Environmental risk factors in multiple sclerosis: The role of active and passive cigarette smoke exposure

Amy Styles

Department of Epidemiology, Biostatistics and Occupational Health

McGill University

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Contributions

Systematic Review

Amy Styles prepared the search strategy, preformed relevance selection, quality assessment and data abstraction of all articles, less one. Christina Wolfson performed the relevance selection, along with AS, and performed quality assessment and data abstraction for 9 articles. Sathya Karunananthan performed quality assessment and data abstraction for 9 articles. Maura Pugliatti performed quality assessment and data abstraction for one article.

Study design and methodology

The EnvIMS study was designed through a collaborative effort involving steering committee members (Trond Riise, Maura Pugliatti, Christina Wolfson and Kjell-Morten Myhr) and researchers from Canada, Norway, Italy, Sweden and Serbia. The means of investigating smoke exposure as a risk factor for MS was discussed amongst Amy Styles, Christina Wolfson, Maura Pugliatti and Trond Riise.

Analysis plan and statistical analysis

Amy Styles created the analysis plan, which was then reviewed by Christina Wolfson and Maura Pugliatti. Amy Styles was responsible for cleaning of the data and all statistical analyses.

Abstract

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease, and although the cause of MS remains unknown, it is widely accepted that both genetic and environmental factors play a role. The purpose of this thesis was to investigate the role of active and passive cigarette smoke exposure, both of which have been recently implicated as possible risk factors for MS. A systematic review was performed to consolidate the existing literature on smoking and MS. This review resulted in 20 published articles, 17 of which reported on active smoke exposure, and 3 that reported on passive smoke exposure as a risk factor for MS. Of the studies reported in these 20 articles, one study was judged to be of excellent quality, 5 studies were of good quality, 9 studies of acceptable quality, and the remaining 5 studies of poor quality. The second part of this thesis investigated active cigarette smoke exposure and passive cigarette smoke exposure (among never-smokers) in the etiology of MS using data from the Norwegian component of the *International Case-Control Study on Environmental risk factors in Multiple Sclerosis* (EnvIMS). Cases (N=807) were frequency matched to controls (N=1716) on sex and age at the time of study. Using a self-administered questionnaire, participants were asked about several environmental exposures, including their active smoke exposure in five year intervals between the ages 11 and 30, and household passive smoke exposure from birth to age 30. Consistent with the literature on active smoking, individuals with MS had a 2.19 (95% CI 1.82-2.63) greater odds of having smoked than controls. The relationship of passive smoke exposure and MS among never-smokers was not statistically significant, OR=1.20 (95% CI 0.83-1.76); however, the magnitude of the effect was consistent with previous literature. The research presented here confirms that active smoke exposure is a risk factor for MS, and although this study was not adequately powered to find a statistically significant effect, the results suggest that passive smoke exposure may also be a risk factor for MS.

Résumé

La sclérose en plaques (SP) est une maladie inflammatoire neurodégénérative. Bien que sa cause demeure inconnue, il est largement accepté que des facteurs à la fois génétiques et environnementaux jouent un rôle dans cette maladie. Cette thèse avait pour but d'examiner le rôle de l'exposition active et passive à la fumée de cigarette; ces deux types d'exposition ayant récemment été identifiés comme étant des facteurs de risque potentiels de la SP. Une revue systématique a été faite afin de regrouper la documentation existante sur l'usage du tabac et la SP. Cette revue a permis d'identifier vingt articles publiés, dont dix-sept traitaient de l'exposition active à la fumée de cigarette et trois traitaient de l'exposition passive à la fumée de cigarette comme facteur de risque de la SP. La qualité de ces vingt articles a été jugée comme suit : une étude était d'excellente qualité, cinq études étaient de bonne qualité, neuf études étaient de qualité acceptable et les cinq études restantes étaient de mauvaise qualité. La deuxième partie de cette thèse a examiné le rôle de l'exposition active à la fumée de cigarette, ainsi que l'exposition active à la fumée de cigarette chez les individus qui n'ont jamais fumé, dans l'étiologie de la SP en utilisant les données de la cohorte norvégienne de l'*International Case-Control Study on Environmental risk factors in Multiple Sclerosis* (EnvIMS). Les cas (N = 807) ont été appariés pour la fréquence à des contrôles (N = 1716) en fonction du sexe et de l'âge au moment de l'étude. À l'aide d'un questionnaire auto-administré, les participants devaient répondre à des questions sur l'exposition à différents risques environnementaux, dont l'exposition active à la fumée de cigarette en intervalles de cinq ans entre 11 et 30 ans, ainsi que l'exposition passive à la fumée de cigarette dans le ménage de la naissance à l'âge de 30 ans. Conformément à la littérature sur l'usage actif du tabac, la probabilité d'avoir fumé était supérieure de 2,19 (95 % IC 1,82-2,63) chez les individus atteints de SP par rapport aux contrôles. La relation entre l'exposition passive à la fumée de cigarette et la SP chez les individus qui n'ont jamais fumé n'était pas significative d'un point de vue statistique, rapport de cote = 1,20 (95 % IC 0,83-1,76); toutefois, l'ampleur de l'effet correspondait aux études déjà publiées. La présente recherche

confirme que l'exposition active à la fumée de cigarette représente un facteur de risque de la SP. Bien que cette étude ne soit pas suffisamment puissante pour détecter un effet significatif d'un point de vue statistique, les résultats suggèrent également que l'exposition passive à la fumée de cigarette pourrait être un facteur de risque de la SP.

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Chapter 1

Introduction

Multiple sclerosis (MS) is an idiopathic neuroinflammatory disease that causes demyelination and axonal damage in the central nervous system. It is widely accepted that MS is a multifactorial disease involving a genetic predisposition and environmental risk factors; however, the cause of MS remains unknown. Many risk factors have been implicated in MS and those that have gained the most confidence are Epstein Bar Virus (EBV) exposure, Vitamin D deficiency and cigarette smoke exposure.

1.1 Cigarette smoke exposure and MS

Cigarette smoke exposure was discovered as a risk factor for lung cancer in 1950[1], and ever since, active cigarette smoking has been implicated as a risk factor for many diseases. More recently, passive smoke exposure has been implicated in the causation of many diseases, and both active and passive smoke exposure have been implicated in the risk of MS[2-8]. This thesis investigates the relationship of active and passive smoke exposure both through a systematic literature review and through analysis of original data.

1.2 Challenges in studying MS risk factors: measurement and misclassification

In order for a risk factor to be etiologically relevant, the exposure must precede not only diagnosis of disease, but also onset of disease. In individuals with MS this is especially relevant as the latent period (from disease onset to diagnosis of disease)[9] can be quite long. The ideal period to study risk factors is what Rothman[9] refers to as the induction period. For environmental risk factors, the induction period is from birth to disease onset, but it is almost impossible to detect the precise point of disease onset and there is almost always overlap of induction and latent periods. Rothman combines both induction and latent periods into a more readily studied “empirical induction period”. In MS research, we use this empirical induction

period to investigate risk factors that precede the first indication of disease. It is essential that a study investigate the exposure period that precedes the age of onset, occurring during the empirical induction period. With respect to cigarette smoke exposure ascertainment, several derived variables have been used in previous studies, including "past" smoke exposure, "current" smoke exposure, and "ever" smoke exposure (which includes both past and current smoke exposure). Clearly the inclusion of current smoking (i.e. smoke exposure following disease onset) is not etiologically relevant and introduces, at the very least, misclassification bias into any analysis. Therefore, in this thesis we collected information on smoke exposure during an individual's empirical induction period (in this case, ages 11 to 30 for active smoke exposure, and from childhood to 30 for passive cigarette smoke exposure), and the analysis plan was created with the intention of ensuring that smoke exposure preceded the onset of MS.

1.3 Study objectives

The objectives of the research presented in this thesis are (1) to conduct a systematic review on the role of active and passive smoke exposure as risk factors for MS, summarizing and assessing the quality of previously published literature; and (2) to estimate the magnitude and direction of the relationship between both active and passive smoke exposure and MS using the Norwegian component of the *International Case-Control study of Environmental Risk Factors in Multiple Sclerosis* (abbreviated as EnvIMS).

This thesis is presented in 6 chapters. Chapter 2 summarizes the clinical course and epidemiology of MS, and covers information on diagnosis, disease course, demographics, risk factors, and biological plausibility for smoke as a risk factor for MS. Chapter 3 reports on a complete systematic review that was conducted in June 2010. This chapter includes the search strategy, and 20 resulting articles that were included in a quality assessment and data abstraction. Chapter 4 describes the methods used in the EnvIMS study to collect data, and the methods for creating "past" smoke exposure variables, as well as the statistical analysis plan. Chapter 5 reports the results of cigarette smoke exposure as a risk factor for MS, from both

active and passive smoke exposure analyses, as well as an investigation of a potential confounder and sensitivity analyses. Finally, Chapter 6 summarizes the findings of the analyses of active and passive smoke exposure as risk factors for MS, and discusses strengths and limitations of the study. The thesis is concluded with recommendations for future research.

Chapter 2

Clinical features and epidemiology of MS

2.1 Introduction

This chapter presents an introduction to MS, beginning with a description of the disease and its diagnostic criteria. This is followed with a summary of the epidemiology of MS, including its geographic distribution, and etiologic factors. The chapter concludes with a review of the biological plausibility of smoke exposure as a risk factor for MS, and a brief summary of smoking behaviours in Norway.

2.2 Multiple sclerosis

MS is an inflammatory neurodegenerative disease affecting the central nervous system (CNS). It is known from pathology that MS is characterized by demyelination of axons as well as axonal degeneration and loss[10, 11]. The myelin that forms a sheath around the axon of a neuron is made primarily of lipids and allows for saltatory conduction, where a signal travels rapidly from the neuron's cell body to the axon terminal. In an individual with MS the myelin sheath is damaged, resulting in a decreased and distorted signal conductance, eventually resulting in complete blockage of the signal[10]. Demyelination can occur throughout the CNS in the optic nerve, brainstem, cerebellum, spinal cord and cerebrum. It is widely accepted that this demyelination is the source of the clinical symptoms of MS including: weakness, fatigue, pain, coordination deficits, vision loss, numbness and tingling, muscle rigidity, bladder and bowel dysfunction, sexual dysfunction and cognitive impairment; however, the cause of this damage remains unknown.

2.3 Diagnostic criteria

There are several disease courses in MS, and this combined with the various clinical symptoms of MS result in a complicated diagnostic process. In the past, diagnostic criteria were used to classify cases as possible, probable or definite MS[12-14] based on clinical findings. With the advent of emerging technology, the diagnostic criteria for MS have evolved over the years to further categorize MS patients. Within

the last 10 years the Poser criteria[15] and the McDonald criteria[16-18] have been the most widely used diagnostic tools. In general, a diagnosis of MS requires dissemination of symptoms in time and in space. Dissemination in space is demonstrated by evidence of neuroinflammation in two different regions of the CNS, and dissemination in time is demonstrated by symptoms occurring at least one month apart, with each symptom lasting for at least 24 hours[15, 16].

The Poser criteria[15] were published in 1983 with the goal of making the diagnosis of MS more consistent. One of the aims of the Poser criteria was to develop a strategy to exclude individuals with 'possible MS', as defined by previous diagnostic criteria, from clinical trials. In 2000 the International Panel on the Diagnosis of Multiple Sclerosis gathered to modify and update the existing diagnostic criteria, resulting in the McDonald criteria[16]. The key addition was the introduction of MRI technology in the identification of lesions, which allows for a quick and reliable diagnosis after the first attack of demyelination, referred to as a clinically isolated syndrome (CIS). The McDonald criteria have been further updated twice, in 2005[17], and again in 2010[18].

2.4 Disease course of MS

The disease course of MS varies from individual to individual, and four disease courses have been described. Three of the disease courses are accepted (relapsing-remitting, secondary progressive, and primary progressive) while the fourth (progressive relapsing) is a less widely accepted form of the disease.

Relapsing-remitting MS (RRMS) is characterized by attacks (relapses) followed by periods of disease remission[19-21]. This relapsing-remitting pattern can continue for years and the time between attacks varies, even within an individual. During the remitting phase the disease does not progress, and after an attack an individual may or may not return to their original level of functioning[19]. Diagnosis of RRMS is accurately made through both the Poser[15] and McDonald criteria[18], and 80 – 85% of MS cases are initially diagnosed with RRMS[20, 21].

Primary Progressive MS (PPMS) is defined by a continually progressing disease course from the time of diagnosis[19, 22]. Although some patients may

experience occasional plateaus and temporary minor improvements, the majority of patients experience a continuous decline in functioning. As there are no distinct relapses, PPMS was difficult to diagnose using the Poser criteria[15], since this criteria require relapses and remitting periods for a definite, rather than probable diagnosis. Now, with the help of MRI, PPMS is more readily diagnosed with approximately 10-15% of MS cases initially being diagnosed with PPMS[22].

Secondary Progressive MS (SPMS) is defined as a disease course with a relapsing remitting-cycle followed by disease progression, which may or may not include relapses, remissions and plateaus. Patients are typically diagnosed with RRMS and later transition to SPMS[19-21]. It can be difficult to determine the exact time of transition from RRMS to SPMS, although it is estimated that approximately 70% of RRMS patients will go on to develop SPMS[20].

Progressive Relapsing MS (PRMS) is the rarest, and less widely accepted, disease course that has been described. The disease course starts with and continues with a steady progression, as in primary progressive MS, with the addition of occasional attacks with or without full recovery as seen in relapsing-remitting MS[21]. PRMS differs from RRMS in that PRMS patients experience a continual steady progression of disease during the remitting phase experienced by individuals with RRMS.

2.5 Epidemiology of MS

2.5.1 Geographic distribution

The World Health Organization conducted a large international study, collecting data from 100 countries in 2005 to 2007. The results of this secondary data reported a worldwide MS prevalence of 1.3 million[23]. The study also reported a worldwide median prevalence of 30 cases per 100 000, and a worldwide median incidence of 2.5 per 100 000 (although it was not clear whether this incidence was an annual incidence or a biannual incidence)[23]. Countries are considered to have a low, medium, or high-frequency of MS depending on the country specific prevalence of MS[24]. Low-frequency areas, such as Africa, Northern South America and most of

Asia have fewer than 5 cases per 100 000. Medium-frequency areas, such as Southern US, Northern Australia, Southern South America, South Africa, Russia and Siberia have 5 to 29 cases per 100 000. Finally, high-frequency areas such as Canada, Southern Australia, Northern United States, New Zealand, Europe, and Israel, have 30+ cases per 100 000, although country specific prevalence of 200 cases per 100 000 population have been reported[23].

The geographical distribution of MS has been described by a latitude gradient in early studies, which suggests that the risk of MS increases with the increasing distance from the equator[25]. In addition, migration studies have suggested potential critical time periods for exposure to risk factors. For example, individuals who emigrate from an area of high risk to an area of low risk before the age of 15 have been shown to adopt the MS risk of the country to which they immigrated; however if individuals emigrate after age of 15 they will maintain the MS risk of their homeland[24, 26]. Both latitude gradient studies and migration studies have been linked to the role of Vitamin D deficiency, an identified risk factor for MS[27].

2.5.2 Age

The mean age of onset for adult onset MS is reported to be 30 years of age, with a peak in age of onset in the mid-20s[28]. It has been estimated that 2-5% of individuals with MS experience their first MS symptom before the age of 16[29], and there are cases of MS patients that have been diagnosed as young as the age of two.

2.5.3 Sex

It is established that women are more likely to develop MS than men, with the current female to male sex ratio reported to range from 2:1 to 3:1[30, 31]. The reason for this sex difference is unknown; however, women are more likely to suffer from autoimmune or immune-mediated diseases, such as lupus erythematosus and rheumatoid arthritis[32].

2.5.4 Interplay of genes and environment

The lifetime risk of MS among the general population is approximately 0.2% and this risk is increased in first, second and third degree relatives of an individual with MS[33, 34]. More specifically the risk of MS increases to 1.3% in half siblings, 3.5%

in full siblings and 30% in monozygotic twins. Although this demonstrates a significant increase from the general population, genetic studies provide evidence that an individual's genes are not solely responsible for their development of MS. Further, studies of adopted individuals who go on to develop MS have shown that the risk of MS in non-biological relatives is the same as the risk of MS in the general population[33, 34]. This provides evidence that environmental factors are not solely responsible for MS development either, and it is now widely accepted both genetic and environmental factors are involved in MS development.

2.6 Risk factors for MS

There have been many environmental factors implicated in the etiology of MS in the past 60 years, over time the three most frequently investigated risk factors are deficiency in Vitamin D, exposure to Epstein Barr Virus (EBV), and exposure to cigarette smoke.

2.6.1 Vitamin D deficiency

Sufficient Vitamin D levels depend on a combination of an individuals' exposure to sunlight, diet and use of dietary supplements.

Exposure through sunlight

The geographical distribution of MS and the latitude hypothesis have led researchers to investigate the role of Vitamin D deficiency as a risk factor for MS. In countries that are further from the equator, not only is the skin less likely to be exposed, but because there are fewer hours of sunlight during the winter months, the exposed skin has a decreased ability to produce Vitamin D due to insufficient intensity of ultraviolet (UV) irradiation which varies with latitude and season[35].

Studies investigating the birth month of individuals with MS have found seasonal trends. In the Northern Hemisphere, a significantly greater number of individuals with MS were born in the month of May, and significantly fewer were born in November[36]. Furthermore, the opposite effects have been found in the Southern Hemisphere, with more MS births in the month of November and fewer MS births in May[37]. These findings suggest that even decreased levels of Vitamin D

exposure during gestation may be linked to an increased risk of MS.

Exposure through diet

Vitamin D is more readily available through sunlight, but may also be obtained through diet and supplements, specifically in certain types of fish oils. Norwegian studies have reported that Northern regions in Norway have a lower prevalence of MS than Southern regions. The difference in MS prevalence was linked to the Northern regions being mainly coastal regions, where fish makes up the majority of the diet[38, 39]. The protective effect of vitamin D intake through diet and supplementation has also been observed in the Nurses Health Cohort Study (NHS)[27].

2.6.2 Epstein Barr Virus (EBV)

There have been several proposed hypotheses suggesting infection as a potential risk factor for MS. The 'polio hypothesis' suggested that there was a specific virus that increased the risk of MS if acquired late in adolescence or adulthood, but was protective if acquired in childhood[40]. The polio hypothesis then evolved into the 'hygiene hypothesis' which suggested that early life exposure to infection in general is protective against MS[40].

The 'EBV paradox' is a prevalent hypothesis that is related to, but not entirely explained by, the hygiene hypothesis. EBV is part of the herpes virus family, with up to 90% of individuals becoming infected by age 40[40-42]. Young children who become infected usually do not demonstrate symptoms different from any other mild childhood illness, whereas older children, adolescents, and adults will present with infectious mononucleosis 35-50% of the time. Evidence in support of the hygiene hypothesis shows that there is a low risk of developing MS amongst those infected with EBV during early childhood, and a higher risk of developing MS amongst those infected with EBV (infectious mononucleosis) in late adolescence or adulthood. However, the lowest risk for MS exists in individuals who were not infected with EBV at all, thus resulting in a paradox not consistent with the hygiene hypothesis[40].

A meta-analysis of published studies on MS and infectious mononucleosis reported a pooled Risk Ratio of 2.3 (95% CI 1.7-3.0) for individuals who reported prior exposure to infectious mononucleosis compared to those EBV positive

individuals with clinically silent infection[43]. This may suggest that it is not *if* you have been exposed to EBV, rather *when* you have been exposed to EBV that is associated with the risk of developing MS. Moreover, infectious mononucleosis and MS share a similar prevalence distribution, in that both have high incidence in Caucasians and both have low incidence among Africans and Asians [40, 41, 44].

2.6.3 Cigarette Smoke exposure

Active cigarette smoke exposure

Since the discovery of an association between active smoke exposure and lung cancer in 1950[1], cigarette smoke exposure has been implicated in the risk of many other diseases, and recently it has been implicated as a risk factor for MS[2-8]. Using a systematic review, the next chapter will focus more closely on how past studies have estimated the relationship between cigarette smoke and the occurrence of MS.

Passive cigarette smoke exposure

Passive cigarette smoke, also referred to as environmental tobacco smoke (ETS) and second hand smoke (SHS) exposure, has also been associated with many negative health outcomes including: lung cancer[45], asthma[46], and several autoimmune diseases[47]. With the recent implication of active cigarette smoke exposure in the etiology of MS, passive cigarette smoke exposure is now being investigated as another potential risk factor for MS[48, 49].

Biological plausibility

We still have a poor understanding of the cellular and molecular mechanism by which smoke exposure, and more specifically the components of cigarettes, increases an individual's risk of MS. The involvement of nicotine as a key harmful component of cigarette smoke leading to MS remains unclear. Nicotine has been shown to have both positive and negative effects on neurons[50]. Also, several studies have suggested neurotoxic effects on the developing nervous system[50]. Other reports have indicated that nicotine weakens the blood brain barrier (BBB)[51, 52], the restrictive network of capillary tissue that separates blood from extracellular fluid in

the brain. This could occur through weakening of the tight junctions that prevent the exchange of certain solutes and immune cells[52]. Surprisingly, nicotine has been shown to have suppressive effects on the Th17 variant of T cells, implicated as the key harmful effector immune cells in MS[53]. A more recent study showed that nicotine attenuates disease in mice with experimental autoimmune encephalomyelitis (EAE)[54], a mouse model of neuroinflammation and MS. These studies suggest a potentially complex biology underlying the interaction between nicotine and MS that needs to be investigated further, and may also point to other components of cigarette smoke as risk factors for MS.

Smoking behaviours in Norway

Statistics Norway has collected data on smoking behaviours since 1973. From 1973 to 1993, smoking among men dropped from 50% to 37%[55], while smoking among women remained at approximately 30% during this 20-year time span. From 1995 to 2004 the percentage of daily smokers aged 16-74 decreased from 33% to 26%[56]. The most recent publication in 2010 reported less than 20% of 16-74 year olds were daily smokers, the lowest it has been since the first report in 1973[57]. With respect to childhood smoke exposure, in 2001, 27% of children under the age of 15 smoked, and this dropped to 10-12% in 2005. Girls were found to smoke more than boys at both time points[58]. Finally, Norway implemented a country wide anti-smoking legislation in 2001, and smoking has been banned in offices since 1988[59].

Chapter 3

A systematic review of cigarette smoke exposure as a risk factor for multiple sclerosis

3.1 Introduction

Systematic reviews are conducted to assess study quality, compile study findings, and to evaluate the consistency of study findings. The purpose of the current systematic review was to compile all existing literature examining active or passive cigarette smoke exposure as etiologic factors for MS. The research question that drives this review is: Are individuals who are exposed to cigarette smoke at a greater risk of developing MS than those who have not been exposed to cigarette smoke? This chapter describes the search strategy, inclusion criteria, quality assessment and data abstraction of relevant studies. The quality of the studies is then summarized and the overall findings are presented with suggestions for future research directions.

3.2 Methods

3.2.1 Study selection and search strategy

A scoping search (a search to find any existing systematic reviews on a particular topic) was performed in May 2010, revealing one previous meta-analysis published in 2007[60]. The meta-analysis included studies published from 1965 to 2005, and it was of interest to examine the evidence published since 2005. For the current review, a search of the medical literature was conducted in June 2010 (inclusion from 1948 to June 2010). The Medical Subject Headings (MeSH) search terms used in Medline were: multiple sclerosis, demyelinating disease, myelitis, and optic neuritis; smoking, tobacco smoke pollution, tobacco, nicotine; cohort studies, case-control studies, and cross-sectional studies. These MeSH terms and their associated keywords were also used to search Embase, CINAHL and AMED using the OvidSP search engine. Web of Science and Proquest thesis dissertations and

abstracts were also searched. Figure 3.1 presents the number of articles included/excluded at each stage. The initial search included 'MS' as a keyword, resulting in 277 journal articles. During the abstract relevance review it became clear that an unexpectedly high proportion of these articles were not relevant. Closer inspection revealed that including MS as a keyword resulted in the inclusion of articles on metabolic syndrome, median survival, and methionine synthase for which the abbreviation MS is also used. Re-running the search without MS as a keyword resulted in 129 articles, and none of 148 excluded articles were relevant to the search of cigarette smoke exposure and multiple sclerosis, justifying the exclusion of this keyword from the search.

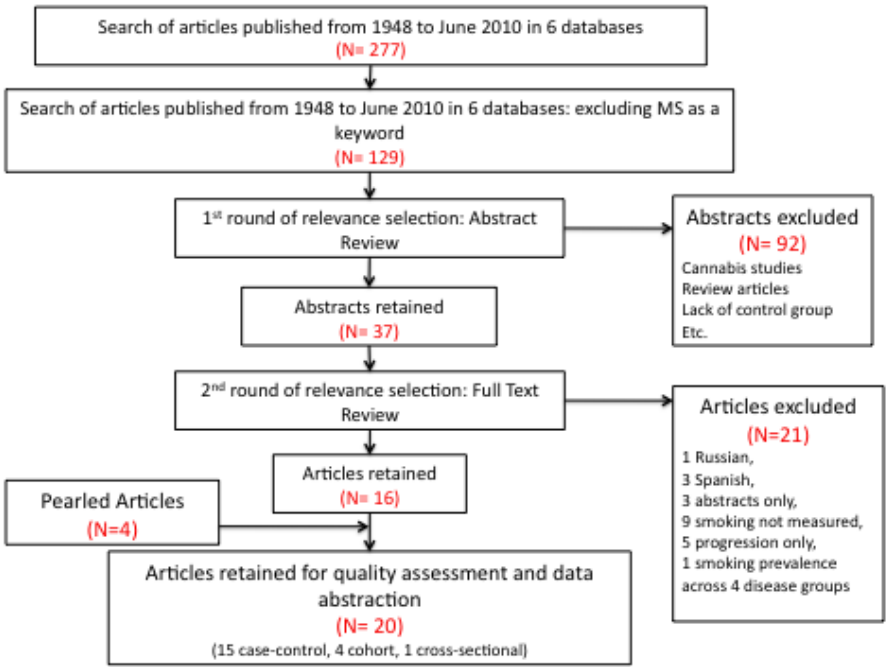


Figure 3.1 Flow chart of studies included in the systematic review of cigarette smoke exposure as a risk factor for MS

Inclusion/exclusion of articles

This review was specifically designed to consolidate studies that examined cigarette smoke exposure as a *risk factor* for MS, therefore studies investigating smoking and disease progression were excluded. Studies that only examined smoke exposure as a confounder or which only studied cannabis smoke exposure were also excluded. In addition, review articles were excluded. Due to language limitations, studies published in a language other than English, French or Italian were excluded.

Studies reporting on either active or passive cigarette smoke exposure were included.

The relevance selection process consisted of abstract (1st round) and full article review (2nd round), and was completed by two reviewers independently (AS and CW). Twenty-one articles were excluded: 1 published in Russian and 3 published in Spanish; 9 studies that did not assess smoke exposure/or only considered smoke exposure as a confounder; 3 studies that were only available as conference abstracts; and 5 studies that only examined progression of MS. Full article review resulted in 16 articles that met inclusion criteria. The references from these 16 articles were then hand searched resulting in four additional relevant articles. The final study selection resulted in 20 articles that were included in the quality assessment and data abstraction.

3.2.2 Quality assessment and data abstraction

There are quality assessment tools available for observational studies, although none are considered to be the "gold standard". Several available quality assessment checklists and scales that have been used in other systematic reviews[61-63] were reviewed, and using these tools as a guide, a tool was developed to specifically assess studies examining cigarette smoke exposure as a risk factor for MS. Three versions of the quality assessment tool were developed to accommodate the review of case control studies, cohort studies and cross sectional studies (see Appendix 1; A, B and C, respectively). Four reviewers with expertise in epidemiology, systematic reviews and neuroepidemiology assessed the articles for quality, with each article receiving two independent reviews (AS, SK, CW, MP). Reviewers also abstracted relevant data including: author, year of publication, study population, description of the study participants, and confounding factors included in adjusted analyses.

Quality assessment

Each article was scored on a scale of A to D (A =excellent, B =good, C=acceptable, D= poor), an A rating indicated that the study was of overall high quality with several positive attributes, and a D rating indicated that the study was

weak in several aspects of design and analysis. Ratings of B and C were used when the studies had design and/or analysis limitations that precluded them from receiving an A rating. The reviewers met to discuss discrepancies in quality assessment and to agree upon the overall quality of each study. As a general rule, studies were given a lower quality rating if authors did not report on important aspects of the study. There are checklists specifically designed to ensure that reporting is done correctly (STROBE checklist on reporting observational studies)[64], and studies that failed to report on important aspects of the study resulted in a lower quality rating since it was indistinguishable whether authors did something poorly or simply choose not to report what they had done.

3.3. Results

Twenty studies met the inclusion criteria, 15 of which were case control studies, 4 were cohort studies, and 1 was a cross-sectional study. Tables 3.1, 3.3, and 3.4 present the methodological characteristics of the case-control, cross-sectional, and cohort studies, respectively. Publication dates ranged from 1965-2010, and although the majority of studies were published since the year 2000, many of the studies collected data before the year 2000. Studies were conducted in North America (Canada and U.S.A), South America (Brazil), Europe (Italy, Netherlands, England, France, Sweden, Norway, Serbia), Southwest Asia (Israel), and Australia. The number of cases in each study ranged from 63 – 902. Each of these studies reported on smoke exposure as a risk factor for MS; however, many of the studies were not specifically designed to evaluate smoke exposure as a risk factor for MS, and may not have been adequately powered to detect a reasonable statistically significant effect.

3.3.1 Quality assessment of case-control studies (N=15)

The case-control quality assessment form (see Appendix 1A) consisted of twenty questions evaluating study quality in four sections: case selection, control selection, smoke exposure measurement, and a general category that included assessment of the appropriateness of the statistical analyses, reporting of response rates and missing data. Each of the 20 questions was given a quality rating of A to D,

and then the study as a whole was given a rating as described in section 3.2.4. The last column in Table 3.1 presents the overall quality score for each of the 15 case-control studies. Five were judged to be of good quality ('B')[2-4, 48, 65], six of acceptable quality ('C')[5, 6, 66-69], and four were of poor quality ('D')[70-73]. None of the studies were considered of excellent quality ('A').

Only 3 of the good (B) quality case-control studies collected data on active cigarette smoke exposure. One study was conducted in Montreal, Canada by Ghadirian and colleagues[2]; one study was conducted in Sweden by Hedstrom and colleagues[3]; and one study was conducted in Sicily, Italy by Ragonese and colleagues[4]. These studies were all rated of high quality for their smoke exposure ascertainment, ensuring that smoke exposure used in the analysis preceded MS onset, and collecting information on intensity (number of cigarettes smoked per day or pack years) of smoke exposure. The studies varied in other sections evaluating study quality. For case selection, Hedstrom and colleagues[3] and Ragonese and colleagues[4], were both rated of high quality for ascertaining neurologist confirmed MS cases from MS clinics, while Ghadirian and colleagues[2] received a lower quality rating for using media announcements to ascertain MS cases. Although the MS status of the cases ascertained by Ghadirian and colleagues[2] was confirmed, this form of case ascertainment may be problematic as several studies have shown smokers to be less likely to participate[74-76] and, as such, non-response bias may be introduced if the response rates were differential for cases and controls.

All three studies used population-based control selection; however, they varied in quality. The study by Hedstrom and colleagues[3] received a lower quality rating for replacing controls if the information they sought could not be found, and the study by Ragonese and colleagues[4] received a lower quality rating for only selecting controls over the age of 60. Finally, while the statistical analysis performed by Hedstrom and colleagues[3] was appropriate, the other 2 high quality studies were given a lower quality rating for failing to take matching of cases with controls into account in the analysis. The remaining 10 active smoke exposure studies were

weaker than these three in one or more aspects of study design, as described below in the quality assessment.

Quality assessment - Case ascertainment

Case ascertainment was judged to be of high quality if cases were diagnosed with definite MS by a neurologist, and if the cases were recruited from reliable sources such as hospital MS databases and countrywide MS registries[5, 48, 65-71, 73]. Lower quality scores were assigned to studies if confirmation of MS diagnosis was unclear or not reported[6, 72], or if case recruitment was done using announcements to the general public, as described above[2].

Quality assessment - Control ascertainment

Control selection is one of the most difficult design aspects of a case-control study as a poor choice of controls can introduce selection bias, distorting the results of the study. The 15 case-control studies reviewed varied widely in the choice of controls, and a low quality rating in this aspect of design was largely responsible for the overall evaluation of many of the studies. Overall, the majority of studies used either population-based controls (N=7) or hospital based controls (N=5). All five good (B) quality (active and passive) studies used population-based controls[2-4, 48, 65], and the 4 poor quality studies used hospital based controls[70, 73], friend or neighbor controls[71], or did not report information on how the controls were selected[72].

Control selection may have introduced selection bias in several studies. One study using population based controls replaced controls if the information sought was unavailable[3]. This replacement may introduce selection bias if the unavailable information was related to smoke exposure, and could have attenuated the effect if smokers were replaced with non-smokers or exaggerated the effect if non-smokers were replaced with smokers, respectively. Another study may have introduced selection bias by only selecting controls over the age of 60 as[4] in an attempt to limit the risk of MS among this comparison group. Among hospital-based controls there was the potential for selection bias through the use of blood donor controls in an Italian study[67]. Blood donation is done on a voluntary basis in Italy and it is suspected that individuals who choose to donate blood may differ from the general

population in relation to lifestyle and health related factors. Using blood donor controls for MS cases in a study examining smoking may result in an exaggeration of the effect, due to the likely lower prevalence of smoking in blood donor controls. Two other studies using hospital-based controls may have been affected by selection bias through the use of Rheumatoid Arthritis (RA) patient controls[68, 73]. Smoking is a known risk factor for RA[77], and using RA controls for MS cases in a study examining smoke exposure may result in attenuation of the effect, due to the likely high prevalence of smoking among RA controls. Lower scores were also assigned to studies that used friend/neighbor controls[71] or sibling controls[69], as these control groups are likely to be more similar to cases than general population controls, when considering a behavioral exposure such as cigarette smoking. Finally, lower quality scores were given when the authors did not report any information on controls[72, 73].

Some investigators chose to conduct matched case-control studies whereby controls were matched to cases on factors thought to be confounders. The design by which authors chose to match controls (individual or frequency matching) to cases was also examined in the quality assessment. All but three studies [6, 69, 72] matched on age and/or sex (11 individually matched, 2 frequency matched; see Table 3.1), which are considered to be fairly strong confounders given the relationship between age, sex and smoking; and the relationship between age, sex and MS. Ten studies were considered of lesser quality for matching on residence at time of study without knowing how long an individual resided at their current residence[2-5, 48, 65, 66, 70]. It was uncertain whether current residence preceded MS onset, and matching on variables not related to risk of MS reduces a study's efficiency[78].

Quality assessment – Active cigarette smoke exposure ascertainment

There were several measures of smoke exposure used in the 15 studies and, the three most common overall smoke exposure measures were: "past smoke exposure" which was defined as smoke exposure prior to MS onset; "current smoke exposure" which was defined as smoke exposure at the time of the study; and "ever smoke exposure" which may include both "past" and "current" smoke exposure. Of these three, "past" smoke exposure would be the best choice for overall smoke

exposure, since it attempts to ensure that smoke exposure precedes MS onset. Other measures of smoke exposure ascertained included levels of smoke exposure (intensity and duration), which provide the most information and can easily be used to create an overall "past" effect estimate.

All 3 good (B) quality active smoke exposure studies[2-4] received a high score for ensuring exposure preceded MS onset, and for ascertaining duration or intensity of smoke exposure. All 4 poor (D) quality studies were given a lower score for not taking into account whether smoke exposure took place prior to MS onset[70-72], or for not reporting on any aspect of smoke exposure[73].

Of the 13 active cigarette smoke exposure case-control studies, ever cigarette smoke exposure was reported in the majority of studies[2-6, 66-70]. Lower scores were given to studies only estimating "ever" cigarette smoke exposure[6, 66-68, 70]; however, studies that collected information on duration (years spent smoking), intensity (number of cigarettes smoked per day) or pack/years (20 cigarettes smoked per day for 1 year) of smoke exposure[2-5, 69] received a higher score (Table 3.2).

Quality assessment – Passive cigarette smoke exposure ascertainment

The two case-control studies that examined passive smoke exposure received high quality scores as the household cigarette smoke exposure was collected in relation to year of MS onset in a pediatric MS cohort[48], and the smoke exposure in utero was estimated via mother's smoking during pregnancy ("ever vs. never" cigarette smoke exposure)[65].

Table 3.1 Methodological data abstraction from case-control studies examining cigarette smoke exposure as a risk factor for MS

Author	Diagnostic Criteria	Cases	Controls	Smoke exposure	Matching (M) & Adjustment (A)	Quality
Active Cigarette smoke exposure						
Ghadirian, et al., 2001[2] Montreal, Canada Data Collection: 1991 - 1994	Not reported	197 Physician referrals; Media announcements	202 Population based; Random digit dialing	Interview; Preceded MS onset	1:1 frequency M: Age (5-year categories), Sex, Residence* A: Age, Sex, education	B
Hedstrom, et al., 2009[3] Sweden Data collection: 2005-2008	McDonald (2005)	902 Hospital Neurology units	1855 Population based; National register	Questionnaire Preceded MS onset	2: 1 individual M: Age, Sex, Residence* A: Matched variables + ancestry	B
Ragonese, et al., 2007[4] Sicily, Italy Data Collection: 2006	McDonald (2001)	100 Consecutive cases at MS centre, Palmero University Hospital	100 Population Based - Age > 60	Interview Preceded MS onset	1:1 frequency M: Sex, Residence* A: weighted by age	B
Antonovsky et al. (1965)[66] Israel Data collection: 1955-1961	Not Reported	241	917 Population based: 1961 Israeli Census	Interview, Preceded MS onset	4:1 individual M: Age, Sex, Region of birth A: None	C
Brosseau et al. (1993)[68] Montreal, Canada Data collection: Not Reported	Poser (1983)	108 Hospital MS register MS onset within 5 years of study	108 Hospital based; rheumatoid arthritis patients	Questionnaire; Preceded MS onset	1:1 individual M: Age, Duration since diagnosis A: Unable to tell	C
Jafari et al (2009)[69] Netherlands Data Collection: Not Reported	McDonald (2001)	36 ErasMS patients & other neurological clinics	204 Unaffected multiplex family sibling controls	Questionnaire Preceded MS onset	M: Sibship A: current age, sex	C
Pekmezovic, et al. (2006)[5] Belgrade, Serbia Data Collection: 1996-2003	Poser (1983)	210 Institute of Neurology	210 Hospital based; Hernia surgery patients	Interview Preceded MS onset	1:1 individual M: Age, Sex, Residence A: None	C
Simon, et al. (2010) [6] Pooled: 1. USA: Nurses' Health Study (NHS) 2. Tasmania, Australia 3. Sweden: National Swedish Health and Disease study (NSHDS) Data Collection: Not Reported	Not Reported	442 TOTAL 1, 210 (incident) 2. 136 (prevalent) Society announcements Neurologist invitation 3. 96 (prevalent) MS registry	865 TOTAL 1. 420 2. 272; Population: voter registration 3. 173 NSHDS participants;	1. Questionnaire 2. Interview 3. Questionnaire	2:1 M: Age, 1. Study (NHS I, NHS II) 2. Birth year 3. Sex, year of blood draw A: None	C
Zorzon et al. (2003)[67] Trieste, Italy Data Collection: 2001	McDonald (2001)	140 Consecutive patients	131 Blood donors	Interview Preceded MS onset	1:1 individual M: Sex, Age ± 2 years A: None	C

Casetta et al. (1994)[70] Ferrara, Italy Data Collection: Not Reported	McAlpine (1972)	104	150 74 hospital based; 76 population based. Randomly selected	Interview	Attempted 2:1, individual M: Age ± 3 years, Sex Residence* A: None	D
da Silva et al. (2009)[71] Rio de Janeiro, Brazil Data Collection: 1995-1997	Poser (1983)	81 Consecutive patients from MS database	81 Non-relative friend OR neighbor controls	Interview; Preceded MS onset	1:1 individual M: Age ± 5 years, Sex, Place of birth A: Matched variables + History of vaccinations, marital status, consumption of animal brain, ancestry, alcohol consumption, history of measles	D
Simpson et al. (1966)[72] Northern England Data Collection: 1958	Not reported Probable MS cases were included, and possible MS cases were excluded	584 probable cases	1958 British tobacco survey Not clear on where the controls were from	Questionnaire:	A: Sex	D
Warren et al. (1982)[73] Calgary, Alberta, Canada Data Collection: 1978	Schumacher (1965)	100 Consecutive MS clinic patients	100 Hospital based: rheumatoid & neurological disorders	Interview Preceded MS onset	1:1 individual M: Age (5-year categories), Sex, Race, residence < 15 years of age (high, medium or low risk zone) A: None	D
Passive cigarette smoke exposure						
Mikaeloff et al. (2007)[48] France Data Collection: 1994 - 2003	McDonald (2001)	129 Incident cases MS patients from KIDSEP neuropediatric cohort Index date (first episode of MS)	1038 Population based: Random selection of French population registry	Questionnaire; - Parental (one or both) smoking within the home before the index date	1:12 individual M: Age (± 6 months), Sex Residence* A: matched variables + family history of MS/other autoimmune diseases, socioprofessional status of the head of the family	B
Montgomery et al. (2008)[65] Sweden Data Collection: up to 2006	Not reported MS register	143 - National Swedish MS Register & National Inpatient Register - Swedish Medical Birth Register.	1730 Population based: Random selection of Swedish population registry	Swedish Medical Birth Register since 1982 Mother's smoking habits during their first prenatal visit.	1:12 Individual M: Date of birth, Age at MS diagnosis, Sex, Residence at MS diagnosis A: Matched variables + Socioeconomic index based on parental education	B

* indicates residence at time of study

Quality assessment - Statistical analysis

When individual level matching is part of a case-control study design, it must be taken into account in the statistical analysis[78] using conditional logistic regression models. In contrast, studies using frequency matching must adjust for variables used in the matching process by adding these variables to the multivariable unconditional logistic regression model. Three good (B) quality studies (one active smoke exposure and two passive smoke exposure) were rated of high quality for statistical analyses, while several studies were given a lower quality score for statistical analysis[2, 4, 5, 66, 67, 70, 72, 73].

Confounders

After age, sex and residence (at time of study, at diagnosis, or at birth), the fourth most common confounder reported was level of education. One study used the individual's education[2], which may not be suitable as smoke exposure (and potentially MS onset) may have preceded education level attainment (this will be further explained in Chapter 4). The two passive smoke exposure studies adjusted for parental education[48, 65], which is acceptable as it gives a representation of childhood socioeconomic environment, which may be related to parental smoking and to MS status.

Missing data and response rates

Very few authors stated if a sample size calculation was performed, what the response rates for cases and controls were, or if there were missing data. Missing data were reported in only 6 studies[5, 6, 48, 68-70], and only four of these reported how they managed missing data, by simple exclusion of individuals with missing data [48, 68-70].

Table 3.2 Case-control study point estimates (OR) and 95% confidence intervals (CI) for cigarette smoke exposure as a risk factor for MS

Author	Quality	Ever vs. never OR (95% CI)	Past/ ex vs. never OR (95% CI)	Current vs. never	Intensity # Cig/day	Pack years	Duration (years)
ACTIVE							
Ghadirian, et al., 2001[2]	B	A: 1.6 (1.0-2.4)			A: 0-10: 0.7 (0.3-1.5) A: 10-20: 1.4 (0.8-2.4) A: 20-40: 1.9 (1.2-3.2) A: 40+: 5.5 (1.7-17.8)		
Hedstrom, et al., 2009[3]	B	A: 1.5 (1.3-1.8)	A: 1.4 (1.1-1.8)	A: 1.6 (1.3-1.9)		A: ≤ 5: 1.3 (1.0-1.6) A: 6-10: 1.5 (1.1-2.0) A: 11-15: 1.7 (1.2-2.4) A: 16+ 1.9 (1.4-2.6)	
Ragonese, et al., 2007[4]	B	A: 1.1 (1.0 4– 1.2)			C: ≤ 10: 3.3 (1.5-7.2) C: > 10: 3.3 (1.6-6.6)		
Antonovsky et al. (1965)[66]	C	C: 1.4(1.05-1.85)°					
Brosseau et al. (1993)[68]	C	C: 0.8 (0.4-1.4)					
Jafari et al (2009)[69]	C	A: 1.1 (0.7-1.7)	A: 1.2 (0.6-2.2)	A: 1.0 (0.6-1.7)		A: < 7.9: 1.0 (0.6-1.8) A: ≥ 7.9: 1.2 (0.6-2.1)	A: < 12: 1.0 (0.6 -1.8) A: ≥ 12: 1.1 (0.6-1.9)
Pekmezovic, et al. (2006)[5]	C	C: 1.6 (1.1-2.4)°			C: ≤15: 1.5 (0.97-2.4)° C: ≥16: 1.7(1.04 – 2.9)°		C: ≤19 1.5 (1.0-2.4)° C: ≥20 1.7 (0.9-3.1)°
Simon, et al. (2010)[6]	C	C: 1.5 (1.1-1.9)					
Zorzon et al. (2003)[67]	C	C: 1.5 (0.9-2.4)		C: 1.9 (1.1-3.2)			
Casetta et al. (1994)[70]	D	C: 1.2 (0.6-2.2)					
da Silva et al. (2009)[71]	D			A: 7.6 (2.1-28.2)			
Simpson et al. (1966)[72]	D			C: M= 1.1 (0.9-1.3)° C: W=0.99 (0.8-1.2)°			
Warren et al. (1982)[73]	D	" Not Significant"					
PASSIVE							
Mikaeloff et al. (2007) [48]	B	A: 2.1 (1.4-3.2)					
Montgomery et al. (2008)[65]	B	A: 0.99 (0.65-1.44)			A: 1-9: 0.9 (0.6-1.5) A: 10+ 1.0 (0.6-1.8)		

C= Crude; A=Adjusted, see table 3.1 for variables used in adjustment); ORs presented as in original article, rounded to one decimal place. °Denotes a 95% CI that was computed using raw data available in the article, since they were not reported by the author; M= Men; W= Women

3.3.2 Quality assessment of a cross-sectional study (N=1)

The cross-sectional assessment form (see Appendix 1B) consisted of fifteen questions evaluating study quality in four sections: population selection, smoke exposure measurement, MS outcome measurement and a general category that included assessment of the appropriateness of the statistical analyses, reporting of response rates and missing data. Each of the 15 questions was given a quality rating of A to D, and then the study as a whole was given a rating as described in section 3.2.4. The one cross sectional study was judged to be of acceptable quality ("C").

Although unusual for studying etiologic factors, Riise and colleagues [8] (Table 3.3) conducted a cross-sectional study and retrospectively estimated smoke exposure. The study population consisted of individuals born between 1953 and 1957 who were living in Norway's Hordaland county in 1997. One concern with this definition of the study population is that only those individuals who remained in the study region during the time frame were included. Without some assurance that emigration was small and that emigration was not related to both the exposure (smoking) and the outcome (MS), there is a potential for selection bias. For this reason, this aspect of the study received a low quality score. The study also received a low quality score for ascertainment of MS status, as it was self-reported (diagnosis and age of onset) and not confirmed clinically, which could lead to misclassification of the outcome. The assessment of cigarette smoke exposure collected information on current and past smoking habits including age at start of smoking, and it was this latter feature that enabled the authors to examine smoke exposure prior to self-reported onset of MS.

Table 3.3 Methodological data abstraction, rate ratio and 95% confidence interval (CI) from the cross-sectional study-examining cigarette smoke exposure as a risk factor for MS

Author	Diagnostic criteria	Cases	Total Cohort	Smoke Exposure	Quality	Estimated Rate Ratio (95%CI)
(16) Riise (2003)[8] Hordaland County Norway	Not reported	87	22 312	Questionnaire; Smoke exposure preceded MS onset	C	1.81 (1.13-2.92)

3.3.3 Quality assessment of cohort studies (N=4)

Twenty-one questions were used to evaluate the quality of cohort studies in three sections: cohort entry, follow-up, and a general category that included assessment of the appropriateness of the statistical analyses and reporting of response rates and missing data (see Appendix 1C). Each question was given a rating of A to D and the study as a whole was given an overall rating as described in section 3.2.4. Table 3.3 presents the overall quality of the 4 cohort studies, one study was judged to be of excellent quality[7], two were of acceptable quality[49, 79], and one was of poor quality[80].

There was one study of excellent quality conducted by Hernan and colleagues[7]. Using data from the Nurses' Health Study, baseline data collection ensured participants were free of MS, and lifetime smoke exposure history was ascertained at baseline and followed-up every 2 years. Smoke exposure was defined as "never", "ex" or "current", and intensity of smoke exposure using the number of pack-years was ascertained. Hernan and colleagues[7] used "current" smoke exposure data from 4 years prior to MS onset in their analysis, making it more similar to "past" smoke exposure than the typical definition of "current" smoke exposure. Participant follow-up was completed every 2 years and if an MS diagnosis was reported it was clinically confirmed. Finally, the statistical analyses used were appropriate.

Quality assessment – Data ascertained from baseline

The excellent quality study[7], and one acceptable quality study[49], were given a high quality score for basing the study on a representative population, confirming that participants were free of MS when they entered the study, and ascertaining lifetime history of smoke exposure at baseline. The poor quality study[80] and the second acceptable quality study[79] were given a lower quality score for only obtaining the current number of cigarettes smoked at the time of study entry (baseline), which may result in misclassification of exposure if smoking status were to change from the time of cohort entry since smoke exposure was not followed-up.

Quality assessment – Data ascertained at follow-ups

The excellent quality study[7] received a high quality score for the way follow-up was carried out, as few individuals were lost to follow-up, duration and intensity of smoke exposure was ascertained between follow-ups, and MS status was confirmed by a physician (Table 3.4). Lower quality scores were given to acceptable and poor quality studies if there were many individuals lost to follow up[80], if smoke exposure was only measured at baseline[79, 80], or if the diagnostic criteria for MS were not reported[49].

Quality assessment - General information

None of the cohort studies described the methodology used for handling missing data, nor reported sample size calculations; however, these factors did not heavily influence overall study quality. The excellent and acceptable quality studies used appropriate statistical analysis, and adjusted for potential confounders (see Table 3.4). Similar to the cross-sectional studies, education was considered a potential confounder, and parental education was appropriately adjusted for in the passive smoke exposure study[49]. Individuals own social class was adjusted for in the poor quality active smoke exposure study[80], which may not be suitable as smoke exposure (and potentially MS onset) would have preceded social class level attainment.

Table 3.4 Methodological data abstraction from cohort studies estimating cigarette smoke exposure as a risk factor for MS

Author & Cohort	MS Diagnosis	Cases	Total Cohort	Smoke exposure	Adjustment	Quality
ACTIVE						
Hernan et al (2001) [7] USA Pooled Nurses' Health Study I & II Follow up: 1976-1995	Poser (1983)	315 incident (Definite and probable)	238 371	Questionnaire	A: Latitude, Ancestry, Age	A
Villard-Mackintosh & Vessey (1993) [79] UK: Oxford family planning association Follow up: 1968-1974	International Classification of Diseases (ICD), 8th Ed. Code 340	63 incident	126	Interview	A: Age & Parity	C
Thorogood & Hannaford (1998) [80] UK: Royal college of general practitioners oral contraception study Follow up: 1968-1996	ICD, 8th Ed. Code 340	114 incident	46 000	Unreported	A: Age, Parity, Social class	D
PASSIVE						
Gardener et al. (2009)[49] USA: Nurses' Health Study I & II Follow up: 1976-2001	Not reported	593 incident 130 prevalent	238, 371	Questionnaire	A: Age (months), Calendar year, Latitude at birth, Paternal occupation, Sibship size; Pack-years of smoking in adulthood, Vitamin D intake	C

A: Variables included in the adjustment

Table 3.5 Cohort study point estimate (risk or rate ratio) and 95% confidence interval (CI) for cigarette smoke exposure as a risk factor for MS

Author	Quality	Past (ex) vs. never Rate[1]/Risk[2] (95% CI)	Current (4 years prior) vs. never Rate Ratio (95% CI)	Intensity # cig/day Rate[1]/Risk[2] Ratio (95% CI)	Other Rate Ratio (95% CI)
Active Smoke Exposure					
Hernan et al. (2001)[7]	A	A: Pooled 1.2 (0.9-1.6)[1]	A: Pooled 1.6 (1.2-2.1)		
Villard-Mackintosh & Vessey (1993) [79]	C	A: 1.5 (0.6-3.3)		A: 1-14 1.6 (0.8-3.1)[2] A: 15+ 1.8 (0.8-3.6)[2]	
Thorogood & Hannaford (1998) [80]	D			A: 1-14: 1.2 (0.8-1.8)[1] A: 15+: 1.4 (0.9-2.2)[1]	
Passive Smoke Exposure					
Gardener et al. (2009) [49]	C	A: 1.24 (1.02-1.51)[1]			In utero exposure from: A: Mother: 0.97 (0.77-1.21) A: Father: 1.50 (0.99-2.28)

A: adjusted (see table 3.4 for variables adjusted for); effect estimates presented as presented in the original article.

3.4 Overall findings from case-control, cohort and cross-sectional studies

Tables 3.2, 3.3 and 3.5 summarize the effect estimates reported in the case-control, cross-sectional and cohort studies, respectively. Studies receiving an overall poor quality rating[70-73, 80] will not be discussed here.

3.4.1 Active cigarette smoke exposure

Four of the higher quality studies (one excellent quality cohort study, and 3 good quality case-control studies) reported statistically significant overall effect estimates ("past" and "ever") that ranged in magnitude from 1.1 to 1.6. The studies were too heterogeneous to pool effect estimates; however, studies implicate smoke exposure as a risk factor, in which MS cases had a greater odds of having smoked than controls.

The most frequent smoke exposure contrast preformed in the analyses was 'ever vs. never' smoke exposure, used in the excellent quality cohort study[7], 3 good quality case-control studies[2-4], and 9 acceptable quality studies[5, 6, 66-69, 79, 80]. The excellent cohort study[7] and two of the good quality case-control studies [3, 4] reported statistically significant effects for "ever" smoke exposure ranging in magnitude from 1.1 to 1.6. Five studies[2-5, 69] further investigated the intensity or pack years of exposure, and the three good quality studies[2-4] reported statistically significant effects, ranging from 1.5 to 5.5 (Table 3.2).

3.4.2 Passive cigarette smoke exposure

Two studies investigated household smoke exposure, one good quality case-control study[48] and one acceptable quality cohort study[49]. Both of these studies found statistically significant effects. Two studies investigating smoke exposure in utero reported non-significant effects[49, 65], with the poorer quality study only reporting estimates stratified by sex[49].

3.5 Conclusion

One observation from this systematic review was that authors of published studies are heterogeneous in how they chose to study this research question. This

heterogeneity made it very difficult to draw a definite conclusion on the findings of this systematic review. At first glance, smoke exposure appears to be a risk factor for MS, with 15 of the 20 published studies reporting one or more smoke exposure variables being associated with MS.

Another observation was that studies lack quality: only 6 of the 20 studies were of good or excellent rigour, and the others lacked quality in one or more aspects of their study design. The six studies of high quality were published from 2001 to 2009[2-4, 7, 48, 65], and the five poor quality studies were published from 1966 to 2009[70-73, 80]. The cohort studies lacked generalizability, as all of the participants were women, more specifically married women only[79, 80], or female nurses[7, 49] who may be less likely to smoke.

Studies were not found to use high quality methodology for the assessment of active smoke exposure, particularly in the timing of the exposure in relation to disease onset. Rothman[9], wrote about the importance of the timing of exposure relative to disease onset and concluded that estimating smoke exposure outside of the empirical induction period results in a dilution of the effect[9]. Despite the importance of pre-onset exposure, "current" smoke exposure was reported in 5 studies (published between 1965 and 2009) although 3 of these also reported on "ever" and/or intensity of smoke exposure, suggesting that authors have come to realize that current smoke exposure is a suboptimal method for estimating smoke exposure as a risk factor for MS. "Ever" smoke exposure was the most prevalent form of smoke exposure that was used in analysis. While "ever" can include "past" smoke exposure, "current" exposure may also be included which again has the potential to dilute estimates of effect. Also, current smoking may classify, as exposed, individuals who started smoking after the onset of MS and potentially introduce protopathic bias.

Furthermore, the findings presented here were from three different study designs. Based on study design, cohort studies are superior to case-control studies which are superior to cross sectional studies. The findings from cohort studies do implicate smoke exposure as a risk factor for MS, although only one study was able to do so with statistical significance, all studies report a magnitude of effect within

the range of 1.2-1.8. The case-control studies further support this evidence, as does the cross-sectional study.

In conducting this systematic review, it was clear that researchers should be more consistent in the terminology used to describe smoke exposure. Hernan and colleagues[7], for example, reported a significant effect for current smoke exposure, and a non-significant effect for past smoke exposure. This finding is misleading though, as past smokers were individuals who had quit smoking, whereas current smokers were based on exposure taken 4 years prior to MS onset, which is more representative of "past" smoke exposure. We are most interested in the individuals who smoked before their age of onset, regardless of whether they are currently a smoker or were able to quit. Furthermore, cohort studies only estimating "past" smoke exposure via exposure at study entry[79, 80] may result in misclassification if individuals who started smoking after recruitment into the cohort go on to develop MS, or if individuals who did not develop MS quit smoking after recruitment into the study.

Comparing this review to the previous meta-analysis assessing smoke exposure as a risk factor for MS[60], an additional 14 studies were included in this review. Four of the six high quality studies reported here were not included in the previous meta-analysis as they were published from 2005-2009. Furthermore, passive smoke exposure studies were not included in the previous study. The magnitude of the pooled estimate reported in the previous meta-analysis is similar to the magnitude of effect estimates reported in the high quality studies reported here. Of note, another review was published in 2011[81]. Problems with this more recent review include combining studies investigating the role of smoking in both etiology and progression of MS, and the systematic review presented here captured more studies than presented in the updated meta-analysis.

Although it appears evident that smoke exposure is a risk factor for MS, further research is required to determine how large the effect really is. This should be done using improved study design, aiming to reduce selection bias. Authors should ensure smoke exposure is ascertained before the age of onset of MS, and should distinguish past and current smoke exposures. It would also be beneficial to investigate

duration and intensity of active smoke exposure as they provide more information than “past” smoke exposure. Finally, it is clear that more evidence is required to determine the actual effect of passive smoke exposure, although with two consistent significant studies reporting negative effects of household smoke exposure, it appears as though this may be an emerging risk factor.

Chapter 4

Methods

4.1 Introduction

In this chapter we describe the overall design of the study from which the data for this thesis are derived. In the latter part of the chapter we pay particular attention to the primary smoking exposure variables and describe in some detail how the active smoking variables and passive smoking among never-smokers variables were defined based on the data available in the study questionnaire. This chapter also contains a section on the statistical analyses used and confirmation of ethical approval.

4.2 Study design

The *International Case-Control Study of Environmental risk factors In Multiple Sclerosis* (EnvIMS) is a population-based case-control study, investigating the role of environmental risk factors in MS in five countries (Canada, Norway, Sweden, Serbia and Italy). The motivation for the EnvIMS study came from the observation that many previous studies have evaluated the role of environmental risk factors in MS, but none of these studies have been adequately powered to investigate the potential interactions among risk factors and many of these studies examined many risk factors without specific hypotheses. In addition, the ability to compare results across countries with differing MS risks and differing risk factor distributions is novel in the study of MS etiology[82]. Once the EnvIMS study is complete, it will be the largest case-control study of MS to date, and will have sufficient power to investigate potential interactions of environmental risk factors. The data collection is now complete in Norway, and the data from the Norwegian component of the study were used in this thesis. At the time of analyses the data from Sweden, Serbia and Italy were still being collected and undergoing data cleaning so were not appropriate for use in this thesis. The Canadian component of the study is now underway.

4.2.1 Questionnaire

In EnvIMS, data are collected purely through self-administered postal questionnaires. The research team, with representatives from each country, developed a common English version of the questionnaire (see Appendix 2). Section 1 contains questions on demographics, including education, ethnicity, and number of siblings. Section 2 contains questions on Vitamin D exposure through sun exposure, and Section 3 contains questions on Vitamin D exposure through diet and supplementation. Section 4 contains questions on medical history, and previous (childhood and adolescence) infections. Section 5 contains questions on smoking habits, exposure to both active and passive smoke, and other lifestyle factors. Finally, Section 6 included questions on hormonal factors, to be completed by women only. The questionnaire was translated into Norwegian, Swedish, Serbian, Italian and French, and in order to accommodate cultural differences the questionnaires were modified for each country. For example, Sami (indigenous people of northern Norway) was a response category for parent's ethnicity on the Norwegian questionnaire, but was not included in any other questionnaire. Also, options for the diet section varied slightly across countries to account for differences in available and common foods. The questionnaires were pilot tested in each country to ensure clarity and ease in understanding the questions.

4.2.2 Eligibility criteria

To be eligible for the study, participants were required to be 18 years of age or older at study entry and cases were to have had onset of MS within 10 years of the study entry (i.e. between 1999 and 2008). Additional details on the diagnostic criteria for cases are presented below.

4.3 Recruitment of cases and controls

As noted above, the data from the Norwegian component of EnvIMS study were used in this thesis. The study period was from May 2009 – September 2009, and participants were required to be living in Norway at the time of mailing of the questionnaires, which took place in May 2009.

A letter of invitation was mailed to cases and randomly selected controls, along with the study package, which included: the questionnaire, a return envelope, and a brochure with the study aims, study relevance and ways to participate, including ethical considerations. Participants were asked to return questionnaires via regular mail, and a completed questionnaire was considered proof of consent to participate. If individuals did not wish to participate, they were asked to return the blank questionnaire.

4.3.1 Case ascertainment

Cases were selected from the Norwegian National Multiple Sclerosis registry, which was established by The Ministry of Health and Social Affairs in 1998[83]. In 2006 the registry consisted of 3300 MS cases, which accounted for 50-60% of all MS cases in Norway[83]. Cases are entered into the national registry at time of MS diagnosis, and cases are confirmed using the Poser criteria[15] or McDonald criteria[16, 17]. All cases with clinical onset of MS within 10 years from time of study were selected from the Norwegian National Multiple Sclerosis registry, which resulted in the selection of 1600 cases. Onset of MS was defined as the first clinically isolated symptom (CIS), and MS cases were to have had their CIS between 1999 and 2008.

4.3.2 Control ascertainment

The Norwegian Tax office, which controls the Norwegian National Population Registry, was provided with information on age at the time of study and sex of the 1600 cases and was asked to select 4 randomly frequency matched individuals per case from the population registry. This matching resulted in 6400 controls that were mailed questionnaires.

4.4 Smoke exposure ascertainment

Section 5 (see Appendix 2) of the questionnaire assessed both smoking habits and other lifestyle factors, and included eleven questions on smoke exposure. Information on lifetime active smoke exposure was collected through four questions, and information on lifetime passive smoke exposure was collected in the remaining seven questions (see Appendix 2, Section 5, questions 1-11).

Age of Onset and Index Age

As highlighted in Chapter 1, in order for a risk factor to be etiologically relevant, the exposure must occur during "the empirical induction period"[9] at some time prior to disease onset. In this thesis we will consider the empirical induction period to span from birth to the first indication of disease (i.e. evidence of CIS). The date of the first clinically isolated symptom is available from the Norwegian National Multiple Sclerosis Registry, so we consider the onset year as the year of CIS, and thus the age at onset as the age at CIS.

To identify a similar period of risk for controls, an 'index age' was assigned to each control to define their empirical induction period. The distribution of the year of onset for the participating cases was used to generate an index year distribution for controls. Then, within this distribution, index years (ranging from 1999-2008) were randomly assigned to controls. The controls 'index age', corresponding to the age of clinical onset in cases, was calculated by subtracting the control's year of birth from their assigned index year.

4.4.1 Active cigarette smoke exposure

Active smokers were defined as individuals who had ever reported being a daily smoker (See Appendix 2, Section 5, question 1). Active smokers were then asked to report how many cigarettes they had smoked per day between the ages of: 11-15, 16-20, 21-25, and 26-30 (See Appendix 2, section 5, question 2). The authors of the questionnaire chose to ascertain past smoke exposure up to 30 years of age as it was expected that the majority of individuals would experience MS onset before this time point. These two questions permitted an estimation of an individual's lifetime active smoke exposure.

Etiologically relevant active cigarette smoke exposure

From Chapter 3, it was observed that many authors used "current" or "ever" (i.e. current + past) smoking as a measure of smoke exposure, and that these are not optimal measures of smoke exposure to assess smoking as a risk factor. Clearly the inclusion of current smoking (i.e. smoke exposure following disease onset) is not etiologically relevant and introduces, at the very least, misclassification bias into any

analysis. Using the data available from the questionnaire we chose to limit the analysis to consideration of "past" smoke exposure, which requires that an individual must have reported that they actively smoked cigarettes before their age of onset, and any reported cigarette smoke exposure after MS onset was not considered.

An individual's age of onset/index age was used to determine cigarette smoke exposure status. For example, for an individual with an age of onset of 24 years, only smoke exposure reported to occur prior to age 24 was included as past exposure. Given that smoke exposure was ascertained in a 5-year age range, this meant that this individual was considered exposed if he or she smoked between the ages of 11-15 and/or 16-20. For this individual, reported smoke exposure in the age range 26-30 years was not included. It was somewhat challenging to decide how to manage reported smoke exposure in an age range that includes the age at onset, in this example between the age range of 21-25 years. Rather than risking the inclusion of smoke exposure that is not etiologically relevant, we chose a conservative approach and if the age at onset fell within an age range of reported smoke exposure that smoke exposure was not considered etiologically relevant. As a second example, if an individual's age of onset/index age was 26 years, then reported cigarette smoking between the ages of 11-15, 16-20, or 21-25 would result in a classification of "past smoker", but exposure during the age range 26-30 was not considered etiologically relevant. Finally, if an individual's age of onset was greater than 30 years of age, then any reported active cigarette smoking classified them as a past smoker, since smoke exposures were only ascertained between ages 11 to 30. Figure 4.1 demonstrates how an individual was considered a past smoker, with the dotted lines representing etiologically relevant exposure periods.

Active smoke exposure was ascertained starting at age 11, in the five-year age category 11-15. If an individual reported smoking during these five years and their age of onset/index age was less than 16 it was impossible to tell if their age of smoke exposure preceded age of onset/index age. As a conservative measure, individuals with an age of onset/index age less than 16 were not included in the active cigarette smoke exposure analyses.

Using 5-year age categories for active cigarette smoke exposure results in a loss of information, and may result in misclassification of exposure compared to using the specific ages at which individuals smoked. For this reason, a sensitivity analysis was performed. Information was not available for each age of smoke exposure, so instead the sensitivity analysis investigates smoke exposure using the midpoint of the age range of smoke exposure (Figure 4.1). This method may also introduce misclassification, but the purpose of this sensitivity analysis was to investigate whether our conservative approach (if the age at onset fell within an age range of reported smoke exposure, that smoke exposure was not etiologically relevant) introduced misclassification when compared with considering the age range of reported smoke exposure that included age at onset as etiologically relevant. From the example above, if an individual's age of onset is 24, any reported cigarette smoke exposure from ages 11-15, 16-20, or 20-25 classified them as a past smoker (since the midpoint of the age category 20-25 is 23 years of age, which precedes the age of onset of 24). Furthermore, the sensitivity analysis also resulted in the inclusion of individuals with an age of onset as young as 13. Individuals 13 years of age and older who reported smoking during the ages of 11-15 were classified as a past smoker in the analysis.

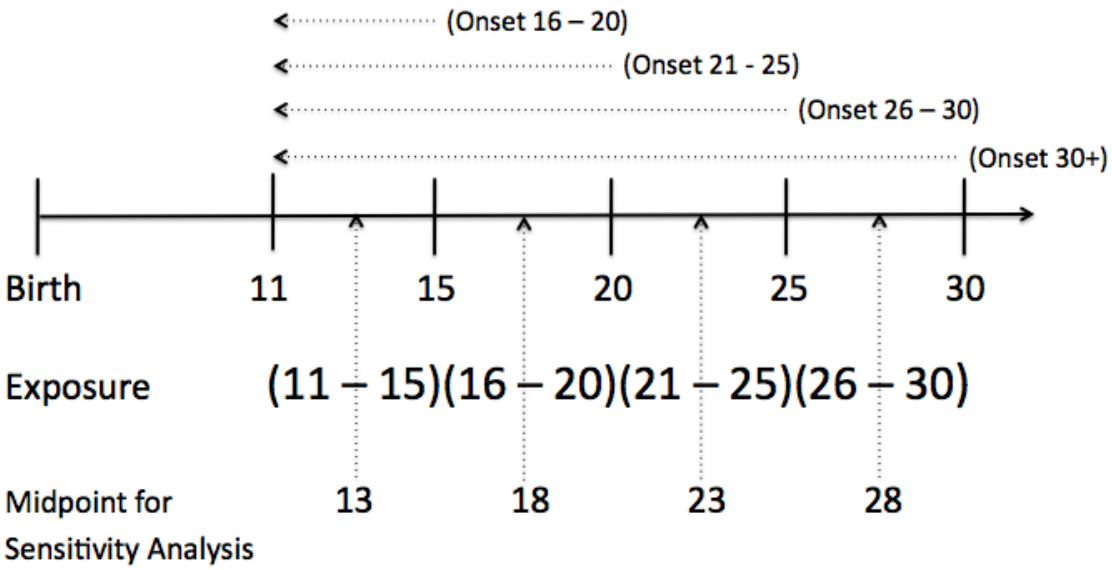


Figure 4.1. Classification of active smoking status based on age of onset/index age

4.4.2 Passive cigarette smoke exposure

Passive smokers were defined as individuals who had ever been exposed to second hand smoke, this was determined by asking individuals if their father or mother smoked in the house while they were a child (see Appendix 2, Section 5, questions 6 and 7 respectively), if they lived with anyone else who smoked in the house when they were under the age of 21 (see Appendix 2, Section 5, question 8), if they lived with someone who smoked in the house from ages 21-25 (see Appendix 2, Section 5, question9), and if they lived with someone who smoked in the house from ages 25-30 (see Appendix 2, Section 5, question 10). As stated above, the authors of the questionnaire chose to ascertain past smoke exposure up to 30 years of age as it was expected that the majority of individuals would experience MS onset before this time point. The relationship between passive smoke and MS was investigated in never-smokers in an attempt to disentangle the effect of passive smoke from active smoke.

Household smoking during childhood

Paternal and maternal smoke exposure were ascertained by asking individuals "Did your father/mother smoke in the house when you were a *child*?". Individuals could then respond: (1) "no, non-smoker"; (2) "yes"; (3) "no, did not smoke inside"; and (4) "don't know". Those who reported option (4) "don't know" were excluded from the passive smoke exposure analysis.

Etiologically relevant passive cigarette smoke exposure

Similar to the method used for defining active smoke exposure, "past" passive smoke exposure was determined, by considering exposure as living with someone who smoked in the house before their age of onset/index age. More specifically, passive smoke exposure was limited to never-smokers.

Passive smoke exposure from paternal and maternal smoking behaviours required choosing an age range for exposure, as it was not explicitly incorporated in the question "Did your father/mother smoke in the house when you were a *child*?", since the word "child" does not suggest a specific age. For this analysis we defined a

"child" to be anyone under the age of 16, using a recent pediatric MS (MS onset during childhood) study as rationale[48]. Mikaeloff and colleagues[48] included MS cases and controls who were under the age of 16, and asked parents if they smoked in the house prior to their child's MS onset. In the study presented here, an individual was classified as exposed if they reported paternal or maternal household smoking behaviour, and their age of onset/index age was 16 years of age or older (Figure 4.2). As a conservative measure, if an individual's age of onset/index age was less than 16 years of age they were not included in the analysis, as it was impossible to tell if their age of smoke exposure preceded their age of onset/index age.

An individual's age of onset/index age was used to assign passive cigarette smoke exposure status. Using the same example from active smoke exposure, an individual with an age of onset at 24 years, only smoke exposure reported to occur prior to age 24 was included as past exposure. Given that the questions were asked in relation to specific time periods, this individual was classified as exposed if their mother or father smoked in the house as a child, or if they lived with someone else who smoked in the house when they were under the age of 21. Smoke exposure reported in the age range of 26 to 30 years was not included. Similar to active smoke exposure, rather than risking the inclusion of smoke exposure that is not etiologically relevant, we chose a conservative approach and if the age at onset fell within an age range of reported smoke exposure we did not include that smoke exposure as etiologically relevant (the age range of 21-25 for this example). Figure 4.2 demonstrates how an individual could be considered exposed to passive smoke in the past, with the dotted lines representing etiologically relevant exposure periods. Finally, if age of onset was greater than 30 years of age, then <u>any</u> reported household smoke exposure under the age of 24 classified an individual as exposed to passive smoke (Figure 4.2).

Again, as in active smoke exposure, using time periods rather than specific ages of exposure to ascertain smoke exposure most certainly results in a loss of information, and may also result in misclassification of exposure. Information was not available for each age of smoke exposure so instead a sensitivity analysis was performed in which the midpoint of the time period was used as the minimum age of

exposure (Figure 4.2). This method may also introduce misclassification, but the purpose of this sensitivity analysis is to investigate whether our conservative approach introduced misclassification when compared with including the age range that included age at onset as etiologically relevant.

From the example above, for an individual with age of onset at 24 years of age, any reported household smoke exposure from father or mother, other household exposure under the age of 21, or household exposure from ages 21-25 would classify them as exposed in the past (since the midpoint of 23 years precedes the individual's the age of onset). This also resulted in individuals with an age of onset as young as 13 being included in the analysis, where any reported maternal or paternal household smoke exposure would classify them as exposed in the past.

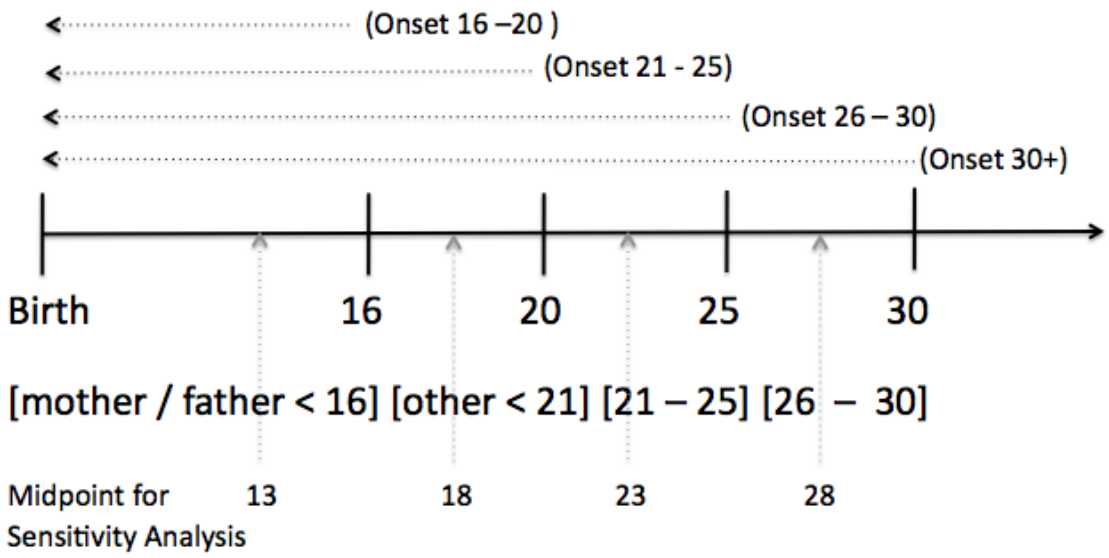


Figure 4.2: Classification of household passive smoke exposure among non-smokers

4.5 Statistical analysis

4.5.1 Descriptive statistics

To summarize the data, descriptive statistics were computed for the variables age at study entry, sex, age of onset, index age, and active and passive smoke exposure.

To examine the magnitude and direction of the relationship between both active smoke exposure and passive smoke exposure among never-smokers, unconditional logistic regression models were used to estimate odds ratios (OR) with 95% confidence intervals (95% CI). The first analysis investigated the relationship between active cigarette smoke exposure and MS, using 'past vs. never' cigarette smoke exposure as the primary exposure variable. The second analysis investigated the relationship between passive cigarette smoke exposure and MS, using "past versus never" cigarette smoke exposure among never-smokers. The relationship between passive smoke and MS was investigated in never-smokers in an attempt to disentangle the effect of passive smoke from active smoke.

4.5.2 Covariates

Matching

Variables used in the frequency matching process (age at study entry and sex) were added to the multivariable unconditional logistic regression models.

Confounders

Socioeconomic position (SEP) has been shown to be associated with both cigarette smoke exposure[84, 85] and MS[86-89]. Moreover, it is widely accepted that SEP is negatively associated with cigarette smoke exposure, whereby lower levels of SEP are associated with higher levels of smoke exposure[84, 85]. While it is accepted that SEP is associated with MS, the direction of this relationship remains unclear. Some studies have reported a positive association between SEP and MS with higher levels of SEP associated with greater risk of MS [86-88], while other studies have reported a negative association between SEP and MS with lower levels of SEP associated with greater risk of MS [89, 90]. Regardless of the direction of this relationship, evidence from the systematic review preformed in Chapter 4 revealed that recent studies have considered SEP to confound the relationship between smoke exposure and MS, and have adjusted for it in their analyses [2, 8, 48, 49, 65, 80].

Education is commonly used as a proxy for socioeconomic position [91] and when investigating life course SEP, parental education is used a proxy for childhood SEP [48, 65] and the individual's education is used as a proxy for adulthood SEP [2, 8, 49, 80].

Although not always available, parental education is the best measure of SEP when investigating an exposure that occurs during childhood. Adjusting for adulthood SEP would result in adjusting for a confounder that follows the exposure, and potentially the outcome, and could result in residual confounding. For example, if smoke exposure (and/or MS onset) occurred during ages 11 through 20 then adjustment for an individual's university level education would not be suitable, as their own university level of education could not have possibly influenced their childhood smoke exposure (and/or MS onset). As a result parental education, as a proxy for childhood SEP, was considered a potential confounder of the relationship between smoke exposure and MS in this thesis.

The EnvIMS questionnaire asked individuals to report the highest level of education attained by their father and mother (less than primary school completed, completed primary to grade 10, completed high school, or completed university). The highest education level attained by either parent was used to represent childhood SEP in these analyses. Parental education was coded using dummy variables in the analysis.

To determine if parental education satisfies the criteria for a confounder, univariate logistic regression models were run to (a) examine the relationship between parental education and past smoke exposure (separately done for active and passive smoking) and (b) examine the relationship between parental education and the outcome (MS status; case or control). The relationship between parental education and smoke exposure was investigated in controls, only to assess the relationship in the source population.

Once the criteria for a confounder were satisfied, two multivariable logistic regression models were run, one including the covariates "past" cigarette smoke exposure, age at time of study, and sex; and the second adding the potential confounder, parental education. We then observed the change in estimate of the beta coefficient for the past smoke exposure variable when parental education was added to the multivariable logistic regression model. Specifically, we observed the absolute value of the difference in beta coefficients divided by the beta coefficient for the smoke exposure.

$$\frac{\left|\hat{\beta}smoke - \left(\hat{\beta}smoke + parental.education\right)\right|}{\hat{\beta}smoke} \times 100$$

If the change in estimate was ≥ 10% then parental education was considered a potential confounder and was included in the final model.

4.5.3 Missing data

For these analyses, missing data were excluded using case-wise deletion. The percent of missing data for each variable of interest can be found in Chapter 5.

4.5.4 Statistical software

All analyses were conducted using STATA Data Analysis and Statistical Software, version 11.2.

4.6 Ethical approval

The Norwegian study received ethics approval from the University of Bergen, Norway regional komité for medisinsk of helsefaglig forskningsetikk, Vest_Norge (REK Vest; Ref No. 2008/11259-ANØL; see Appendix 3A). All data have been anonymized such that participant names and contact information are not included in the Norwegian dataset. McGill University's Faculty of Medicine Institutional Review Board approved the secondary analysis of this dataset (research project A02-E15-11A; Institutional Review Board Assurance Number: FWA 00004545; see Appendix 3B).

Chapter 5

Results

5.1 Introduction

This chapter reports the results from statistical analyses conducted to investigate smoke exposure (both active and passive) as a risk factor for MS. Primary exclusions and missing data are disclosed, followed by presentation of descriptive statistics. Results of active cigarette smoke exposure are reported first, investigating the role of parental education as a potential confounder, followed by logistic regression models, and sensitivity analyses. Then the results of passive smoke exposure among non-smokers are reported. The chapter concludes with a summary of the findings.

5.2 Primary exclusions and missing data

Primary exclusions

The study successfully recruited 2526 participants, 809 cases (54% response rate) and 1717 controls (29% response rate). There were 2 cases and 1 control excluded from the analyses because they were under the age of 18 at study entry and thus did not meet the study inclusion criteria. Individuals were also excluded if they did not report whether or not they were ever a daily smoker (25 cases and 45 controls). This exclusion was necessary as it was impossible to tell if these individuals were exposed or unexposed, which was required for both active and passive cigarette smoke exposure analyses. Therefore, there were 782 cases and 1671 controls (N=2453) considered for the analyses.

Missing data

For the scope of this thesis all missing data were excluded using case-wise deletion. There were no missing data for age at time of study or age of onset/index age. For each covariate, approximately the same proportion of data was missing for cases and controls. As mentioned above, individuals were excluded for not reporting whether they were ever a daily smoker, representing 3% of both cases and controls.

Combining the active smoke exposure variables to create a composite "past" active cigarette smoke exposure variable resulted in missing data for 2% of cases and 3% of controls. The combined variable for "past" passive cigarette smoke exposure among never-smokers resulted in missing data for 11% of cases and 13% of controls. When "past" passive cigarette smoke exposure data included individuals who reported parent's non-household smoke behaviours, this variable was missing for only 5% of cases and 7% of controls. Finally, missing data for the confounder parental education was 6% among cases and 8% among controls.

5.3 Descriptive statistics

Table 5.1 provides descriptive statistics for the overall sample (782 cases and 1671 controls). Sample sizes vary for each variable reported depending on missing data as described above. The distribution of the covariate "age at study entry" suggest that the frequency matching process was successful, while the distribution of the covariate "female" (sex) may suggest that the frequency matching for this covariate was compromised by the low response rates of controls (29%). The index ages for controls were assigned using the distribution of year of MS onset (see section 4.4), and as a result we see that the mean index age of the controls was similar to the mean age at onset of the cases. The highest level of parental education (mother's or father's) and the individual's own education is also presented. Parents of cases and controls attained similar levels of education, and controls appear to have higher education than cases with 88% of controls having completed high school or university as compared to 83% of cases. The majority of the sample was of Norwegian, European or western origin. The active and passive smoke exposure variables show the number of participants exposed to smoke during each time category, prior to their age of onset/index age.

Table 5.1 Descriptive statistics for the overall sample

Variable - Total N=2453	Cases (N=782)	Controls (N=1671)
Female - N (%)	541 (69%)	1223 (73%)
Age at study entry - Mean (SD)	44.1 (10.2)	45.0 (10.9)
Age at MS onset - Mean (SD)	37.6 (9.95)	
Index age* - Mean (SD)		38.5 (11.2)
Highest level of completed parental education - N (%)	*N=721*	*N=1565*
< Primary	211 (29)	500 (32)
Primary-grade 10	110 (15)	224 (14)
High school	223 (31)	409 (26)
University	177 (25)	432 (28)
Individual's highest level of completed education – N (%)	*N=771*	*N=1629*
< Primary	19 (3)	42 (3)
Primary-grade 10	112 (14)	155 (10)
High school	319 (41)	568 (35)
University	321 (42)	864 (53)
Father's ethnicity - N (%)	*N= 779*	*N=1663*
Norwegian / European / other Western	769 (98.7)	1623 (97.6)
Sami	3 (0.4)	8 (0.5)
Asian	1 (0.13)	21 (1.3)
African	1 (0.13)	2 (0.1)
Middle Eastern	5 (0.64)	4 (0.2)
Latin American	0	5 (0.3)
Mother's ethnicity - N (%)	*N=778*	*N=1666*
Norwegian / European / other Western	770 (99)	1621 (97.3)
Sami	4 (0.5)	14 (0.8)
Asian	1 (0.1)	21 (1.3)
African	3 (0.4)	2 (0.1)
Middle Eastern	0	4 (0.2)
Latin American	0	4 (0.2)
Active Smoke exposure		
Age of exposure N (%)		
11-15 (N=2119)	130 (19)	150 (10)
16-20 (N=2220)	412 (58)	638 (42)
21-25 (N= 2073)	436 (66)	673 (48)
26-30 (N=1774)	355 (63)	540 (44)
Composite "past" active cigarette smoke exposure	519 (68)	816 (50)
Passive smoke exposure among never-smokers (N= 1028)	*N=232*	*N=796*
Childhood household exposure: Father (N=941)		
Yes, inside	119 (54)	367 (51)
Yes, not inside	16 (7)	46 (6)
Childhood Household exposure: Mother (N=959)		
Yes, inside	80 (37)	220 (30)
Yes, not inside	13 (6)	27 (4)
Other household exposure age < 21 (N=950)	26 (12)	115 (16)
Household exposure age 21-25 (N=885)	38 (19)	119 (17)
Household exposure age 26-30 (N=762)	25 (15)	95 (16)
Composite "past" passive cigarette smoke exposure	156 (75)	501 (72)

*** An index age was assigned to controls based on the distribution of year of clinical onset for MS cases. The total sample (N=2453) is used for each variable unless otherwise noted. Active and passive smoke exposure variables do not add to 100% as participants may be placed in more than one category.**

5.4 Active cigarette smoke exposure analysis

5.4.1 Classification of "past" active cigarette smoke exposure

The variables used to gather information on active smoke exposure across the four 5-year age categories were combined to create an overall "past" active cigarette smoke exposure variable (see section 4.4.1). Table 5.1 includes the number and percentage of exposed cases and controls for each variable, with respect to their age of onset/index age.

5.4.2 Potential confounder: Highest level of parental education

As described in section 4.3, several analyses were run to determine if the highest level of education achieved by either parent confounds the relationship between active cigarette smoke exposure and MS. Two models were run to determine if parental education meets the criteria for a confounder. First, a univariate logistic regression model was run to investigate the relationship between parental education (as the independent variable) and "past" active smoke exposure ("past" or "never" as the binary outcome). This analysis was conducted among controls to avoid disease status inducing a relationship between the confounder and the exposure. A statistically significant negative association was revealed between the highest level of parental education and active smoke exposure (Table 5.2), with the odds of smoking decreasing with increasing levels of parental education. Second, a univariate logistic regression model was run investigating the relationship between parental education and MS status. This univariate regression revealed an inconclusive finding, with only the high school level of education resulting in a statistically significant relationship with MS status (Table 5.3).

Although the relationship between parental education and MS status was inconclusive and the criteria for a confounder were not completely met, we decided to observe the change in estimate, rather than run the risk of not adjusting for a confounder. Two multivariable logistic regression models were run, one including the covariates "past" active cigarette smoke exposure, age at time of study, and sex; and the second adding the potential confounder, parental education. The beta

coefficients for "past" smoke exposure from these two models were then compared, and the change in estimate that occurred when adding parental education variable was determined. The change in estimate observation revealed there was no change in the estimated β when parental education was added to the multivariable model of active smoke exposure as a risk factor for MS (Table 5.4). Taking all of these results into account, there does not appear to be sufficient evidence to conclude that parental education is a strong confounder in the relationship between active smoke exposure and MS, and was not included in the final model.

Table 5.2 The relationship between highest level of parental education completed and "past" active cigarette smoke exposure among controls

	OR (95%CI)
Parental education (N*=1521)	
< Primary	1.0
Primary – grade 10	0.58 (0.42 - 0.79)
High school	0.62 (0.47 – 0.81)
University	0.54 (0.41– 0.70)

Total N = 1671
N* = Total N – missing parental education – missing "past" active cigarette smoke exposure

Table 5.3 The relationship between highest level of completed parental education and MS status.

	OR (95%CI)
Parental education (N*=2228)	
< Primary	1.0
Primary – grade 10	1.16 (0.88 – 1.54)
High school	1.32 (1.04 – 1.66)
University	0.97 (0.76 – 1.24)

Total N = 2453
***N = Total N – missing parental education – missing "past" active cigarette smoke exposure**

Table 5.4 Highest level of completed parental education as a potential confounder of the relationship between "past" active cigarette smoke exposure and MS: Percent change in estimate.

Potential confounder: Parental Education	β estimate Multivariable** model	β estimate Multivariable** model + potential confounder	Percent change in estimate
Past active cigarette smoke exposure (N*=2228)	0.76	0.76	0%

Total N = 2453
N* = Total N – missing parental education – missing "past" active cigarette smoke exposure
****Multivariable model includes: "past" active cigarette smoke exposure, age at time of study, and sex.**

5.4.3 “Past vs. Never” active cigarette smoke exposure.

Classification of “past” active cigarette smoke exposure

The four variables used to define active smoke exposure during 5-year age categories were combined to create an overall binary variable for “past” active cigarette smoke exposure. Three cases and 30 controls were excluded from the analysis if MS onset/index age was less than 16 years of age as it was impossible to tell if their age of smoke exposure preceded age of onset/index age. Eleven cases and 16 controls were excluded from the analysis if age of exposure was not reported. The analysis included 768 cases and 1625 controls (N= 2393). Table 5.5 displays the number of exposed and unexposed cases and controls.

Table 5.5 “Past” (exposed) vs. Never (Unexposed) active cigarette smoke exposure

Overall active cigarette smoke exposure (N=2393)	Cases (n=768)	Controls (n=1625)
Exposed (“Past”)	519	816
Unexposed (“Never”)	249	809

The results from this 2X2 table are presented in Table 5.7

5.4.4 Sensitivity analysis for the use of 5-year age categories of smoke exposure

A sensitivity analysis was run to investigate the potential impact of using the limit of 5-year age category of smoke exposure when classifying an individual as exposed to smoke prior to their MS onset. If an individual’s age of onset fell within one of the four 5-year age categories, the limit of the age category was compared with the midpoint of the age category to determine relevant smoke exposure (see section 4.4.1). This resulted in 20 individuals added to the analysis (5 cases, 15 controls). Of these, 16 individuals were between the ages of 13-15 and had previously been excluded (2 unexposed cases, 13 unexposed controls, and 1 exposed control; Table 5.6), and 4 individuals whose first report of smoke exposure occurred during the age category that included of their age of MS onset. (3 exposed cases and 1 exposed control). Furthermore, among the individuals already included in the analysis, the exposure status of 6 cases and 12 controls changed from unexposed to exposed.

Table 5.6 Past (Exposed) vs. Never (Unexposed) active cigarette smoke exposure, using the midpoint of each 5-year age category of smoke exposure

Overall active cigarette smoke exposure	Cases (n=773)	Controls (n=1640)
Exposed ("Past")	528	830
Unexposed ("Never")	245	810

The results from this 2X2 table are presented in Table 5.8

5.4.5 Logistic regression of "past" active cigarette exposure as a risk factor for MS

Table 5.7 reports odds ratios (OR) from the crude and adjusted logistic regression models, and Table 5.8 reports the sensitivity analysis ORs from the crude and adjusted logistic regression models. Model 1, the crude model, includes a single covariate: "past" active smoke exposure. Model 2 adds in the covariates used in the frequency matching (age at time of study and sex). Model 2, the most informative model, indicates MS cases have a 2.19 (95% CI 1.82-1.63) greater odds of having smoked than controls. Age at time of study was not a strong covariate in the multivariable logistic regression model; however, sex remains a strong variable 1.27 (1.04-1.53), indicating that MS cases had a greater odds of being male than controls. This is not expected since it is widely reported that females have a greater risk of MS than males[30, 31]. This may be due to the lack of success in the recruitment process after frequency matching, in which 31% of cases were males, while only 27% of controls were males.

The sensitivity analysis shows only a marginal increase in the effect estimate when using the midpoint of the 5-year age category instead of the upper limit of the age category when determining an individual's exposure status based on their age of onset/index age. This indicates that effect of "past vs. never" active smoke exposure is robust, and using the upper limit of a 5-year age category of smoke exposure for analysis (a conservative measure) does not add substantial misclassification (Table 5.7).

The finding from this analysis is consistent with good quality previous literature, which has implicated smoke exposure as a risk factor for MS[2-4].

Table 5.7 Logistic regression of past. Vs. never active cigarette smoke exposure on the risk of MS (N= 2393)

Covariate	Model 1 OR (95%CI)	Model 2 OR (95%CI)
"Past" cigarette smoke exposure	2.07 (1.73-2.47)	2.19 (1.82 -2.63)
Age at study entry		0.98 (0.97- 0.99)
Sex - male		1.27 (1.04-1.53)

Table 5.8 Sensitivity analysis: Logistic regression of past vs. never active cigarette smoke exposure on the risk of MS (N=2453)

Covariate	Model 1 OR (95%CI)	Model 2 OR (95%CI)
"Past" cigarette smoke exposure	2.10 (1.76-2.52)	2.21 (1.84-2.65)
Age at study entry		0.98 (0.98-0.99)
Sex		1.27 (1.04 – 1.53)

5.5 Passive cigarette smoke exposure among never-smokers analysis

The relationship between passive cigarette smoke exposure and MS was investigated in never-smokers in an attempt to disentangle the effect of passive smoke from active smoke. Passive smoke exposure was ascertained in 232 never-smoking cases and 796 never-smoking controls (N=1028). Figure 5.1 displays the number of individuals included at each stage of analysis.

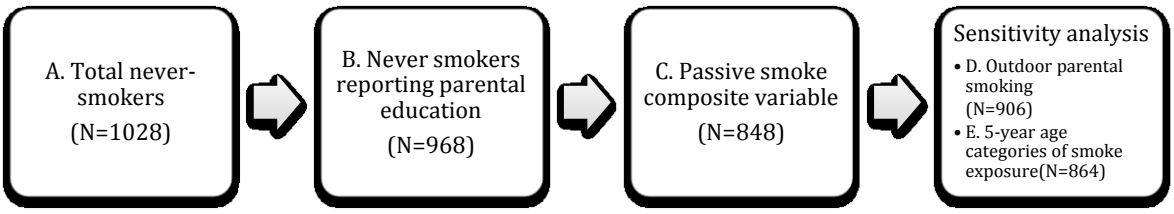


Figure 5.1 Flow chart of sample sizes after exclusions and sensitivity analyses

5.5.1 Descriptive statistics for never-smokers

Table 5. 9 presents descriptive statistics for the subgroup of never-smokers. The covariate "female" (sex) shows a near equal distribution for cases and controls for this subsample. Mean ages (at onset for cases, and associated index age for

controls) were also similar in the subsample of never-smokers just as it was in the overall sample. The distribution of parental education demonstrates that parents of controls attained a higher level of education than parents of cases, which could provide evidence to support the negative relationship between parental education and MS (lower levels of parental education being associated with greater prevalence of MS).

Table 5.9 Descriptive statistics of covariates for the never-smokers subgroup

Covariate	Cases (N=232)	Controls (N=796)
Female - N (%)	165 (71%)	576 (72%)
Age at study entry - Mean (SD)	42.2 (9.9)	43.5 (10.9)
Age at MS onset - Mean (SD)	35.8 (9.7)	
Index age* - Mean (SD)		37.0 (11.2)
Highest level of completed parental education - N(%)		
< Primary	53 (24)	195 (26)
Primary-grade 10	35 (16)	116 (15)
High school	72 (33)	209 (28)
University	59 (27)	229 (31)

*** An index age was assigned to controls based on the distribution of year of clinical onset for MS cases**

5.5.2 Classification of "past" passive cigarette smoke exposure

The variables used to gather information on lifetime passive smoke exposure were combined to create an overall "past" passive cigarette smoke exposure variable (see section 4.4.1). Table 5.1 includes the number and percentage of exposed cases and controls for each variable, with respect to their age of onset/index age. Using this composite variable, individuals were classified as exposed for the analysis if they reported passive smoke exposure during any time period that preceded age of onset/index age. Individuals were considered unexposed if they reported never being exposed to passive smoke before their age of onset/index age.

5.5.3 Potential confounder: Parental education

As described in section 4.3, and again for active smoke exposure in section 5.3.1 above, several analyses were run to determine if the highest level of education achieved by either parent confounds the relationship between passive cigarette smoke exposure and MS in the subgroup never-smokers.

Two models were run to determine if parental education meets the criteria for a confounder. First a univariate logistic regression model was run to investigate the relationship between parental education and "past" passive smoke exposure ("past" or "never" as the binary outcome). This analysis was conducted among controls to avoid disease status inducing a relationship between the confounder and the exposure. A statistically significant negative association was revealed between the highest level of parental education and passive smoke exposure (Table 5.10), with the odds of smoking decreasing with increasing levels of parental education. Second, a univariate logistic regression model was run investigating the relationship between parental education and MS status. This univariate regression revealed an inconclusive finding, as none of the levels of education were found to have a statistically significant association with MS status (Table 5.11). Finally, although the relationship between parental education and MS status was inconclusive and the criteria for a confounder were not completely met, we decided to observe the change in estimate, rather than run the risk of not adjusting for the confounder. This revealed a 17% change in estimated β when parental education was added to the multivariable model of passive cigarette smoke exposure as a risk factor for MS (Table 5.11). Taking all observations into account, the large change in estimate provided sufficient evidence to conclude that parental education is a confounder of the relationship between passive smoke exposure and MS and is included in the final model.

Individuals not reporting their mother or father's level of education were excluded from the analyses investigating "past" passive cigarette smoke exposure as a risk factor for MS, as this confounder was adjusted for in the multivariable logistic

regression model. Therefore, the final analyses of passive smoke among never-smokers included 219 cases and 749 controls (N=968; Figure 5.1B).

Table 5.10 The relationship between highest level of completed parental education and "past" passive cigarette smoke exposure

Among Controls (N=796)	OR (95%CI)
Highest level of completed parental education (N*=654)	
< Primary	1.0
Primary – grade 10	1.01 (0.55 -1.84)
High school	0.73 (0.45 – 1.20)
University	0.39 (0.25 – 0.62)

Total N = 796
N* = Total N – missing parental – missing "past" passive cigarette smoke exposure

Table 5.11 The relationship between highest level of completed parental education and MS status.

	OR (95%CI)
Highest level of completed parental education (N*=968)	
< Primary	1.0
Primary – grade 10	1.27 (0.77 – 2.10)
High school	1.39 (0.91 – 2.14)
University	0.94 (0.60 – 1.48)

Total N = 1028
N* = Total N – parental education – missing "past" passive cigarette smoke exposure

Table 5.12 Highest level of completed parental education as a potential confounder of the relationship between "past" passive cigarette smoke exposure and MS: Percent change in estimate.

Potential confounder: Parental education	β estimate Multivariable** model	β estimate Multivariable** model + potential confounder	Percent change in estimate
Past passive cigarette smoke exposure (N*=968)	0.23	0.19	17%

Total N = 1028
N* = Total N – missing childhood SEP – missing "past" passive cigarette smoke exposure
**** Multivariable model includes: "past" passive smoke exposure among never-smokers, age at time of study, and sex**

5.5.4 "Past vs. Never" passive cigarette smoke exposure among never-smokers

Classification of "past" passive cigarette smoke exposure

The lifetime passive smoke exposure variables used to define passive smoke exposure were combined to create an overall binary variable for "past" passive

cigarette smoke exposure. Using this classification of "past" passive cigarette smoke exposure, 25 cases and 95 controls were excluded from the analysis. Three cases and 23 controls were excluded if age of MS onset/index was less than 16 years of age. Seven cases and 25 controls were excluded for reporting they did not know if their mother or father smoked. Fifteen cases and 43 controls were excluded for reporting that their mother or father smoked, but not inside the house. Finally, 4 controls were excluded for not reporting their household smoke exposure prior to their age of MS onset/index (4 controls). Therefore, the analysis included 194 cases and 654 controls (N=848; Figure 5.1C). Table 5.13 displays the number of exposed and unexposed cases and controls.

Table 5.13 Past (Exposed) vs. Never (Unexposed) passive cigarette smoke exposure among non-smokers (excluding parents who did not smoke inside)

Overall passive smoke exposure among non smokers N=848	Cases (n=194)	Controls (n=654)
Exposed ("Past")	147	471
Unexposed ("Never")	47	183

Results from this 2X2 table are presented in Table 5.16

5.5.5 Sensitivity analysis: Non-household parental smoke exposure

The first sensitivity analysis was run to investigate the potential impact of excluding individuals if they indicated that a parent had smoked, but not inside the house. Although the smoke exposure may not have occurred in the household, parents may have exposed their children to passive smoke while in a vehicle, or while being in close proximity while smoking outside. Individuals indicating that a parent had smoked, but not inside the house, were considered exposed for this sensitivity analysis, and resulted in 56 individuals added to the analyses (N=906; 15 cases and 43 controls; Figure 5.1D; Table 5.14).

Table 5.14 Past (Exposed) vs. Never (Unexposed) passive cigarette smoke exposure among non-smokers (including parents who did not smoke inside)

Overall passive smoke exposure among non smokers (education covariates) N= 906	Cases (n=209)	Controls (n=697)
Exposed ("Past")	162	514
Unexposed ("Never")	47	183

Results from this 2X2 table are presented in Table 5.17

5.5.6 Sensitivity analysis: Age categories of smoke exposure

A second sensitivity analysis was run to investigate the potential impact of using the limit of the time period of smoke exposure when classifying an individual as exposed to smoke prior to their MS onset. If an individual's age of onset fell within one of the age categories, the upper limit of the age category was compared to the midpoint to determine relevant smoke exposure (see section 4.4.2). This resulted in 16 individuals added to the analysis (3 cases, 13 controls; see Table 5.15). Fifteen of these individuals had an age of onset/index age between 13-15 and were previously excluded because their age of onset/index age was less than 16 (1 exposed case, 9 exposed controls, 1 unexposed case, and 4 unexposed controls), and one exposed case first reported smoke exposure occurred during the age category that included their age of MS onset. This resulted in a total sample size of 864 (197 cases, 667 controls; Figure 5.1E)

Table 5.15 Past (Exposed) vs. Never (Unexposed) passive cigarette smoke exposure among non-smokers (excluding parents who did not smoke inside) using the midpoint of each age category of smoke exposure

Sensitivity Analysis using midpoint of age category: Overall passive cigarette smoke exposure N=864	Cases (n=197)	Controls (n=667)
Exposed ("Past")	149	187
Unexposed ("Never")	48	480

Results from this 2X2 table are presented in Table 5.18

5.5.7 Logistic regression of "past" passive cigarette exposure as a risk factor for MS among never-smokers

Table 5.16 reports estimated ORs from the crude and adjusted logistic regression models for "past" passive smoke exposure as a risk factor for MS. Model 1, the crude model, includes only "past" passive cigarette smoke exposure. Model 2 adds in the covariates used in the matching process, age at time of study and sex. Finally, Model 3 adjusts for the confounder, parental education. All 3 models report non-significant odds ratios. The subsample used to explore the relationship of passive smoke among never-smokers was small, and a post-hoc power calculation revealed that the study was underpowered (power, $1-\beta = 20\%$) to detect a

statistically significant odds ratio. With the sample size presented here, and a power of 0.80, the statistically significant odds ratio that could have been detected was 4.75.

Tables 5.17 and 5.18 report ORs from the sensitivity analyses of household parental smoke exposure, and 5-year age categories, respectively. Both sensitivity analyses reveal little or no change in the effect estimate, and effect estimates remain not statistically significant. This indicates that the effect of "past vs. never" passive smoke exposure is robust, and using the upper limit of an age category of smoke exposure does not add substantial misclassification, nor does excluding individuals who were exposed to non-household passive smoke exposure.

Table 5.16 Logistic regression of past Vs. never passive cigarette smoke exposure on the risk of MS (N=848)

Covariate	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
"Past" passive cigarette smoke exposure	1.22 (0.84-1.76)	1.26 (0.87-1.84)	1.20 (0.83-1.76)
Age at study entry		0.98 (0.96- 1.00)	0.98 (0.96- 1.00)
Sex - male		1.17 (0.82-1.68)	1.18 (0.82-1.68)
Highest level of completed parental education			
< Primary			1.0
Primary – grade 10			1.16 (0.69-1.94)
High school			1.24 (0.80-1.94)
University			0.84 (0.52-1.36)

Table 5.17 Household smoke exposure sensitivity analysis: Logistic regression of past vs. never passive smoke exposure (N=906)

Covariate	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
"Past" passive cigarette smoke exposure	1.23 (0.85-1.77)	1.26 (0.87 -1.82)	1.23 (0.85 – 1.78)
Age at study entry		0.98 (0.97 – 1.0)	0.98 (0.97- 1.0)
Sex - male		1.14 (0.81 – 1.61)	1.14 (0.81-1.61)
Highest level of completed parental education			
< Primary			1.0
Primary – grade 10			1.04 (0.63-1.71)
High school			1.10 (0.72-1.71)

University	0.84 (0.53-1.32)

Table 5.18 Age-category of smoke exposure sensitivity analysis: Logistic regression of past vs. never passive smoke exposure (N=864)

Covariate	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
"Past" passive cigarette smoke exposure	1.21 (0.83-1.74)	1.25 (0.86-1.82)	1.20 (0.83-1.76)
Age at study entry		0.98 (0.97- 1.00)	0.98 (0.97- 1.00)
Sex – male		1.20 (0.84-1.71)	1.20 (0.85-1.72)
Highest level of completed parental education			
< Primary			1.0
Primary – grade 10			1.15 (0.69-1.92)
High school			1.23 (0.79-1.93)
University			0.87 (0.54-1.40)

5.6 Summary of results

Overall active cigarette smoke exposure was found to be a statistically significant risk factor for MS, and passive smoke exposure findings were non-significant; however, the passive smoke exposure analysis was not powered to find a statistically significant finding of this magnitude among never-smokers. The next chapter will discuss these results further, along with the limitations and strengths of this study.

Chapter 6

Discussion

6.1 Introduction

This discussion summarizes the findings from the Norwegian component of the EnvIMS study analyzing smoke exposure for a risk factor for MS. The findings for "past" active cigarette smoke exposure are presented first, followed by the findings for "past" passive cigarette smoke exposure among never-smokers. The strengths and limitations are then discussed, followed by recommendations for future research.

6.2 Findings

6.2.1 Active cigarette smoke exposure

Individuals with MS had a 2.19 (95% CI: 1.82-2.63) greater odds of having smoked than controls. This result is in line with previously published high quality studies that were presented in the systematic review in Chapter 3[2-4, 7], and implicates active cigarette smoke exposure as a risk factor for MS. The magnitude of effect found in our study was greater than the largest effect estimate presented in the systematic review (OR 1.6)[2], and may have been a result of ascertaining history of smoke exposure in 5-year age categories. Given the nature of the composite smoke exposure variable, an individual's exposure was only included if it occurred a minimum of 1-5 years prior to MS onset. However, previous high quality studies used various time periods of smoke exposure (ranging from any time period prior to MS onset[3, 4], within 1 year of MS onset[2], or at least 4 years prior to MS onset [7]) which does not support the possibility that the 5-year age categories of smoke exposure was responsible for the increase in magnitude. Other possibilities for an increased magnitude of effect are presented below, along with the discussion of potential biases that may have been encountered (e.g. recall bias, non-response bias).

6.2.2 Passive cigarette smoke exposure among never-smokers

The analysis of passive cigarette smoke exposure among never-smokers resulted in a non-significant odds ratio (1.20; 95% CI 0.83-1.76). The subsample of

never-smokers used to explore the relationship was small (N= 848; 194 cases, 654 controls), and a post-hoc power calculation revealed that the study was underpowered to find a statistically significant OR of 1.20 (see section 5.5.7), rather, the sample size used here resulted in an analysis that was powered to find a statistically significant OR of at least 4.75. Few studies have investigated the relationship between passive smoke exposure and MS. As described in Chapter 3, Mikaeloff and colleagues [48], with a sample size of 1167 (129 cases and 1038 controls), reported a statistically significant OR of 2.1 (95% CI: 1.4-3.2) for pediatric MS patients; and Gardener and colleagues[49], with 622 cases and an unaffected cohort of 185 103, reported a RR of 1.24 (95% CI: 1.02-1.51;) for women from the Nurses' Health Study who were exposed to parental smoke. In a more recent study by Hedstrom and colleagues[92], MS cases (N=695) and matched controls (N=1635) were interviewed, and information on workplace or household daily passive smoke exposure prior to MS onset was collected. An OR of 1.3 (95% CI: 1.1-1.6) for daily passive smoke exposure was obtained. The authors also found a statistically significant trend with increasing duration of smoke exposure increasing the odds of MS.

From the three studies mentioned above, passive household cigarette smoke exposure appears to be a risk factor for MS. Although the Norwegian component of the EnvIMS study was underpowered for this specific analysis, once data collection is complete, the results of pooled data from 5 countries should be adequately powered to detect a statistically significant effect with a magnitude comparable to that found in this study (OR=1.20).

6.3 Strengths

6.3.1 Case and control ascertainment

Case ascertainment was completed using The Norwegian National MS Registry, which includes 50-60% of all MS cases in Norway and requires that patients have been diagnosed by a neurologist. Cases selected for this study were 10-year "incident" cases, in which MS onset occurred within 10 years of the study

start date. Using the criteria from the quality assessment of cases in the systematic review (Chapter 3), the case selection performed in the EnvIMS study would be considered of high quality.

Control ascertainment was completed using the Norwegian National Population Register. Population-based controls were frequency matched to cases on age and sex, and these variables were adjusted for during the statistical analysis. Case and control ascertainment were carried out in the same time period (May-September 2009). Using the criteria from the quality assessment of controls in the systematic review (Chapter 3), the control selection performed in the EnvIMS study would also be considered of high quality.

6.3.2 Smoke exposure ascertainment

Smoke exposure was ascertained in cases and controls using several age categories of exposure and smoke exposure ascertainment was done independent of MS status. Individuals were classified as exposed if their age of MS onset (or control index age) was after their age of smoke exposure, and given the 5-year age categories of smoke exposure, individuals were exposed a minimum of 1-5 years prior to their onset of MS. Using the criteria from the quality assessment of smoke exposure ascertainment in the systematic review (Chapter 3), the smoke exposure ascertainment performed in the EnvIMS study would be considered to be of high quality.

The sensitivity analysis of smoke exposure ascertainment used the midpoint of each 5-year age of exposure category and resulted in a minimal change in effect estimate (see section 5.4.5 and 5.5.7). This indicates that the conservative method of smoke exposure ascertainment chosen for this study should not have biased the results with respect to excluding individuals if their age of onset fell within one of the 5-year age categories of smoke exposure. These two methods of smoke exposure ascertainment are sub-optimal, with the "gold standard" being smoke exposure data from each year of exposure. Although, misclassification of smoke exposure may be even greater if using smoke exposure from every year of exposure as recall at specific ages is likely to be more difficult than recall for periods of life. In

ascertaining smoke exposure data in specific age categories of exposure, future analyses can investigate critical time periods of smoke exposure, and ascertaining intensity of smoke exposure at each age category will allow for a dose-response analysis.

6.3.3 Parental education as a potential confounder

Collecting data on parental education is a strength of this study. Many studies use parental education as a proxy for childhood socioeconomic position (SEP)[84, 85], as parental education represents the socioeconomic living conditions experienced during childhood. Passive smoke exposure studies typically adjust for childhood SEP using parental education[48, 65], or parental occupation[49]. Several studies analyzing active smoke exposure have adjusted for the individual's own education level (as a proxy measure for adulthood SEP)[2, 8, 80], this is problematic since the level of education attained could occur after smoke exposure. For example, our study ascertained smoke exposure from age 11, while education level ranges from less than primary school to university, and the assumption that obtaining a university level education influenced an individual's smoking behaviour at age 11 (or any age prior to their educational achievement) is problematic. In this case, adjusting for adult education would result in adjusting for something that is not a confounder and would reduce precision, resulting in residual confounding.

6.3.4 The EnvIMS Questionnaire

The extensive EnvIMS questionnaire is another strength of this study. The questionnaire investigated several potential environmental risk factors, and asked 50 multi-part questions in 6 sections: demographics, sun exposure, diet, medical history, smoking habits and lifestyle factors, and hormonal factors. Finally, the questionnaire development, and the use of a single (translated) questionnaire across five countries is novel and once completed other countries may adopt this questionnaire, and enable direct comparisons to the findings from the five countries.

6.4 Limitations

6.4.1 Selection bias

Response rates

This study originally planned to recruit 4 controls per case frequency matching on age at time of study and sex. However, low response rates among controls resulted in slightly more than two controls per case. The response rates were 51% among cases (809/1600) and only 27% among controls (1717/6400). Low response rates are undesirable and can lead to non-response bias if responders and non-responders differ on factors related to the exposure, and if the distribution of responders and non-responders differs across comparison groups. Previous literature investigating non-response bias indicates that typical non-responders are young men, who smoke, and are of lower socioeconomic status[74-76]. In the current study, non-response was greater among controls and non-response bias may have been responsible for the larger effect observed in our study for active smoke exposure if the non-responders were more likely to smoke.

The possible non-response bias may also be associated with survival bias, which may occur if individuals who participate are healthier than individuals who refused to participate[93]. MS has several symptoms associated with the disease course, including cognitive impairments[10]. If cases exposed to cigarette smoke declined participation due to cognitive or memory impairments, the effect estimate may have been attenuated. Furthermore, if the MS cases that responded were healthier and less likely to smoke, the effect estimate would also have been attenuated. The EnvIMS study attempted to minimize survival bias due to cognitive impairment by only including MS cases with a relatively recent clinical onset (within the past 10 years).

Prevalent cases

In attempts to limit the number of cases with cognitive impairment, and limit poor recall, the EnvIMS study recruited MS cases with a clinical onset within 10 years of the study. Although these are the most recent onset cases, they are still prevalent cases, and there is a potential for survival bias if the cases that survive and

are included in the study were less likely to smoke. MS results in a decreasing quality of life, as opposed to being a life-threatening disease, and it is not likely that individuals within 10 years of disease onset would not have survived to be included in the EnvIMS study.

6.4.2 Misclassification

Recall bias is always a concern in case-control studies, in which disease status may affect reporting of exposures and may result in a differential misclassification of exposure[93]. The effect estimate may be exaggerated if disease status results in improved recall or provokes false recall among cases. On the other hand, the effect estimate may be attenuated if disease status results in impaired memory among cases.

Poor recall is also a concern when requiring individuals to report on past exposures. This results in a non-differential misclassification of exposure for cases and controls, which results in an underestimate of the effect. Previous literature has shown that recall of past active[94] and passive [95, 96]cigarette smoke exposure is reliable, indicating a low likelihood of poor recall.

One way to prevent poor recall and recall bias is through framing of the questions to promote recall[97]. The EnvIMS questionnaire ascertained smoking habits in 5-year age categories, priming individuals to consider specific periods in their life. Again, the EnvIMS study further tried to reduce recall bias due to cognitive impairments by selecting cases with only 10 years since clinical onset

6.4.3 Residual confounding

There is potential for residual confounding by unmeasured variables[98], and it is possible that the relationship between cigarette smoke exposure and MS may be confounded by other factors. Smoking could be a product of poor lifestyle habits, which may be associated MS, although studies investigating coffee and alcohol consumption and MS have only found a statistically significant negative effect for smoke exposure[5]. Studies investigating body mass index (BMI) have proven to be inconclusive with one study reporting increased risk of MS among women with high BMI[99], while another study reported a protective effect of high BMI[100].

Residual confounding may also result from misclassification of a confounder if the confounder is not measured properly. Adjusting for an inaccurate confounder is the same as not adjusting for a confounder, resulting in reduced precision. The ability to recall either parent's education is quite reliable, and it is unlikely that misclassification of this confounder occurred in the EnvIMS study.

6.4.4 Missing data

Data were assumed to be missing completely at random, and a case-wise deletion was used to handle any missing data. Missing data may bias the results if the proportion of missing data is differential among cases and controls. Given the means of dealing with missing data, selection bias may result if missing data for the exposure variable is different among cases and controls. This may result in an exaggerated effect estimate if controls are missing a greater proportion of smoke exposure data than cases, while the effect may be attenuated if the opposite is true. The EnvIMS resulted in less than 2% missing data for cases and controls when asked if they were ever a daily smoker.

Furthermore, a confounding variable that has missing data may result in a selection bias, given how we decided to use case-wise deletion for dealing with missing data. The use of mother's or father's highest level of education limited the missing data for the confounder parental education.

6.5 Future research and recommendations

6.5.1 Active cigarette smoke exposure

Evidence from the systematic review presented in Chapter 3 indicates that future research should focus on the role of intensity and duration of smoke exposure when investigating the relationship between smoke exposure and MS. Critical age periods may also be of interest, specifically the effect of exposure at younger ages. The lag time associated with smoke exposure could also be investigated, restricting smoke exposure to at least 5 years prior to MS onset which would allow sufficient time for smoke exposure to cause a biological effect.

6.5.2 Passive cigarette smoke exposure

More evidence is needed to confirm that passive smoke exposure is a risk factor for MS. There are currently four studies which have evaluated household passive smoke exposure as a risk factor for MS. Three of these found statistically significant harmful effect estimates, although two of these were not easily generalizable (one study investigated pediatric MS cases only[48], and one study investigated female nurses and their mothers[65]). Similar to active smoke exposure, critical time periods should also be investigated. One study investigating critical time periods for passive smoke exposure and lung cancer found a greater risk if individuals were exposed between 0 - 25 years of age as compared to exposures at ages > 25 years[101], and the same critical time periods may exist for MS.

6.5.3 Smoking behaviours

As mentioned in Chapter 2, data on smoking behaviours was collected in Norway starting in 1973, when 50% of men smoked and 30% of women smoked. Smoking behaviours have changed and in 2010 smoking prevalence was reported at an all time low of 20% among men and women combined. It may be beneficial to investigate the lag time between smoke exposure and MS incidence. In Norway, the incidence of MS has increased steadily from 1953-1997, only decreasing to an annual incidence of 3.0/ 100 000 in 1998-2002[102]. This decrease in MS incidence may be associated with the decrease in smoking behaviours in Norway that have been observed in the last 30 years and it will be of interest to see if MS incidence continues to decrease as smoking behaviours decrease.

As a recommendation, future studies should investigate how changes in population level smoking behaviours affect MS incidence, and the lag time associated with a decrease in smoking behaviours. If MS incidence declines over time, it may provide further evidence of smoke exposure as a risk factor for MS, although the lag time required to observe such an effect could be lengthy.

6.5.4 Other potential research

Interactions between other MS risk factors, EBV and vitamin D deficiency should also be investigated. In the past, studies have been underpowered to investigate interactions. Once completed, the EnvIMS study will be the largest case-control study collecting information on environmental risk factors for MS, and will be adequately powered to investigate interactions of potential environmental risk factors.

The biological plausibility for smoke exposure as a risk factor for MS should be further investigated focusing on the mechanism of components of cigarette smoke.

6.6 Conclusion

The results of this thesis suggest that active smoke exposure is a risk factor for MS. The role of passive smoke exposure in MS development is less clear, although there appears to be a relationship from the 3 studies that have published statistically significant effect estimates.

References

1. Doll R, Hill B. Smoking and carcinoma of the lung. *Br. Med. J.* 1950;221:739-748.

2. Ghadirian P, Dadgostar B, Azani R, Maisonneuve P. A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis. *Canadian Journal of Public Health.* 2001;92(4):281-285.

3. Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology.* Sep 2009;73(9):696-701.

4. Ragonese P, Castiglia G, Cusimano V, Battaglieri F, Salemi G. Cigarette smoking, coffee consumption and multiple sclerosis risk: A case-control study. *Acta Medica Mediterranea.* 2007;23(3):133-140.

5. Pekmezovic T, Drulovic J, Milenkovic M, et al. Lifestyle factors and multiple sclerosis: A case-control study in Belgrade. *Neuroepidemiology.* 2006;27(4):212-216.

6. Simon KC, van der Mei IAF, Munger KL, et al. Combined effects of smoking, anti-EBNA antibodies, and HLA-DRB1*1501 on multiple sclerosis risk. *Neurology.* Apr 2010;74(17):1365-1371.

7. Hernan MA, Olek MJ, Ascherio A. Cigarette smoking and incidence of multiple sclerosis. *Am. J. Epidemiol.* Jul 2001;154(1):69-74.

8. Riise T, Nortvedt MW, Ascherio A. Smoking is a risk factor for multiple sclerosis. *Neurology.* 2003;61(8):1122-1124.

9. Rothman KJ. Induction and latent periods. *Am. J. Epidemiol.* 1981;114(2):253-260.

10. Compston A, Coles A. Multiple sclerosis. *The Lancet.* 2008;372(9648):1502-1516.

11. Trapp B, Nave KL. Multiple sclerosis: An immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 2008;31:247-259.

12. Allison R, Millar J. Prevalence and familial incidence of disseminated sclerosis. *Ulster Med. J.* 1954;23:1-92.

13. Schumacher G, Beebe G, Kibler R, Kurland L, Kurtzke J, McDowell F. Problems of experimental trials of therapy in multiple sclerosis. *Annals of New York Academy of Sciences.* 1965;122:552-568.

14. McAlpine D, Lumsden C, Acheson E, eds. *Multiple sclerosis: a reappraisal.* Baltimore: Williams and Wilkins; 1972.

15. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis. *Ann. Neurol.* 1983;13(3):227-231.

16. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple aclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. *Ann. Neurol.* 2001;50:121-127.

17. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann. Neurol.* 2005;58:840-846.
18. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann. Neurol.* 2010;69:292-301.
19. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: Results of an international survey. *Neurology.* 1996;46:907-911.
20. Noseworthy JH, Lucchnetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N. Engl. J. Med.* 2000;343:938-952.
21. Courtney AM, Treadaway K, Remmington G, Frohman E. Multiple sclerosis. *Med. Clin. North Am.* 2009;93:451-476.
22. Miller DH, Leary SM. Primary-progressive multiple sclerosis. *The Lancet Neurology.* 2007;6(10):903-911.
23. *Atlas: Multiple sclerosis resources in the world.* Geneva: World Health Organization 2008.
24. Kurtzke JF. Multiple sclerosis in time and space - geographic clues to cause. *J. Neurovirol.* 2000;6(Supplement 2):S134-140.
25. Kurtzke J. Geography in multiple sclerosis. *J. Neurol.* 1977;215:1-26.
26. Hammond SR, English DR, McLeod JG. The age-range of risk of developing multiple sclerosis: Evidence from a migrant population in Australia. *Brain.* 2000;123:968-974.
27. Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. *Neurology.* 2004;62(60):60-66.
28. Handel AE, Giovannoni G, Ebers GC, Ramagopalan SV. Environmental factors and their timing in adult-onset multiple sclerosis. *Nature Reviews Neurology.* 2010;6:156-166.
29. Ness JM, Chabas D, Sadovnick AD, Phol D, Banwell B, Weinstock-Guttman B. Clinical features of children and adolescents with multiple sclerosis. *Neurology.* 2007;68(Supplement 2):S37-45.
30. Orton SM, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. *The Lancet Neurology.* 2006;5(11):932-936.
31. Alonso A, Hernan MA. Temporal trends in the incidence of multiple sclerosis. *Neurology.* 2008;71:129-137.
32. Whitacre CC. Sex differences in autoimmune disease. *Nature Immunology.* 2001;2(9):777-781.
33. Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *The Lancet Neurology.* 2004;3(2):104-110.
34. Ebers GC. Environmental factors in multiple sclerosis. *The Lancet Neurology.* 2008;7:268-277.
35. Raghuwanshi A, Joshi SS, Christakos S. Vitamin D and multiple sclerosis. *J. Cell. Biochem.* 2008;105:338-344.
36. Willer CJ, Dyment DA, Sadovnick AD, Rothwell PM, Murray TJ, Ebers GC. Timing of birth and risk of multiple sclerosis: population based study. *Br. Med. J.* 2005;330(7483):120-124.

37. Staples J, Ponsonby A-L, Lim L. Low maternal exposure to ultraviolet radiation in pregnancy, month of birth, and risk of multiple sclerosis in offspring: longitudinal analysis. *Br. Med. J.* 2010;340:c1640.
38. Kampman MT, Brustad M. Vitamin D: A candidate for the environmental effect in multiple sclerosis - Observations from Norway. *Neuroepidemiology.* 2008;30:140-146.
39. Hayes CE. Vitamin D: A natural inhibitor of multiple sclerosis. *Proc. Nutr. Soc.* 2000;59:531-535.
40. Ascherio A, Munger K. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann. Neurol.* 2007;61(4):288-299.
41. Bagert BA. Epstein-barr virus in multiple sclerosis. *Current Neurology and Neuroscience Reports.* 2009;9:405-410.
42. Lunemann JD, Kamradt T, Martin R, Munz C. Epstein-barr virus: Environmental trigger of multiple sclerosis. *J. Virol.* 2007;81(13):6777-6785
43. Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: A meta-analysis. *Ann. Neurol.* 2006;59(3):499-504.
44. Pohl D. Epstein-Barr virus and multiple sclerosis. *J. Neurol. Sci.* 2009;286:62-64.
45. Correa P, Fontham E, Williams Pickle L, Lin Y, Haenszel W. Passive smoking and lung cancer. *The Lancet.* 1983;233(8350):595-597.
46. Evans D, Levison M, Feldman C, et al. The impact of passive smoking on emergency room visits of urban children with asthma. *American Reviews of Respiratory Disease.* 1987;135(3):567-572.
47. Costenbader KH, Karlson EW. Cigarette smoking and autoimmune disease: What can we learn from epidemiology? *Lupus.* 2006;15:737-745.
48. Mikaeloff Y, Caridade G, Tardieu M, Suissa S. Parental smoking at home and the risk of childhood-onset multiple sclerosis in children. *Brain.* 2007;130(10):2589-2595.
49. Gardener H, Munger KL, Chitnis T, Michels KB, Spiegelman D, Ascherio A. Prenatal and Perinatal Factors and Risk of Multiple Sclerosis. *Epidemiology.* Jul 2009;20(4):611-618.
50. Ferrea S, Winterer G. Neuroprotective and neurotoxic effects of nicotine. *Pharmacopsychiatry.* 2009;42:255-265.
51. Hans FJ, Wei L, Bereczki D, et al. Nicotine increases microvascular blood flow and flow velocity in three groups of brain areas. *Am. J. Physiol.* 1993;265(6):H2142-2150.
52. Hawkins BT, Abbruscato TJ, Engleton RD, et al. Nicotine increases in vivo blood-brain barrier permeability and alters cerebral microvascular tight junction protein distribution. *Brain Res.* 2004;1027:48-58.
53. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urteger A, Lavi E, Brenner T. Activation of cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. *The Journal of Immunology.* 2009;183(10):6681-6688.
54. Hao J, Simard AR, Turner GH, et al. Attenuation of CNS inflammatory responses by nicotine involves α7 and non-α7nicotine receptors. *Exp. Neurol.* 2011;277:110-119.

55. Smedslund G, Ahn DK. Smoking initiation and cessation in Norway and the United States: A comparison of two cross-sectional surveys. *J. Subst. Abuse.* 1998;10(4):397-410.
56. *Smoking in Norway, 2004*: Statistics Norway;2004.
57. *Smoking habits 2010*: Statistics Norway;2011.
58. *Smoking in Norway - fact sheet.* Nydalen: Norwegian Institute of Public Health;2010.
59. *Norway's ban on smoking in bars and restaurants - A review of the first year.* Oslo: Directorate for Health and Social Affairs;2005.
60. Hawkes CH. Smoking is a risk factor for multiple sclerosis: a metanalysis. *Mult. Scler.* Jun 2007;13(5):610-615.
61. Jadad AR, Moore A, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? *Control. Clin. Trials.* 1996;17:1-12.
62. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. Paper presented at: Proceedings of the Third Symposium on Systematic Reviews. Beyond the basics: improving quality and impact; July 3-5, 2000; Oxford.
63. Karunananthan S, Wolfson C, Bergman H, Beland F, Hogan DB. A multidisciplinary systematic literature review on frailty: Overview of the methodology used by the Canadian Initiative on Frailty and Aging. *BMC Medical Research Methodolgy.* 12 October 2009;9(68).
64. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J. Clin. Epidemiol.* 2008;61:344-349.
65. Montgomery SM, Bahmanyar S, Hillert J, Ekbom A, Olsson T. Maternal smoking during pregnancy and multiple sclerosis amongst offspring. *Eur. J. Neurol.* 2008;15(12):1395-1399.
66. Antonovsky A, Leibowitz U, Smith HA, et al. Epidemiologic study of multiple sclerosis in Israel. *Arch. Neurol.* 1965;13:183-193.
67. Zorzon M, Zivadinov R, Nasuelli D, et al. Risk factors in multiple sclerosis: a case-control study. *Neurological Sciences.* 2003;24:242-247.
68. Brosseau L, Philippe P, Methot G, Duquette P, Haraoui B. Drug abuse as a risk factor of multiple sclerosis: case-control analysis and a study of heterogeneity. *Neuroepidemiology.* 1993;12(1):6-14.
69. Jafari N, Hoppenbrouwers IA, Hop WCJ, Breteler MMB, Hintzen RQ. Cigarette smoking and risk of MS in multiplex families. *Mult. Scler.* 2009;15(11):1363-1367.
70. Casetta I, Granieri E, Malagu S, et al. Environmental risk factors and multiple sclerosis: A community-based, case-control study in the province of Ferrara, Italy. *Neuroepidemiology.* 1994;13:120-128.
71. da Silva KRP, Alvarenga RMP, Fernandez OFY, Alvarenga H, Thuler LCS. Potential risk factors for multiple sclerosis in Rio de Janeiro: A case-control study. *Arq. Neuropsiquiatr.* 2009;67(2A):229-234.

72. Simpson CA, Newell DJ, Schapira K. Smoking and multiple sclerosis. *Neurology.* 1966;16(10):1041-1043.
73. Warren SA, Warren KG, Greenhill S, Paterson M. How multiple sclerosis is related to animal illness, stress and diabetes. *Can. Med. Assoc. J.* 1982;126:377-385.
74. Korkeila K, Suominen S, Ahvenainen J, et al. Non-response and related factors in a nation-wide health survey. *Eur. J. Epidemiol.* 2002;17:991-999.
75. Van Loon AJM, Tijhuis M, Picavet S, Surtees PG, Ormel J. Survey non-response in the Netherlands: Effects on prevalence estimates and associations. *Ann. Epidemiol.* 2003;13(2):105-110.
76. Kotaniemi J-T, Hassi J, Kataja M, et al. Does non-responder bias have a significant effect on the results in a postal questionnaire study? *Eur. J. Epidemiol.* 2002;17:809-817.
77. Aho K, Heliovaara M. Risk factors for rheumatoid arthritis. *Ann. Med.* 2004;36:242-251.
78. Wacholder S, Silverman DT, McLaughlin JK, Mandel JS. Selection of controls in case-control studies. III. Design options. *Am. J. Epidemiol.* 1992;135(9):1042-1050.
79. Villard-Mackintosh L, Vessey MP. Oral contraceptives and reproductive factors in multiple sclerosis incidence. *Contraception.* 1993;47(2):161-168.
80. Thorogood M, Hannaford PC. The influence of oral contraceptives on the risk of multiple sclerosis. *Br. J. Obstet. Gynaecol.* 1998;105(12):1296-1299.
81. Handel AE, Williamson AJ, Disanto G, Dobson R, Giovannoni G, Ramagopalan SV. Smoking and multiple sclerosis: An updated meta-analysis. *PLoS ONE.* 2011;6(1):e16149. doi:16110.11371/journal.pone.0016149.
82. Pugliatti M, Harbo HF, Holmoy T, et al. Environmental risk factors in multiple sclerosis. *Acta Neurol. Scand.* 2008;117:34-40.
83. Myhr KM, Grytten N, Aarseth J, Nyland H. The Norwegian Multiple Sclerosis National Competence Centre and National Sclerosis registry - a resource for clinical practice and research. *Acta Neurol. Scand.* 2006;113(Suppl. 183):37-40.
84. Barbeau EM, Krieger N, Soobader M-J. Working class matters: Socioeconoimic disadvantage, race/ethnicity, gender and smoking in NHIS 2000. *Am. J. Public Health.* 2004;94(2):269-278.
85. Huisman M, Kunst A, Mackenbach J. Educational inequalities in smoking among men and women aged 16 years and older in 11 European countries. *Tob. Control.* 2005;14:106-113.
86. MIller H, Ridley A, Schapira K. Multiple sclerosis: A note of social incidence. *British Medical Journal.* July 30, 1960 1930.
87. Hammond SR, McLeod JG, Macaskill P, English DR. Multiple sclerosis in Australia: socioeconomic factors. *J. Neurol. Neurosurg. Psychiatry.* 1996;61:311-313.
88. Russell WR. Multiple sclerosis: occupation and social group at onset. *The Lancet.* 1971;298(7729):823-834.

89. Antonovsky A, Leibowitz U, Medalie JM, Smith HA, Halpern L, Alter M. Reappraisal of possible etiologic factors in multiple sclerosis. *Am. J. Public Health Nations Health.* 1968;58(5):836-848.
90. Rosati G, Granieri E, Pinna L, Aiello I, De Bastiani P, Tola R. The geographical distribution of multiple sclerosis, rheumatoid arthritis, rheumatic heart disease and poststreptococcal nephritis in Sardinia: climatic and socioeconomic factors. *J. Neurol.* Sep 12 1978;219(1):27-35.
91. Galobardes B, Shaw M, Lawlor DA, Lynch JW, Smith GD. Indicators of socioeconomic position (part 1). *Journal of Epidemiology and Community Health.* 2006;60:7-12.
92. Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L. Exposure to environmental tobacco smoke is associated with increased risk for multiple sclerosis. *Multiple Sclerosis Journal.* 2011;17(7):788-793.
93. Rothman KJ, Greenland S, Lash TL. Case-control studies. *Modern Epidemiology*. 3 ed. Philadelphia: Lippincott, Williams & Wilkins; 2008:111.
94. Brigham J, Lessov-Schlaggar CN, Javitz HS, et al. Validity of recall of tabacco use in two prospective cohorts. *Am. J. Epidemiol.* 2010;172(7):828-835.
95. Brownson RC, Alavanja MCR, Hock ET. Reliability of passive smoke exposure histories in a case-control study of lung cancer. *Int. J. Epidemiol.* 1993;22(5):804-808.
96. Coultas DB, Peake GT, Samet JM. Questionnaire assessment of lifetime and recent exposure to environmental tobacco smoke. *Am. J. Epidemiol.* 1989;130(2):338-347.
97. Rothman KJ. Biases in study design. *Epidemiology: An introduction*. New York: Oxford University Press; 2002.
98. Szklo M, Nieto FJ. Identifying non-causal associations: Confounding. *Epidemiology: Beyond the basics*. 2 ed. Sudbury, MA: Jones and Bartlett Publishers; 2007:173.
99. Munger K, Chitnis T, Ascherio A. Body size and risk of MS in two cohorts of US women. *Neurology.* 2009;73:1543-1550.
100. Ghadirian P, Jain M, Ducic S, Shatenstein B, Morisset R. Nutritional factors in the aetiology of multiple sclerosis: a case-control study in Montreal, Canada. *Int. J. Epidemiol.* 1998;27:845-852.
101. Asomaning K, Miller DP, Liu G, et al. Second hand smoke, age of exposure and lung cancer risk. *Lung Cancer.* 2008;61:13-20.
102. Grytten N, Glad SB, Araseth JH, Nyland HI, Midgard R, Myhr KM. A 50-year follow up of the incidence of multiple sclerosis in Hordaland County, Norway. *Neurology.* 2006;66(2):182-186.

Appendix 1

Quality Assessment Forms

A. Case-control study quality assessment form

Paper #:
Author:

Case Ascertainment

Quality Assessment	Data Abstraction
1. Are all cases MS patients who have been clinically diagnosed by a physician? ☐ Yes ☐ No ☐ Unable to tell	How was MS diagnosed? (e.g. Poser criteria, McDonald criteria, self report)
2. More specifically, were the MS cases diagnosed by a neurologist? ☐ Yes ☐ No ☐ Unable to tell	
3. Are the MS cases representative of all cases with MS? ☐ Yes ☐ No ☐ Unable to tell	What is the source population of cases? Source and means of case recruitment: (E.g. Hospital: Consecutive, Random selection)
4. Are MS cases incident cases? ☐ Yes ☐ No ☐ Unable to tell	Number of Cases? If prevalent, cases were selected within how many years of diagnosis?

Control Ascertainment

Quality Assessment	Data Abstraction
5. Are controls drawn from the same source population as cases? ☐ Yes ☐ No ☐ Unable to tell	If no, What was the source population of controls? What kind of controls were used? (E.g. Hospital Based, Population Based)
6. Was it certain that controls did NOT have MS? ☐ Yes ☐ No ☐ Unable to tell	How many controls were there?
7. Was recruitment of cases and controls done over the same time period? ☐ Yes ☐ No ☐ Unable to tell	
8. Were cases and controls matched? ☐ Yes ☐ No ☐ Unable to tell	What variables were cases and controls matched on? [E.g. Sex, Age (+/- how many years)]
9. Is it possible that overmatching occurred, in that cases and controls were matched on factors related to exposure? ☐ Yes ☐ No ☐ Unable to tell	How many controls were there for each Case? (E.g. 1:1, 2:1, 4:1)
10. Was matching taken into account during the analysis? (I.e. Were appropriate statistical methods used) ☐ Yes ☐ No ☐ Unable to tell	What analysis was done? What other confounders were addressed/adjusted for in the analysis?

11. Were other confounders that were not matched on taken into account, through the analysis or restriction?
☐ Yes
☐ No
☐ Unable to tell

Smoke Exposure Ascertainment

Quality Assessment	Data Abstraction
12. Was smoking exposure clearly defined? ☐ Yes ☐ No ☐ Unable to tell	What was smoking exposure defined as? What was the method of smoke exposure? (E.g. Passive, active)
13. Was the method of ascertainment of smoking status same for cases and controls? ☐ Yes ☐ No ☐ Unable to tell	How was smoking status ascertained? (E.g. Biological markers, self-report, interview)
14. Was smoking status ascertainment done independent of case status (i.e. blinded to disease status)? ☐ Yes ☐ No ☐ Unable to tell	
15. Was it clear whether smoking exposure preceded onset of MS? ☐ Yes ☐ No ☐ Unable to tell	What levels of Smoking status were measured and what were the definitions for each level? (E.g. Current, ever, never)
16. Was the duration of smoke exposure assessed? ☐ Yes ☐ No ☐ Unable to tell	What were the durations of smoke exposure? (E.g. pack years, number of cigarettes smoked per day, years of smoke exposure)

Other data ascertainment:

Quality Assessment	Data Abstraction
17. What was the response rate of cases? ☐ _____% ☐ Not Reported What was the response rate of controls? ☐ _____% ☐ Not Reported	
18. Was the non-response rate the same for Cases and controls? ☐ Yes ☐ No ☐ Unable to tell	
19. Was there a sample size calculation done? ☐ Yes ☐ No ☐ Unable to tell	
20. Were there missing data? ☐ Yes ☐ No ☐ Unable to tell	How were missing data handled in the analysis?
21. If yes, was the missing data addressed in the analysis? ☐ Yes ☐ No ☐ Unable to tell	

B. Cross-sectional study quality assessment form

Paper #
Author:

Study population quality ascertainment

Quality Assessment	Data Abstraction
1. Was the sample population clearly described in terms of demographic characteristics: ☐ Yes ☐ No ☐ Unable to tell	What is the source population
2. Are the participants of the study representative of the population from which they were recruited? ☐ Yes ☐ No ☐ Unable to tell	How was the study sample selected? (random, haphazard, consecutive patients)
3. Were study participants selected at random? ☐ Yes ☐ No ☐ Unable to tell	Inclusion/exclusion criteria
4. Was the study inclusion/exclusion criteria clearly described? ☐ Yes ☐ No ☐ Unable to tell	
5. What was the response rate? ☐ _____ ☐ Unable to tell	

Smoke exposure ascertainment

Quality Assessment	Data Abstraction
6. Was smoking exposure clearly defined? ☐ Yes ☐ No ☐ Unable to tell	What was the method of smoke exposure? (E.g. Passive, active) How was smoking status ascertained? (E.g. Biological markers, self-report, interview)
7. Was it clear whether smoking exposure preceded onset of MS? ☐ Yes ☐ No ☐ Unable to tell	What levels of Smoking status were measured and what were the definitions for each level? (E.g. Current, ever, never)
8. Was the dosage/duration of smoke exposure assessed? ☐ Yes ☐ No ☐ Unable to tell	What were the dosage/durations of smoke exposure? (E.g. pack years, number of cigarettes smoked per day, years of smoke exposure)

Outcome (MS) ascertainment

Quality Assessment	Data Abstraction
9. Have all MS cases been clinically diagnosed by a physician, or a neurologist? ☐ Yes, neurologist ☐ Yes, physician ☐ No ☐ Unable to tell	How was MS diagnosed? (E.g. Poser criteria, McDonald criteria, self report)
10. Was MS ascertainment influenced by knowledge of the exposure status (lack of blinding)? ☐ Yes ☐ No ☐ Unable to tell	

Other data ascertainment

11. Were potential confounders measured and adequately addressed in the analysis? ☐ Yes ☐ No ☐ Unable to tell	Confounders adjusted for:
12. Were there missing data? ☐ Yes ☐ No ☐ Unable to tell	
13. If yes, were the missing data handled appropriately? ☐ Yes ☐ No ☐ Unable to tell	
14. Was the statistical analysis appropriate: ☐ Yes ☐ No ☐ Unable to tell	Statistical analysis preformed:
15. Was a sample size calculation done? ☐ Yes ☐ No ☐ Unable to tell	

C. Cohort study quality assessment form

Paper #
Author:

Baseline study entry data ascertainment

Quality Assessment	Data Abstraction
1. Do the authors clearly describe the population from which the participants were drawn? ☐ Yes ☐ No	What was the time period for recruitment? (Simply for data abstraction, the years devoted to recruitment)
2. Are the participants in the study representative of the population from which they were recruited? ☐ Yes ☐ No ☐ Unable to tell	Study Population:
3. Were study inclusion/exclusion criteria clearly described? ☐ Yes ☐ No ☐ Unable to tell	Inclusion/exclusion criteria:
4. What proportion of eligible subjects was included in the study? ☐ _____ ☐ Unable to tell	What made participants ineligible?
5. Were efforts taken to confirm participants were free of MS at baseline? ☐ Yes ☐ No ☐ Unable to tell	
6. Was lifetime history of smoking exposure assessed at baseline? ☐ Yes ☐ No ☐ Unable to tell	How was prior smoking exposure ascertained? (age at starting smoking; number of years of pre-cohort smoking exposure)
7. Was amount of lifetime history of smoking exposure assessed at baseline? ☐ Yes ☐ No ☐ Unable to tell	How was amount of smoke exposure ascertained? (pack years; # of cigarettes/day/year)

Follow-up data ascertainment:

Quality Assessment	Data Abstraction
9. Was a reasonable proportion of the baseline sample followed up? ☐ _____% followed up ☐ Unable to tell	Length of follow up: (mean, median, range of follow-up):
10. Have the characteristics of participants lost to follow up been described? ☐ Yes ☐ No ☐ Unable to tell	Characteristics of those lost to follow up:
11. Was there any indication that loss to follow up was associated with smoking exposure? ☐ Yes ☐ No ☐ Unable to tell	How was smoking status ascertained at follow up? (Biological Markers, self-report, interview)
12. Was duration of smoking exposure ascertained for time between follow-ups? ☐ Yes ☐ No ☐ Unable to tell	What was the smoking duration between follow-ups? (Yes, smoked between follow up; quit during follow up etc.)

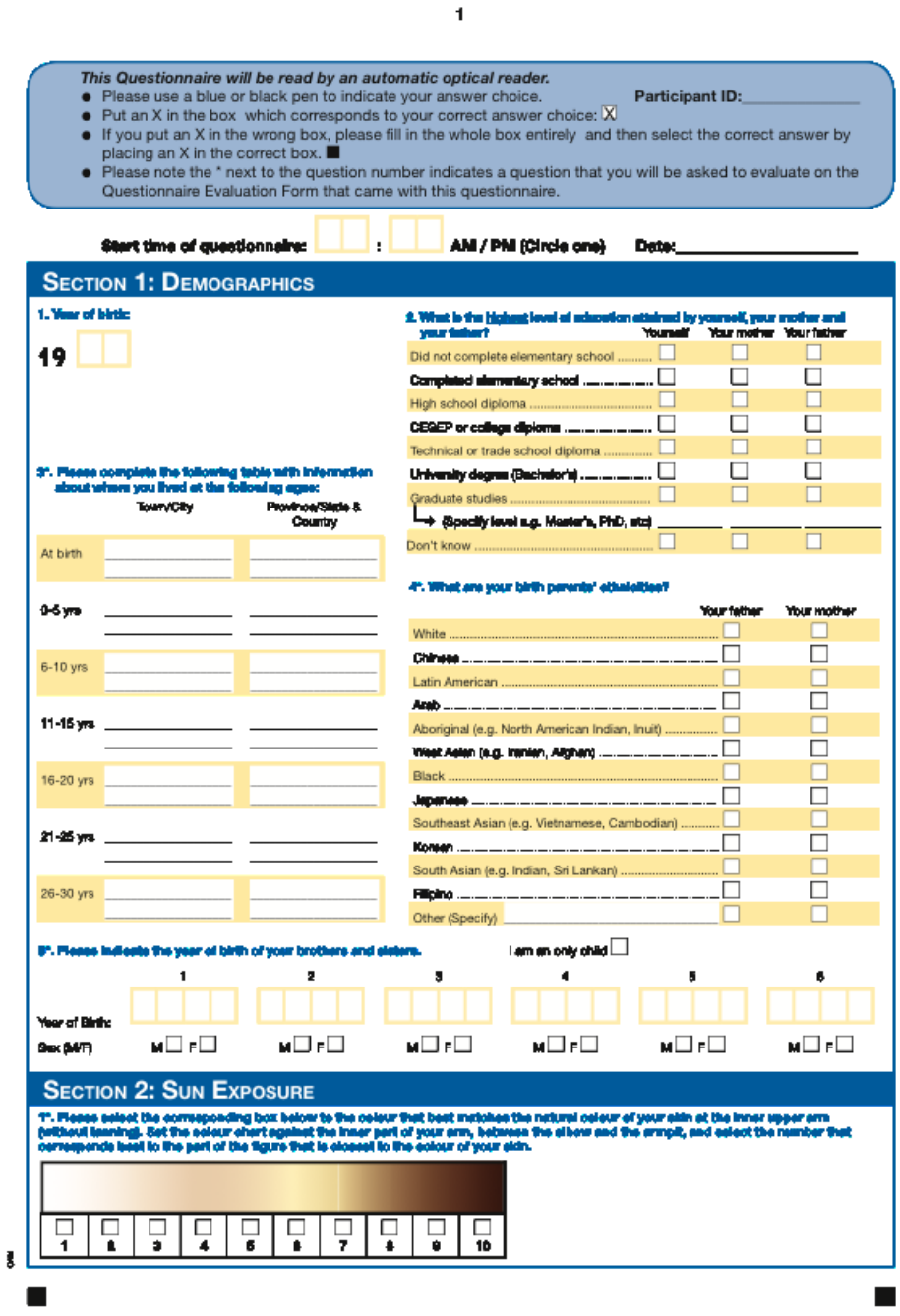
13, Was amount of smoke exposure assessed at each follow up? ☐ Yes ☐ No ☐ Unable to tell	How was amount of smoke exposure ascertained during follow up? (E.g. number of cigarettes smoked/day; pack years; etc)
14. Were all participants who developed MS clinically diagnosed by a physician, or a neurologist? ☐ Yes, neurologist ☐ Yes, physician ☐ No ☐ Unable to tell	How was MS status identified at follow up? (Self-report; medical records; clinical tests; etc) How was MS diagnosed? (E.g. Poser criteria, McDonald criteria, self report)
15. Was follow up long enough for MS outcome to occur? ☐ Yes ☐ No ☐ Unable to tell	Was MS diagnosed the same way at all follow up points? How often was MS status reviewed? (Yearly; biennially; every 5 years)

Other data ascertainment:

16. Was the statistical analysis appropriate: ☐ Yes ☐ No ☐ Unable to tell	Statistical analysis preformed:
17. Were potential confounders adequately addressed in the analysis? ☐ Yes ☐ No ☐ Unable to tell 18. Did the analytic techniques take into account different lengths of follow up of participants? ☐ Yes ☐ No ☐ Unable to tell	Confounders adjusted for:
19. Were missing data handled appropriately? (I.e. case deletion, imputation methods)- ☐ Yes ☐ No ☐ Unable to tell ☐ Not Applicable/ No missing data	How were missing data handled in the analyses?
20. Was there a sample size calculation done? ☐ Yes ☐ No ☐ Unable to tell 21. Did the study have sufficient power to detect clinically meaningful difference in the outcomes: ☐ Yes ☐ No ☐ Unable to tell	Sample size for different analyses and subgroups:

Appendix 2

English version of the EnvIMS questionnaire



1

This Questionnaire will be read by an automatic optical reader.

- Please use a blue or black pen to indicate your answer choice.
- Put an X in the box which corresponds to your correct answer choice: X
- If you put an X in the wrong box, please fill in the whole box entirely and then select the correct answer by placing an X in the correct box. ■
- Please note the * next to the question number indicates a question that you will be asked to evaluate on the Questionnaire Evaluation Form that came with this questionnaire.

Participant ID:_____

Start time of questionnaire: ☐☐ : ☐☐ AM / PM (Circle one) Date:_____

SECTION 1: DEMOGRAPHICS

1. Year of birth:

19 ☐☐

2. What is the highest level of education attained by yourself, your mother and your father?

	Yourself	Your mother	Your father
Did not complete elementary school	☐	☐	☐
Completed elementary school	☐	☐	☐
High school diploma	☐	☐	☐
CEGEP or college diploma	☐	☐	☐
Technical or trade school diploma	☐	☐	☐
University degree (Bachelor's)	☐	☐	☐
Graduate studies	☐	☐	☐
↳ (Specify level e.g. Master's, PhD, etc)	_____	_____	_____
Don't know	☐	☐	☐

3*. Please complete the following table with information about where you lived at the following ages:

	Town/City	Province/State & Country
At birth	_____	_____
0-5 yrs	_____	_____
6-10 yrs	_____	_____
11-15 yrs	_____	_____
16-20 yrs	_____	_____
21-25 yrs	_____	_____
26-30 yrs	_____	_____

4*. What are your birth parents' ethnicities?

	Your father	Your mother
White	☐	☐
Chinese	☐	☐
Latin American	☐	☐
Arab	☐	☐
Aboriginal (e.g. North American Indian, Inuit)	☐	☐
West Asian (e.g. Iranian, Afghan)	☐	☐
Black	☐	☐
Japanese	☐	☐
Southeast Asian (e.g. Vietnamese, Cambodian)	☐	☐
Korean	☐	☐
South Asian (e.g. Indian, Sri Lankan)	☐	☐
Filipino	☐	☐
Other (Specify) _____	☐	☐

5*. Please indicate the year of birth of your brothers and sisters. I am an only child ☐

	1	2	3	4	5	6
Year of Birth:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Sex (M/F)	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐	M☐ F☐

SECTION 2: SUN EXPOSURE

1*. Please select the corresponding box below to the colour that best matches the natural colour of your skin at the inner upper arm (without tanning). Set the colour chart against the inner part of your arm, between the elbow and the armpit, and select the number that corresponds best to the part of the figure that is closest to the colour of your skin.

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10

2*. What is the tanning reaction of your skin to its first sun exposure in the summer, with no use of sunscreen?

1. Always burn, never tan ☐
2. Usually burn, tan less than average (with difficulty) ☐
3. Sometimes mild burn, tan about average ☐
4. Rarely burn, tan more than average (with ease) ☐
5. Don't know ☐

3*. What is the natural colour of your hair as an adult?

1. Black ☐
2. Dark Brown ☐
3. Light Brown ☐
4. Blonde ☐
5. Red ☐

4*. What colour are your eyes?

1. Black ☐
2. Brown ☐
3. Grey, green ☐
4. Blue ☐
5. Hazel ☐

5*. In the past, in summer, how often did your activities (playing, participating in sports, watching sports, gardening, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐
In the past 3 years	☐	☐	☐	☐

6*. In the past, in winter, how often did your activities (playing, participating in sports, watching sports, shovelling snow, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐
In the past 3 years	☐	☐	☐	☐

7*. At the following ages, where have your work and occupational activities (including parenting, caregiving, etc.) been carried out

	Mainly indoors	Mainly outdoors	Same time spent indoors and outdoors
16-20 yrs	☐	☐	☐
21-25 yrs	☐	☐	☐
26-30 yrs	☐	☐	☐

8*. On weekends and holidays, how much time did you normally spend outside at these ages:

	Never	<1 hour/day	1-2 hours/day	3-4 hours/day	>4 hours/day
0-5 yrs	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐	☐

9*. How often did you go on vacation to sunny places during winter months at these ages?

	Never/seldom	1 week/year or less	1-2 weeks/year	4+ weeks/year
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

10*. How often did you use sun protection (sunscreen or protective clothing such as hats, long sleeves) at these ages?

	Never/seldom	Sometimes	Quite often	Almost always
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

11. How often did you use sunlamps or tanning beds at these ages?

	Never/Seldom	Less than once/year	Less than once/month	Once or more/month
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

SECTION 3: DIET

We would like to ask you information about your diet when you were a "teenager" (between 13 and 19 years old). If your diet changed substantially during this period of time, please try to report the average consumption for the period.

1*. Please indicate in which season(s) you generally consumed the following foods while you were a teenager (age 13-19 years)?
(you may choose more than one checkbox per row).

	Winter	Spring	Summer	Fall	Never/ seldom
Cows' milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐
Fresh cheeses (e.g. fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐
Aged cheeses (e.g. Parmesan, strong cheddar)	☐	☐	☐	☐	☐
Smoked cheeses	☐	☐	☐	☐	☐
Other cheeses (e.g. cheddar, marble, feta, havarti, mozzarella, Monterey jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐
Red meat (e.g. Beef, lamb, venison, bison) or Cold cuts (of all types)	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐
Hot dogs, frankfurters, weiners	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐
Shellfish					
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐

2a*. Please indicate how often you generally consumed the following foods while you were a teenager (age 13-19 years).
(please select only one box per row).

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Cows' milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐	☐
Fresh cheeses (e.g. fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐	☐
Aged cheeses (e.g. Parmesan, strong cheddar)	☐	☐	☐	☐	☐	☐

Continues next page

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Smoked cheeses	☐	☐	☐	☐	☐	☐
Other cheeses (e.g. cheddar, marble, feta, havarti, mozzarella, Monterey jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐	☐
Red meat (e.g. Beef, lamb, venison, bison) or Cold cuts (of all types)	☐	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐	☐
Hot dogs, frankfurters, weiners	☐	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐	☐
Shellfish						
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐	☐

2b*. We are particularly interested in how often you consumed the following specific types of fish as a teenager:

	Never	Less than once/month	1-3 times/ month	Once/week	2-3 times/ week	More than 3 times/week
Canned salmon	☐	☐	☐	☐	☐	☐
Fresh or frozen salmon (not including smoked)	☐	☐	☐	☐	☐	☐
Canned tuna	☐	☐	☐	☐	☐	☐
Fresh or frozen tuna	☐	☐	☐	☐	☐	☐
Trout, Carp	☐	☐	☐	☐	☐	☐
Halibut	☐	☐	☐	☐	☐	☐
Sardines, anchovies	☐	☐	☐	☐	☐	☐
Fresh or frozen mackerel	☐	☐	☐	☐	☐	☐
Cod	☐	☐	☐	☐	☐	☐
Herring	☐	☐	☐	☐	☐	☐
Grouper, swordfish	☐	☐	☐	☐	☐	☐
Flounder, sole, smelt	☐	☐	☐	☐	☐	☐
Pickerel, snapper, perch	☐	☐	☐	☐	☐	☐
Other: specify _____	☐	☐	☐	☐	☐	☐

3*. What type of water did you usually drink when you were a teenager? *(you can check more than one box per row)*

	No Consumption	For drinking	For cooking	To make coffee/tea/hot drinks	Don't remember
Well water, spring water	☐	☐	☐	☐	☐
Tap water	☐	☐	☐	☐	☐
Bottled water	☐	☐	☐	☐	☐
Don't know	☐	☐	☐	☐	☐

4*. How often did you use the following condiments and oils as a teenager (including as dressings, or sauces, and for cooking)?
(Please check only one box per row)

	Never	Less than once/ mth	1-3 times/ mth	Once/ week	2-3 times/ week	4-6 times/ week	More than 6 times/week
Butter	☐	☐	☐	☐	☐	☐	☐
Margarine	☐	☐	☐	☐	☐	☐	☐
Lard	☐	☐	☐	☐	☐	☐	☐
Mayonnaise	☐	☐	☐	☐	☐	☐	☐
Vegetable oil							
(i) Corn, sesame, walnut, sunflower, rapeseed, safflower oil	☐	☐	☐	☐	☐	☐	☐
(ii) Canola, peanut, olive, coconut, avocado, almond oil	☐	☐	☐	☐	☐	☐	☐
(iii) Other vegetable oils. Specify: _____	☐	☐	☐	☐	☐	☐	☐

5*. Did you take any of the following dietary supplements while you were a teenager?

	Yes	No	Don't know
Cod liver oil liquid	☐	☐	☐
Cod liver oil capsules	☐	☐	☐
Fish oil capsules	☐	☐	☐
Multivitamins	☐	☐	☐
Calcium	☐	☐	☐
Vitamin B12	☐	☐	☐
Vitamin C	☐	☐	☐
Vitamin D	☐	☐	☐

6*. Please report what type of milk you were given when you were a baby. *(You can select more than one box per column and row).*

	At Birth	From 1-3 mths	From 4-6 mths	From 7-9 mths	From 10 mths & older
Breast milk	☐	☐	☐	☐	☐
Artificial formula	☐	☐	☐	☐	☐
Other milk (e.g. cow, soy, etc.) Specify:_____	☐	☐	☐	☐	☐
Don't know	☐	☐	☐	☐	☐

SECTION 4: MEDICAL HISTORY

The following questions concern illnesses that you may have had when you were younger.

1*. Please indicate at what age you had the following illnesses or surgical interventions. To help you remember, think about which school grade you were in when you had the illness/surgery. Check all that apply.

	Didn't have	Don't know	Did have	Age at diagnosis: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Tonsillectomy (tonsil removal)	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Measles	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Mumps	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Rubella (German Measles)	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Chicken pox	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Pneumonia (check as many times as applies)	☐	☐	☐→	☐	☐	☐	☐	☐	☐

2a*. Have you had infectious mononucleosis (also called "mono" or "the kissing disease")?

Yes ☐ →go to question 2b

No	Don't know
☐	☐

→ If no or don't know, skip to question 3a

2b*. If yes, do you recall if you had a blood test done to verify the diagnosis?

Yes	No	Don't remember
☐	☐	☐

2c. At what age did you have mononucleosis?

0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐	☐	☐	☐

3a*.Do you remember in which month you were diagnosed with mono?

☐☐ 01-12 → If you know the month skip to question #4.

3b. If you don't remember the exact month, can you recall in which season you had mono?

Spring	Summer	Fall	Winter	Don't Remember
☐	☐	☐	☐	☐

4. Have you ever had a urinary tract infection (UTI)? If yes, please give your best estimate of the age(s) when it/they occurred.

No	Don't know	Yes	Ages when UTI occurred. *(you can check more than one box in the same row)*: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐→	☐	☐	☐	☐	☐	☐

5*. Have you ever had a parasitic infection (eg. tenia or tapeworm, oxiuri, ascarides, giardia, cryptosporidium, etc.)? If yes, please give your best estimate of your age when it first occurred.

No	Don't know	Yes	Age of first infection: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐→	☐	☐	☐	☐	☐	☐

6*. Do you have a history of allergy (such as conjunctivitis or red itchy watery eyes, rhinitis or runny nose, eczema, hives, asthma) to any of the following? If yes, please estimate the approximate age at which you experienced the first symptoms (i.e., when did the allergies begin?)

	No	Don't know	Yes	0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Pollens	☐	☐	☐→	☐	☐	☐	☐	☐	☐
House dust	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Animal dander / fur	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Any food	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Other allergies Specify: _____	☐	☐	☐→	☐	☐	☐	☐	☐	☐

7. Has a doctor ever told you that you had any of the following disorders?

	No	Don't know	Yes	Age at first diagnosis		No	Don't know	Yes	Age at first diagnosis	
Systemic Lupus erythematosus (Lupus)	☐	☐	☐→		yrs	Celiac disease	☐	☐	☐→	yrs
Rheumatoid arthritis	☐	☐	☐→		yrs	Psoriasis	☐	☐	☐→	yrs
Hypothyroidism	☐	☐	☐→		yrs	Leukemia	☐	☐	☐→	yrs
Hyperthyroidism	☐	☐	☐→		yrs	Hodgkin's lymphoma	☐	☐	☐→	yrs
Multiple sclerosis	☐	☐	☐→		yrs	Non Hodgkin's lymphoma	☐	☐	☐→	yrs
Optic neuritis	☐	☐	☐→		yrs	Melanoma skin cancer	☐	☐	☐→	yrs
Crohn's disease	☐	☐	☐→		yrs	Non-melanoma skin cancer	☐	☐	☐→	yrs
Ulcerative colitis	☐	☐	☐→		yrs	Kidney disorders	☐	☐	☐→	yrs
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐→		yrs	Other medical disorders, specify: _____	☐	☐	☐→	yrs

8. To your knowledge, does anyone in your family have a history of any of the following diseases?

	No	Father	Mother	Brother/Sister	Child	Don't know
Systemic lupus erythematosus (lupus)	☐	☐	☐	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐	☐	☐	☐
Hypothyroidism	☐	☐	☐	☐	☐	☐
Hyperthyroidism	☐	☐	☐	☐	☐	☐
Multiple sclerosis	☐	☐	☐	☐	☐	☐
Optic neuritis	☐	☐	☐	☐	☐	☐
Crohn's disease	☐	☐	☐	☐	☐	☐
Ulcerative colitis	☐	☐	☐	☐	☐	☐
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐	☐	☐	☐
Celiac disease	☐	☐	☐	☐	☐	☐
Psoriasis	☐	☐	☐	☐	☐	☐
Leukemia	☐	☐	☐	☐	☐	☐
Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐
Non Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐

SECTION 5: SMOKING HABITS AND LIFESTYLE FACTORS

1. Have you ever been a regular smoker ("regular" = smoked one or more cigarettes per day for 6 months or longer)?

Yes ☐ No ☐→ If your answer is no skip to question #5

2. If yes, how many cigarettes per day on average did you smoke at the following ages?

	Never smoked	1-4 cig./day	5-10 cig./day	11-20 cig./day	21+ cig./day
11-15 yrs	☐	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐	☐

3. At what age did you begin to smoke cigarettes daily? (Age)

4. How many years have you smoked in total? (Total years)

5. Did your mother smoke while she was pregnant with you?

No ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did she smoke?

< 10 ☐ 10+ ☐

6. Did your father smoke inside the house when you were a child?

He was a non smoker ☐ No, he didn't ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did he smoke inside the house?

< 10 ☐ 10+ ☐

7. Did your mother smoke inside the house when you were a child?

She was a non smoker ☐ No, she didn't ☐ Don't know ☐ Yes ☐ → How many cigarettes per day did she smoke inside the house?

< 10 ☐ 10+ ☐

8. Did you live with anybody else who smoked inside the house before you were age 21?

No ☐ Yes ☐ →

Who?	How many cigarettes a day were smoked inside the house?	
Brother ☐	< 10 ☐	10+ ☐
Sister ☐	< 10 ☐	10+ ☐
Other ☐	< 10 ☐	10+ ☐

9. Did you live with anybody else who smoked inside the house when you were between the ages of 21-25?

No ☐ Yes ☐ → How many cigarettes per day were smoked inside the house?

< 10 ☐ 10+ ☐

10. Did you live with anybody else who smoked inside the house when you were between the ages of 26-30?

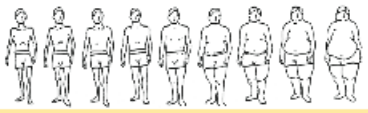
No ☐ Yes ☐ → How many cigarettes per day were smoked inside the house?

< 10 ☐ 10+ ☐

11. Have you ever worked in an environment where someone regularly smoked inside your workplace?

No ☐ Yes ☐

12*. MEN and WOMEN. What figure best depicts the shape of your body at the different ages. Also, please indicate approximately how long you were in this shape?



	# of years
At 5-years	
At 10-years	
At 15-years	
At 20-years	
At 25-years	
At 30-years	
Today	

13. What is your current weight? (Pounds) or (Kilograms)

14. What is your current height? (Feet & Inches) or (Centimeters)

15. What was your level of physical activity per week when you were a teenager (between 13 and 19 years old)? (For example, light physical activities refer to activities that require light physical effort such as walking leisurely, stretching, vacuuming or light yard work. Vigorous physical activities refer to activities that take heavy physical effort such as jogging, running, stair machine, sports (e.g. tennis, basketball, soccer, etc.)).

	None	< once/week	1-2 times/week	3 or more times/week
Light physical activity (your heart beats slightly faster than normal)	☐	☐	☐	☐
Vigorous physical activity (your heart rate increases a lot)	☐	☐	☐	☐

SECTION 6: HORMONAL FACTORS — WOMEN ONLY.
Men, please proceed to the last question (#14) on page 8.

1*. How old were you when you started menstruation? (started getting your period)? ☐☐ (Age)

2*. Are you pregnant now? Yes ☐ No ☐

3*. Have you ever been pregnant? Yes ☐ No ☐→ If no skip to question #5.

4*. If yes, please provide the following information on the outcome of each pregnancy and the year(s).

	1st pregnancy	2nd pregnancy	3rd pregnancy	4th pregnancy	5th pregnancy	6th pregnancy
Born alive	☐	☐	☐	☐	☐	☐
Breastfeeding for at least 1 month	☐	☐	☐	☐	☐	☐
Lost pregnancy (spontaneous or induced abortion, interuterine death, still born)	☐	☐	☐	☐	☐	☐
Lost at # weeks:	_____	_____	_____	_____	_____	_____
Year of outcome:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐

5*. Have you ever undergone hormonal treatment for infertility? Yes ☐ No ☐→ If no skip to question #7.

6*. If yes, please indicate the year(s) you received treatment and the number of cycles per year.

Year:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
No. of cycles/year:	☐☐	☐☐	☐☐	☐☐	☐☐

7*. Have you ever used a birth control pill (not the "mini-pill" that contains progesterone only, but the type that is taken for 3 weeks, followed by 1 week replacement with "sugar-pills"), hormonal patches, vaginal hormonal rings, or hormonal intrauterine devices (IUD)?
Yes ☐ No ☐→ If no skip to question #10.

8*. How old were you when you started using these contraceptives? ☐☐ Age

9*. For how long did you/have you used these contraceptives?

<1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

10*. Have you ever suffered from hirsutism, that is, from an excess of coarse hair in areas of the body where it is not normally found (e.g. face, chest, back, abdomen)? Yes ☐ Don't know ☐ No ☐→ If no/don't know skip to last question #14.

11. If yes, have you ever been given hormonal therapies to treat this? Yes ☐ No ☐→ If no skip to last question #14.

12. At what age did you start these therapies? ☐☐ Age

13. For how long did you take these therapies?

<1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

14. Lastly, we would like to know if someone else helped you fill out the questionnaire.

No ☐	Yes ☐→ Who?	Mother	Father	Sibling	Husband/Wife	Child
		☐	☐	☐	☐	☐

End time of completed questionnaire: ☐☐ : ☐☐ **AM / PM (Circle one)**

Thank you for your participation!