SMOKING AND MULTIPLE SCLEROSIS: A SYSTEMATIC REVIEW AND
META-ANALYSIS USING THE BRADFORD HILL CRITERIA FOR CAUSATION

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by

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Michelle Leigh Degelman, candidate for the degree of Master of Science in Kinesiology and Health Studies, has presented a thesis titled, *Smoking and Multiple Sclerosis: A Systematic Review and Meta-Analysis Using the Bradford Hill Criteria for Causation*, in an oral examination held on December 14, 2016. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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*Via teleconference*
Abstract

**Background.** Multiple sclerosis (MS) is one of the most common neurological disorders in the world. However, the cause(s) of MS is unknown. Studies have suggested that smoking may be related to the development and progression of MS. The Bradford Hill criteria are commonly used to assess potentially causal associations. However, to date, no systematic reviews and meta-analyses had used these criteria to comprehensively evaluate the relationship between smoking and MS. The objective of this research was to assess the relationship between smoking and both MS risk and MS progression through a systematic review and meta-analysis, subsequently applying Hill’s criteria to further assess the likelihood of these associations being causal. **Methods.** Databases including Medline, EMBASE, CINAHL, PsycInfo, and Cochrane Library were searched for relevant studies up until July 28, 2015. A random-effects meta-analysis was conducted for three outcomes: MS risk, conversion from clinically isolated syndrome (CIS) to clinically definite multiple sclerosis (CDMS), and progression from relapsing-remitting multiple sclerosis (RRMS) to secondary-progressive multiple sclerosis (SPMS). Consideration was given to dose-response relationships and risk factor interactions, and discussions of mechanisms and analogous associations presented in the studies. Hill’s criteria were applied to assess causality of the relationships between smoking and each outcome. The effect of second-hand smoke exposure was also briefly reviewed. **Results.** Smoking had a statistically significant association with both MS risk (conservative: OR/RR 1.54, 95% CI [1.46-1.63]) and SPMS risk (HR 1.80, 95% CI [1.04-3.10]), but the association with progression from CIS to CDMS was non-significant (HR 1.13, 95% CI [0.73-1.76]). Based on Hill’s criteria, there was strong evidence of a causal role of
smoking in MS risk, but only moderate evidence of a causal association between smoking and MS progression. Heterogeneity in study designs and target populations, inconsistent results, and an overall scarcity of studies point to the need for more research in the area of second-hand smoke exposure in relation to MS prior to conducting a detailed meta-analysis. **Conclusion.** This first review to supplement systematic review and meta-analytic methods with the application of Hill’s criteria to analyze the smoking-MS association provided evidence supporting the causal involvement of smoking in the development and progression of MS. As such, smoking cessation programs and policies should highlight MS as an additional health risk to deter the public from smoking.

**Keywords:** Multiple sclerosis; cigarette smoking; clinically isolated syndrome; Bradford Hill
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<th>Description</th>
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<tbody>
<tr>
<td>CCSVI</td>
<td>Chronic Cerebrospinal Venous Insufficiency</td>
</tr>
<tr>
<td>CDMS</td>
<td>Clinically Definite Multiple Sclerosis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CIS</td>
<td>Clinically Isolated Syndrome</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr Virus</td>
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<tr>
<td>GRADE</td>
<td>Grading of Recommendations Assessment, Development, and Evaluation</td>
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<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<tr>
<td>HR</td>
<td>Hazard Ratio</td>
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<tr>
<td>IV</td>
<td>Inverse Variance</td>
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<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>NARCOMS</td>
<td>North American Research Committee on Multiple Sclerosis</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PPMS</td>
<td>Primary-Progressive Multiple Sclerosis</td>
</tr>
<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic Reviews and Meta-Analyses</td>
</tr>
<tr>
<td>RR</td>
<td>Risk Ratio</td>
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<tr>
<td>RRMS</td>
<td>Relapsing-Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
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<tr>
<td>SPMS</td>
<td>Secondary-Progressive Multiple Sclerosis</td>
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CHAPTER 1: General Introduction

1.1 Introduction

Multiple sclerosis (MS) is a common neurological disorder of the central nervous system (CNS). The prevalence of MS in Canada is one of the highest in the world at 291 cases per 100,000 people compared to the global median prevalence of 33 cases per 100,000 people. MS is an extremely unpredictable disease with a challenging diagnosis as it exhibits many different symptoms and a variable disease course. Furthermore, the disease has been shown to have a profound adverse effect on the well-being of patients, family members, and society in general.

The cause of MS remains unknown. However, a number of factors, including genetics, infectious agents, and lifestyle behaviours, have been shown to increase one’s risk of developing MS. Included among lifestyle behaviours is smoking, a risk factor that has been increasingly studied over the past decade.

The Bradford Hill criteria for causation encompass a list of nine criteria that are widely known for evaluating causation between an exposure and a given outcome. Five previous systematic reviews and meta-analyses, as well as two systematic reviews without meta-analyses, have been published that examine the relationship between smoking and MS. However, none of the reviews methodically used Hill’s criteria to evaluate the relationship between smoking and MS. While two recent narrative reviews used Hill’s criteria to examine this relationship, they lacked systematic methods. Conducting an updated systematic review and meta-analysis and subsequently using Hill’s criteria to examine the association between smoking and MS would provide
valuable insight into this relationship as this combination of methods makes it possible to evaluate causation at a more comprehensive level.

1.1.1 Definitions and Concepts

*MS* is an autoimmune disorder of the CNS.\(^1\) The disease process is primarily characterized by peripheral immune activation whereby activated immune cells in the blood cross the blood-brain barrier, triggering an immune reaction that leads to patchy myelin damage and axonal injury.\(^1\) The demyelination, inflammation, and scarring are what characterize lesions (or plaques).\(^1\) Myelin, the tissue that wraps around the nerve axons in the CNS, is responsible for conducting impulses quickly along nerves to different parts of the body, but lesions disrupt this conduction which ultimately leads to the symptoms of MS.\(^1\)

A *risk factor*, a term first coined by the original investigators of the Framingham Heart Study,\(^2^2\) is defined as an “exposure, behaviour, or attribute that, if present and active, clearly increases the probability of a particular disease in a group of people who have the risk factor compared with an otherwise similar group of people who do not”.\(^2^3\) A *cause* of a particular disease has been defined as an “antecedent event, condition, or characteristic that was necessary for the occurrence of the disease at the moment it occurred, given that other conditions are fixed”.\(^2^4\)

*Smoking* is generally described as “the inhalation of the smoke of burning tobacco of cigarettes, pipes or cigars, [which] may be an occasional habit or, more often, a smoking habit involving a physical addiction to tobacco products, primarily nicotine”.\(^2^5\) Smoking is considered the most important avoidable health risk in developed countries,
and is linked to a variety of diseases such as cancer, coronary heart disease, stroke, chronic obstructive pulmonary disease, and peptic ulcer disease, as well as abnormal fetal and neonatal growth and development during pregnancy. Furthermore, second-hand smoke or environmental tobacco smoke, which involves the inhalation of the smoke of burning tobacco by non-smokers, is linked to many of the same smoking-related diseases as previously stated.

Systematic reviews strive to “collate all empirical evidence that fits pre-specified eligibility criteria in order to answer a specific research question. [These reviews use] explicit, systematic methods that are selected with a view to minimizing bias, thus providing more reliable findings from which conclusions can be drawn and decisions made”. In general, a systematic review involves locating and assessing all relevant studies, and obtaining and synthesizing data from such studies. A systematic review may or may not incorporate a meta-analysis, which involves “the use of statistical methods to combine the results of independent studies [and therefore] … can provide more precise [effect] estimates … than those derived from the individual studies included within a review”. One meta-analysis does not necessarily need to combine the results of all of the studies included in a systematic review, especially if studies are at risk of bias or are varied in terms of exposures and outcomes. Such a meta-analysis would likely generate an erroneous effect estimate. Given that systematic reviews consider the entire research literature available on a selected topic, and meta-analyses summarize the quantitative results from such studies, systematic reviews and meta-analyses are important for establishing clinical practice guidelines and keeping health care providers informed of up-to-date medical evidence.
1.1.2 What is Known – What is Needed

A potential MS risk factor that is currently receiving considerable attention is smoking. At the population-level, it has been demonstrated that smoking prevalence is associated with MS prevalence to some degree. In addition, several studies have shown that smoking is generally associated with increased MS risk and severe and rapid disease progression, with greater risk and progression associated with greater smoking exposure. Conversely, smoking cessation is generally associated with a lower risk of developing MS as well as a slower disease progression. The following are some progression outcomes that have been associated with smoking: 1) Conversion from a first episode of CNS demyelination, known as clinically isolated syndrome (CIS), to clinically definite multiple sclerosis (CDMS); 2) disease progression as measured by the conversion from a relapsing-remitting multiple sclerosis (RRMS) disease course to a secondary-progressive multiple sclerosis (SPMS) disease course; 3) progression of disability; and 4) progression of disease activity, including lesions.

Three previous systematic reviews and meta-analyses published in 2007, 2011, and 2014 concluded that smoking was associated with MS risk. The review conducted in 2011 involved a further analysis examining MS progression and showed a statistically non-significant relationship between smoking and SPMS risk. Two more recent systematic reviews with meta-analyses published in 2016 concluded that different smoking statuses (current, past, and ever-smoking, the latter comprised of current and past smokers in combination) were associated with a higher risk of MS. While one of the reviews showed a relationship between passive smoke exposure and MS risk, the other review did not. In addition, two systematic reviews without meta-analyses were
recently published, one of which showed a relationship between smoking and MS risk and progression,\textsuperscript{18} while the other review, exploring MS risk in more detail, did not demonstrate a consistent relationship between smoking and a certain course of MS.\textsuperscript{19} There are also several narrative reviews examining the association between smoking and MS,\textsuperscript{20, 21, 38, 39} all of which provided evidence of the adverse effects of smoking on MS risk and/or progression. While there is an abundance of research on the smoking-MS relationship, there is a critical need to comprehensively evaluate the likelihood that smoking is truly playing a causal role in MS.

*The Bradford Hill criteria for causation*\textsuperscript{12} are used to evaluate a causal association between an exposure and a particular disease. Hill listed nine criteria that together increase the probability that an association is causal. The criteria are described below:

1. **Strength**: stronger relationships between an exposure and a disease have a greater likelihood of being causal compared to weaker relationships; strength is commonly conveyed by an odds ratio (OR) or risk ratio (RR), whereby a larger magnitude of the estimate indicates a greater probability of the association being causal, though weaker associations do not necessarily indicate a non-causal relationship.

2. **Consistency**: repetition of a relationship across studies undertaken by different researchers, in different places and times, and using different population groups and methods, strengthens the likelihood that the relationship is causal.

3. **Specificity**: a causal association is more likely to exist if an exposure is limited to one disease; however, the importance of this criterion should not be over-emphasized, given that one-to-one relationships are rare and generally non-applicable to most chronic diseases.
4. **Temporality**: an exposure must always precede the disease in time in order for a causal association to exist; this is the one criterion that always must be met in order to infer causation.

5. **Biological Gradient**: the presence of a dose-response curve strengthens the likelihood of a causal relationship (i.e., an increase in the level of an exposure is followed by an increase in disease risk).

6. **Plausibility**: a causal relationship is consistent with current biological knowledge of disease processes (i.e., it is supported by known biological mechanisms underlying the relationship); however, plausibility is limited by existing biological knowledge, and as such, the absence of knowledge to support a new relationship does not necessarily indicate a non-causal relationship.

7. **Coherence**: a causal relationship will likely not deviate from known facts related to the natural history and biology of the disease.

8. **Experimental Evidence**: a causal relationship is more likely to exist if experimental evidence under controlled conditions shows that changing the exposure results in a change in the outcome. In many cases, it is not possible to obtain experimental evidence ethically when examining disease causation.

9. **Analogy**: a causal relationship is more likely to be accepted if elsewhere a similar exposure results in a similar outcome.

Hill developed these criteria during the rise of non-communicable disease epidemiology. A portion of these criteria was used in assessing the relationship between cigarette smoking and lung cancer in a landmark report to the Surgeon General of the United States. Hill’s criteria are still viewed as a valuable tool against which
characteristics of an association can be tested to determine if causal inference is a reasonable conclusion.\textsuperscript{40} The criteria have been applied in many areas of epidemiology to establish support for causality, such as the link between Vitamin D and breast cancer,\textsuperscript{42} dietary acid and osteoporosis,\textsuperscript{43} and alcohol consumption and cardiovascular disease outcomes.\textsuperscript{44} In addition, Hill’s criteria have been used in narrative reviews to independently assess the causality of Vitamin D insufficiency,\textsuperscript{45} the Hepatitis B vaccine,\textsuperscript{46} and smoking\textsuperscript{20, 21} in relation to MS. Overall, relationships between an exposure and a particular disease that meet a greater number of criteria have a greater likelihood of being causal than those meeting fewer criteria.\textsuperscript{47}

However, the Bradford Hill criteria have attracted some criticism. Hill himself noted that the criteria do not provide absolute evidence for determining whether a relationship is causal.\textsuperscript{12} For instance, associations that do not satisfy the criteria do not necessarily provide definite support for non-causation (e.g., causal relationships can be weak, non-specific, inconsistent, odd). Similarly, associations satisfying Hill’s criteria do not provide definite support for causation (i.e., a strong relationship could be due to a strong underlying confounder). Building on Hill’s argument, there is agreement that satisfying the criteria neither deductively nor inductively supports a causal claim.\textsuperscript{48} Specifically, one cannot detect the aspects of the criteria that guarantee that a causal claim is true (deduction) or that a false claim is improbable (induction). Furthermore, given that Hill’s criteria are used to analyze epidemiological associations that may be the result of bias or confounding, such data are not likely to undeniably demonstrate causation.\textsuperscript{49} It has also been argued that the application of Hill’s criteria involves a complex causal system with many components rather than a simple one-to-one
relationship, limiting the value of the criteria.\textsuperscript{50} Nevertheless, the Bradford Hill criteria are important in establishing justified causal links between an exposure and a given disease that represents the best explanation based on study data.\textsuperscript{48, 49}

To date, two narrative reviews used Hill’s criteria in their reviews of studies published up to and including 2011\textsuperscript{21} and 2014\textsuperscript{20} respectively. Arguably, utilizing Hill’s criteria to extensively evaluate the causal nature of the smoking-MS relationship based on the most reliable findings identified in a rigorous systematic review and meta-analysis provides even greater insight into this relationship, and would greatly assist in establishing if smoking is playing a causal role in MS.

\subsection*{1.1.3 Conceptual Framework}

The conceptual framework used as the underlying basis for this review of smoking as a cause for MS is Rothman’s \textit{Model of Necessary and Sufficient Causes},\textsuperscript{51} a well-known model conceptualizing chronic disease etiology presented in Figure 1-1.

Rothman’s model shows how a hypothetical disease may have multiple \textit{sufficient causes} (I, II, III, etc.), each consisting of different combinations of \textit{component causes} (A, B, C, etc.). Any component cause that is removed from a sufficient cause makes the remaining components insufficient, thus preventing disease onset by that sufficient cause. Furthermore, a \textit{necessary cause} is a component cause that appears in every sufficient cause (e.g., A in Figure 1-1). As such, researchers always hope to identify a necessary cause of the disease in question as removing it would lead to complete disease prevention.
MS fits well into Rothman’s model given that this chronic disease is considered to be a multi-causal process. In other words, there is no single factor that has been found to be sufficient to cause MS. However, a necessary cause of MS has yet to be identified. With regards to smoking, it is neither a necessary cause of MS (i.e., smoking is not represented by A in Figure 1-1 since non-smokers can develop MS) nor is smoking a sufficient cause of MS (i.e., I, II, III in Figure 1-1 do not consist solely of smoking since not all smokers develop MS). Therefore, it is envisioned that smoking could represent a component cause that may be part of one or several sufficient causes of MS (i.e., smoking could be represented by B, C, D, etc. in Figure 1-1).

1.2 Overview and Overall Objective

Hill’s criteria have previously been used in two narrative reviews to evaluate the association between smoking and MS. However, the criteria have not been used in an updated systematic review and meta-analysis to evaluate this relationship. The overall objective of this research was to examine the relationship between smoking and MS through a systematic review and meta-analysis, incorporating the Bradford Hill criteria for causation.

1.2.1 Specific Objectives

1) To analyze the relationship between smoking and MS risk in a systematic review and meta-analysis that incorporates the Bradford Hill criteria for causation;

2) To analyze the relationship between smoking and MS progression, including the conversion from CIS to CDMS, and the conversion from RRMS to SPMS, in a
systematic review and meta-analysis that incorporates the Bradford Hill criteria for causation; and

3) To provide a brief summary of the literature as it pertains to the relationship between other forms of smoke exposure (i.e., passive, prenatal) and MS, in the form of a systematic review.

**Rationale:** The present research serves to expand on previous systematic reviews and meta-analyses that have examined the effect of smoking on MS risk. Additional relevant studies are included and a more detailed evaluation of causation is conducted.

There is also a need to examine the relationship between smoking and MS progression in greater detail, given that only one previous systematic review and meta-analysis analyzed the effect of smoking on the conversion from RRMS to SPMS,\textsuperscript{13} and none examined the influence of smoking on the conversion from CIS to CDMS. This comprehensive examination will help clarify the role of smoking in important stages of MS. To date, Hill’s criteria have not been methodically used in a systematic review and meta-analysis to evaluate the association between smoking and MS. In the present review, Hill’s criteria provide a basis for thoroughly evaluating the causal nature of the relationship, offering new insight into the role of smoking in MS.

Two recent reviews were the first to use both systematic review and meta-analytic methods to evaluate the relationship between passive smoking and MS.\textsuperscript{16,17} Given this new research on second-hand smoke exposure and the possibility that such exposures are important in the development of MS, providing a summary of the literature to date in this area, as well as summarizing the MS literature on other forms of smoke exposure (e.g., prenatal smoking exposure) is essential for stimulating further research.
1.3 References


CHAPTER 2: Review of the Literature

2.1 Multiple Sclerosis

MS is among the most common neurological diseases in the world, and one of the primary causes of non-traumatic disability in the young adult population.\(^1\) According to an extensive international study, approximately 2.3 million people were diagnosed with MS worldwide in 2013.\(^1\) While MS is considered a global disease, numerous studies have confirmed that Canada has one of the highest prevalences of MS in the world,\(^2,4\) with some of the highest prevalences being reported in Alberta,\(^5\) Saskatchewan,\(^6\) Manitoba,\(^7\) and Nova Scotia.\(^8\) In 2013, over 97,000 Canadians were living with MS.\(^1\)

Although MS affects every demographic, this chronic disease primarily has its onset in adults between 20 and 40 years of age,\(^9\) and is twice as common in women as it is in men.\(^1\) In addition, MS is more common among Caucasians with a northern European background than among Asians, Africans, and Native Americans.\(^10\) Studies have suggested the existence of a latitudinal gradient in MS prevalence, with countries located further away from the equator, such as Denmark, Ireland, and Canada, having higher MS prevalences compared to countries located closer to the equator, such as China, Thailand, and Argentina.\(^4,11\) There is currently no cure for MS.\(^12\)

2.1.1 Symptoms

MS is highly unpredictable as the disease has a variety of different symptoms, some of the most common being extreme fatigue and sensory disturbances.\(^13\) According to a study that used the North American Research Committee on Multiple Sclerosis (NARCOMS) Registry, a database containing symptom information on 36,000 MS
patients, sensory symptoms (85%) and unexplained fatigue (81%) were the most common symptoms reported within the first year after disease onset.\textsuperscript{14} When it comes to sensory symptoms, many MS patients experience numbness, tingling, pricking, and sensitivity loss in the trunk as well as in the upper and lower extremities.\textsuperscript{15}

Many MS patients will also experience motor impairment and vision problems. Specifically, motor symptoms can occur in different parts of the body, including spasticity in the upper and lower limbs, and tremors occurring in the arms, legs, head, and trunk.\textsuperscript{16,17} Such symptoms may lead to mobility issues, balance problems, postural instability, and wheelchair dependency.\textsuperscript{16,18} NARCOMS participants reported some degree of spasticity (54%) and tremor (38%) within the first year of MS onset.\textsuperscript{14} In addition, 35\% of participants reported that their mobility was noticeably affected in the first year, with an additional 15\% of participants occasionally requiring a mobility device.\textsuperscript{14} With respect to vision problems, an eye condition known as optic neuritis is a frequent symptom of MS. Optic neuritis is an inflammation of one or both optic nerves, which usually leads to temporary loss of vision.\textsuperscript{19} Other visual disturbances that MS patients may experience include diplopia (double vision), loss of color vision, nystagmus (involuntary movement of the eyes), and blurred vision.\textsuperscript{20,21} Within the first year of disease onset, 55\% of NARCOMS participants reported some degree of a visual disability.\textsuperscript{14}

Pain is another frequent symptom of MS. Among the NARCOMS participants, 59\% reported some degree of pain immediately after MS onset.\textsuperscript{14} Specifically, central neuropathic pain and trigeminal neuralgia are among the most common neuropathic pain syndromes experienced by MS patients.\textsuperscript{22} In addition, pain often accompanies sensory,
motor, and visual symptoms. For example, many MS patients suffer from painful sensory symptoms such as burning, aching, pricking, and electric-shock sensations.15 Also, MS patients often describe spasms as feeling similar to a cramping and pulling pain.22 Those MS patients who are wheelchair users may suffer from pain due to improper posture, incorrect use of their mobility aid, and persistent wheelchair use.22 Furthermore, MS patients suffering from optic neuritis often experience eye pain, especially when they move their eyes.19

Other symptoms that MS patients may experience include loss of cognition such as memory loss and dementia,13,23 speech and swallowing difficulties,24 bladder complications including urgency and urinary infections,25 and sexual dysfunction such as loss of interest in sex and failure of arousal.26 Overall, many patients feel extremely anxious and uncertain about their symptoms, especially once diagnosed with MS.27

2.1.2 Diagnosis

Correctly diagnosing MS is particularly challenging. For example, some symptoms of MS, such as fatigue and sensory disturbances, are non-specific to MS, and therefore may be associated with other chronic diseases.28 In addition, there is no single clinical feature or laboratory test that is entirely sufficient to make an MS diagnosis.29 However, various sets of diagnostic criteria have been developed throughout the years to support a clinical diagnosis of MS, including the Schumacher criteria,30 the Poser criteria,31 and the McDonald criteria.32 In general, these criteria are based on the following principles: (1) dissemination in space of CNS lesions, (2) dissemination in time
of CNS lesions, and (3) exclusion of other similar diseases that may have caused the symptoms.  

These principles were first introduced in the Schumacher criteria, the first official standardized set of criteria developed in 1965 for diagnosing definite MS in a purely clinical manner. In general, a clinical diagnosis of definite MS can be made using the Schumacher criteria if two separate attacks (dissemination in time) are located in two different areas of the CNS (dissemination in space), and the signs and symptoms cannot be better explained by a different disease.

The Schumacher criteria were modified and a more advanced set of diagnostic criteria, known as the Poser criteria, were published in 1983. These criteria incorporate laboratory tests (evoked potentials and cerebrospinal fluid analysis) to locate asymptomatic CNS lesions. Furthermore, the Poser criteria allow for a diagnosis of probable MS in addition to a diagnosis of CDMS that was already included in the Schumacher criteria. Overall, these advancements aid in further confirming the diagnosis of MS.

The most recent set of MS diagnostic criteria, the McDonald criteria, were published in 2001. While the McDonald criteria still focus on demonstrating the MS diagnostic principles, they rely on magnetic resonance imaging in addition to evoked potentials and cerebrospinal fluid analysis to facilitate an earlier MS diagnosis. This advancement also assists in diagnosing patients that exhibit various MS subtypes (described in the section that follows). Furthermore, the terms CDMS and probable MS were replaced by the terms MS and possible MS. In an effort to improve accuracy and efficiency in the diagnosis of MS, the McDonald criteria were revised in 2005 and again
in 2010. According to a global survey conducted by the World Health Organization and the MS International Federation, the Schumacher, Poser, and McDonald criteria have all been used in recent years to diagnose MS.

2.1.3 Types

Prior to being diagnosed with MS, approximately 85% of MS patients would have suffered from CIS, which is the first clinical episode of neurological symptoms due to inflammatory demyelination of the CNS. While CIS is a disease course suggestive of MS, the syndrome may or may not develop into MS. Specifically, the risk of developing MS is high if brain lesions are found on a magnetic resonance imaging scan of a CIS patient.

Besides CIS, the International Advisory Committee on Clinical Trials of MS outlined other MS phenotypes, including relapsing-remitting multiple sclerosis (RRMS), secondary-progressive multiple sclerosis (SPMS), and primary-progressive multiple sclerosis (PPMS).

Most patients present with RRMS, which represents approximately 85% of MS cases. RRMS is characterized by episodes of symptom exacerbation and recovery. These periods of relapse and remission can continue for many months or years without disease progression. Eventually, about 80% of RRMS patients will develop SPMS. Specifically, following the RRMS course, a patient may experience a gradual increase in disability either with or without exacerbations. Lastly, a less common type of MS is PPMS, representing 10-15% of all MS cases. From the onset, PPMS patients will experience a gradual increase in disability without prior exacerbations.
2.1.4 Impact

Once diagnosed with a specific MS type, living with the symptoms of MS can have a negative impact on patients and their families, as well as on society as a whole. Specifically, the symptoms can adversely affect the quality of life of patients and family members, including their physical, psychological, social, and economic well-being.\textsuperscript{40}

Many MS patients who suffer from mobility problems, spasticity, instability, or fatigue experience a pronounced decline in daily physical functioning.\textsuperscript{41} In addition, pain and sensory disturbances have been classified as physically disabling symptoms given that such symptoms can impair one’s ability to undertake daily activities at work and at home.\textsuperscript{42} Furthermore, difficulty in participating in social activities and maintaining relationships is common among MS patients, especially if suffering from severe pain, limited mobility, fatigue, or a severe disability.\textsuperscript{43-45} MS patients may also choose to isolate themselves from social activities if they suffer from bladder control problems to avoid embarrassment.\textsuperscript{44} Not only do the symptoms of MS place severe limitations on social participation, they can also adversely impact one’s sexual relationship with a spouse. For instance, MS patients may demonstrate decreased affection and intimacy towards their spouse, as well as dissatisfaction with sexual activity.\textsuperscript{46}

MS patients may also find it difficult to maintain employment due to cognitive impairment, mobility problems, fatigue, loss of vision, or accumulating disability.\textsuperscript{44, 47, 48} It has been reported that approximately 80\% of MS patients face unemployment within 10 years of being diagnosed.\textsuperscript{35} Finally, the presence of depression and anxiety among MS patients is commonly reported in the literature, especially among those patients who suffer from a severe disability or have functional limitations.\textsuperscript{49-51}
The various issues that accompany an MS diagnosis can impact the health and well-being of family members charged with caring for an MS patient. One study showed that a majority of caregivers experience back problems, fatigue, and general worry about their own health. In addition, a large proportion of the family members in the study reported that the patients’ illnesses influenced their careers. These influences included declining a job promotion, opting for part-time employment or a lower-grade job, or quitting their job in order to find more time to assist their relative with MS. Furthermore, family caregivers who experience disruptions in their daily activities, financial problems, or long-hours of caregiving can be at risk of developing depression and anxiety. Several studies have shown that the greater the disability of MS patients, the greater the caregiver burden.

The economic burden of MS on patients, families, the health care system, and Canadian society in general is extremely high. Based on data collected by the multinational Treatment Experience, Burden, and Unmet Needs study, the mean annual cost for each MS patient in Canada was $37,672 in 2009 dollars. This amount was attributable to direct healthcare costs (e.g., professional care and consultations), therapies, and indirect costs of productivity loss, including a patient’s sick leave and retirement due to MS. Greater disease severity led to higher costs. Another study estimated the lifetime cost of MS for one patient in Canada to be approximately $1.6 million in 1995 dollars. According to the Public Health Agency of Canada, the estimated total cost of MS was approximately $1 billion in 2000-2001, which included approximately $139 million in direct costs (e.g., hospital care, physician care, and drug costs) and $811 million in indirect costs (e.g., mortality and morbidity costs).
2.1.5 Etiology

Despite the various symptoms, disease courses, and adverse effects that are associated with the diagnosis of MS, the exact cause of MS has yet to be discovered. Over the years, researchers have identified a number of different risk factors that could be potential causes of MS. These include genetics, viruses, infections, immune factors, vascular disturbances, psychological aspects, environmental triggers, and lifestyle behaviours. Currently, factors such as the Human Leukocyte Antigen (HLA) genetic complex, the Epstein-Barr virus (EBV), Vitamin D deficiency, Chronic Cerebrospinal Venous Insufficiency (CCSVI), and smoking are attracting considerable attention from researchers and scientists. Other risk factors that have been investigated include psychological stress, organic solvents, dietary fat intake, the Hepatitis B vaccination, and dog exposure.

MS is likely a multi-factorial disease process involving an interaction between a genetic predisposition and multiple environmental factors, which can be illustrated by Rothman’s Model of Necessary and Sufficient Causes previously presented in Figure 1-1 (Chapter 1). This interaction may be responsible for triggering an autoimmune process that eventually leads to MS. As previously stated, MS usually develops in adulthood, which may be an indication that an environmental component is required to trigger disease onset later in life. Numerous studies provide evidence that the interaction of risk factors could increase the risk of MS, such as when HLA genes interact with EBV antibodies and cigarette smoke.
2.1.5.1 Smoking

In 2014, approximately 5.8 trillion cigarettes were smoked globally. Nationally representative survey data from 187 countries showed that the prevalence of daily smoking is approximately five times higher in men (31.1%) compared to women (6.2%), with the highest prevalence observed at 45-49 years of age among men and 50-54 years of age among women. According to the World Lung Foundation and the American Cancer Society, the prevalence of cigarette consumption is the highest in the European Region (e.g., Russia, Germany, Turkey), followed by the Americas (e.g., United States, Canada, Argentina), South-East Asia (e.g., Indonesia, India, Singapore), the Western Pacific (e.g., China, Japan, Vietnam), and finally, the Eastern Mediterranean and Africa.

In Canada, 21.4% of men and 14.8% of women reported being smokers in 2014, down from 24.2% and 17.4% reported in each respective group in 2010.

In the 1960s, case-control studies and surveys began to investigate the relationship between smoking and MS. Results from these early studies were mixed. For instance, a nationwide case-control study in Israel showed a larger proportion of MS patients had smoked prior to MS onset compared to the control group. Conversely, a national survey in Britain demonstrated no relationship between smoking and MS. The past decade has witnessed a dramatic increase in the number of studies examining the association between smoking and MS.

Numerous cohort and case-control studies demonstrated evidence that smoking is associated with MS susceptibility. For example, two cohort studies of American women showed a 60% greater MS rate among current smokers than among individuals who never smoked. This study also showed that the MS rate increased with cumulative
smoking exposure, with the greatest risk (1.7 times) among those who had smoked for 25 years or more. The large prospective design of this study confirms that smoking exposure was evaluated prior to an MS diagnosis, establishing a temporal relationship. In addition, a nested case-control study verified a higher risk of MS among current smokers and ex-smokers compared to never-smokers.\textsuperscript{84} Given that the study used a sample taken from a well-defined prospective cohort, they were also able to establish temporality, as well as minimal bias in the selection of participants. Furthermore, two Swedish case-control studies showed that an increase in smoking dose resulted in increased risk of MS; individuals classified as heavy smokers had a greater MS risk compared to light smokers.\textsuperscript{86} The study also showed that after a decade of smoking cessation, the risk of MS subsided, regardless of timing and cumulative dose of smoking. However, these results should be taken with caution given that smoking history was collected retrospectively, making the study prone to recall errors, and the presence of a large proportion of non-respondent controls may have introduced selection bias.

There have been mixed results from studies investigating the relationship between different forms of second-hand smoke exposure and MS risk. For instance, a case-control study in France showed a relationship between exposure to household parental smoke and increased MS risk among children.\textsuperscript{87} However, a Canadian case-control study did not demonstrate a relationship between exposure to household smoke and MS among adults.\textsuperscript{88} Similarly, while a case-control study showed an effect of maternal prenatal smoke exposure on MS risk among offspring,\textsuperscript{89} a different study using two large cohorts of female nurses did not reveal a relationship.\textsuperscript{90}

Studies on the relationship between smoking and MS progression show mixed
results. For instance, a cohort study utilizing smoking data from medical records and interviews showed an association between smoking and the conversion from CIS to CDMS. On the other hand, a different cohort study that used cotinine as a measure of smoking exposure did not show a relationship. In addition, a study utilizing self-reported survey data showed that ever-smokers (current and past smokers in combination) had a greater likelihood of developing progressive MS compared to never-smokers. This association was more pronounced among smokers who started smoking at an earlier age (15 years of age or younger) than smokers who started later. A cross-sectional and longitudinal follow-up study that used a considerably larger sample concluded that current smokers had more severe MS compared to never-smokers. In addition, the current smokers in the study experienced a faster progression from RRMS to SPMS compared to those who never smoked. Furthermore, a population-based cohort study in the United Kingdom demonstrated that ex-smokers had reduced disability progression compared to current smokers, regardless of whether smoking cessation occurred before or after MS onset. However, a different cohort study conducted in the Netherlands revealed no effect of smoking on disability or disease progression among MS patients, including the development of SPMS. Rather than being population-based, this study cohort was hospital-based, and as such, may not have been representative of the general MS population.

The relationship between smoking and MS was examined in two systematic reviews without meta-analyses. Based on 14 articles published no later than 2012, one of the reviews showed that smoking led to an increase in MS risk, as well as a faster disease progression. After examining three studies identified in a search ending in June
2014, the second review did not demonstrate a consistent relationship between smoking and an MS disease course, namely RRMS and PPMS. However, the authors emphasized that while the research on MS risk factors is extensive, findings were rarely published according to MS disease course.

Five systematic reviews with meta-analyses have examined the association between smoking and MS. The earlier reviews all demonstrated a statistically significant relationship between smoking and MS risk based on six studies published up to and including 2005, 14 studies published no later than May 2010, and 26 studies published up to and including 2014 respectively. Based on four studies, one of the reviews also showed that the association between smoking and risk of SPMS approached statistical significance with a RR of 1.88 and a 95% confidence interval (CI) of 0.98-3.61. However, more recent studies have now been published investigating the association between smoking and the progression from RRMS to SPMS that need to be incorporated into the greater assessment of the literature. In addition, studies investigating the progression from CIS to CDMS in relation to smoking have thus far not been examined in a systematic review with a meta-analysis. Arguably, such a critical stage in the disease process also warrants a further investigation.

Out of the five systematic reviews with meta-analyses, the two most recent included 26 studies (published to around mid-2015) investigating the association between ever-smoking and MS risk. Both reviews showed a statistically significant relationship, with ORs of 1.46 (95% CI [1.33-1.59]), and 1.57 (95% CI [1.50-1.64]). In both reviews, the risk of MS was greater among current smokers than among past smokers, and one of the reviews demonstrated a dose-response relationship between
cigarette smoking and MS risk. However, some studies directly examining the relationship between smoking and MS were missing from at least one of the reviews, and indirect studies providing appropriate smoking data that are important in the relationship were also missing. Lastly, in examining the effect of passive smoke exposure on MS risk, one review showed a statistically significant relationship based on three studies (OR 1.24, 95% CI [1.03-1.49]), while the other, also based on three studies, did not (OR 1.12, 95% CI [0.87-1.36]). Given that this was the first time that the relationship between second-hand smoke exposure and MS has been examined in a systematic review and meta-analysis, there is a need to summarize the literature in this area in greater detail that includes both passive and prenatal smoke exposure. Such exposures could be critical MS risk factors that require further investigation.

Overall, while the reviews outlined above provide important data evaluating the association between smoking and MS, the use of Hill’s criteria as a framework to determine the likely causality of this relationship would further advance the understanding of this risk factor in relation to the outcome.

Several narrative reviews have also confirmed the growing evidence supporting the association between smoking and MS. However, authors of narrative reviews may selectively cite the literature to support a preconceived argument, rather than use comprehensive procedures to identify and analyze all studies relevant to a certain research topic as in a systematic review and meta-analysis. As such, narrative reviews may be more prone to bias and authors may draw misleading conclusions.

Overall, there is value to conducting a new systematic review and meta-analysis that includes both more recently published and previously missed studies on smoking and
MS risk, and also examines the effect of smoking on important MS progression outcomes. Relevant outcomes include expanding on the analysis of secondary progression, as well as, for the first time in a systematic review and meta-analysis, examining the conversion from CIS to CDMS. In addition, providing a summary of the literature to date on the association between different forms of second-hand smoke exposure and MS is informative as it represents an important area of inquiry. Moreover, incorporating Hill’s criteria to determine the likelihood of a causal relationship between smoking and MS would further enhance this more timely and comprehensive review.

2.2 Overview of Systematic Review and Meta-Analytic Methods

A systematic review involves utilizing a methodical procedure to find, evaluate, and synthesize data from multiple studies. Prior to conducting a systematic review, it is recommended to first register a detailed review protocol. An example of a database used for registration is PROSPERO, which compiles registered reviews in areas such as health, welfare, education, and justice. By keeping a record of pre-registered reviews, PROSPERO strives to help reviewers avoid duplicating an existing review, as well as to reduce the risk of reporting biases by allowing important elements highlighted in a published review and those recorded in the registered protocol to be compared.

A systematic review begins with the development of eligibility criteria (e.g., study design, nature of independent and dependent variables, population and participant characteristics) in order to determine which studies are to be included in a specific review, with the intention of finding every study satisfying the select criteria. From there, bibliographic databases, conference abstracts, theses or dissertations, and/or other
sources are searched for studies applicable to the review. A search for additional studies identified in the reference lists of associated studies and review articles can also be conducted. Once the studies are retrieved, they are individually screened in order to determine if they meet the eligibility criteria, and therefore, are relevant to the review. As such, it is imperative to consult the established criteria to ensure that they are consistently applied. In order to assist researchers in reporting their reviews, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement was established; this includes a detailed flow diagram which, by convention, is included by many authors in their publications. Specifically, the PRISMA flow diagram allows review authors to document the number of records located in their search, screened, and excluded from the review. The full-text publications of those records not excluded are then retrieved. Subsequently, review authors can use the PRISMA flow diagram to document the number of full-text studies screened, excluded, and finally, included in the review.

Data applicable to the review (e.g., study design, participant characteristics, results) are then extracted from each included study, which may involve using a data extraction form to ensure that the procedure is undertaken as consistently as possible. It is essential that the form first be piloted on a small group of included studies to confirm that the form is appropriately customized to the review topic, and is efficient in obtaining all necessary data.

Another important component of a systematic review involves evaluating the risk of bias and quality of the included studies, which can include assessing for such elements as selection bias (e.g., sample representativeness, loss to follow-up, volunteer and
response bias) and confounder control. Several instruments have been identified as being helpful in facilitating such an evaluation process, including the Downs and Black instrument and the Newcastle-Ottawa Scale. Reviewers can also evaluate the quality of evidence overall using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach, which involves evaluating factors such as study limitations, inconsistency and imprecision of results, and publication bias.

Besides reducing bias through the use of systematic methods as presented above, systematic reviews are beneficial in terms of rectifying conflicting findings, investigating clinical practice alternatives, and verifying suitable clinical practice to assist individuals (e.g., health professionals, healthcare users) in making sensible healthcare choices in the face of an overwhelming amount of health data. In terms of synthesizing the results, study findings can be summarized by means of a narrative synthesis, or, if results are displayed quantitatively and studies are relatively similar, results from individual studies can be combined using a meta-analysis.

A meta-analysis involves utilizing statistical procedures to integrate the results of separate studies; these results (e.g., individual study effect estimates and summary meta-analytic effect estimate with their corresponding CIs) are graphically displayed in forest plots accompanied by a listing of the included studies. Such a statistical analysis is beneficial as it can contribute to increased precision of effect estimates and higher power as a result of establishing a larger total sample size.

A meta-analysis can be performed using either a fixed-effects or a random-effects model. Specifically, a fixed-effects model assumes that one true effect is present in every study, which is estimated by the summary effect. Therefore, the only source of
error in this model is random sampling variation. A fixed-effects model can be conducted if it is assumed that the included studies are the same (e.g., same researchers, one pool of participants), with the intention of estimating the effect strictly for the population under study as opposed to making broad generalizations. However, since the results are usually obtained from independent studies, it is likely that the studies vary in their design and other characteristics, and such differences are likely to have influenced the results. As such, an assumption of one true effect should not be made. Instead, a random-effects model can be conducted, which assumes that a distribution of true effects is present rather than one effect, with the intention of extrapolating to different circumstances (e.g., populations). The summary effect estimate represents the mean of the individual effects. Under this model, the two sources of error include within-study sampling variation and between-study variation in the true effects.

An odds ratio (OR) is a common measure of the effect of an exposure on a given disease outcome. Specifically, the OR signifies the odds of a given disease developing in the presence of an exposure in comparison to the odds of the disease developing without the exposure. The OR is calculated using the following formula:

\[
\text{OR} = \frac{\frac{a}{c}}{\frac{b}{d}}
\]

An OR > 1 signifies that an exposure increases the odds of a given disease outcome, an OR < 1 signifies that an exposure decreases the odds of the disease, and an
OR = 1 signifies that an exposure has no effect on the odds of the disease.\textsuperscript{121} In a similar context, a risk ratio (\(RR=(a/a+b)/(c/c+d)\)) signifies the risk of an outcome in an exposed group in comparison to the risk of the outcome in the non-exposed group.\textsuperscript{112} Traditionally, RRs were calculated for cohort studies and ORs for case-control studies. However, ORs are now commonly used with various study designs involving dichotomous outcomes.

Finally, a hazard ratio (HR) is an effect measure that is commonly used to analyze time-to-event outcomes (e.g., time to symptom appearance, time to death, etc.).\textsuperscript{121} Specifically, a HR considers both the number of events and their timing, which are critical when analyzing such outcomes. On the other hand, an OR and RR only consider the number of events, and therefore, such effect measures should not be used to investigate time-to-event outcomes.

In summary, systematic reviews and meta-analyses comprise comprehensive research methods whereby all studies relevant to a particular research question can be identified, assessed, and synthesized (quantitatively and/or qualitatively), providing reliable and precise results that can be confidently used to draw important conclusions.

\textbf{2.3 Previous Use of Hill’s Criteria}

\textbf{2.3.1 Previous Use of Hill’s Criteria in Narrative Reviews of MS Causation}

Several narrative reviews have applied the Bradford Hill criteria for causation as a framework to assess the causal nature of risk factors in relation to MS, including Vitamin D insufficiency\textsuperscript{122} and the Hepatitis B vaccine.\textsuperscript{123} After applying Hill’s criteria, the authors of these reviews reported that both relationships may be causal. Specifically, it
was reported that the findings available from the literature on the relationship between Vitamin D insufficiency and MS fulfilled all of Hill’s criteria with the exception of Experimental Evidence; experimental evidence demonstrating whether optimizing Vitamin D levels reduced MS risk was not available. The authors concluded that specificity between Vitamin D status and MS existed in terms of biological mechanisms involving inflammatory pathways in general, despite Vitamin D insufficiency being linked to many other diseases. On the other hand, the authors examining findings from the literature on the Hepatitis B vaccine and MS reported that the association fulfilled all of Hill’s criteria except Biological Gradient and Specificity; the relationship did not follow a dose-response curve, and it was non-specific given that other risk factors are likely involved in the development of MS (besides Hepatitis B). The authors argued that experimental evidence supporting the relationship between the Hepatitis B vaccine and MS was available; however, the evidence was based on studies that investigated immune responses generated in immunized animals rather than humans.

The Bradford Hill criteria for causation have also been used to evaluate the relationship between smoking and MS in two narrative reviews. An earlier review was based on approximately 50 studies published up to and including 2011, including case-control and cohort studies, as well as systematic review and meta-analysis data. Based on these studies, the author reported that the effect of smoking on MS was small, consistent across different populations and study designs, non-specific, plausible, and coherent. On the other hand, the Experimental Evidence criterion could not be applied given that randomized controlled trials of smoking and MS were unavailable (and would be unethical to conduct). The Analogy criterion was also regarded as weak. In addition,
methodological problems across studies made it challenging to evaluate Temporality, as well as compromised the validity of data needed to evaluate Biological Gradient—despite many studies providing evidence of a dose-response relationship. Overall, the author concluded that while smoking was associated with MS risk, as well as disease progression and disability, more research was needed to provide further support for the causal nature of the smoking-MS relationship. On the other hand, the more recent narrative review included original studies and review articles published up to September 2014 that investigated many MS risk factors, including approximately 10 studies related to smoking. From these studies, the authors reported that the smoking-MS relationship was weak, consistent, plausible, and coherent, and exhibited a temporal and dose-response association. Overall, the authors concluded that smoking was associated with increased MS risk, while its impact on disease activity was more uncertain. The authors emphasized the need for more evidence to support causation.

Unfortunately, a major issue with narrative reviews is the ability of authors to be selective in reporting studies that support their preconceived views surrounding a relationship, therefore allowing the possibility that authors would draw misleading conclusions. This limitation is addressed with a systematic review and meta-analysis—an approach utilizing methodical procedures for reviewing all known studies on smoking and MS in the literature in order to obtain the most reliable findings. Subsequently applying Hill’s criteria to these systematically obtained findings to comprehensively assess the causal nature of the smoking-MS relationship thus further enhances the understanding generated by the review.
2.3.2 Previous Use of Hill’s Criteria in Systematic Reviews and Meta-Analyses

The Bradford Hill criteria for causation have been used in systematic reviews and meta-analyses to evaluate whether an association is causal, including the relationship between dietary factors and coronary heart disease,\textsuperscript{124} and the association between knee loading and knee osteoarthritis,\textsuperscript{125} but not the relationship between smoking and MS. Whether or not each criterion was satisfied was based on pre-established parameters specific to the reviews. To determine the Strength of the associations, both reviews examined the magnitude, direction, and statistical significance of the pooled effect estimates (OR or RR). In addition, replication of strong or moderate associations across studies provided the authors with evidence of Consistency. Temporality was also established given that prospective cohort studies were included in the analyses. Evidence of a Biological Gradient was determined by examination of tests for a trend in the relationship between the exposures and outcomes in question. To establish Coherence, the authors of the diet/coronary heart disease review stated that there needed to be evidence supporting an association of dietary exposures with surrogate risk factors for cardiovascular disease outcomes, as well as with indicators of such outcomes.\textsuperscript{124} With regards to Experimental Evidence, randomized controlled trials were analyzed for evidence of statistically significant associations between dietary exposures and coronary outcomes.

While the Specificity, Plausibility, and Analogy criteria were not evaluated in these two reviews, the authors of the diet/coronary heart disease review discussed how the dietary exposures would be associated with many cardiovascular disease outcomes given the interconnectedness of these outcomes, making it a non-specific relationship.\textsuperscript{124}
In terms of Plausibility, it was stated from the onset that the relationship met the criterion given that credible mechanisms existed that explained the relationship. Furthermore, the authors of the knee loading/osteoarthritis review noted the possibility of associations existing that were analogous to the relationship between knee loading and knee osteoarthritis, such as hip or hand osteoarthritis.\(^\text{125}\) Overall, Hill’s criteria were used by these reviews to develop a causation score for the association in question, illustrating whether there was strong, moderate, or weak evidence supporting a cause-and-effect relationship.\(^\text{124, 125}\) Employing a similar methodology to evaluate smoking as an MS risk factor would greatly assist in determining if smoking is playing a causal role in this chronic disease.

### 2.4 Summary

MS is a neurological condition with a high burden of disease in Canada, and is an important issue on a global scale. Especially concerning is the unpredictable nature of the disease, with its variable symptoms and disease courses, as well as challenging diagnosis, and substantial impact on patients, family members, the health care system, and society in general. While the cause of MS is unknown, numerous observational studies and several systematic reviews and meta-analyses have established that smoking is a risk factor for the disease. However, recent systematic reviews and meta-analyses failed to include some studies relevant to the association between smoking and MS risk. Systematic review and meta-analysis research examining important MS progression outcomes in relation to smoking is also lacking, including only one dated review investigating the relationship between smoking and the conversion from RRMS to SPMS and none.
examining the association between smoking and the conversion from CIS to CDMS. In addition, systematic reviews with meta-analyses have only recently begun to examine the relationship between passive smoke exposure and MS risk. A new, more inclusive review is warranted, specifically examining different forms of second-hand smoke exposure that could represent important risk factors in the development of MS. Finally, the Bradford Hill criteria for causation, an important tool that can be used to decipher whether smoking is likely playing a causal role in MS, have not been optimally used in previous systematic reviews and meta-analyses. The research presented in this thesis aims to examine the association between smoking and MS through a systematic review and meta-analysis incorporating the Bradford Hill criteria for causation, with a particular focus on MS risk including additional relevant studies not included in previously published reviews, as well as a more comprehensive focus on MS progression outcomes (i.e., CIS to CDMS, and RRMS to SPMS). The research also aims to summarize the literature on the relationship between various forms of second-hand smoke exposure (i.e., passive, prenatal) and MS within a systematic review.
2.5 References


CHAPTER 3: Manuscript

*Smoking and Multiple Sclerosis: A Systematic Review and Meta-Analysis Using the Bradford Hill Criteria for Causation*
Abstract

**Background.** Multiple sclerosis (MS) is one of the most common neurological disorders in the world. However, the cause(s) of MS is unknown. Studies have suggested that smoking may be related to the development and progression of MS. The Bradford Hill criteria are commonly used to assess potentially causal associations. However, to date, no systematic reviews and meta-analyses had used these criteria to comprehensively evaluate the relationship between smoking and MS. The objective of this research was to assess the relationship between smoking and both MS risk and MS progression through a systematic review and meta-analysis, subsequently applying Hill’s criteria to further assess the likelihood of these associations being causal. **Methods.** Databases including Medline, EMBASE, CINAHL, PsycInfo, and Cochrane Library were searched for relevant studies up until July 28, 2015. A random-effects meta-analysis was conducted for three outcomes: MS risk, conversion from clinically isolated syndrome (CIS) to clinically definite multiple sclerosis (CDMS), and progression from relapsing-remitting multiple sclerosis (RRMS) to secondary-progressive multiple sclerosis (SPMS). Consideration was given to dose-response relationships and risk factor interactions, and discussions of mechanisms and analogous associations presented in the studies. Hill’s criteria were applied to assess causality of the relationships between smoking and each outcome. The effect of second-hand smoke exposure was also briefly reviewed. **Results.** Smoking had a statistically significant association with both MS risk (conservative: OR/RR 1.54, 95% CI [1.46-1.63]) and SPMS risk (HR 1.80, 95% CI [1.04-3.10]), but the association with progression from CIS to CDMS was non-significant (HR 1.13, 95% CI [0.73-1.76]). Based on Hill’s criteria, there was strong evidence of a causal role of
smoking in MS risk, but only moderate evidence of a causal association between smoking and MS progression. Heterogeneity in study designs and target populations, inconsistent results, and an overall scarcity of studies point to the need for more research in the area of second-hand smoke exposure in relation to MS prior to conducting a detailed meta-analysis. **Conclusion.** This first review to supplement systematic review and meta-analytic methods with the application of Hill’s criteria to analyze the smoking-MS association provided evidence supporting the causal involvement of smoking in the development and progression of MS. As such, smoking cessation programs and policies should highlight MS as an additional health risk to deter the public from smoking.

**Keywords:** Multiple sclerosis; cigarette smoking; clinically isolated syndrome; Bradford Hill
1. Introduction

Multiple sclerosis (MS) is among the most common neurological diseases in the world. The disease affected approximately 2.3 million people worldwide in 2013, with the highest prevalence reported in North America and Europe. MS is characterized by damage to the myelin surrounding the nerves of the central nervous system (CNS), as well as damage to the nerve axons, contributing to a disruption in impulse conduction which ultimately leads to MS symptoms. The cause of MS remains unknown. However, MS likely involves an interaction between genetic and environmental factors. Currently, the Human Leukocyte Antigen (HLA), Epstein-Barr virus (EBV), Vitamin D deficiency, and cigarette smoking are thought to be involved.

The past decade witnessed an increase in the number of studies examining the association between smoking and MS. Numerous studies have shown that smoking is associated with MS risk. However, studies on the relationship between smoking and MS progression show mixed results. Specifically, some studies demonstrate an effect of smoking on the conversion from a first episode of CNS demyelination—clinically isolated syndrome (CIS)—to clinically definite multiple sclerosis (CDMS), as well as on the conversion from a relapsing-remitting multiple sclerosis (RRMS) course to a secondary-progressive multiple sclerosis (SPMS) course. Other studies show no effect. There have been mixed results from studies investigating the relationship between different forms of second-hand smoke exposure and MS. While some studies demonstrate an increased MS risk in relation to passive and maternal prenatal smoke exposure, others do not.

Previous systematic reviews with meta-analyses examining the association
between smoking and MS risk have demonstrated a statistically significant relationship with an effect estimate of approximately 1.5.\textsuperscript{31-35} The two most recent reviews examined the effect of passive smoking on MS, and while one showed a statistically significant relationship,\textsuperscript{35} the other did not.\textsuperscript{34} Only one previous review examined the association between smoking and MS progression, and did not show a statistically significant association between smoking and risk of SPMS.\textsuperscript{31}

Previous reviews have several limitations and research gaps remain. Specifically, there is a need to include both more recently published and previously missed studies that provide direct and indirect evidence relevant to the smoking-MS risk association. Also, systematic review and meta-analysis examination of the smoking-MS progression association is minimal and non-current; studies investigating the progression from CIS to CDMS (a critical stage in the disease process) in relation to smoking have not yet been subjected to systematic review and meta-analysis. In addition, systematic reviews with meta-analyses have only recently begun to examine the relationship between passive smoking and MS. This creates an even greater need to collate and summarize all studies that investigate different forms of second-hand smoke exposure, which could represent important MS risk factors that require further examination. Finally, the Bradford Hill criteria for causation\textsuperscript{36} provide a useful tool consisting of nine criteria used to evaluate research evidence to assess whether a relationship between an exposure and a disease outcome is likely to be causal. Hill’s criteria include Strength, Consistency, Specificity, Temporality, Biological Gradient, Plausibility, Coherence, Experimental Evidence, and Analogy. However, Hill’s criteria have previously only been used in narrative reviews\textsuperscript{37,38} evaluating the smoking-MS association. While Hill’s criteria have been used in
systematic reviews and meta-analyses assessing other relationships, this has not been done for the smoking-MS relationship. Applying Hill’s criteria to data compiled through systematic review and meta-analytic methods would be a more comprehensive approach to evaluating the potential causality of the association.

Therefore, the primary objective of this research was to a) examine the association between smoking and both MS risk and MS progression (i.e., CIS to CDMS, and RRMS to SPMS) through a systematic review and meta-analysis and b) use Hill’s criteria to more comprehensively assess the causality of each association. A secondary objective was to briefly summarize the literature on the relationship between different forms of second-hand smoke exposure (i.e., passive, prenatal) and MS through a systematic review.

2. Methods

A review protocol is available through PROSPERO: International Prospective Register of Systematic Reviews and can be retrieved at http://www.crd.york.ac.uk/PROSPERO/. (Registration number: CRD42015025039)

2.1 Search Strategy

The following databases were searched for published studies up until July 28, 2015: Medline via PubMed (1965-current), EMBASE: Excerpta Medica & EMBASE Classic via Ovid (1947-current), CINAHL Plus with Full Text via EBSCO (1937-current), PsycInfo via Ovid (1806-current), and Cochrane Library (1992-current). The search was restricted to English-language articles and used the following phrase:
(“multiple sclerosis” OR “disseminated sclerosis” OR “encephalomyelitis disseminata” OR “clinically isolated syndrome” OR “clinically isolated syndromes” OR demyelination*) AND (smoke* OR cigarette OR pipe OR cigarettes OR tobacco* OR nicotine* OR cotinine*).

No publication period limits were applied to the search. Reference lists of relevant studies and review articles were scanned for additional studies.

2.2 Eligibility Criteria

The review included peer-reviewed original research studies on humans with a case-control, cohort, or cross-sectional design that examined the relationship between smoking and MS. Specifically, studies investigating MS risk or progression (i.e., CIS to CDMS, and RRMS to SPMS) for active, passive, or prenatal smoking versus non-smoking, using participants with a confirmed or self-reported MS diagnosis were included. Studies were also included in the review if they examined the interaction between smoking and other MS risk factors; this formed part of the evaluation of causality. For case-control studies to be included in the review, an appropriate comparator group must have been utilized, such as a group that is healthy or drawn from a general population, to minimize the risk of particular diseases or conditions confounding the relationship of interest.

2.3 Study Selection

All studies retrieved from the search were exported from the databases and imported to EndNote X3.0.1 (Thomson Reuters, New York City, NY, USA), where duplicate studies were located and removed. One reviewer (M.D.) screened the studies
using a piloted list of questions (e.g., Does the study investigate MS? Is the study an original research study? Appendix A) to ensure that studies complied with the eligibility criteria. First, the titles of all studies were screened, followed by the abstracts of those studies not excluded based on their respective titles. A second reviewer (K.H.) screened a random selection of 10% of the same titles and abstracts to verify inclusion and exclusion decisions, and establish an appropriate level of inter-rater reliability. The level of inter-rater reliability was determined as the percentage of titles and abstracts on which the reviewers agreed (the number of agreements divided by the total number of titles and abstracts screened by the two reviewers). The full-text publications of potentially relevant studies whose abstracts were not excluded were retrieved and assessed for inclusion by M.D., and any uncertainties were discussed between M.D. and K.H. to reach a consensus. Opinions provided by both reviewers were given equal weight during all discussions of uncertainties.

2.4 Data Extraction

Data were extracted from the included studies using a piloted Microsoft Excel spreadsheet. Data obtained from the eligible studies and stored in the spreadsheet included: First author and year of publication, study objective and design, country of origin, sample size (initial number of participants and final number with outcome of interest, where applicable), age, sex (female to male ratio), source and disease history of study groups, smoking status and method for ascertaining smoking, MS course (initial MS types or sequence of progression if provided and applicable), method of MS diagnosis, confounders controlled (matched or adjusted), and reported odds ratio (OR),
risk ratio (RR), or hazard ratio (HR) with 95% confidence interval (CI). If a study provided an effect estimate, either alone or alongside raw data (e.g., an OR representing the odds of developing MS among smokers compared to the odds of developing MS among non-smokers), this value was used in the analysis (Appendix B). However, if effect estimates were not provided, either raw data (if available from the studies) were used to calculate an effect estimate and 95% CI (i.e., total number of cases and controls, and number of smokers in each participant group), or results were qualitatively reported. Analyses of dose-response relationships (statistically tested or observed) and tests for interactions between smoking and other MS risk factors were considered and results were noted. Consideration was also given to investigations and discussions of possible mechanisms underlying the relationship between smoking and MS, as well as associations analogous to the relationship of interest.

2.5 Quality Assessment

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used by one reviewer (M.D.) to evaluate the quality of evidence overall for each outcome of interest, namely MS risk, and the conversion from CIS to CDMS, and RRMS to SPMS. The second reviewer (K.H.) was consulted in cases where uncertainty existed around whether grade modifications were warranted. Only studies investigating active smoking were assessed. A detailed description of the GRADE framework has been published. Briefly, the quality of evidence for the three outcomes was assigned a grade of high, moderate, low, or very low depending on the following factors outlined by GRADE: Study design, study limitations, inconsistency of results,
indirectness of evidence, imprecision of results, publication bias, a large effect estimate, presence of a dose-response relationship, and residual confounding. Specifically, observational studies were initially assigned a low quality of evidence grade. The grade was reduced if there were limitations in design and execution, inconsistent effect estimates, indirect evidence, imprecise effect estimates (and therefore wider CIs), or publication bias. On the other hand, the grade was increased if there was a large or very large effect estimate, dose-response relationship, or an effect of residual confounding (further details in Appendix C).

2.6 Meta-Analysis

A random-effects meta-analysis was conducted using the generic inverse variance (IV) method to calculate the pooled effect estimates (OR/RR or HR) and 95% CIs for the effect of active smoking on both MS risk and progression. Greater weight (software generated) was typically given to studies whose standard errors (SEs) were smaller, which tended to be larger studies, with the aim of achieving more precise pooled estimates.\(^{42}\) Previously reported formulas\(^ {43}\) were utilized to obtain the data necessary to use the generic IV method, including converting the individual effect estimates and their corresponding errors into natural logarithms for input into the software program, which would then convert them back into ratio measurements (OR/RR or HR) and 95% CIs for display. A random-effects meta-analysis is a desirable approach for the current review, which assumes that the effect estimates across individual studies differ.\(^ {44}\) Such heterogeneity is found to be a common occurrence, but using a random-effects approach
allows for heterogeneity to be integrated in an analysis, resulting in a larger amount of variation in the overall effect estimate.\textsuperscript{44}

Separate analyses were conducted for each of the three outcomes of interest, with forest plots created. As in previous reviews,\textsuperscript{31,35} for MS risk, both a conservative (only studies in which smoking was considered before MS onset) and non-conservative (all studies) analysis were conducted. The conservative analysis establishes temporality from smoking to MS risk, which is required to infer causality.\textsuperscript{45} The non-conservative analysis allows for the inclusion of additional evidence which, given similar results, strengthens the overall conclusions drawn. A subgroup analysis was also carried out to compare the risk estimates for different study designs, smoking statuses, MS diagnostic methods, and methods for ascertaining self-reported smoking, in line with categories or variations investigated in previous reviews.\textsuperscript{31,33,35}

Given that MS is a rare condition, the rare disease assumption\textsuperscript{46} was used to combine ORs and RRs from the individual studies. Under this assumption, the OR approaches the RR when the prevalence of a disease is low. If the studies did not provide effect estimates and 95% CIs to be used in the analysis, these were calculated based on the reported number of smoking events in each participant group if provided. Where possible, estimates for current smoking and past smoking provided by a study were combined to obtain a measure of ever-smoking to be used in the appropriate analysis (see Appendix B for individual provided and calculated effect estimates for smoking-MS risk association). The $I^2$ statistic\textsuperscript{47,48} was used to detect and interpret heterogeneity among the studies included in each analysis. Specifically, the value of $I^2$ determined whether heterogeneity was possibly unimportant (0\%-40\%), moderate (30\%-60\%), substantial
The possible presence of publication bias was assessed using funnel plots, which graph the individual study effect estimates against their SEs, whereby a relatively symmetrical inverted funnel shape indicates a lower likelihood of bias. Funnel plots were not provided for analyses of <10 studies as the plots could be misleading due to difficulty in assessing symmetry. If applicable, analyses were repeated with and without outliers.

The meta-analysis was performed using Review Manager 5.3 (Cochrane Collaboration, Copenhagen, DK).

### 2.7 Application of Hill’s Criteria

The Bradford Hill criteria for causation were applied to data from the systematic review and meta-analysis to evaluate the causal nature of the relationship between active smoking and each outcome (MS risk; CIS to CDMS; RRMS to SPMS). As Hill’s criteria are originally stated very generally, the criteria were further defined for purposes of the present review by adding specific parameters to be used to assess whether or not each criterion was satisfied:

1. **Strength**: The pooled effect estimate for each relationship was interpreted as a statistically significant strong (3.0-8.0), moderate (1.8-3.0), modest (1.4-1.7), or weak (1.1-1.3) relationship in the expected direction; a statistically non-significant effect estimate was interpreted as no relationship.

2. **Consistency**: ≥50% vs. <50% of all included studies for each association showed an effect of similar strength in the expected direction.
3. **Temporality**: ≥50% vs. <50% of all included studies for each association established a temporal direction by design.

4. **Biological Gradient**: ≥50% vs. <50% of tests for a dose-response relationship either showed a statistically significant trend or an apparent change in the magnitude of effect in the expected direction.

5. **Plausibility**: Possible mechanisms underlying each relationship, and statistically significant interactions between smoking and other MS risk factors existed vs. did not exist.

6. **Coherence**: The associations did not conflict with existing MS knowledge vs. conflict observed.

7. **Analogy**: Analogous relationships existed vs. did not exist.

In a scoring system created for this review, one point was given for each criterion satisfied; criteria not satisfied were given zero points. The number of criteria satisfied were summed together to produce an overall causation score for each association ranging from 0-7. A causation score of 7 or 6, 5 or 4, and ≤3 represented strong, moderate, and weak evidence, respectively, supporting a cause-and-effect association. A similar evaluation process using different scoring systems has been previously employed by other systematic reviews and meta-analyses assessing other relationships.\(^{39,40}\)

From the original nine criteria, the Specificity criterion was deemed non-applicable to the current review given that there are known relationships between smoking and other diseases besides MS.\(^{53,54}\) Furthermore, the Experimental Evidence criterion was non-applicable since studying smoking as a cause of disease under controlled conditions is unethical.
3. Results

3.1 Search Results

The search strategy initially produced 1870 citations. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram illustrating the study selection process is presented in Figure 1. In terms of the level of inter-rater reliability achieved, the two reviewers had 90% agreement on the selection of titles and abstracts. After discussion of selection disagreements, full consensus was reached. Following the exclusion of irrelevant titles, abstracts, and full-text articles, 56 studies were left to be included in the review (publication dates 1965-2015). Specifically, the studies were included in the relevant quantitative and/or qualitative synthesis, depending on the area of investigation in each study. The following meta-analyses were included in the current review: 1) Smoking vs. MS risk; 2) Smoking vs. CIS to CDMS; 3) Smoking vs. RRMS to SPMS. The following qualitative analyses were included: 1) Smoking vs. CIS to CDMS; 2) Smoking vs. RRMS to SPMS; 3) Passive and prenatal smoke exposure vs. MS risk; and 4) Risk factor interactions. Appendix D provides a list of study references included under each analysis. Studies listed in the forest plots are not referenced in this manuscript if not discussed in the manuscript, but are listed with the other included studies in Appendix D. Characteristics of the studies included in each meta-analysis, such as the study design, sample sizes, and details pertaining to the population groups, exposure and outcomes of interest, are provided in Appendix E. There were 66 full-text articles excluded from the review, with reasons for exclusion listed in Figure 1. Appendix F provides a list of excluded full-text study references and corresponding reasons for their exclusion.
Figure 1. Flow diagram of study selection process depicting number of studies identified, excluded (including titles, abstracts, full-text articles), and included in review
CIS = clinically isolated syndrome; CDMS = clinically definite multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis
3.2 Smoking and MS Risk

The meta-analysis of the association between smoking and MS risk included 36 studies. Case-control and cross-sectional studies contributed 21,996 cases and 9,199,028 controls; another 764 cases developed in a population of 562,854 individuals contributed by cohort studies.

The conservative analysis included 23 studies in which smoking was considered before MS, and showed a statistically significant association between smoking and MS risk (OR/RR 1.54, 95% CI [1.46-1.63]; Figure 2) with statistically non-significant heterogeneity, $\chi^2(22)=21.20$, $p=0.51$; $I^2 = 0\%$. Of the total weight, the large study by Hedstrom et al., 2013\textsuperscript{14} took 32.9\%, likely due to its narrow 95% CI of 1.36-1.65. The non-conservative analysis including all 36 studies produced similar results (OR/RR 1.46, 95% CI [1.32-1.61]; Figure 3), with the exception of demonstrating substantial heterogeneity, $\chi^2(35)=137.20$, $p<0.00001$; $I^2 = 74\%$. Compared to the conservative analysis, the total weight was distributed more evenly across the individual studies included in the non-conservative analysis, likely due to the inclusion of more studies. The funnel plots for the conservative (Figure 4) and non-conservative (Figure 5) analyses were generally symmetrical, indicating that publication bias was unlikely.
Figure 2. Meta-analysis and forest plot: Relationship between smoking and MS risk (conservative*)
IV = inverse variance; CI = confidence interval
Studies identified by first author and year. *Includes 23 studies where smoking considered before MS onset
### Figure 3. Meta-analysis and forest plot: Relationship between smoking and MS risk (non-conservative*)

IV = inverse variance; CI = confidence interval  
Studies identified by first author and year. *Includes all 36 studies
Figure 4. Funnel plot: Conservative* analysis, association of smoking with MS risk
OR = odds ratio; SE = standard error
*Includes 23 studies where smoking considered before MS onset
Figure 5. Funnel plot: Non-conservative* analysis, association of smoking with MS risk
OR = odds ratio; SE = standard error
*Includes all 36 studies
The subgroup analyses presented in Table 1 showed no statistically significant differences among study designs, MS diagnostic methods, and methods for ascertaining self-reported smoking; however, the risk of MS was greater among studies comparing ever-smokers with never-smokers than among those comparing current smokers with past and never-smokers in combination (p=0.02). There was one outlier identified among the studies included in the non-conservative analysis whose effect estimate was especially distant from the other estimates. Repeating the non-conservative and subgroup analyses without this outlier did not change the overall results.

Ten studies included in the meta-analysis presented a dose-response relationship between smoking and MS risk either in the form of a statistically significant trend or change in effect estimates in the expected direction; however, three additional studies that tested for a dose-response relationship were not able to demonstrate it.

Based on the GRADE approach, the overall quality of evidence for MS risk was low (Table 2). While this assessment could have been upgraded from low to moderate quality due to the presence of a dose-response relationship, there were serious study limitations that prevented upgrading. One example is the inclusion of studies in which the large majority had a retrospective case-control design that is prone to recall bias, a limitation highlighted in several of the studies themselves. Other examples of study limitations include the considerable use of smoking data based on self-report, as well as either a low response/participation rate or a noticeably different rate between cases and controls as demonstrated by some studies.
Table 1. Smoking-MS risk effect estimates with 95% CIs for study subgroups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Study Count</th>
<th>OR/RR</th>
<th>95% CI</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case-control</td>
<td>28</td>
<td>1.50</td>
<td>1.38-1.64</td>
<td></td>
</tr>
<tr>
<td>Cohort</td>
<td>4</td>
<td>1.52</td>
<td>1.27-1.81</td>
<td>0.93</td>
</tr>
<tr>
<td>Cohort</td>
<td>4</td>
<td>1.52</td>
<td>1.27-1.81</td>
<td></td>
</tr>
<tr>
<td>Case-control/cross-sectional</td>
<td>32</td>
<td>1.45</td>
<td>1.30-1.62</td>
<td>0.69</td>
</tr>
<tr>
<td>Ever vs. never smoking</td>
<td>28</td>
<td>1.57</td>
<td>1.46-1.69</td>
<td></td>
</tr>
<tr>
<td>Current vs. past and never smoking</td>
<td>8</td>
<td>1.11</td>
<td>0.83-1.49</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking ascertainment using medical records/interview</td>
<td>20</td>
<td>1.42</td>
<td>1.28-1.57</td>
<td></td>
</tr>
<tr>
<td>Smoking ascertainment using questionnaire</td>
<td>15</td>
<td>1.50</td>
<td>1.27-1.78</td>
<td>0.56</td>
</tr>
<tr>
<td>MS diagnostic criteria</td>
<td>26</td>
<td>1.51</td>
<td>1.39-1.65</td>
<td></td>
</tr>
<tr>
<td>Other diagnostic methods</td>
<td>7</td>
<td>1.30</td>
<td>0.87-1.94</td>
<td>0.47</td>
</tr>
</tbody>
</table>

OR = odds ratio; RR = risk ratio; CI = confidence interval

*χ² test for differences between subgroups. p<0.05 statistically significant difference between subgroups
Table 2. Quality of evidence evaluation for MS risk and MS progression outcomes using the GRADE approach

<table>
<thead>
<tr>
<th>OUTCOME</th>
<th>GRADE FACTORS</th>
<th>QUALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study Limitations*</td>
<td></td>
</tr>
<tr>
<td>MS risk</td>
<td>Serious limitations (-1)†</td>
<td>++, low</td>
</tr>
<tr>
<td></td>
<td>Inconsistency Way</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indirectness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imprecision</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Publication Bias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Factors That Increase Quality</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quality</td>
<td></td>
</tr>
<tr>
<td>CIS→CDMS</td>
<td>Serious limitations (-1)‡</td>
<td></td>
</tr>
<tr>
<td>RRMS→SPMS</td>
<td>Serious limitations (-1)§</td>
<td></td>
</tr>
</tbody>
</table>
| GRADE = Grading of Recommendations Assessment, Development, and Evaluation; CIS = clinically isolated syndrome; CDMS = clinically definite multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; CI = confidence interval

*All outcomes: Each outcome contained at least one retrospective study (e.g., case-control, retrospective cohort), most studies relied on self-reported smoking data, many studies did not specify any blinding procedures used in the exposure or outcome assessments (where appropriate).

†Limitations include those that apply to all outcomes, and the following that apply to this outcome only: Self-reported MS diagnosis in one study, either response/participation rate < 80% or rate notably different between cases and controls in nine studies, > 20% lost to follow-up in one study, type of smoking ascertainment not stated in one study, MS diagnostic method not stated in three studies.

‡Limitations include those that apply to all outcomes, and the following that apply to this outcome only: Source of cohort not stated in one study, MS diagnostic method not stated in one study.

§Limitations include those that apply to all outcomes, and the following that apply to this outcome only: Source of cohort not stated in one study, response/participation rate < 80% in two studies, > 20% lost to follow-up in one study.

‖95% CI in meta-analysis included no effect and notable benefit and harm.

¶While not including no effect, 95% CI in meta-analysis conveyed borderline significance up to notable harm; many individual studies produced wide CIs.
3.2.1 Risk Factor Interactions

In addition to the 36 smoking-MS risk studies, four studies specifically investigated interactions between MS risk factors, and some of the 36 studies conducted further analyses on interactions pertaining to MS risk. While there were studies that demonstrated no statistically significant interactions between smoking and both EBV\textsuperscript{68-70} and HLA alleles,\textsuperscript{60,69,70} others showed that these risk factors interacted with smoking.\textsuperscript{60,71} Recent studies also showed significant interactions between smoking and both non-consumption of alcohol\textsuperscript{72} and non-HLA alleles.\textsuperscript{67}

3.3 Smoking and Conversion from CIS to CDMS

The meta-analysis of the effect of smoking on the conversion from CIS to CDMS included three studies comprised of 755 CDMS patients developed from 1261 CIS patients. The analysis showed a statistically non-significant association (HR 1.13, 95\% CI [0.73-1.76]; Figure 6) with substantial heterogeneity, $\chi^2(2)=7.68$, $p=0.02$; $I^2 = 74\%$.

Two prospective cohort studies were not included in the meta-analysis due to insufficient data to calculate the unreported HR or other appropriate statistics,\textsuperscript{75} or calculation of the required statistics from provided data\textsuperscript{19} not performed for this meta-analysis due to statistical methods previously cited as likely being less reliable than other methods.\textsuperscript{76} One of these studies using self-reported smoking data from 125 CIS patients showed that smoking increased the risk of progression from CIS to CDMS.\textsuperscript{19} However, the second study of 194 CIS patients used cotinine as a measure of smoke exposure and showed that smoking had no effect.\textsuperscript{75}
Figure 6. Meta-analysis and forest plot: Relationship between smoking and conversion from CIS to CDMS

CIS = clinically isolated syndrome; CDMS = clinically definite multiple sclerosis; IV = inverse variance; CI = confidence interval

Studies identified by first author and year.
Only one study investigated a possible dose-response effect for the conversion from CIS to CDMS, and found none.\textsuperscript{23} Due to individual study limitations, including important study characteristics not stated in some studies,\textsuperscript{19,75} as well as inconsistent results across studies and imprecise meta-analysis results, the quality of evidence for the progression from CIS to CDMS was downgraded from low to very low (Table 2).

### 3.4 Smoking and Conversion from RRMS to SPMS

The meta-analysis of the association of smoking with SPMS risk included five studies comprised of 379 SPMS patients developed from 1837 RRMS patients. The analysis yielded an association just achieving statistical significance (HR 1.80, 95% CI [1.04-3.10]; Figure 7) with substantial heterogeneity, \( \chi^2(4)=12.68, p=0.01; I^2 = 68\% \).

Two prospective cohort studies were not included in this meta-analysis. As previously noted in one case, potentially unreliable methods for calculating the required statistics (e.g., HR) were avoided.\textsuperscript{19} The second study for this outcome measured the association between smoking and the conversion from RRMS to SPMS per pack year smoked\textsuperscript{77} rather than comparing smoking and non-smoking as in all other studies. Nonetheless, both studies demonstrated a statistically significant effect of smoking on the conversion from RRMS to SPMS by either comparing smokers and non-smokers in 203 RRMS patients,\textsuperscript{19} or per pack-year smoked in 148 RRMS patients.\textsuperscript{77}
**Figure 7.** Meta-analysis and forest plot: Relationship between smoking and conversion from RRMS to SPMS

RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; IV = inverse variance; CI = confidence interval

Studies identified by first author and year.
While one study did not demonstrate a dose-response relationship between smoking and SPMS risk, three studies showed evidence of a dose-response relationship. However, the quality of evidence for the association between smoking and SPMS risk was downgraded from low to very low given the presence of study limitations, as well as inconsistent and imprecise results (Table 2). Some examples of study limitations include the use of self-reported smoking data and a low response/participation rate as shown by some studies.

### 3.5 Assessment of Causality Using Hill’s Criteria

An evaluation of the causal nature of the relationship between active smoking and both MS risk and MS progression outcomes using Hill’s criteria is displayed in Table 3. Based on data from the systematic review and meta-analysis, and the scoring protocol developed for this review, there is strong evidence supporting a causal association between smoking and MS risk (causation score of 6 out of 7), while there is only moderate evidence of a causal role of smoking in the progression from CIS to CDMS (4/7) and RRMS to SPMS (5/7).
Table 3. Causality evaluation of the association of smoking with MS risk and MS progression outcomes using Hill’s criteria

<table>
<thead>
<tr>
<th>Hill’s Criteria</th>
<th>MS Risk</th>
<th>CIS→CDMS</th>
<th>RRMS→SPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Consistency</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Temporality</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Biological Gradient</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Plausibility</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Coherence</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Analogy</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Causation Score</strong></td>
<td><strong>6</strong></td>
<td><strong>4</strong></td>
<td><strong>5</strong></td>
</tr>
<tr>
<td><strong>Interpretation</strong></td>
<td><strong>Strong evidence of causality</strong></td>
<td><strong>Moderate evidence of causality</strong></td>
<td><strong>Moderate evidence of causality</strong></td>
</tr>
</tbody>
</table>

CIS = clinically isolated syndrome; CDMS = clinically definite multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis
3.6 Passive/Prenatal Smoke Exposure and MS Risk

Given that passive and prenatal smoke exposure may play a potential role in MS risk, the relationship between second-hand smoke exposure and MS risk was briefly explored. The association between passive smoke exposure and MS risk was examined by eight studies, with mixed results. Four studies did not show a statistically significant relationship between passive smoking and MS, including three with a case-control design that investigated exposure primarily among adults,14,30,79 and one that specifically investigated childhood exposure in a cohort of female nurses.80 Among the four remaining case-control studies that showed a statistically significant relationship between passive smoking and MS, one examined exposure strictly among children,81 one used cotinine as a marker of smoke exposure,61 and two demonstrated a statistically significant dose-response relationship between duration of passive smoke exposure and MS.26,27

Four studies examined the relationship between maternal prenatal smoke exposure and MS risk in offspring. One case-control study involving 35 hospitalized MS cases with smoking data demonstrated a strong association.28 The other three studies did not show a statistically significant relationship, including two larger case-control studies,29,30 and a large prospective cohort study.80 One study examined a possible dose-response effect, but did not demonstrate that MS risk increased with cumulative number of cigarettes smoked by the mother.29

4. Discussion

This systematic review and meta-analysis examined the relationship between smoking and both MS risk and MS progression (i.e., CIS to CDMS, and RRMS to
SPMS), and subsequently used the Bradford Hill criteria to further evaluate causation. Overall, a statistically significant association exists between smoking and both MS risk and the conversion from RRMS to SPMS. However, there is a statistically non-significant association between smoking and the progression from CIS to CDMS. Based on data from the review, as well as the application of Hill’s criteria, there is strong evidence of a causal relationship between smoking and MS risk, but only moderate evidence of a causal role of smoking in MS progression.

While the findings were generally consistent with those from the narrative reviews that also used Hill’s criteria, the present review involved a more rigorous and objective methodology to review all relevant studies and apply Hill’s criteria to the most reliable study findings to evaluate causality. Overall, the large majority of studies included for each of the three outcomes firmly established a temporal direction from smoking to the outcome of interest, which is necessary to infer causation. In this review, temporality was established in many studies either through the use of a prospective cohort design, or smoking was considered prior to MS onset in case-control and cross-sectional designs.

4.1 Smoking and MS Risk

Based on the meta-analysis, smoking led to a modest 50% increase in MS risk in comparison to non-smoking, which is in agreement with previous meta-analyses. The conservative and non-conservative analyses yielded similar results, both of which showed a statistically significant relationship between smoking and MS risk. In addition, the possibility of publication bias was unlikely given that the funnel plots for both analyses
were, for the most part, symmetrical, lending further weight to the results. Moreover, the majority of individual studies that tested for a dose-response relationship provided evidence of such a gradient in the expected direction, thus supporting a causal role of smoking in MS. In general, studies showed that MS risk increased with greater duration of smoking, higher levels of cotinine, cumulative numbers of cigarettes smoked daily, cumulative numbers of packs smoked daily, and greater pack-years smoked as evidenced by a greater MS risk among heavier smokers.

There was substantial heterogeneity in the non-conservative analysis, meaning that most of the variation in the individual effect estimates was more likely the result of true differences as opposed to chance alone. Using a subgroup analysis to explore the possible sources of heterogeneity, it was found that different study designs, MS diagnostic methods, and methods for ascertaining self-reported smoking had no statistically significant effect on the association between smoking and MS risk. However, an approximately 2.5 times greater MS risk was observed among the larger group of studies that compared ever-smokers with never-smokers than among the studies that compared current smokers with the combination of past and never-smokers. Classifying a past smoker as a non-smoker may lead to a weaker association between smoking and MS given that those who have quit smoking are still at a higher risk of MS in comparison to non-smokers. It is also likely that some heterogeneity resulted from the one outlying study included in the non-conservative analysis, which had no major effect on the overall results. Despite being cross-sectional, its results suggested a statistically significant protective effect of smoking on MS risk. On the other hand, the findings showed that heterogeneity was likely unimportant in the conservative analysis, whereby
virtually all included studies compared ever-smokers with never-smokers, and the outlier had been removed. Overall, the estimates in the conservative analysis were consistent in that a modest effect of smoking on MS risk was evident across the majority of studies, and all studies demonstrated an effect in the expected direction. This consistency provides additional evidence of a causal role of smoking in MS risk.

4.2 Smoking and MS Progression

Regarding MS progression, the meta-analysis showed that smoking contributed to a moderate 80% increase in SPMS risk compared to non-smoking, which is in close agreement with a previous meta-analysis. However, while the relationship in the previous review was statistically non-significant, the relationship in the current review was statistically significant, likely due to the inclusion of a more recent study showing a statistically significant relationship between smoking and SPMS risk. The pooled effect estimate in the current review was corroborated by the results from the two additional studies not included in the meta-analysis that also demonstrated a statistically significant association between smoking and the progression from RRMS to SPMS. It was also supported by evidence of a dose-response relationship between secondary progression and the number of cigarettes smoked daily and pack-years smoked as evidenced by tests for such a relationship, providing further evidence of causality.

On the other hand, the meta-analysis showed no statistically significant relationship between smoking and the conversion from CIS to CDMS, and no individual studies provided evidence of a dose-response gradient. Mixed results were evident in the two studies not included in the meta-analysis as one study showed a statistically
significant effect of smoking,\textsuperscript{19} while the second study did not,\textsuperscript{75} further confirming that insufficient evidence exists to support a causal role of smoking in the progression from CIS to CDMS.

While the MS progression outcomes exist along the same disease continuum, their results differed in this review. Since MS presents more neurological damage (i.e., characterized by multiple regions of CNS demyelination) compared to CIS (i.e., characterized by a first episode of CNS demyelination),\textsuperscript{82} it is possible that the greater damage could make RRMS patients more susceptible to the harms of cigarette smoke compared to CIS patients. Alternatively, results may have been impacted by the use of different study methods between the two outcomes, such as the smoking status under study (e.g., predominantly current smokers for CIS to CDMS, and ever-smokers for RRMS to SPMS), methods for ascertaining smoking (e.g., self-report for both outcomes, but measures of cotinine included for CIS to CDMS as well), and confounders controlled (e.g., CIS symptoms and lesions for CIS to CDMS, and disease duration and relapses for RRMS to SPMS).

Overall, the meta-analysis for both progression outcomes was based on a small number of studies, and as such, funnel plots were not generated given their potential to be misleading in such circumstances.\textsuperscript{51} As such, there is a possibility that publication bias exists, meaning that published studies in this area, especially in the area of smoking and SPMS risk, may have been more likely to show an effect, while studies not showing an effect may have failed to be published.\textsuperscript{50} Furthermore, the meta-analysis for both outcomes yielded substantial heterogeneity across studies, indicating that most of the differences in the effect estimates were not likely the result of chance alone.\textsuperscript{47} Instead,
they may have been due to variation in the setting, study design, population groups, and type of smoke exposure measures used in the individual studies. As a result, the findings were generally inconsistent across the studies included for each outcome. The strength of the association between smoking and SPMS risk varied considerably from weak, to moderate, to very strong. Similarly, alternate directions of an effect and levels of statistical significance were observed among the individual estimates and statistical tests for the association between smoking and the progression from CIS to CDMS. Overall, these factors make it difficult to reach a definite conclusion for the two progression outcomes regarding the causal role of smoking.

4.3 Mechanisms, Interactions, Analogies Relevant to the Smoking-MS Association

The relationship between smoking and both MS risk and MS progression is plausible and coherent given that there are possible biological mechanisms underlying each relationship, and the relationships are consistent with what is already known about MS. This provides some evidence of a causal role of smoking towards these outcomes. Mechanistically, it has been shown that nicotine increases the permeability of the blood-brain barrier,\textsuperscript{83} which may represent an early step in MS.\textsuperscript{84} Other chemical components of cigarette smoke may also be involved. For instance, it has been found that cyanide contributes to CNS demyelination,\textsuperscript{85} and nitric oxide has been linked to degeneration and conduction blockage of axons,\textsuperscript{86} axonal loss is representative of MS progression.\textsuperscript{87} There is also evidence of statistically significant interactions between smoking and known MS risk factors, including HLA alleles and EBV. Specifically, smoking may elicit autoimmunity in those who are genetically susceptible.\textsuperscript{88} However, the interaction
between smoking and HLA may not always occur, depending on the presence of other genetic factors. For instance, while an interaction was found between smoking and the HLA-DRB1*15 allele, it was only observed among those without the more protective HLA-A*02 allele.\(^71\) Regarding EBV, both smoking and EBV infection have been shown to stimulate cellular activity that may play a critical role in MS development.\(^60\) However, the interaction between smoking and EBV may be affected by age given the evidence of a negative interaction among younger subjects\(^68\) and a positive interaction among older subjects,\(^60,68\) again suggesting that an interaction may only occur under certain circumstances.

Relationships analogous to the association between smoking and both MS risk and MS progression exist, as shown by research demonstrating a relationship between smoking and other autoimmune diseases. Specifically, associations have been found between smoking and rheumatoid arthritis, Crohn’s disease, and ulcerative colitis risk,\(^89\) as well as increased risk of systemic lupus erythematosus\(^90\) and Graves’ disease.\(^91\) It was also found that smoking is associated with disease severity in several autoimmune disorders.\(^91,92\) These analogies provide further evidence to support causality.

### 4.4 Passive/Prenatal Smoke Exposure and MS Risk

The relationship between both passive and prenatal smoke exposure and MS risk was briefly explored to draw attention to other factors that could also play an important role in MS. Regarding passive smoke exposure, results were mixed and heterogeneity existed among the studies in terms of age groups studied and types of smoking data used. Likewise, there were mixed findings from two systematic reviews and meta-analyses.
regarding whether or not a statistically significant relationship existed.\textsuperscript{34, 35} This area of research would benefit from additional studies and a subsequent subgroup analysis to determine if the relationship between passive smoking and MS only exists in certain circumstances. When it comes to maternal prenatal smoke exposure, the evidence thus far points to no association with MS risk. However, additional studies need to be conducted with a larger and more representative sample of MS cases prior to conducting an informative meta-analysis.

**Strengths and limitations.** The current review used a comprehensive search strategy, and included several databases, reducing the likelihood of missing important studies. Another strength of the review was the inclusion of a thorough quality assessment (GRADE) of included studies, allowing for a more meticulous evaluation of study evidence. Furthermore, distinguishing itself from previous systematic reviews and meta-analyses, the current review used Hill’s criteria to evaluate causation. This evaluation, in turn, allowed for a more complete examination of important aspects of the association between smoking and both MS risk and progression based on all relevant studies.

While the association between smoking and MS risk was based on many studies that included a large sample of participants, the number of studies included in the analysis of MS progression was comparatively small. As such, the results pertaining to MS progression should be taken with caution. In addition, the quality of study evidence for each outcome was either low or very low. However, this can be largely attributed to the observational nature of studies examining the health effects of smoking, which is unavoidable given that such an investigation under controlled conditions is unethical (i.e.,
one cannot force participants to smoke in order to study its effects). As such, an initial quality grade of low is unavoidable. Furthermore, it may be challenging to improve this initial grade, especially given a GRADE factor such as study limitations, which can be difficult for study authors to avoid. One such limitation was the use of self-reported smoking data in most individual studies included in the review. However, while it has been shown that self-reported smoking habits tend to be underreported, such data is primarily relied upon in tobacco surveillance. Given such circumstances, the low and very low quality of evidence grade determined by the review should not be interpreted to mean that evidence was derived from poorly conducted studies. In fact, rather than being solely based on the methodological execution of individual studies, the grade was largely based on the collective examination of studies to evaluate a group of highly diverse factors (e.g., the inconsistency of effect estimates across studies, imprecision of effect estimates conveyed by the 95% CI in the meta-analysis, publication bias detected using funnel plots, dose-response relationship based on its frequency in studies). As such, despite the lower grades, the evidence obtained from the present review is essential in determining the causal role of smoking in MS.

Conclusions and implications. There is strong evidence of a causal role of smoking in MS risk. However, this review suggests only moderate evidence of a causal relationship between smoking and MS progression, namely from CIS to CDMS and RRMS to SPMS. Overall, this review supports the addition of MS to the list of the more commonly known health outcomes related to smoking, such as the development and advancement of heart and lung diseases. As such, anti-smoking government policies and
smoking cessation programs should underscore the risk of developing MS and other neurological disorders as a further means of discouraging the public to smoke.
5. References


CHAPTER 4: General Summary and Future Directions

The primary objective of this research was to examine the association between smoking and MS risk and MS progression through a systematic review and meta-analysis, and subsequently use the Bradford Hill criteria\(^1\) to further and more comprehensively evaluate causality. The results from this review suggest a statistically significant association between smoking and both MS risk and the progression from RRMS to SPMS; however, the relationship between smoking and the conversion from CIS to CDMS was statistically non-significant. Overall, there is strong evidence of a causal role of smoking in MS risk, and moderate evidence of a causal relationship between smoking and MS progression. However, it is unlikely that smoking plays an independent role in MS.

Rothman’s Model of Necessary and Sufficient Causes\(^2\) was used as the theoretical framework for this systematic review and meta-analysis as it offers a clear depiction of chronic disease causation. Past research suggests that MS is likely a multi-causal process in that it involves an interaction between a combination of genetic and environmental factors.\(^3\) As such, Rothman’s model is well suited to illustrate MS causation.

A depiction of two possible causal pathways involving MS risk factors is presented in Figure 4-1 (others may exist). Research has shown an interaction between smoking and the HLA-DRB1*15 allele, but only among those individuals without the more protective HLA-A*02 allele.\(^4\) In addition, a positive interaction between smoking and EBV was observed, but only among older individuals.\(^5\) Figure 4-1 illustrates two possible sufficient causes of MS (I, II), each consisting of different combinations of component causes (e.g., A, B, C, etc.) potentially including smoking. If a component
Figure 4-1. Potential causal pathways for MS using currently suspected component causes. Adapted from “Causes,” by K. J. Rothman, 1976, Am J Epidemiol, 104(6), p. 589. EBV = Epstein-Barr Virus; HLA = Human Leukocyte Antigen; ? = unknown component cause
cause is removed from one of the sufficient causes, such as having the protective HLA-A*02 allele, being a younger age, or not smoking, the remaining components may become insufficient for causing MS. Given that not everyone who possess all the known component causes in either sufficient cause develops MS, it is very likely that other unknown yet to be discovered component causes also play a role in these sufficient causes (e.g., ? in Figure 4-1). Further research on MS risk factors is pertinent to the discovery of such component causes, as well as the discovery of other possible causal pathways in MS. For instance, in a pediatric study, a mere 5% of children without three MS risk factors—HLA-DRB1*15, EBV, low vitamin D levels—had an MS diagnosis as opposed to 57% of children who had the three risk factors. While interactions between the risk factors were not found, the study itself highlighted the possibility of detecting the interactions with a larger sample. Overall, future research should include a more in depth investigation of the role of smoking in MS, while additional studies investigating interactions between risk factors are also needed. These investigations would be critical steps hopefully contributing to MS prevention.

In retrospect, this research provided a valuable opportunity to collate and extensively analyze a large number of studies relevant to the association between smoking and MS, enhancing the reliability of the results. Furthermore, a variety of research skills were acquired and practiced as a result of completing such an in depth meticulous and comprehensive review, including skills in database searching, compilation of evidence, critical thinking and analysis, and meta-analytic techniques. A limitation of this project was the involvement of only one primary reviewer, which may have resulted in a higher risk of errors in study selection and data extraction; however,
reliability checks and consultations to address uncertainties were done with a 2\textsuperscript{nd} reviewer to limit these errors and increase both the internal and external validity of the review. In addition, there were times when the volume of work was overwhelming for one reviewer, and as such, certain steps in the review took longer than originally anticipated. Should the opportunity to conduct another systematic review and meta-analysis present itself, the ideal is to undertake such a project as part of a research team, both in terms of reducing bias and in terms of conducting the review in a more time-efficient manner.
4.1 References


**APPENDIX A: Title, Abstract, and Full-Text Screening Questions Specific to Thesis**

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<td>Move to Question 2</td>
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<td>2. Does the study investigate MS?</td>
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<td>Move to Question 3</td>
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<td>3. Does the study investigate smoking?</td>
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# APPENDIX B: Studies Included in Smoking-MS Risk Meta-Analysis and Corresponding Effect Estimates

<table>
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<tr>
<th>First Author, Year</th>
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<td>Al-Afasy, 2013</td>
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<td>Antonovsky, 1965</td>
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<td>Bjornevik, 2015</td>
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<td>Carlens, 2010</td>
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<td>Gustavsen, 2014</td>
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<td>Current smoker: 1.6 RR [1.2-2.1]†</td>
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<td>Zorzon, 2003</td>
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OR = odds ratio; RR = risk ratio
*Unadjusted, otherwise adjusted
†Estimates initially combined in separate meta-analysis to derive average summary effect for input into main meta-analysis
### APPENDIX C: GRADE Approach

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<th>Factor</th>
<th>Description</th>
<th>Grade Change</th>
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<td>1. Study Limitations</td>
<td>Limitations in observational studies include: Selection bias, measurement bias (exposure and outcome), lack of proper confounder control, loss to follow-up</td>
<td>Serious (-1)/very serious (-2) limitations in design and execution (risk of bias)</td>
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<td>2. Inconsistency</td>
<td>Differences in effect estimates across studies, which might be due to different populations, exposures, or outcomes</td>
<td>Serious (-1)/very serious (-2) unexplained heterogeneity or inconsistency</td>
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<td>3. Indirectness</td>
<td>Variability in populations, exposures, comparators, and outcomes of interest to review authors and those available from the evidence</td>
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<td>4. Imprecision</td>
<td>Studies have a small sample size and a small number of events, and therefore, CIs are wide; the 95% CI does not show an effect as well as conveys considerable benefit or harm</td>
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<td>5. Publication Bias</td>
<td>Researchers do not disclose their studies, especially those that do not show an effect</td>
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<td>6. Large Effect Estimate</td>
<td>Large (RR &gt; 2.0 or &lt; 0.5) or very large (RR &gt; 5.0 or &lt; 0.2) and consistent effect estimates are produced by observational studies of high methodological quality</td>
<td>Large (+1)/very large (+2)</td>
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<td>7. Dose-Response Relationship</td>
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<td>8. Residual Confounding</td>
<td>Possible confounders or biases would lower an observed effect, or produce a false effect when results do not show an effect</td>
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CI = confidence interval; GRADE = Grading of Recommendations Assessment, Development, and Evaluation; RR = risk ratio

APPENDIX D: Included Studies in Each Quantitative and Qualitative Analysis

1. Smoking and MS Risk (Meta-Analysis)


**Duplicate data excluded**


**Duplicate data excluded**


**Duplicate data excluded**


2. **Risk Factor Interactions (Qualitative)**


   **Duplicate data excluded**


   **Duplicate data excluded**


   **Duplicate data excluded**


**Duplicate data excluded**

3. **Smoking and Progression from CIS to CDMS (Meta-Analysis)**


4. **Smoking and Progression from CIS to CDMS (Qualitative)**


5. **Smoking and Progression from RRMS to SPMS (Meta-Analysis)**


6. **Smoking and Progression from RRMS to SPMS (Qualitative)**


7. Passive Smoke Exposure and MS Risk (Qualitative)


   **Duplicate data excluded**


   **Duplicate data excluded**


8. **Maternal Prenatal Smoke Exposure and MS Risk (Qualitative)**


To determine whether smoking, in combination with other risk factors, increases the risk of MS, a case-control study was conducted. The study included 101 cases and 202 non-smoking controls, matched for age, sex, and ethnicity. Cases were recruited from the Oslo MS Registry, and controls were selected from the general population. Smoking status was confirmed through self-report.

Results indicated a significant association between smoking and the risk of MS, with an odds ratio of 1.29 (95% CI 1.03-1.60). Smoking appeared to be a stronger risk factor for RRMS (odds ratio 1.42) compared to SPMS (odds ratio 0.86).

Conclusion: Smoking is a significant risk factor for MS, with a higher risk for RRMS than SPMS. Further research is needed to understand the mechanisms behind this association and to develop strategies to reduce smoking as a risk factor for MS.
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| USA         | Prospective cohort | To determine the extent to which smoking and smoking-never status are associated with MS, neurological symptoms, and other factors | University of Texas MS patients; Boston University MS patients /
| USA         | Prospective cohort | To determine the relationship between smoking and MS risk and MS patients controls | Blood donors from local blood transfusion centre                                           | Blood donors from local blood transfusion centre controls |
| USA         | Prospective cohort | To determine the relationship between smoking and MS risk and MS patients controls | University of Texas MS patients; Boston University MS patients                            | University of Texas MS patients; Boston University MS patients controls |

**Notes:**
- **RRMS:** Relapsing-remitting MS
- **SPMS:** Secondary progressive MS
- **PPMS:** Primary progressive MS
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<th>Reference</th>
<th>Study Design</th>
<th>Aim</th>
<th>Methods</th>
<th>Results/Findings</th>
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APPENDIX F: Excluded Full-Text Studies, with Corresponding Reasons

1. Smoking Not Investigated


2. Smoking Not Investigated as Independent Variable in Interaction


3. Effects of Smoking on MS Not Reported


4. **Impossible to Isolate Effects of Smoking**


5. **Participants Matched on Smoking**


6. **Outcomes of Interest Not Investigated**


7. **Diagnosed MS Not Investigated**


8. **Unoriginal Research Study**


9. **Ecological Study**


10. **No Comparator Group**


11. **Unhealthy Control Group**


12. **Duplicate Data**


13. **Comparison with Previously Published Data**