.04- 2.54) (Lill et al., 2013), and this genotype is typically present in about 15.6% of unaffected populations (Brynedal et al., 2007). Of the main environmental or lifestyle-related factors with the best evidence for an association with MS risk, serum 25(OH) D levels and smoking may have the greatest public health implications. It is estimated that 1 billion people worldwide are vitamin D 'deficient' (Holick, 2007), and smoking is still common globally (Warren et al., 2006). Although it was not always clear as to whether the effects of vitamin D were entirely mediated through sunlight exposure, or if sunlight exposure inferred additional benefits, the evidence has been convincing enough for interventional studies involving vitamin D supplementation in at risk populations to begin in the hopes of preventing MS (Derakhshandi et al., 2013; Thouvenot et al., 2013; Hutchinson et al., 2013).

While EBV exposure might be considered less amenable to modification (related to lack of a suitable vaccine (Cohen et al., 2013), and its ubiquitous and long history of co-existence in human populations), findings provide valuable insights into the disease mechanisms involved in MS, and have been used by some groups exploring causal pathways or even developing an algorithm to predict MS risk (De Jager et al., 2009; Ramagopalan et al., 2010).

While earlier studies focused on the influence of diet on MS risk (Lauer, 1997), there has been a shift more recently to the assessment of body mass index (BMI) as a possible risk factor (Gotay et al., 2013; Ogden et al., 2012). The suggestion that high BMI might increase the risk of MS is intriguing and of potential concern given the recent 'obesity epidemic' in children, adolescents and adults alike (Gotay et al., 2013; Ogden et al., 2012). An increased risk of MS within specific disease groups, or an increased risk of specific diseases before MS onset were found for conditions such as COPD (Egesten et al., 2008), inflammatory bowel disease (Gupta et al., 2005; Langer-Gould et al., 2010), and type I diabetes (Nielsen et al., 2006), which is also of interest; many of these are considered to have an autoimmune component and the prevalence of autoimmune diseases has reportedly been rising in the general population (Moroni et al., 2012). However, the evidence was sometimes limited (few studies) or mixed and others reported no association, depending on the condition. Oral contraceptive use (Hernán et al., 2000; Thorogood and Hannaford 1998; Villard-Mackintosh and Vessey, 1993), standard vaccinations (Farez and Correale 2011; Mikaeloff et al., 2009b) and exposure to other viral infections (with the exception of EBV) (Granieri et al., 1997; Berr et al., 1989; Bager et al., 2004; Massa et al., 2009) were typically not associated with an altered risk of developing MS. It was unclear whether factors such as diet (Ghadirian et al., 1998; Zhang et al., 2000; Zhang et al., 2001), coffee and alcohol consumption (Pekmezovic et al., 2006; da Silva et al., 2009), exposure to house pets (Read et al., 1982; Cook et al., 1978; Warren et al., 1991; Bansil et al., 1997; De Keyser and Zwanikken, 1997; Hernán et al., 2001), stress (Riise et al., 2011; Li et al., 2004; Grant et al., 1989; Berr et al., 1989; Warren et al., 1982), or antibiotic use (Nørgaard et al., 2011; Alonso et al., 2006) might alter MS risk; each

have been associated with an increased MS risk in at least one study, and not associated in another. Most of these studies included prevalent cases, with only a few including incident cases or being able to collect information of the exposure independent of the outcome, i.e before onset of MS (Hernán et al., 2001; Riise et al., 2011; Li et al., 2004; Nørgaard et al., 2011; Alonso et al., 2006). Work-related organic solvent exposure was consistently associated with an increased MS risk (Landtblom et al., 1996; Riise et al., 2002; Landtblom et al., 2006), but few other occupational exposures emerged as potential candidates for triggering MS (Stenager et al., 2003; Johansen et al., 1999). Disruption to the circadian rhythm might play a role in MS, with one recent study indicating that shift work may be associated with an increased risk of MS (Hedstrom et al., 2011c). Shift work has previously been linked to an increased risk of developing other autoimmune diseases (Magrini et al., 2006; Knutsson, 2003). Whether this also relates to reduced exposure to sunlight (vitamin D), or sleep deprivation, and/or is mediated through disruption to melatonin secretion which can impact the immune system is unknown (Hedstrom et al., 2011c). Confounding also cannot be ruled out as shift-work in general has been associated with other adverse health behaviors, including smoking, poor diet, reduced physical activity, and lower socioeconomic status (Ponsonby and Lucas, 2011; Knutsson, 2003).

Other possible pathways or hypothesized mechanisms of action for the risk factors identified above have been wide ranging, including the 'hygiene hypothesis,' in relation to exposure to infections, or the immune-modulatory effects of the HLA allele (Sundqvist et al., 2012; De Jager et al., 2008; Simon et al., 2010), lack of vitamin D (Raghuwanshi et al., 2008; Pierrot-Deseilligny et al., 2013) and smoking (Wingerchuk, 2011) Possible interactions between smoking, high EBV titers and the HLA allele were considered in some studies (Simon et al., 2010; De Jager et al., 2008; Hedstrom et al., 2011a). It was postulated that smoking may increase the association between EBV (high EBV titres) and the risk of developing MS (Simon et al., 2010). Carriers of the HLA-DRB1*1501 allele who also had elevated levels of EBV titers were at a significantly increased risk of MS (De Jager et al., 2008). Smokers with both the presence of HLA-DRB1*15 and absence of HLA-A*02 were at a particularly high risk of MS (compared to non-smokers without these risk variants) (Hedstrom et al., 2011a).

4.2 Factors influencing the MS disease activity: relapses and progression

There were some commonalities between the factors associated with the risk of developing MS and those associated with the subsequent disease activity in patients with established MS. There was evidence suggesting that low vitamin D serum levels or low sunlight exposure increased the risk of an MS relapse; although as with MS onset, the independent contribution of each was not always clear (Simpson et al., 2010; Tremlett et al., 2008; Smolders et al., 2008; Correale et al., 2009). Nonetheless, interventional studies involving vitamin D supplementation are underway and a limited number have been completed; although results to date have been rather disappointing (Jagannath et al., 2010; Stein et al., 2011). The presence of infections was also correlated with an increased relapse rate; however, rather

than EBV, the focus was more on common infections, particularly upper respiratory tract infections (possibly because by the time of MS onset, virtually all those with MS will be EBV positive). There appears to be a period of increased risk of a relapse surrounding the time of infection with most studies exploring the two weeks before and five weeks after infection as 'at risk' periods (Sibley et al., 1985; Correale et al., 2006; Andersen et al., 1993; Buljevac et al., 2002; Buljevac et al., 2003, Panitch, 1994). Some also reported a seasonal trend in relapse rates; possibly mediated through seasonal variation in vitamin D/sunlight levels as well as infection rates, although findings were not always consistent (Jin et al., 2000; Tremlett et al., 2008; Ogawa et al., 2003; Fonseca et al., 2009; Koch et al., 2008; Panitch, 1994; Buljevac et al., 2002). Preliminary work has suggested that parasitic gut infections might alter the risk of a relapse as well as possibly longer-term disability outcomes (Correale and Farez, 2007). While of interest, this requires confirmation, and interestingly is currently under investigation in a Phase 2 clinical trial (Constantinescu and Gran, 2013). Several studies examined the role of various vaccines in relation to MS onset; typically no associations were found, aside from vaccination against tetanus and diphtheria, both of which were associated with a 30-40% decreased risk of MS (OR 0.60 to 0.68, (Hernán et al., 2006; Farez and Correale, 2011). Further, one study implicated a specific brand of hepatitis B vaccine (Engerix B) as increasing MS risk in children (Mikaeloff et al., 2009b). Whether this related to a preservative commonly used in vaccines (the mercury based thiomersal/ thimerosal) is unclear, however its use is being phased out because of broader concerns surrounding neurotoxicity in children, with thiomersal-free vaccines available. Only one study could be found exploring the relationship between vaccination and disease activity in those already with MS (Confavreux et al., 2001). Of the several common vaccines examined in this study (which were verified by evidence of a vaccination or medical record), none appeared to trigger relapses (Confavreux et al., 2001). Various forms of stress (including psychosocial and war-related), often examined retrospectively were associated with increased relapse rates in some studies (Yamout et al., 2010; Schwartz et al., 1999), however, no 'secure conclusions' could be drawn from the one systematic review in this area (Artemiadis et al., 2011). While we did not identify any studies implicating pregnancy as a factor triggering MS, pregnancy did appear to influence the risk of a relapse in those with established disease. A reduced risk was typically observed in the third trimester, followed by a slight increased risk post-partum (Finkelsztejn et al., 2011; Runmarker and Anderson, 1995; Vukusic et al., 2004; Neuteboom et al., 2012). Pregnancy itself did not appear to alter long-term disability progression, although there were fewer studies in this area (Worthington et al., 1994; Koch et al., 2009; Runmarker and Anderson, 1995; Stenager et al., 1994).

Similar to disease onset, cigarette smoking was implicated in influencing the long-term disability progression in MS (Handel et al., 2011; Pittas et al., 2009; Zivadinov et al., 2009). Otherwise there were generally few observational studies investigating factors which might influence disease progression *and* be amenable to modification; there may be merit in pursuing the influence of modifiable lifestyle factors, such as body mass index, diet or exercise.

Age and sex were implicated in the progression of the disease in some studies, with a faster progression associated with an older current age, older age at onset (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011), and male sex (Confavreux et al., 2006; Tremlett et al., 2010). Although these were not identified as strong risk factors in a systematic review focused on relapsing-onset MS (Langer-Gould et al., 2006) and was not always true when the age at which patients reached disability milestones or progression were taken into account (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011). Further, risk factors may differ in paediatric MS, with girls reported as progressing faster than boys (Banwell et al., 2007) in contrast to the faster progression of adult men vs. adult women with MS (when measured from symptom onset). Presence of primary-progressive MS (versus relapsing-onset) in adults was associated with a faster progression (Confavreux et al., 2006; Tremlett et al., 2010; Renoux, 2011).

As with MS onset, because we included only human observational studies within this review, we did not explore possible mechanisms of action of the risk factors on disease progression. A possible pathway is via immunomodulation. There is evidence implicating infections, low serum vitamin D levels, the HLA allele, elevated stress, and smoking in the activation of an altered immune response (Correale et al., 2009; Lill et al., 2013; Padgett and Glaser, 2003; Sopori, 2002).

4.3 Timing of risk factors in relation to MS onset

Timing of environmental exposures has been found to relate to MS onset (McDowell et al., 2010). The season of birth effect (Torkildsen et al., 2012; Disanto et al., 2012), lower UV exposure in the first trimester of pregnancy (Staples, 2010) and reduced maternal vitamin D intake (Mirzaei et al., 2011) increasing MS risk, as well as the observation of the association between maternal smoking and increased risk of MS in offspring (Gardener et al., 2009a), could highlight exposure *in utero* as a critical time point. Although a recent study has questioned whether the season/month of birth effect in diseases such as MS is actually an artifact, or chance finding, related to natural variation in both regional and temporal months of birth in any underlying (control) population (Fiddes et al., 2013).

Much discussion has surrounded the possible timing of environmental exposures after birth which might trigger MS onset, with no definitive answers (Handel et al., 2010b; van der Mei et al., 2011). Evidence from studies included in our review suggested that early childhood through until adolescence represent ‘at risk’ periods, with increased sun exposure, vitamin D intake or exposure to infant siblings during these times associated with a reduced risk of multiple sclerosis (Islam et al., 2007; Dalmay et al., 2010; van der Mei et al., 2003; Ponsonby et al., 2005; McDowell et al., 2011). Further, high BMI during late adolescence has been associated with an increased risk (Munger et al., 2009; Hedstrom et al., 2012), although only one study was able to include incident cases, with BMI measured before a person’s MS status was known (Munger et al., 2009). Childhood exposure to dogs was also associated with MS risk (De Keyser and Zwanikken, 1997; Cook et al., 1987), though these (and other) studies were reliant on self-report and susceptible to recall bias. Older age of exposure to vaccination was considered a trigger for onset of MS by some (Berr et al., 1989; Hernán et al., 2001), as was later exposure

to common childhood infections (Hays et al., 1992; Compston et al., 1986; Bachmann and Kesselring 1998; Hernán et al., 2001). However, 'at risk' periods might also exist through until adulthood – one example being the increased risk of MS associated with severe stress in parents - specifically death of a child (Li et al., 2004). Low levels of serum vitamin D even in early adulthood have also been implicated with an increased risk of MS (Munger et al., 2004; Munger et al., 2006).

4.4 Strengths and Limitations

The strengths of this review include its comprehensive and systematic approach, applying an exhaustive search strategy, relevant MeSH terminology and accessing seven large databases to reduce the chance of missing relevant articles. Further, a large number of titles, abstracts and full articles were screened by a limited number of trained reviewers. Any disagreement between reviewers was resolved by consensus, reducing the possibility of bias. The high reliability score (Kappa > 7) suggested methodological consistency.

It is recognized however that specific studies may have been missed. The literature on MS is vast and rapidly expanding. Not all journals or relevant publications may be contained within the data sources accessed and time and cost restraints meant it was not feasible to access the sizable 'grey literature.' Publication bias cannot be fully ruled out; it remains highly possible that studies with positive findings or associations are more likely to be submitted by authors and accepted by Editors for publication. Testing for publication bias is possible to a certain degree using funnel plots in relation to a meta-analysis. While reports on study heterogeneity from other such systematic reviews were included, this review is so broad that it would require a series of complex meta-analyses which was not feasible, and whose value has been questioned in the context of observational studies (Egger et al., 1998).

This systematic review focuses on observational studies rather than interventional studies or randomized controlled trials. While this broadens the horizon to consider the impact of many exposures on MS, including those less amenable to a clinical trial (e.g. smoking); other exposures, such as vitamin D supplementation, which span both observational and interventional studies, may not be comprehensively captured here. Further, the inherent limitations associated with observational studies must be taken into consideration, especially challenges when inferring causation. Multiple studies were susceptible to the possibility of reverse causation or recall bias. This is especially relevant in a disease such as MS which might remain subclinical for many years, making assessing risk factors truly associated with triggering the disease (versus just triggering a clinically apparent attack or relapse) difficult. Taking vitamin D as an example; a number of studies measured serum vitamin D levels, but in a cross-sectional manner (i.e. at one point in time), and retrospectively examined the association with either MS onset or disease activity (relapses or progression). It is typically not possible to rule out reverse causation from these types of study designs.

The clinical importance or population impact of some of the statistically significant findings reported in studies can be challenging to interpret (Kazdin, 1999). The magnitude of the association between exposure (risk factor) and outcome (MS onset or progression) could be modest (OR between 1 and 2), but still statistically significant, especially in studies with particularly large sample sizes (Hochster, 2008). Further, multiple studies reported only a p-value, without a measure of effect size (odds ratio or relative risk). A p-value indicates the statistical significance of an association, but does not specify the strength of that association. Without knowledge of the strength of association, inferring clinical importance can be even more challenging.

Many of the studies were heavily reliant on self-reported behaviors. Exposures such as sunlight and history of breast feeding or stressful life events are difficult to document, and susceptible to recall and interviewer bias. Although a number of studies went to considerable lengths to mitigate this by using objective measures, such as skin casts to record sun-related damage (van der Mei et al., 2003), or by accessing prospective cohorts who were followed before clinical signs of MS emerged (e.g. The Nurses' Health Study) (Munger et al., 2003; Munger et al., 2004). Overall, of the original studies included in our review which examined risk factors associated with developing MS, over half (66/119 - 55%) focused on prevalent cases, while the remainder were able to include incident cases with the exposure largely collected before onset of clinically identifiable symptoms of MS. Inferring causation is particularly challenging when examining prevalent (existing) cases with the exposure collected after MS onset. Although reverse causation can often never be fully ruled out, even in incidence studies, because although the exposure (e.g. serum levels of a specific infection) might have been collected pre-onset of clinical symptoms of MS, the actual (i.e. subclinical) onset of MS is still unknown and may have already occurred.

Further, the risk factor or exposure being studied was not always clearly defined or varied across studies. For instance, some studies compared 'never smokers' with 'ever smokers' while others grouped individuals into 'low', 'medium' and 'high' levels of smoking exposure. These differences in categorization of smoking could also lead to residual confounding and make comparisons between studies challenging (Fewell et al., 2007).

The majority of studies which examined factors associated with the risk of developing MS were of a case-control design (101/125, 81%). The method of control selection varied widely across studies; some were nested within large defined cohorts, such as the USA's Nurses' Health Study (Alonso et al., 2008; Ascherio et al., 2001; De Jager et al., 2008; Gardener et al., 2009a; Hernán et al., 2000; Hernán et al., 2001; Kister et al., 2012; Massa et al., 2007; Massa et al., 2009; Mirzaei et al., 2011; Munger et al., 2003; Munger et al., 2004; Munger et al., 2009, Simon et al., 2010; Simon et al., 2011; Wagner et al., 2004; Zhang et al., 2000; Zhang et al., 2001), while others relied on controls that may not have been representative of the 'at risk' population, such as hospital personnel or spouses (Kragt et al., 2009; Ramagopalan et al., 2008a, Ramagopalan et al., 2008b; Ramagopalan et al., 2009; Ramagopalan et al., 2010; Ramagopalan et al., 2011;

Shaygannejad et al., 2010; Smolders et al., 2008; Soilu-Hänninen et al., 2008). The source of controls was unclear in some studies, which limited the interpretation and potentially the validity of results.

There were multiple methods for measuring disease activity in MS which differed between studies. While progression was typically measured using well-recognized scales (such as the DSS, EDSS or MSSS), relapses were often less well defined, sometimes involving subjective self-report. Further, as MS can be a slowly evolving disease such that long-term follow-up is typically needed to truly measure disease progression, but for many studies this was not the case. Perhaps for this reason, two-thirds of the studies which examined the influence of environmental or lifestyle factors on MS disease activity focused on relapses rather than the longer-term progression.

Overall, it seemed that the quality of the MS literature has markedly improved over time, evidenced by a general trend for an increase in the quality scoring over time – for instance, for studies published between 1988 to 1998, the mean quality score was 15.04, increasing to 16.38 (1999 – 2005), and then 16.68 for 2006 to 2012 studies. This has been aided by improved study design, implementation of robust epidemiological methodology, and the use of validated and standardized diagnostic measurements.

While the comprehensive coverage of this review is considered a strength, it has also led to certain limitations. This literature is so vast that it was often challenging to give each study the full discussion that it deserved. For instance, certain authors reported adjustments for various potential confounders (eg ethnicity, socioeconomic status, etc); however, to report all of these details was not feasible. Some studies also had significant limitations, but because they met our inclusion criteria they were given seemingly equal weight (space) to studies with greater validity. We tried to detail the methodology of each study using short descriptions, but to address the strengths and limitations of each paper was simply not practical.

4.5 Generalizability of findings

Not unexpectedly, most of the factors (for both onset and disease activity) were largely explored in cohorts dominated by women or those with relapsing-onset MS. It remains possible that many of the factors identified in our review are less applicable to men or those with PPMS. One example might be the month of birth effect and MS risk; one group re-examined this association by disease course and found the association to exist only in those with relapsing-onset MS (Willer et al., 2005; Sadovnick et al., 2007), not progressive MS. A substantial proportion (around two-thirds) of studies examining risk factors associated with MS onset did not report the disease course - presumably because of difficulties in accessing clinical information. However, by focusing on observational studies, it could be argued that the overall external validity is high, in that within each study, the inclusion criteria were generally broad, with many studies examining patients from large population-based cohorts. Finally, the ‘special cohorts’ such as patients with clinically isolated syndrome,

who are at risk of developing MS were purposely not included. Nor were the outcomes such as death or biomarkers such as imaging, or interventions such as drug treatments considered.

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****TO BE USED AS A DIVIDER PAGE TO INTRODUCE THE MAIN PAPER****

Multiple Sclerosis

Key Scientific Facts

Multiple sclerosis (MS) is a chronic neurological disease characterized by inflammation, demyelination, and axonal degeneration of the central nervous system (CNS). In MS, the immune system attacks the myelin sheath surrounding the nerves of the CNS, ultimately resulting in disruption of communication between the brain and other parts of the body.

A wide variety of MS symptoms can be experienced, including sensory symptoms, cognitive difficulties, depression, pain, fatigue, bladder dysfunction, balance and walking difficulties.

MS is the most common cause of neurological disability among young adults with MS incidence peaking around age 30. The prevalence of MS is 1-2 cases per 1000 people in North America, Europe, and Australasia.

The aetiology of MS is not well understood but epidemiological studies indicate a complex interplay between genetic and environmental factors in the onset, and perhaps also in subsequent relapses and progression of MS.

This study identified exposure to Epstein-Barr virus, smoking, low serum vitamin D levels and obesity in adolescence as risk factors for the onset of MS.

Common infections, low vitamin D serum levels or low sunlight exposure, and stress were associated with an increased risk of relapse, while cigarette smoking was associated with long-term disability progression of MS.

UPDATE

The field of MS is evolving rapidly. Several modifiable risk factors have received particular attention since this systematic review was completed, which may shape the future landscape of MS research. Advances in the study of genetics led to the identification of 48 new susceptibility loci associated with MS risk (International Genetics Consortium, 2013); further compelling evidence from studies largely accessing prospectively collected data implicated obesity in childhood / early adolescence with future MS risk (Munger et al., 2013; Langer-Gould et al., 2013), and emerging basic science suggested that salt could be a contender in altering MS risk (Kleinewietfeld et al., 2013; Wu et al., 2013), and sodium levels in the brain may also be associated with disease progression (Paling et al., 2013; Zaaraoui et al., 2012).

Beyond these original studies, a number of systematic reviews and meta-analyses have been published since the search date of our systematic review, which this update aimed to identify. A supplementary search of the Medline database was performed to identify recently published studies that may be of interest to readers. Our supplementary search returned 169 citations published between January 1, 2013 and December 31, 2015, 9 of which met criteria for inclusion. The majority focused on risk factors for the development of MS (8 articles), while two focused on risk factors for disease progression (one study examined both onset and progression). No reviews addressing risk factors for MS relapses were identified. A list of the citations is provided below.

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Figure 1 Screening Process for Articles related to Onset and Progression of Multiple Sclerosis

Table 1. Summary of the Systematic Reviews Assessing the Association between Risk Factors and MS Onset

Author, Year	AMSTAR Score*	Objective	Methodological design(s) of included studies	Years included in search strategy	Database searched	No. of studies included	Main findings	Heterogeneity (significant p-value [<0.05] suggestive of heterogeneity)
Aminzadeh, 2007	5	To determine the association between dental amalgam restorations and MS risk	Case-control, Cohort	1966-2006	Embase, MEDLINE	3	Exposure to amalgam fillings was associated with a modest, but not statistically significant increased risk of MS compared to non-amalgam fillings (OR: 1.24; 0.96–1.61).	Q = 13.7, p = 0.004

Farez, 2011	6	To estimate the effect of immunizations on the risk of adult-onset MS	Cohort Case-control Randomized controlled trial	1961-2011	MEDLINE, Embase, Cochrane Central Register of Controlled Trials	14	No significant change in the risk of developing MS after vaccination for BCG (OR: 0.96; 0.69–1.34), Hepatitis B (OR: 1.00; 0.74–1.37), Influenza (OR: 0.97; 0.77–1.23), Measles–Mumps– Rubella (MMR) (OR: 1.02; 0.64–1.61), Polio (OR: 0.87; 0.61–1.25) or Typhoid fever (OR: 1.05; 0.72–1.53). Decreased risk of developing MS after diphtheria (OR: 0.60; 0.40–0.90) or tetanus (OR: 0.68; 0.54–0.84) vaccinations.	BCG (I^2=0.0%, p = 0.95), diphtheria (I^2=0.0%, p = 0.63), Hepatitis B (I^2=0.0%, p = 0.92), Influenza (I^2=5.1%, p = 0.37), MMR (I^2=0.0%, p =0.62), Polio (I^2=28%, p = 0.21), Tetanus (I^2=8.3%,p = 0.37), Typhoid fever (I^2=14.5X, p = 0.32)

Handel, 2010	5	To update Thacker et al. 2006 and investigate the association between infectious mononucleosis and MS onset and to ascertain whether the association was latitude or sex dependent	Case-control Cohort	2006-2010	MEDLINE	18	History of infectious mononucleosis was associated with an increased risk of developing MS (RR: 2.17; 1.97-2.39).	I^2= 0%, p = 0.47
Handel, 2011	6	To determine the association between cigarette smoking and MS risk	Case-control Cohort	1960-2010	OLDMEDLINE, MEDLINE	10	History of smoking prior to disease onset was associated with an increased risk of MS (RR: 1.48; 1.35-1.63).	X^2 = 7.38, p = 0.60

Hawkes, 2007	6	To determine the association between cigarette smoking and MS risk	Case-control	1964-2006	PubMed, Embase, CINAHL, Google, Google Scholar, Cochrane, textbooks, conference proceedings	6	'Ever smoking' was associated with an increased risk of MS when compared to 'never smoking' (OR: 1.34; 1.17-1.54).	Not reported
Hernán, 2006	7	To investigate the association between tetanus vaccination and MS risk	Case-control	1996-2005	MEDLINE, Embase, LILACS, Science citation index	9	Tetanus vaccination was associated with a reduced risk of developing MS (OR: 0.67; 0.55-0.81).	I^2= 2.5%, p = 0.41
Landtblom, 1996	4	To investigate the association between organic solvent exposure and MS risk	Case-control	1966 – 1994	MEDLARS	9	Organic solvent exposure was associated with an increased risk of MS (RR: 2.1; 1.6 – 2.7)	Not reported

Thacker, 2006	4	To determine the association between infectious mononucleosis and risk of MS	Case-control	1965-2005	MEDLINE	14	History of infectious mononucleosis was associated with an increased risk of MS (OR: 2.3; 1.7-3.0).	$Q_{df=13}$ = 16.07, p = 0.25
Torkildsen, 2012	4	To determine the association between month of birth and risk of MS.	Not pre-specified	Up until 2012	MEDLINE, PubMed	15	Studies based in the Northern Hemisphere reported an excess of MS births in April and May, with a nadir in November and December. Studies based in the Southern Hemisphere reported a peak in November births with a nadir in May.	Not reported
*AMSTAR scores of 4-7 indicate moderate quality, OR=Odds Ratio; RR=Relative risk								

Table 2. Summary of the Systematic Reviews Assessing the Association between Risk Factors and Disease Activity in MS (Relapses or Progression)

Author, Year	AMSTAR * Score	Outcome: Relapse or Progression	Objective	Methodological design(s) of included studies	Years included in search strategy	Database(s) searched	No. of studies included	Main findings	Heterogeneity (significant p-value [<0.05] suggestive of heterogeneity)
Artemia dis, 2011	6	Relapse	To examine the relationship between psychological stress and MS relapses.	Case-control Cohort	1980-2010	MEDLINE	12	10 of 12 studies found an association between stress and relapse rate. However, because of heterogeneity in the measurement of stress, the authors could draw no firm conclusions.	Not reported

Finkelsztejn, 2011	8	Relapse	To assess evidence regarding the effects of pregnancy on MS relapse rates	Not explicitly stated; only original studies included	1983-2009	Embase/ Excerpta Medica, MEDLINE, PubMed, Scopus, Index Medicus, Biomed, Central, Ebsco Fulltext, LILACS, SciELO, Cochrane Database of Systematic Reviews	13 studies (specific to relapses)	A significant decrease in relapse rate was observed during pregnancy (vs. the year pre-pregnancy), followed by a significant increase in the 3-12 months after delivery. Pooled pre-pregnancy relapse rate/year: 0.44 (95% CI 0.39–0.48); during pregnancy: 0.26 (95% CI 0.19–0.32); after pregnancy: 0.76 (95% CI 0.64–0.87)	I^2 = 82.35%, p = 0.000
Handel, 2011	7	Progression (SPMS)	To assess the effect of smoking on the risk of developing SPMS	Case-control Cohort	1960-2010	OLDMEDLINE MEDLINE	4	Smokers had an increased , though not statistically significant, risk of reaching SPMS relative to non-smokers, (RR: 1.88; 95%CI, 0.98 – 3.61).	X^2= 13.7, p = 0.003)

Jin, 2000	6	Relapse	To assess seasonal variation in relapses	Not stated	1966-1999	MEDLINE	10	Ratio of highest to lowest seasonal proportions was 1.10 (95%CI, 1.07 – 1.13) with the highest relapses in spring and the lowest in winter.	p-value = 0.136)
Langer-Gould, 2006	7	Progression	To identify clinical and demographic factors associated with long-term disability in RRMS	Cohort Cross-sectional	1996-2005	MEDLINE, Embase, CINAHL, Cochrane, PsycINFO	27	Sphincter symptoms at onset (hazard ratio, 1.1-3.1), incomplete recovery from the first attack (hazard ratio, 1.3-3.3), and a short interval between the first and second attack (hazard ratio, 1.6-1.9) were most strongly and consistently associated with poor prognosis.	Not reported
Pakpoor, 2012	5	Relapse	To assess the association between breastfeeding and relapse rates	Not stated	1988-2011	PubMed	12	Women who breastfed were at a reduced risk of post-partum relapse (OR: 0.53; 95%CI, 0.34-0.82)	I^2 = 63%, p = 0.002)

*AMSTAR scores of 4-7 indicate moderate quality, OR=Odds Ratio; RR=Relative risk				

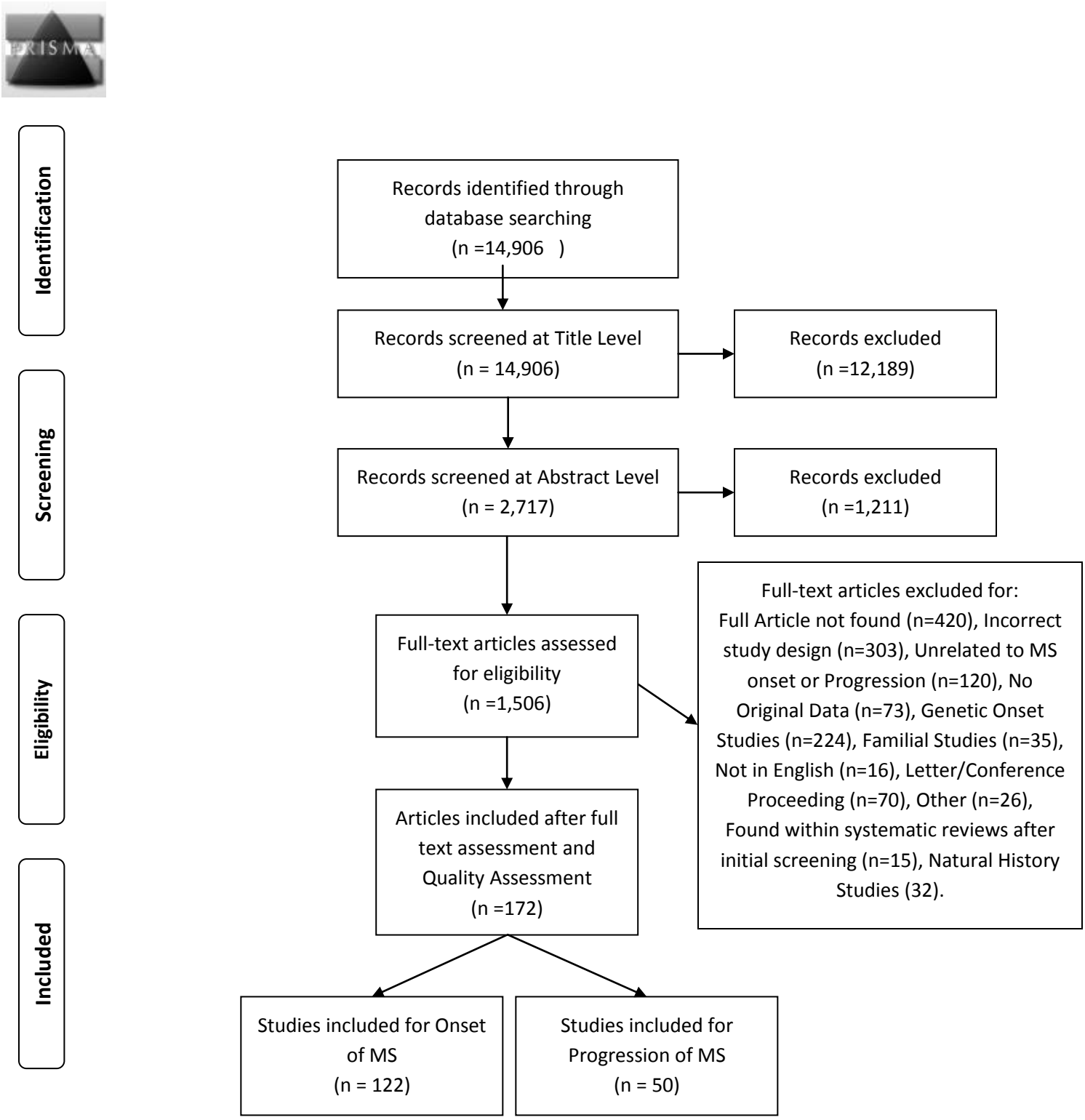

Figure 1. Screening Process for Articles related to Onset and Progression of Multiple Sclerosis